Department of Earth and Environmental Sciences (DISAT)

PhD program in Marine Sciences, Technology and Management (MTM)

Cycle XXXVIII

OCEAN BIOPROSPECTING: EXPLORING THE PHARMACEUTICAL POTENTIAL OF TROPICAL MARINE ORGANISMS

Phytochemical composition and biological activities of the mangroves *Sonneratia caseolaris* and *Avicennia marina*

CERRI FEDERICO

Registration number 830602

Tutor: Prof. GALLI PAOLO

Co-tutor: Prof. COLOMBO MIRIAM

Coordinator: Prof. GALLI PAOLO

ACADEMIC YEAR 2024/2025

Finanziato dall'Unione europea NextGenerationEU

Ministero dell'Università e della Ricerca

Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA

ABSTRACT

Mangroves thrive at land-sea interface, combining the bioprospecting potential of both terrestrial plants and marine environments. Their ability to withstand extreme environmental conditions is potentially linked to the producing of stress-induced secondary metabolites with unique and rare structures. Despite their ethnomedicinal significance, mangroves remain neglected in natural product research and drug discovery, with phytochemical and pharmacological investigations still limited.

This thesis project explored the bioprospecting of two mangrove species from underexplored regions: *Avicennia marina* from the United Arab Emirates (UAE) and *Sonneratia caseolaris* from the Maldives. Both species hold ethnomedicinal relevance, yet detailed chemical and bioactivity data remain scarce, particularly from these regions, where unique environmental pressures may influence secondary metabolism. The study aimed to provide comprehensive phytochemical profiles of different plant parts, evaluate their antioxidant capacity, and, in the case of *A. marina*, assess cytotoxic potential against human cell lines. This integrative approach aims to identify the most promising extracts and link bioactivities to tissue-specific metabolites.

Hydroalcoholic extracts from roots, leaves, propagules, and cotyledons of *A. marina*, together with roots and leaves of *S. caseolaris*, were characterized using ultra-performance liquid chromatography coupled with high-resolution mass spectrometry (UPLC-HRMS). Antioxidant capacity was assessed through DPPH, ABTS, and ORAC, while the cytotoxic activity of *A. marina* extracts was evaluated via MTT assays against five human cancer cell lines, SW480 and E705 (colorectal cancer), MDA-MB-231 (triple-negative breast cancer), U-87 (glioblastoma), and HeLa (cervical cancer), and two normal cell lines (CCD841 and MRC-5).

Our literature review revealed that *A. marina* produces several classes of secondary metabolites and exhibits both antioxidant and anticancer potential. However, most previous studies have been geographically restricted and focused primarily on leaves. In this context, our work represents the first comprehensive phytochemical and pharmacological investigation of *A. marina* from the UAE, within the Arabian Gulf, a region characterized by high salinity, extreme temperature, and aridity that may drive distinctive metabolic adaptations. We identified 49 metabolites, mainly belonging to phenylethanoid glycosides, flavonoid glycosides, iridoid glycosides, and triterpene saponins, compound classes widely reported in *A. marina* from other regions and known for their ecological roles in protection against abiotic stress. In contrast, several compounds were newly reported for the species, particularly among phenylethanoid

glycosides and triterpene saponins, suggesting region-specific metabolic adaptations. The pericarp and root extracts exhibited the strongest antioxidant activity (DPPH: 187.14 ± 2.87 and 128.25 ± 1.12; ABTS: 217.16 ± 2.67 and 147.21 ± 2.42 µmol TE/g, respectively), correlating with their high phenylethanoid content. The root extract also displayed the highest cytotoxicity, particularly against MDA-MB-231, SW480, and E705 (IC_{50} values of 58.46, 81.98, and 108.10 µg/mL, respectively), while showing lower activity against normal cells. The triterpene saponins identified in roots, including medicoside G, esculentoside C, and azukisaponin III, are likely contributors to this activity, as supported by *in silico* predictions.

S. *caseolaris*, in contrast, has received far less attention in mangrove bioprospecting. Our literature review analysis revealed a rich and diverse chemical composition, along with consistently reported antioxidant activity. However, as with *A. marina*, existing studies have been geographically limited and focused mainly on leaves. In this context, we present the first detailed phytochemical characterization of *S. caseolaris* from the Maldives and, notably, the first analysis of its roots. A total of 45 metabolites were identified, mainly flavonoid glycosides, hydrolysable tannins, catechins, and phenolic acids. Most of these compounds, including two sulphated flavonoids typical of plants inhabiting swampy environments, are newly reported for this species, suggesting a unique phytochemical profile potentially shaped by the Maldivian habitat. Both leaf and root extracts exhibited strong antioxidant capacity in DPPH, ABTS, and ORAC assays, comparable to or exceeding that of ascorbic acid.

A comparative evaluation revealed distinct phytochemical patterns between the two species, reflecting both phylogenetic and ecological differences. Both species, however, shared a subset of flavonoid glycosides, highlighting their conserved role in protection against oxidative stress. Overall, this thesis expands the phytochemical and pharmacological knowledge of mangroves by characterizing two species from regions that remain understudied. More than twenty compounds identified here are newly reported for mangroves, reinforcing the untapped potential of these ecosystems for natural product research. The findings demonstrate that *A. marina* roots represent a promising source of cytotoxic compounds, while *S. caseolaris* extracts display significant antioxidant potential. Future investigations should focus on bioactivity-guided fractionation, quantification, mechanistic studies, and the evaluation of other bioactivities to validate these compounds as potential leads for *in vivo* testing.

CHAPTER 1

Introduction: mangroves as target for bioprospecting

1.1. GENERAL OVERVIEW OF MANGROVES

Mangroves are a specialized group of salt-tolerant woody plants that inhabit intertidal areas where seawater and freshwater mix. These ecosystems are distributed across 123 tropical and subtropical countries, predominantly within latitudes of 30° north and 30° south (Kandasamy and Bingham, 2001; Kerry et al., 2018). Mangroves species have evolved unique adaptations that enable them to survive in environments characterized by high and fluctuating salinity, high temperatures, periodic tidal inundation, storm surges, intense coastal winds, and oxygen-deficient soils. These adaptations span morphological, physiological, ecological, and reproductive traits (Kandasamy and Bingham 2001; Srikanth et al., 2016). Specialized aerial root systems, including pneumatophores, knee roots and stilt roots, function as breathing roots that facilitate gas exchange in anoxic substrates. Stilt roots also provide mechanical stability, anchoring the plants against tidal currents and monsoon-driven surges. Additionally, mangroves display vivipary, where propagules germinate while still attached to the parent tree, enhancing seedling survival in challenging intertidal conditions. Physiologically, mangroves regulate internal salt concentrations through mechanisms of salt exclusion at the root level and salt secretion via specialized leaf glands (Scholander 1968; Shi et al., 2005; Srikanth et al., 2016).

Globally, mangrove ecosystems are among the most biologically, ecologically, and economically important natural systems. They provide critical habitat and food resources for a variety of terrestrial, estuarine, and marine organisms. Furthermore, mangroves play a vital role in supporting adjacent ecosystems such as coral reef and seagrass meadows by facilitating nutrient and sediment exchange (Polidoro et al., 2010; Agardy et al., 2017). The ecosystem services provided by mangroves are manifold and have been valued at an estimated US$1.6 billion annually (Costanza et al., 1997; Zhang et al., 2018). These services include shoreline stabilization, mitigation of coastal erosion, buffering against extreme weather events like tsunami, and supporting fisheries, with approximately 80% of global fish catch relying on mangrove-associated habitats during some life stage. Additionally, mangroves are critical for carbon sequestration, capturing up to 25.5 million tonnes of carbon annually. They also contribute to nutrient cycling, detoxification of pollutants, and regulation of salinity balances withing coastal waters. Socio-economically, mangroves provide essential resources such as timber, firewood, medicinal compounds, and materials for constructions, alongside enhancing ecotourism and recreational opportunities (Eong, 1993; Dahdouh-Guebas et al., 2015; Das and

Vincent, 2009; Polidoro et al., 2010; Agardy et al., 2017; Spalding and Parrett, 2019; Cerri et al., 2022).

Mangrove species are broadly classified in two categories: 'true mangrove' and 'mangrove associates'. True mangrove species are strictly confined to intertidal mangrove habitats, whereas mangrove associates may occur in both mangrove habitats and adjacent terrestrial or freshwater environments (Parani et al., 1998; Polidoro et al., 2010). Tomlinson (1986) proposed stringent criteria for defining true mangrove species, emphasizing their exclusive occurrence in mangrove ecosystems, the presence of morphological adaptations such as aerial roots and vivipary, physiological mechanisms for salt exclusion and/or secretion, and distinct taxonomic separation from terrestrial relatives (Wang et al., 2010; Kandasamy and Bingham, 2001). Additionally, Duke (1992) characterized a true mangrove species as a tree, shrub, palm or ground fern exceeding 0.5 m in height, and which normally grows above mean sea level in the intertidal zone of coastal or estuarine environments (Polidoro et al., 2010). Kandasamy and Bingham (2001) after reviewing historical classifications, recognized 65 true mangrove species distributed across 22 genera and 16 families worldwide. However, for several species, their classification remains a subject of scientific debate. Wang et al. (2010) referred to these as 'controversial' species, which lack a universal consensus regarding their placement as either true mangroves or associates. These species are often distinguished based on detailed assessments of leaf morphology and osmotic adjustment mechanisms.

Note: This section is extrapolated, restructured, and modified from content previously published in Cerri, F., Louis, Y.D., Fallati, L. *et al*. (2024). Mangroves of the Maldives: a review of their distribution, diversity, ecological importance and biodiversity of associated flora and fauna. *Aquatic Sciences* 86, 44

1.2. MANGROVES AS STRATEGIC RESOURCES FOR BIOPROSPECTING

Mangroves represent a distinctive and underexplored group of plants that offer lines of justification for their consideration in natural product discovery. Their unique ecological positioning at the interface between marine and terrestrial environments provides access to two of the most prolific sources of natural products. At the same time, their ability to withstand extreme abiotic stresses has driven the production of unique metabolites. Beyond their ecological and biochemical adaptations, mangroves have a long-standing role in traditional medicine, where they are widely employed for treating a broad range of ailments. Despite this ethnopharmacological significance and the increasing number of reports on their bioactive metabolites, mangroves remain comparatively neglected in bioprospecting studies. The

following subsections expand on these four interrelated aspects, ecological positioning, stress-driven chemistry, ethnomedicinal relevance, and research neglect, that together define the rationale for targeting mangroves in bioprospecting.

1.2.1. At the intersection of two promising worlds: marine ecosystems and terrestrial plants

Mangroves occupy a unique ecological niche at the interface between land and sea, combining the biological complexity of both marine ecosystems and terrestrial plant life. Marine environments are among the most biodiverse and chemically rich ecosystems of Earth (Fisher et al., 2015). This biodiversity arises from the complex interplay of biotic and abiotic factors, leading to the evolution of a vast array of organisms capable of producing structurally diverse and bioactive natural compounds. Over the past few decades, marine biodiversity has been widely investigated for their pharmacological potential. As of 2017, more than 30,000 marine natural products had been identified, with over 1,000 new compounds discovered each year (Altmann, 2017). To date, 16 marine-derived drugs have been approved for therapeutic uses (www.marinepharmacology.org).

Mangroves, a specialized group of angiosperm plants, add an important botanical dimension to this marine-terrestrial interface. As sessile organisms, plants produce a wide range of secondary metabolites to adapt and overcome abiotic and biotic stresses (Muhlemann et al., 2014; Teoh, 2016; Zaynab et al., 2018). Within the broader plant kingdom, more than 100,000 secondary metabolites have been identified across various taxa (Aggarwal et al., 2024). Thanks to this metabolic richness, plants have historically played a central role in drug discovery, offering a vast and chemically diverse repertoire of bioactive compounds. Despite the advances in synthetic chemistry, natural products and their derivatives still account for one-third of all FDA-approved new molecular entities (NMEs) (Patridge et al. 2016). Between 1981 and 2010, more than 50% of approved pharmaceuticals were either natural products, derivatives, or inspired by natural scaffolds, while only 36% were entirely synthetic (Newman and Cragg, 2012; Davis and Choisy, 2024). Plant-derived compounds remain especially important in this context. Globally, approximately 25% of approved drugs are derived directly or indirectly from plants (Davis and Choisy, 2024). Although the relative percentage of plant-based NMEs has declined over the decades, from 22% before 1950 to around 8.7% in recent years, plant products continue to represent 5.6% of all NMEs approved since 2000, highlighting their enduring value in pharmaceutical innovation (Patridge et al., 2016). This longstanding relevance calls for

renewed exploration of understudied plant groups, particularly those inhabiting extreme environments. Mangroves, which embody a convergence of two prolific bioprospecting domains, marine biodiversity and terrestrial phytochemistry, offer a promising yet underexplored source of novel bioactive compounds.

1.2.2. Extreme habitats as drivers of chemical novelty

From a bioprospecting perspective, mangroves represent a highly promising group of plants, not only due to their valuable genes, the rich biodiversity of their ecosystems and their traditional use in ethnomedicine, but above all because of their remarkable ability to adapt to extreme environmental conditions (Kathiresan, 2020; Audah et al., 2022; Cerri et al., 2022). Mangroves are continuously exposed to high salinity, fluctuating water levels, hypoxia, high temperature, and UV radiation. In response to these harsh environments, mangrove plants produce abundant secondary metabolites that help them survive in such environmental conditions (Murthy et al., 2025). These compounds, often unique or rare in structure, are not only crucial for the plant's survival, but also offer potential therapeutic applications.

It is well established that the chemical profile of plants is heavily influenced by geographical, environmental and climate factors (Jin et al., 2009; Abd-Elgawad et al., 2019; Rozirwan et al., 2022). Plants subjected to abiotic stresses often exhibit an increased accumulation of bioactive secondary metabolites. Numerous studies have shown that exposure to salinity, drought, temperature extremes, or UV radiation can stimulate the biosynthesis of flavonoids, iridoid glycosides, phenylethanoid glycosides, and other antioxidant compounds with known pharmacological activities (Wang et al., 2010; Ferdinando et al., 2012; Neugart et al., 2016; Falahi et al., 2018; Franzoni et al., 2019; Sarri et al., 2021). These stress-induced metabolites serve a dual purpose: promoting plant survival while offering novel scaffolds for drug discovery (Toscano et al., 2019). Given their ability to thrive in extreme habitats, mangroves stand out as particularly attractive candidates for modern bioprospecting efforts.

1.2.3. Ethnomedicinal significance

Since ancient times, plants played a fundamental role in the health care systems of human civilizations, serving as primary remedies for various ailments across cultures and continents. This is especially in Asia, Africa, and Latin America, where traditional knowledge has been passed down through generations, relying on the natural flora for treating diseases. Plants recognized for their therapeutic properties, commonly referred to as "medicinal plants" have

long been used to alleviate human suffering and are regarded as valuable sources of bioactive compounds with pharmacological potential (Gurib-Fakim, 2006; Saranraj et al., 2015; Wyk and Prinsloo, 2020; Marrelli, 2021; Miranda, 2021).

Among the diverse ecosystems that harbor medicinal plants, mangrove forests have emerged as particularly important. For centuries, mangroves species have been used in folk medicine throughout tropical and subtropical coastal regions, including South, Southeast and East Asia, Africa, South America, the Pacific islands, and Australia. Ethnomedicinal surveys reveal that the majority of these traditional uses are documented in Asian countries, with India being the most prominent, followed by Bangladesh, Malaysia, China, Indonesia, and the Philippines (Govindasamy and Kannan, 2012; Bibi et al., 2019; Genilar et al., 2021).

Out of 65 true mangrove species, about 30 are cited for their traditional medicinal applications. Among them, species such as *Bruguiera gymnorhiza*, *Rizophora mucronata*, *Achanthus ilicifolius*, *Heritiera fomes*, and *Avicennia marina* are widely used and exhibit a broad range of potential medicinal values compared to other species (Bandaranayake, 1998; Liebezeit and Rau, 2006; Govindasamy and Kannan, 2012; Bibi et al., 2019; Genilar et al., 2021).

Mangrove plants have traditionally been employed to treat a wide spectrum of human ailments, reflecting their vital role in folk medicine. Different parts of these plants, including leaves, roots, bark, stems, and fruits, are used to address diseases such as leprosy, elephantiasis, tuberculosis, malaria, and various skin diseases. Their medicinal application also extends to the management of diabetes, hypertension, ulcers, and a range of gastrointestinal disorders including constipation, diarrhoea, dysentery, dyspepsia, haematuria, and abdominal pain. These represent only a fraction of the many ethnomedicinal uses documented, underscoring the rich traditional knowledge associated with mangrove species (Govindasamy and Kannan, 2012; Bibi et al., 2019).

Within this ethnopharmacological context, *Avicennia marina* and *Sonneratia caseolaris* stand out to their broad spectrum of traditional uses and prominence in local medical practices. *A. marina* has been used across countries such as Pakistan, Iran, Indonesia, Egypt, and China to treat ailments including smallpox, ulcers, rheumatism, burns, infections, skin disorders, respiratory ailments, and even as a contraceptive. The leaves have been the most widely used part, traditionally applied in the management of smallpox, malaria fever, food poisoning, diarrhoea, ulcers, rheumatism, burns, wounds, skin disorders, abscesses, and throat pain. The bark has also been used for treating smallpox, diarrhoea, abscesses, ulcers, skin diseases, and

throat pain, and has additionally been used as a natural aphrodisiac. The fruits have been administered for colds, sore throats, diarrhoea, inflammation, constipation, and skin ailments. Other plant parts have been less frequently reported but still noteworthy: the wax has been used as a natural aphrodisiac and toothache remedy, the roots for wound healing and aphrodisiac purposes, and the wood in the treatment of snakebites. Collectively, these traditional applications highlight the versatile therapeutic role of *A. marina*, with the leaves standing out as the most extensively utilized part. (Liebezeit and Rau, 2006; Bibi et al., 2019; Zhou et al., 2025).

Similarly, *S. caseolaris* is traditionally employed in Bangladesh, Myanmar, and Malaysia as an astringent, antiseptic, and treatment for sprains, swellings, haemorrhages, coughs, helminthic infections, diabetes, and skin conditions such as smallpox and haematuria. Its fruit is used to relieve pain, reduce swelling, and stop bleeding (Sadhu et al., 2006; Govindasamy and Kannan, 2012; Audah et al., 2024).

1.2.4. Underexplored taxa

The bioprospecting of mangroves is a relative recent and promising area of research, with the number of related publications increasing in recent years. However, the exploration of mangroves for drug discovery remains limited (Patra and Thatoi, 2011; Parthiban et al., 2022). Several challenging continues to hinder the comprehensive exploration and utilization of mangrove species for drug discovery purposes.

One of the primary constraints is the challenging nature of mangrove habitats. These ecosystems are typically harsh, waterlogged, densely vegetated, and often difficult to access. Fieldwork in such environments demands significant logistical effort, specialized equipment, and expertise, which limits the frequency and scale of scientific expeditions. Additionally, many mangrove forests fall under strict conservation regulations, restricting sample collection and research access. These access challenges are intensified by the alarming projection that over 50% of the world's mangrove forests are at risk of collapse by 2050 due to anthropogenic pressures such as pollution, infrastructure development, illegal aquaculture, and unsustainable resource exploitation (IUCN, 2024).

Mangrove ecosystems have historically received less attention than other marine or coastal systems in natural product research. Marine bioprospecting efforts have predominantly targeted organisms such as sponges, corals, and algae, which are more accessible in open marine environment and often perceived as more prolific in terms of unique metabolite production. In

contrast, mangroves, situated at the land-sea interface, have not benefitted from the same level of scientific interest, despite their unique adaptations and diverse chemical profile.

Despite the known richness of secondary metabolites in mangrove plants, current research tends to focus on only a handful of widely studied species. As a result, a vast number of mangrove species remain phytochemically underexplored. For example, Nebula et al. (2013) highlighted that within the Rhizophoraceae family, half the species were not subjected to detailed chemical investigation. Furthermore, Parthiban et al. (2022) reported that only 40% of Indian mangroves have been screened. This selective attention leaves much of the mangrove biodiversity unexploited for potential therapeutic applications.

Another significant issue is the research bias toward associated microbial communities, particularly endophytic fungi and actinobacteria found in mangrove soils and roots. While these organisms have shown great promise in producing novel bioactive compounds, the emphasis on microbial sources has led to the underrepresentation of plant-derived metabolites in the literature. Consequently, the potential of mangrove plants themselves is often overlooked or treated as secondary in bioprospecting studies.

Geographical and regulatory limitations also play a crucial role in hampering the global scope of mangrove bioprospecting. Many of the biodiverse tropical regions where mangroves thrive face infrastructural, administrative, and logistic barriers to scientific research. Regulatory hurdles, including lengthy and complex procedures for obtaining research permits and challenges in establishing international collaboration agreements all contribute to slowing down progress. This restricted geographic scope is problematic, as the chemical composition of plants is influenced by environmental, climatic and geographic variables (Jin et al., 2009; Tran et al., 2023). Thus, there is a urgent need to expand research efforts into underexplored regions to capture the full spectrum of phytochemical diversity.

1.3. UNDEREXPORED AND UNIQUE MANGROVE HABITATS FOR BIOPROSPECTING

As discussed in the previous sections, two primary factors should guide the selection of study areas for mangrove bioprospecting: ecosystems that remain scientifically underexplored, and habitats characterized by unique environmental conditions that may influence phytochemical profiles. Two geographical areas stand out as fitting both criteria: the Maldives and the United Arab Emirates. The solid institutional presence of the Marine Research and Higher Education

Center (MaRHE Center) in the Republic of Maldives, has been instrumental in facilitating fieldwork and collaborations for the study of mangroves within the Maldivian archipelago. Additionally, collaborations with institutions in the United Arab Emirates (UAE) have enabled the extension of this study to the unique mangrove habitats of the Arabian Gulf. This allows for a comprehensive investigation of mangroves growing under diverse yet extreme environmental conditions, providing a valuable framework for exploring their phytochemical potential for bioprospecting purposes.

1.3.1. The Republic of Maldives

The Maldives is an archipelago of 1,192 islands grouped into 26 atolls, spanning 870 km across the equatorial region of the Indian Ocean, from 7° north to 0.5° south. Despite the ecological importance of its mangroves, scientific research in this domain remains notably limited, especially in terms of phytochemical and pharmacological investigations. Unlike coral reefs, which have been extensively studies, mangroves in the Maldives have received minimal academic attention. Nevertheless, they play a crucial role in safeguarding coastal biodiversity and delivering essential ecosystem services, which are vital for a Small Island Developing State declared vulnerable by the 2010 UNDP's Assessment of Development Results (Cerri et al., 2025). Their functions include coastal protection against tidal events, monsoonal storm surges, and potential tsunamis (Guannel et al., 2016; Agardi et al., 2017), water purification, and supporting livelihoods trough fisheries and other resource-based activities (Shadiya et al., 2016; IDEAS, 2017). Despite these significant ecological roles, awareness and scientific understanding of Maldivian mangroves remain limited. This knowledge gap is reflected in their recent classification as critically endangered, largely due to poor management practices, aggressive land reclamation, unregulated aquaculture, and overexploitation of natural resources (Cerri et al., 2025). To address this fragmentation of information, we conducted a comprehensive review aimed at consolidating existing data to enhance understanding, guide conservation efforts, and support sustainable management strategies (Cerri et al., 2024)

Our survey revealed the presence of mangroves in 108 islands, representing approximately 9% of the Maldivian archipelago (**Figure 1.1**). Notably, half of these islands are inhabited, emphasizing the direct socio-economic relevance of these ecosystems. Although 23 islands have established protected mangrove areas, current conservation measures remain insufficient to halt the ongoing decline. Furthermore, the Maldivian mangrove flora includes 14 sepcies: *Avicennia marina*, *Bruguiera cylindrica*, *Bruguiera gymnorrhiza*, *Bruguiera hainesii*,

Bruguiera sexangula, *Ceriops tagal*, *Excoecaria agallocha*, *Heritiera littoralis*, *Lumnitzera racemosa*, *Pemphis acidula*, *Rhizophora apiculata*, *Rhizophora mucronata*, *Sonneratia caseolaris*, and *Xylocarpus moluccensis* of which three are considered controversial species (*E. agallocha*, *H. littoralis*, and *P. acidula*) (Cerri et al., 2024).

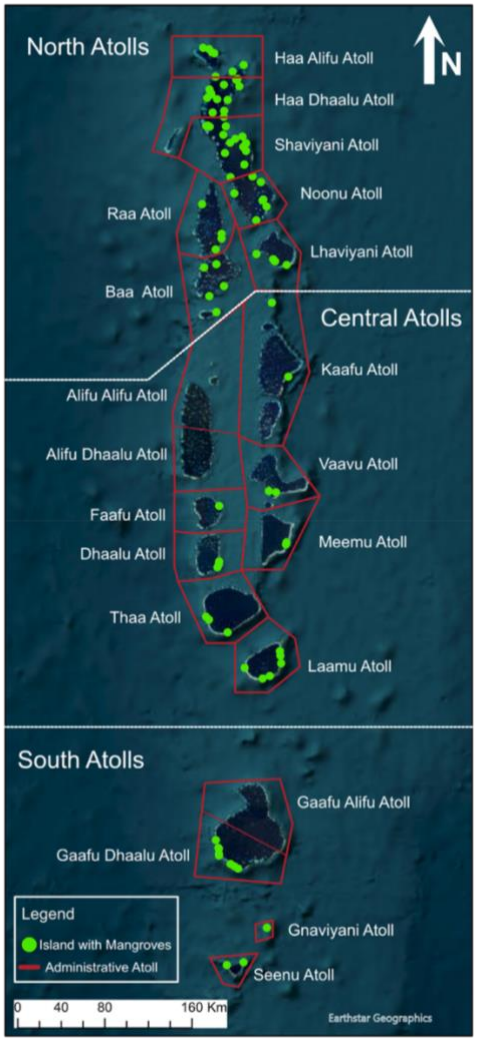

Figure 1.1. Distribution of islands with a reported presence of mangroves (green points) in the Maldives according to our review of the literature. The different atolls are indicated in red boxes. (*Cerri et al., 2024*)

Beyond their underexplored status, Maldivian mangroves also thrive in distinctive ecological settings. Unlike typical estuarine mangrove systems, the Maldives lacks rivers. Consequently, mangroves are confined to small patches associated with closed or semi-enclosed water bodies. We categorized these ecosystems into four habitat types, which can coexist in a single island: (1) inland lake-based mangroves, (2) inland marsh-based mangroves, (3) coastal fringe mangroves, and (4) embayment mangroves (Cerri et al., 2024). These unique formations

suggest the potential for distinct ecological and phytochemical characteristics yet to be explored.

1.3.2. The United Arab Emirates

The Arabian Gulf, specifically along the coasts of the United Arab Emirates (UAE), Bahrain, Qatar, and Iran, hosts more extensively documented mangrove populations, though significant knowledge gaps persist (El-Tarabily et al., 2021). In UAE, which is a federation of seven emirates spanning along the northern coast of the Arabian Peninsula from 22°50' and 26° north latitude, mangroves create unique habitats that support diverse biological communities and provide vital ecosystem services, including carbon sequestration, coastal protection, and acting as nurseries for commercially important fish species (Friis and Killilea, 2023).

Mangroves in the Arabian Gulf, however, thrives under some of the most extreme environmental conditions recorded for mangrove ecosystems. The UEA's climate is characterized by sharp seasonal temperature fluctuations, with summer air temperatures often exceeding 50°C and winter nights dropping below 8 °C (climateknowledgeportal.worldbank.org; Patlakas et al., 2019). In addition to these extreme temperatures, there is high solar radiation, nutrient-poor soils, and severe aridity, with annual rainfall generally below 150 mm (Ab Habshi et al., 2007). Furthermore, limited freshwater inflow and high evaporation rates cause salinity levels in coastal lagoons and bays to reach values as high as 65-70 ppt, well beyond the typical salinity tolerance of mangroves (Ab Habshi et al., 2007; Friis and Killilea, 2023).

A. marina is the only mangrove species naturally occurring in the UAE, forming evergreen coastal forests along its 850km of shoreline and covering approximately 4,000 hectares of mangrove (Moore et al., 2015; Friis and Killilea, 2023). The distribution of mangroves in the UAE is shown in **Figure 1.2** according to Friis and Killilea (2023) and Raihan et al. (2023).

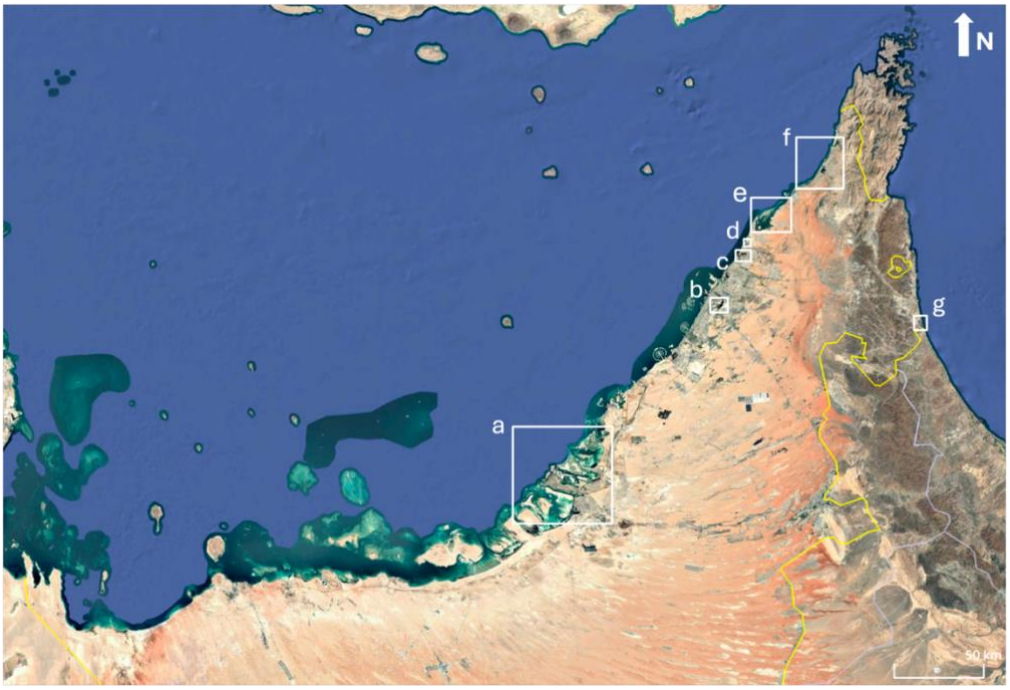

Figure 1.2. UAE mangrove distribution. **a** Abu Dhabi mangroves; **b** Dubai's mangrove at Ras Al Khor; **c** Al Zorah Nature Reserve in Ajman Emirate; **d** Al Hamriyah Mangroves Reserve; **e** Umm Al Quwain mangroves; **f** Ras Al Khaimah mangroves; **g** Khor Fakkan mangroves.

1.4. TARGET SPECIES

Following the discussion of underexplored mangrove habitats and their distinctive environmental conditions, this section introduces the two mangrove species selected for this study: *Avicennia marina* and *Sonneratia caseolaris*. Both species are of particular interest due to their ecological prominence, unique physiological adaptations, and traditional ethnomedicinal uses. The following subsections provide a detailed overview of their taxonomy, morphology, distribution, and ecological characteristics, laying the groundwork for understanding their potential as sources of bioactive compounds.

1.4.1. *Avicennia marina*

The genus *Avicennia* (L.) is named after the Persian philosopher and physician Abdallah Ibn Sīnā, widely known in the West as Avicenna (981–1037 CE) (Quattrocchi, 2000; Friis and Killilea, 2023). Taxonomically, *Avicennia* belongs to the division of Tracheophyta, sub-division Spermatophytina, class Magnoliopsida, and order Lamiales. The assignment of its family, however, has been a subject of longstanding debate. Initially, *Avicennia* was classified under Verbencacea, and subsequently, several botanists advocated for its placement in a distinct

family, Avicenniaceae. Modern molecular phylogenetic studies have resolved this taxonomic ambiguity, leading to a consensus that places *Avicennia* within the family Acanthaceae. Advancements in phylogenetic research have clarified the composition of the genus, which is now recognized to include eight distinct species: *Avicennia balanophora*, *A. bicolor*, *A. germinans*, *A. integra*, *A. marina*, *A. officinalis*, *A. schaueriana*, and *A. tonduzii* (Thatoi et al., 2016). *Avicennia* holds a unique ecological distinction as the only mangrove genus with a global distribution, forming dominant mangrove communities along tropical and subtropical coastlines (Thatoi et al., 2016).

Among these species, *A. marina* (Forssk.) Vierh, commonly known as grey mangrove, is the most widely distributed mangrove species globally (Martínez-Díaz and Reef, 2022). Its extensive range spans the tropical coastlines of East Africa, South and Southeast Asia, Australia, the North Island of New Zealand, and islands of the Pacific Ocean, including Fiji. In addition, *A. marina* is prevalent along the western Red Sea and Southwestern Asian coastlines, particularly dominating the mangrove flora of the Arabian Gulf. In this region, it represents the principal coastal vegetation along the shores of the United Arab Emirates (UAE), Saudi Arabia, Bahrain, Qatar, and Iran (Cerri et al., 2022)

Morphologically, *A. marina* growths either as shrubs or medium-size trees that typically attain heights from three to fourteen meters. The species is characterized by its light grey bark, which is thin, smooth, and composed of delicate scales. Its root system includes specialized aerial structures known as pneumatophores, The bark is light grey in colour, with thin, smooth, and delicate scales. Its root system includes specialized aerial structures known as pneumatophores, which protrude vertically from the substrate to facilitate gas exchange in anoxic conditions. These pneumatophores are relatively short, measuring approximately ten to fifteen centimeters in length, and end in pointed tips. The leaves of *A. marina* are yellowish-green, elliptic in shape, and measure around six to seven centimeters long, with a dense covering of fine hairs on the underside. The inflorescences consist of small clusters bearing three to five yellow flowers, each about one centimeter in diameter. The dispersal unit of *A. marina* is not a true fruit but a propagule. In the literature, these structures are sometimes also referred as “fruits” or “seeds”. Propagules are compressed, oval capsules, light grey-green in color, and measure between twenty to twenty-five millimeters in diameter (**Figure 1.3**) (Tomlinson et al., 1986; Baba et al., 2016; Thatoi et al., 2016; Cerri et al., 2022; Dhawi et al., 2025).

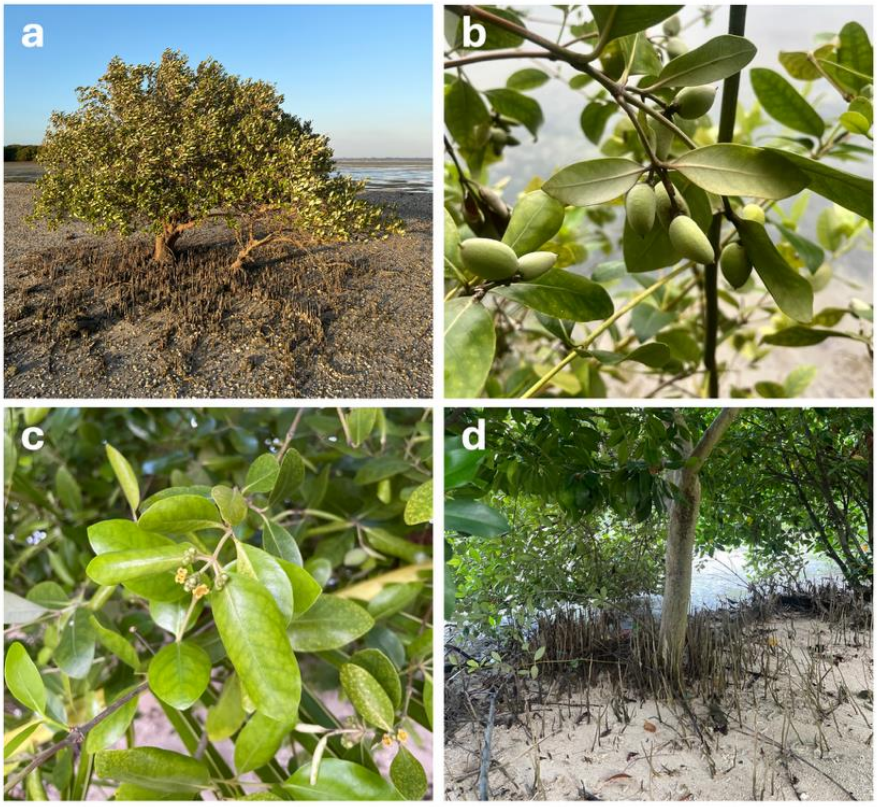

Figure 1.3. Representation of the plant (**a**), propagules (**b**), flowers (**c**) and pneumatophores (**d**) of *Avicennia marina*.

The ecological success of *A. marina* is largely attributable to its suite of morphological and physiological adaptations that enable survival under extreme environmental stresses. The species thrives in habitats characterized by wide temperature fluctuations, high salinity, intense ultraviolet radiation, strong winds, arid conditions, and oxygen-deficient soils (Eldohaji et al., 2020; Das et al., 2016). These challenging environments have driven *A. marina* to develop efficient salt regulation mechanism, including the presence of salt glands on its leaves that excrete excess salts, thereby maintaining cellular ionic balance (Ma et al., 2022; Guo et al., 2023), making it one of the most salt-tolerant mangrove species (Sudhir et al., 2022). Furthermore, its pneumatophores, which are equipped with lenticels, facilitate the diffusion of atmospheric oxygen to submerged root tissues, allowing, respiration in waterlogged, anoxic substrates (Hao et al., 2021; Friis and Killilea, 2023). Reproductively, as one-third of all mangrove species, *A. marina* produces propagules and exhibits a form of vivipary, where seeds begin germination while still attached to the maternal plant (Guo et al., 2023). This strategy facilitates rapid seedling establishment upon dispersal, enhancing survival in dynamic tidal environments. Additionally, by allowing early development to occur with the protective fruit, the propagules can establish in saltwater environments, where initial growth stages benefit from transient low-salinity conditions. Specifically, *A. marina* follows a crypto-viviparous strategy,

where the embryo breaks through the seed coat but remains enclosed within the fruit until detachment (Liu et al., 2025). Additional physiological mechanisms, such as salt exclusion at the root level and compartmentalization of excess salts in senescent leaves, further contribute to the plant's ability to withstand saline stress (Cram et al., 2002; Cheng et al., 2020). Notably, populations of *A. marina* in the United Arab Emirates exhibit distinct adaptations that are not commonly observed in other regions because of the unique extreme environmental conditions. These include the development of more intricate cuticular wax compositions, which enhance water retention under arid conditions, as well as other physiological adjustments to cope with extreme salinity and temperature fluctuations (Dodd et al., 1999; Friis and Killilea, 2023). Such unique traits suggest the possibility of specialized phytochemical profiles in these populations, which could be of significant interest for bioprospecting initiatives.

The diverse morphological and physiological adaptations of *A. marina* are often associated with the phytochemical variations that may lead to the production of unique secondary metabolites with pharmacological potential. Across its range, various *Avicennia* species, including *A. marina*, have been traditionally used in ethnomedicine to several ailments such as inflammation, skin disorders, and infections. In recent years, scientific investigations have substantiated many of these traditional claims, identifying a plethora of bioactive compounds with antioxidant, anticancer, antiviral, antimicrobial, anti-inflammatory, and antidiabetic properties (Thatoi et al., 2016). These findings underscore the relevance of *A. marina* as a promising candidate for pharmacological bioprospecting, particularly in regions like the Arabian Gulf, where its unique environmental adaptations may be reflected in its secondary metabolite profiles.

1.4.2. *Sonneratia caseolaris*

Among the fourteen species recorded in the Maldives, *S. caseolaris* was selected as the primary subject for bioprospecting in this study. This choice was driven by several interrelated factors. Firstly, species within the *Sonneratia* genus bare recognized for their ethnomedicinal applications and the production of unique bioactive secondary metabolites with reported pharmacological potential (Nguyen et al., 2024). Despite this, *S. caseolaris* remains underexplored, compared to other species including *Avicennia* spp. and *Rhizopora* spp., particularly in the Maldives, where no comprehensive phytochemical or pharmacological studies have been conducted to date. Globally, research on *S. caseolaris* has predominantly

focused on its leaves and edible fruits, while other organs, such as roots, which could possess distinct and untapped phytochemical profiles, have largely been neglected.

Another important rationale for selecting *S. caseolaris* lies in its distinct ecological niche within the Maldivian archipelago. Unlike other species, that predominantly colonize coastal margins, *S. caseolaris* typically inhabits deep muddy soils and tidal areas associated with inland mangrove wetlands (**Figure 1.4**). These habitats are often situated in shallow depressions away from the immediate shoreline, characterized by mud banks influenced by transient freshwater inflows, creating brackish to low-salinity microenvironments (Selvam, 2007; Rahim, 2018; Tatongjai et al., 2021; Farhath et al., 2025), reflecting the low salinity tolerance of *S. caseolaris* (Sudhir et al., 2022) . Furthemore, in contrast to other Maldivian mangrove species, which are categorized as 'controversial species', *S. caseolaris* is a true mangrove species. Additionally, is it not listed as critically endangered, thus making it a sustainable candidate for bioprospecting activities.

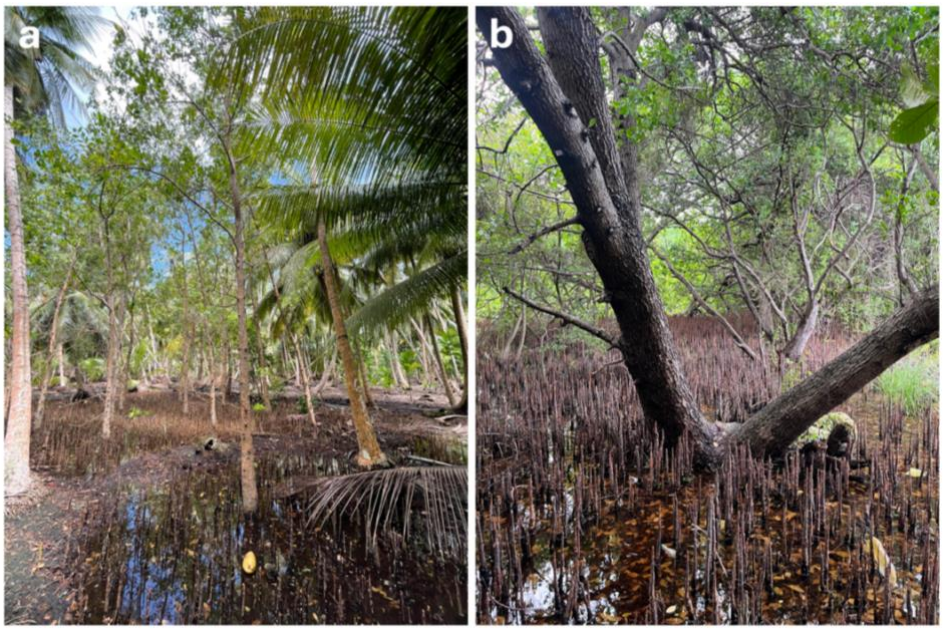

Figure 1.4. Habitats of *Sonneratia caseolaris* on Kashidhoo Island (**a**) and Khuludhuffushi Island (**b**) in the Maldives.

Taxonomically, *Sonneratia* belongs to the division Tracheophyta, sub-division Spermatophytina, class Magnoliopsida, and order Myrtales. Historically, the genus was placed within the family Sonneratiaceae (Bingham and Kandasamy, 2001). However, advances in molecular phylogenetic analyses have since led to its reclassification under the family Lythraceae (APG IV, 2016). This taxonomic divergence is significant when compared to other common Maldivian mangrove species such as *Rhizophora* spp., *Bruguiera* spp., and *Ceriops tagal*, which all belong to the family Rhizophoraceae. The evolutionary distance may reflect

distinct biosynthetic pathways, potentially resulting in a unique profile of secondary metabolites not found in Rhizophoraceae species.

Commonly known as the mangrove apple, *S. caseolaris* is an evergreen tree that typically reaches heights of 8–10 meters but can grow up to 20 meters. The species is characterized by horizontal spreading branches, slender twigs, and prominent pneumatophores that can extend up to 90 cm above the substrate to facilitate exchange in anoxic muddy soils. The inflorescences are borne terminally on outer twigs and typically consists of a single or few-flowered cymes with large, red-petalled blossoms (8–10 cm in diameter) that open nocturnally and last only for one night. The leathery, elliptic leaves and, rounded fruits (12–20 cm in diameter) are also notable, with approximately 92% of the fruit's weight comprised of the seeds enclosed within (Primavera et al., 2004; Sadhu et al., 2006; Selvam, 2007; Rahim, 2018; Dev et al., 2021) (**Figure 1.5**). Geographically, *S. caseolaris* has a broad distribution across various regions including the Maldives, Sri Lanka, China, Bangladesh, the Malay Peninsula, Indonesia, Borneo, the Philippines, Timor, New Guinea, the Solomon Islands, and northern Australia (Sadhu et al., 2006; Tian et al., 2009; Yang et al., 2016 Dev et al., 2021; Cerri et al., 2024).

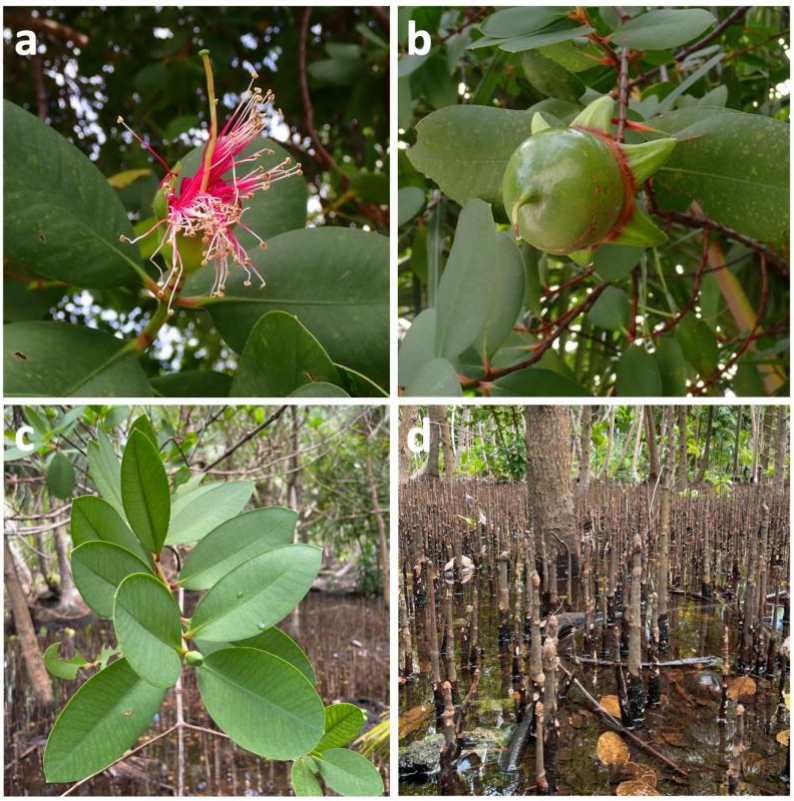

Figure 1.5. Flower (**a**), fruit (**b**), leaves (**c**), and pneumatophores (**d**) of *Sonneratia caseolaris*.

Adaptively, *S. caseolaris* thrives in anoxic muddy substrates by producing dense arrays of cone-shaped pneumatophores that support efficient gas exchange (Rahim, 2018). Additionally, the species exhibits a salt-exclusion mechanism at the root level where the root periderm acts as a

barrier to prevent excessive salt uptake, allowing survival in moderately saline environments (Tatongjai et al., 2021). Reproductively, this species employs a non-viviparous strategy, releasing seeds that germinate upon settling in a suitable substrate (Liu et al., 2025).

Given its unique ecological adaptations, taxonomic distinctiveness, and underexplored phytochemistry, *S. caseolaris* represents a promising candidate for bioprospecting efforts aimed at discovering novel bioactive compounds.

1.5. ANALYTICAL AND EXTRACTION STRATEGIES FOR UNTARGETED METABOLOMIC PROFILING OF MANGROVE SPECIES

Recent advances in analytical techniques, combined with innovative bioinformatics tools, have significantly enhanced the capacity to investigate the chemical complexity of biological samples. Particularly, developments in mass spectrometry have markedly improved analyte detection accuracy, even in highly complex matrices, by reducing confounding factors that can enhance or suppress ion signals. Untargeted metabolomic profiling, which does not require prior knowledge of the compounds present, has become an essential strategy in bioprospecting, especially when exploring under-investigated species. This approach aims to capture the broadest possible spectrum of metabolites linked to biological activities (Zanatta et al., 2021; Kodikara et al., 2024; Lee et al., 2025). In this context, ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) has emerged as a pivotal tool for untargeted metabolomic profiling of complex plant matrices, such as mangrove species (Zanatta et al., 2021; Kodikara et al., 2024; Singh and Choudhary et al., 2025; Lee et al., 2025). This methodology offers superior sensitivity, selectivity, and mass accuracy, enabling the detection and identification of a wide array of secondary metabolites, particularly phenolic compounds. Phenolics are widely distributed in mangroves and are of considerable interest due to their potent antioxidant activity and other bioactive properties (Bibi et al., 2019; Kodikara et al., 2024; Botosoa and Shahidi., 2025).

Equally crucial to the success of metabolomic studies is the choice of an appropriate extraction solvent. In recent years, there has been a growing shift towards the use of “green solvents” that are safer for both human health and the environment. This reflects increasing awareness of the environmental impact of traditional toxic solvents in terms of pollution, energy consumption, and contributions to climate change (Clarke et al., 2018; Plaskova and Mlcek, 2023). Water is the safest and most economical solvent, but its extraction efficiency is limited to highly polar

compounds. Consequently, binary solvent systems, particularly mixtures of water and organic solvents, are favoured to efficiently extract a higher range of bioactive compounds present in plant tissues. Binary mixtures, combine the strengths of both solvents: water facilitates the diffusion of extractable components through plant cell walls and efficiently solubilizes polar molecules, while organic solvent in recovering less polar constituents (Plaskova and Mlcek, 2023; Kim et al., 2019). Ethanol, in particular, is considered a preferable solvent for applications involving food, nutraceuticals, and pharmaceuticals, due to its low toxicity and ease of handling. For example, an 80:20 ethanol-water mixture has a relative polarity closer to methanol's polarity but without the associated toxicity, making it highly effective for a broad spectrum of phytochemicals (Kim et al., 2019; Plaskova and Mlcek, 2023).

This solvent combination is especially efficient in extracting polyphenols, a major class of secondary metabolites abundantly produced by mangroves. Polyphenols are chemically divers, and their solubility profiles vary depending on the number of the hydroxyl groups and molecular size. Thus, hydroalcoholic mixtures provide a more comprehensive extraction profile, accommodating both hydrophilic and moderately lipophilic phenolic compounds (Lezoul et al., 2020; Plaskova and Mlcek, 2023; Palaiogiannis et al., 2023; Huamán-Castilla et al., 2024).

Beyond solvent selection, the integrity and efficiency of metabolite extraction are also influenced by pre-extraction processing methods. Drying is a critical step to preserve bioactive compounds, particularly to prevent degradation of heat-sensitive molecules. Freeze-drying (lyophilization) is widely regarded as the most suitable technique for preserving phytochemical integrity, as it avoids thermal degradation. Additionally, reducing plant material to a fine powder increases the surface area in contact with the solvent, thereby enhancing extraction yields (Plaskova and Mlcek, 2023).

In terms of extraction techniques, non-conventional methods such as ultrasound-assisted extraction have gained prominence. This extraction offers advantages over traditional methods, including reduced extraction time, lower energy and solvent consumption, and improved extraction efficiency. The application of ultrasonic waves enhances solvent penetration into plant tissues, facilitating the release of bioactive compounds (Farahmandfar et al., 2019; Plaskova and Mlcek, 2023). These combined strategies reflect a holistic approach to maximizing metabolite recovery while adhering to principles of green chemistry.

1.6. ANTIOXIDANT AND CYTOTOXIC ASSESSMENTS AS KEY SCREENING STRATEGIES IN MANGROVE BIOPROSPECTING

Evaluating antioxidant and cytotoxic activities has become a fundamental approach in the initial bioprospecting of plant species, especially when exploring their therapeutic potential. It is now standard practice to pair phytochemical profiling with antioxidant assays during the initial screening of plant extracts, as these two analyses collectively provide insights into both chemical composition and functional bioactivity (Barros et al., 2011; Grzegorczyk-Karolak et al., 2020; Yu et al., 2021; Bang et al., 2021). In many cases, cytotoxicity assessments are added to this workflow to explore the potential anticancer properties of plant-derived compounds, making the combined evaluation of phytochemistry, antioxidant capacity, and anticancer effect a systematic and effective strategy for identifying bioactive molecules with pharmacological relevance (Grover at al., 2021; Jabbar, 2021; Ganamé et al., 2021; Mohamed, 2022; Burlec et al., 2021; Alzandi et al., 2021).

Phytochemical characterization offers insights into the classes of secondary metabolites present, such as phenolics, flavonoids, terpenoids, which are often linked to biological activities. Antioxidant assays (e.g., DPPH, ABTS, ORAC) serve as high-throughput indicators of a plant's ability to neutralize free radicals, which is relevant because oxidative stress contribute to a wide range of chronic diseases and cancer. Cytotoxicity testing (e.g., MTT assays on cancer and normal cell lines) provides direct evidence of anticancer potential and selectivity of the extracts.

In mangrove bioprospecting, these combined assessments are particularly relevant. Also in this context, studies have focused on the phytochemical composition and antioxidant activity of mangrove species (Bulan et al., 2022; Rozirwan et al., 2023), while others have extended this investigation to include anticancer evaluations (Thao et al., 2015; Youssef et al., 2022), verifying whether the same chemical scaffold drives both activities, and identify extracts that are both potent and selective. The results of these studies allow us to prioritise the most promising extracts and compounds, focusing subsequent follow-up efforts solely on these, thereby maximising efficiency in the early discovery of promising bioactive compounds.

For species that have not been previously studied, or for those sampled from different geographical regions, it is crucial to integrate phytochemical analyses with antioxidant and cytotoxicity assays. This integrated approach allows researchers to capture variations in secondary metabolite profiles and correlate them with potential bioactivities, providing a rational basis for further pharmacological investigations.

1.6.1. Antioxidant activity: relevance and mangrove adaptations

Free radicals, defined as molecules with one or more unpaired electrons, are produced naturally in the human body during physiologically process and include, among the others, reactive oxygen species (ROS). Under normal conditions, the antioxidant defence system maintains a balance by neutralizing excess free radicals. However, factors such as environmental stress, aging, and disease can disrupt this balance, leading to oxidative stress. Persistent oxidative stress is implicated in the development of numerous diseases, including neurodegenerative disorders, cardiovascular diseases, inflammatory conditions, and cancer (Thatoi et al., 2014; Ser et al., 2017). Given the potential toxicity of synthetic antioxidants, increasing attention has been directed towards natural antioxidants derived from plants, particularly polyphenols and flavonoids, which are known for their radical-scavenging properties (Olszowy, 2021). Plant-based antioxidants are considered safer alternatives and play a critical role in managing oxidative stress-related diseases (Stagos, 2020).

In plants, ROS are produced abundantly during normal growth and metabolic processes (Bhattacharjee, 2005). To mitigate the resulting oxidative stress, plants evolved robust antioxidant defense systems, comprising both enzymatic and non-enzymatic components, including phenolic compounds (Asada, 2006). Mangrove plants, in particular, are rich in phenolic compounds due to the extreme environmental conditions they endure. These stressors trigger enhanced production of ROS within plant cells, necessitating a robust antioxidant defense system (Mittler, 2002; Mittler, 2004). Mangroves respond by accumulating high levels of enzymatic and non-enzymatic antioxidants, including phenolic compounds and other phytoconstituents (Wang et al., 2014; Thatoi et al., 2014). Indeed, mangroves produce high level of polyphenolic compounds, which are known for their strong antioxidant activities (Botosa et al., 2025) making them promising candidates for bioprospecting antioxidant potential evaluation (Thatoi et al., 2014).

Species such as *S. caseolaris* and *A. marina* have been reported to possess significant antioxidant activities, attributed to their rich content of phenolic and flavonoid compounds (Wetwitayaklung et al., 2013; Eldohaji et al., 2020). These bioactivities are commonly evaluated through *in vitro* assays like DPPH, ABTS, and ORAC, which are among the most widely applied methods for evaluating the antioxidant activity of mangrove extracts (Thatoi et al., 2014). These assays operate through distinct mechanisms: ORAC is based on hydrogen atom transfer (HAT) and measure the ability of antioxidants to neutralize peroxyl radicals, whereas DPPH and ABTS are single electron transfer (SET) assays that detect electron transfer

leading to radical reduction (Platzer et al., 2021). Because their responded depend on pH and solvent conditions, each assay may favour different classes of compounds. DPPH dissolves in organic solvents such as methanol and ethanol and is widely used for screening plant extracts, while ABTS, which reacts in aqueous media, accommodates both hydrophilic and lipophilic antioxidants (Thatoi et al., 2014; Munteanu and Apetrei, 2021). As no single assay can capture the full range of antioxidant mechanisms present in complex plant extracts, combining ABTS, DPPH, and ORAC provides a more reliable and comprehensive assessment of antioxidant potential. Despite the recognized medicinal value of mangroves in traditional practices, scientific assessments of their antioxidant potential remain limited to a few species, underscoring the need for broader bioprospecting studies.

1.6.2. Cytotoxic activity: exploring mangrove-derived compounds as anticancer agents

Cancer remains one of the world's major health problems, accounting for 19.2 million new cases and approximately 10 million deaths worldwide in 2020, consisting of nearly one-sixth of the total deaths globally (second cause for death after strokes) with projections indicating a continuous rise in incidence over the coming decades (Sung et al., 2021). While conventional chemotherapies have improved cancer survival rates, their limitations, including toxicity and drug resistance, have prompted the search for safer and more effective alternatives.

Natural products have long served as a reservoir for anticancer drug discovery. Historically, plant-derived compounds like vincristine, taxanes, and camptothecins have been pivotal in cancer treatment (Cerri et al., 2022). The structural diversity and biological specificity of phytochemicals make them promising candidates for novel anticancer agents.

Mangrove plants are increasingly being recognized for their anticancer properties considering their rich diversity of bioactive compounds, their ethnobotanical significance, particularly for communities in native adjacent coastal regions, and their unique adaptations to harsh environmental conditions (Mahmud et al., 2014; Kerry et al., 2018; Dey et al., 2021; Chowdhury et al., 2024). Phytochemical analyses have revealed that mangrove-derived compounds can induce apoptosis, inhibit cell proliferation, arrest the cell cycle, generate oxidative stress and disrupt mitochondrial function, and modulate pathways involved in metastasis and DNA repair (Chowdhury et al., 2024). However, despite their pharmacological profile, the cytotoxic potential of mangrove species remains underexplored, with the number of studies having grown in recent years but still remaining limited (Tian et al., 2009; Thao et al., 2015; Huang et al., 2016; Yang et al., 2018; Saroyo and Sapturi, 2021; Youssef et al., 2022).

Cytotoxicity screening, particularly using viability assay such as MTT, represents a practical and informative first step in assessing the anticancer potential of mangrove extracts. These assays measure the extract's ability to reduce cell viability in cancer cell lines, while concurrently evaluating selectively by testing on normal cells. Identifying promising cytotoxic extracts is crucial for developing lead compounds with therapeutic relevance (Satiawati et al., 2022; Chowdhury et al., 2024). Moreover, early-stage cytotoxicity data help in prioritizing extracts for further in-depth mechanistic studies and potential *in vivo* validation. Given the pharmacological uniqueness of mangrove metabolites and their traditional medicinal uses in coastal communities, expanding cytotoxicity studies in this plant group could uncover new anticancer candidates.

1.7. SUSTAINABILITY CONSIDERATIONS IN THE DEVELOPMENT OF MANGROVE-DERIVED BIOACTIVE COMPOUNDS

Given the unique ecological fragility of mangrove forests, with more than half of mangrove ecosystems at risk of collapse by 2050 (IUCN, 2024), and the growing interest in mangrove-derived natural products, it is crucial to ensure that the development of any promising metabolites aligns with biodiversity conservation and sustainable resource use. Importantly, once a bioactive compound has been identified, its further development does not require large-scale biomass collection. Modern drug discovery typically employs natural compounds as lead scaffolds for synthetic or semi-synthetic chemistry, allowing laboratory-based production and structural optimization that is independent of further plant extraction (Newman and Cragg, 2012; Davis and Choisy, 2024). A well-known example is the antitumor agent taxol (paclitaxel), naturally occurring in the bark of *Taxus brevifolia*, for which semi-synthetic production from renewable precursors in *Taxus baccata* needles prevented large-scale destruction of Pacific yew tree (Li and Vederas, 2009). Structural modification of natural leads has also yielded more potent or safer analogues, including vincristine, vinblastine, topotecan, and numerous emerging anticancer and anti-infective agents (Khazir et al., 2013), while rational scaffold simplification can improve drug-likeness and synthetic accessibility (Wang et al., 2019).

Beyond synthetic and semi-synthetic chemistry, advances in genomics and metabolic engineering now allow the identification of plant biosynthetic genes and their heterologous expression in microbial organisms such as yeast or bacteria, providing renewable production of complex metabolites. A hallmark example is the engineering of *E. coli* and yeast to produce

artemisinic acid, the precursor of the antimalarial artemisinin (Li and Vederas, 2009; Newman and Cragg, 2012; Paddon et al., 2013; Newman and Cragg, 2020; Davis and Choisy, 2024). Microbial cell factories increasingly support sustainable biosynthesis of plant polyphenols and other specialized metabolites through optimized pathway integration (Kallscheuer et al., 2019; Milke et al., 2018), while plant synthetic biology has expanded engineered biosynthesis from single-celled microbes to model plant systems such as Nicotiana sp. (Zhu et al., 2021). A Complementary strategy relies on plant cell and tissue culture, including stem-like and meristem-derived cultured maintained in vitro, which allow high-value metabolite production under controlled conditions while reducing pressure on natural populations (Ochoa-Villareal et al., 2016; Abdulhafiz et al., 2022).

Collectively, these approaches demonstrate that the downstream development of promising mangrove metabolites is fully compatible with biodiversity conservation. Even if novel or rare compounds are discovered in ecologically sensitive mangrove taxa, their future production can rely on renewable, laboratory-based systems, ensuring that drug-development efforts do not exert additional pressure on mangrove ecosystems.

1.8. AIMS OF THE STUDY

Among the plant kingdom, mangroves stand out as promising candidates for bioprospecting due to their rich genetic diversity, the ecological complexity of their habitats, and their remarkable resilience to extreme environmental conditions, which drives the production of unique bioactive compounds. Their longstanding use in traditional medicine, coupled with their recognized pharmacological potential, further underscores their importance as a source of novel therapeutic agents (Simlai and Roi, 2013; Bibi et al., 2019; Kathiresan, 2020; Sudhir et al., 2022; Murthy et al., 2025). However, despite these attributes, the exploration of mangroves as reservoirs of new drug candidates remains significantly underexplored (Patra and Thatoi, 2011; Parthiban et al., 2022), particularly in under-studied regions such as the Maldivian archipelago and the United Arab Emirates (UAE), where the unique environmental pressures of these locations are likely to drive the synthesis of specialized secondary metabolites (Jin et al., 2009; Tran et al., 2023).

In this context, our study focuses on two ecologically and pharmacologically significant mangrove species: *A. marina*, the only species forming extensive mangrove forests in the UAE

(Friis and Killilea, 2023), which remains underexplored in this region despite its historical use in traditional medicine (Zhou et al., 2025) and documented pharmacological properties (Thatoi et al., 2016); and *S. caseolaris*, a key species in the Maldivian mangrove ecosystems, which is considered highly promising due to its habitat specificity (Farhath et al., 2025), limited phytochemical characterization, and known ethnomedicinal relevance (Audah et al., 2024) and pharmacological potential (Nguyen et al., 2024).

The primary aim of this study is to conduct a comprehensive phytochemical investigation of various plant parts, including leaves, roots, propagules, and cotyledons, of *A. marina* collected from the UAE, and leaves and roots of *S. caseolaris* collected from the Maldives. By employing an untargeted analytical approach that combines ultra-performance liquid chromatography (ULPL) with high-resolution mass spectrometry (HRMS), we aim to generate detailed phytochemical profiles for each tissues type, facilitating the identification and spatial localization of specific bioactive compounds.

Given that mangrove species are renowned for their phenolic constituents with potential antioxidant activities (Botosa et al., 2025), we further assessed the antioxidant capacity of the extracts using established *in vitro* assays (DPPH, ABTS, and ORAC), providing a thorough comparison across different plant parts and correlating bioactivity with specific phytochemical constituents. Additionally, considering the reported anticancer potential of *A. marina*, we performed a comprehensive cytotoxicity evaluation using MTT assays, testing extracts against five cancer cell lines and two normal cell lines to compare their activity, assess selectivity and investigate potential molecule-specific effects. This approach enabled the preliminary identification of the most promising extract, which will serve as the focus of future fractionation, quantification, and mechanistic studies aimed at elucidating its anticancer potential and identifying prospective drug candidates.

In addition to the experimental objectives, we aimed to critically review the scientific literature on *A. marina* and *S. caseolaris*, in order to contextualize our findings and highlight both the confirmation and novelty of the experimental results.

Overall, the goal of this study was to expand the phytochemical knowledge and confirm the pharmacological potential of these mangrove species. The integrative design enabled comparison o different plant extracts, identification of the most promising ones in terms of bioactivity and preliminarily linking of observed activity to tissue-specific classes of compounds, providing a first step toward more focused mechanistic investigations. The novelty

of this study lies not only in the geographic specificity of the plant material collected, explored underrepresented regions like the UAE and the Maldives, but also in the holistic evaluation of phytochemistry and pharmacological activities across multiple plant tissues. Notably, this represents the first detailed UPLC-ESI-HRMS-based phytochemical profiling of different plant parts of both *A. marina* and *S. caseolaris*, and to our knowledge, it is the fist phytochemical investigation of *S. caseolaris* roots. This work significantly expands current knowledge on the chemical diversity and bioactivity of these mangrove species, highlighting their potential and providing a fundamental framework for future pharmacological and nutraceutical applications.

1.9. REFERENCES

Abd-ElGawad, A. M., Elshamy, A. I., Al-Rowaily, S. L., & El-Amier, Y. A. (2019). Habitat affects the chemical profile, allelopathy, and antioxidant properties of essential oils and phenolic enriched extracts of the invasive plant *Heliotropium curassavicum*. Plants, 8(11), 482.

Abdulhafiz, F., Mohammed, A., Reduan, M. F. H., Kari, Z. A., Wei, L. S., & Goh, K. W. (2022). Plant cell culture technologies: A promising alternatives to produce high-value secondary metabolites. Arabian Journal of Chemistry, 15(11), 104161.

Agardy, T., Hicks, F., Nistharan, F., Fisam, A., Abdulla, A., Schmidt, A., & Grimsditch, G. (2017). Ecosystem Services Assessment of North Ari Atoll Maldives.

Aggarwal, G., Sharma, M., Singh, R., & Sharma, U. (2024). Plant-based natural product chemistry: An overview of the multistep journey involved in scientific validation of traditional knowledge. Studies in Natural Products Chemistry, 80, 327-377.

Altmann, K. H. (2017). Drugs from the oceans: Marine natural products as leads for drug discovery. Chimia, 71(10), 646-646.

Alzandi, A. A., Taher, E. A., Al-Sagheer, N. A., Al-Khulaidi, A. W., Azizi, M., & Naguib, D. M. (2021). Phytochemical components, antioxidant and anticancer activity of 18 major medicinal plants in Albaha region, Saudi Arabia. Biocatalysis and Agricultural Biotechnology, 34, 102020.

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant physiology, 141(2), 391-396.

Audah, K. A., & Anisa, A. S. (2024). The Potential of *Sonneratia caseolaris* Mangrove Plant as Functional Food and Medicine. Journal of Functional Food and Nutraceutical, 45-58.

Audah, K. A., Ettin, J., Darmadi, J., Azizah, N. N., Anisa, A. S., Hermawan, T. D. F., et al. (2022). Indonesian mangrove Sonneratia caseolaris leaves ethanol extract is a potential super antioxidant and anti methicillin-resistant Staphylococcus aureus drug. Molecules, 27(23), 8369.

Baba, S., Chan, H. T., Oshiro, N., Maxwell, G. S., Inoue, T., & Chan, E. W. (2016). Botany, uses, chemistry and bioactivities of mangrove plants IV: *Avicennia marina*. ISME/GLOMIS Electron. J, 14, 5-10.

Bandaranayake, W. (1998). Traditional and medicinal uses of mangroves. Mangroves and salt marshes, 2(3), 133-148.

Bang, J. H., Lee, K. J., Jeong, W. T., Han, S., Jo, I. H., Choi, S. H., et al. (2021). Antioxidant activity and phytochemical content of nine *Amaranthus* species. Agronomy, 11(6), 1032.

Barros, L., Cabrita, L., Boas, M. V., Carvalho, A. M., & Ferreira, I. C. (2011). Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. Food Chemistry, 127(4), 1600-1608.

Bhattacharjee, S. (2005). Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transducation in plants. Current Science, 1113-1121.

Botosoa, E. P., & Shahidi, F. (2025). Phenolics and polyphenolics in mangrove plants: antioxidant activity and recent trends in food application–a review. Critical Reviews in Food Science and Nutrition, 1-35.

Bulan, D. E., Nurfadilah, N., Syahrir, M. R., Mismawati, A., Torambung, A. K., & Rachmawati, M. (2022). Phytochemical Composition and Antioxidant Activity of Leaf Extracts from Three *Rhizophora* Species from Bontang Waters, Indonesia. Tropical Journal of Natural Product Research, 6(8).

Burlec, A. F., Pecio, L., Kozachok, S., Mircea, C., Corciovă, A., Vereștiuc, L., et al. (2021). Phytochemical profile, antioxidant activity, and cytotoxicity assessment of *Tagetes erecta* L. flowers. Molecules, 26(5), 1201.

Cerri, F., Giustra, M., Anadol, Y., Tomaino, G., Galli, P., Labra, M., et al. (2022). Natural products from mangroves: an overview of the anticancer potential of *Avicennia marina*. Pharmaceutics, 14(12), 2793.

Cerri, F., Louis, Y. D., Fallati, L., Siena, F., Mazumdar, A., Nicolai, R., et al. (2024). Mangroves of the Maldives: A review of their distribution, diversity, ecological importance and biodiversity of associated flora and fauna. Aquatic Sciences, 86(2), 44.

Cerri, F., Mohamed, S., & Galli, P. (2025). Mangrove forests as a natural trap for marine plastic litter: Insights from the Maldives. Marine Pollution Bulletin, 213, 117677.

Cerri, F., Saliu, F., Maggioni, D., Montano, S., Seveso, D., Lavorano, S., et al. (2022). Cytotoxic compounds from Alcyoniidae: An overview of the last 30 years. Marine Drugs, 20(2), 134.

Cerri, F., Giustra, M., Anadol, Y., Tomaino, G., Galli, P., Labra, M., et al. (2022). Natural products from mangroves: an overview of the anticancer potential of *Avicennia marina*. Pharmaceutics, 14(12), 2793.

Cheng, H., Inyang, A., Li, C. D., Fei, J., Zhou, Y. W., & Wang, Y. S. (2020). Salt tolerance and exclusion in the mangrove plant *Avicennia marina* in relation to root apoplastic barriers. Ecotoxicology, 29(6), 676-683.

Chowdhury, R., Bhuia, M. S., Al Hasan, M. S., Hossain Snigdha, S., Afrin, S., Büsselberg, D., et al. (2024). Anticancer potential of phytochemicals derived from mangrove plants: Comprehensive mechanistic insights. Food Science & Nutrition, 12(9), 6174-6205.

Clarke, C. J., Tu, W. C., Levers, O., Brohl, A., & Hallett, J. P. (2018). Green and sustainable solvents in chemical processes. Chemical reviews, 118(2), 747-800.

Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., et al. (1997). The value of the world's ecosystem services and natural capital. nature, 387(6630), 253-260.

Cram, J. W., Torr, P. G., & Rose, D. A. (2002). Salt allocation during leaf development and leaf fall in mangroves. Trees, 16(2), 112-119.

Dahdouh-Guebas, F., Jayatissa, L. P., Di Nitto, D., Bosire, J. O., Seen, D. L., & Koedam, N. (2005). How effective were mangroves as a defence against the recent tsunami?. Current biology, 15(12), R443-R447.

Das, S., & Vincent, J. R. (2009). Mangroves protected villages and reduced death toll during Indian super cyclone. Proceedings of the National Academy of Sciences, 106(18), 7357-7360.

Davis, C. C., & Choisy, P. (2024). Medicinal plants meet modern biodiversity science. Current Biology, 34(4), R158-R173.

Dev, S., Acharyya, R. N., Akter, S., Al Bari, M. A., Asma, K., Hossain, H., et al. (2021). Toxicological screening and evaluation of anti-allergic and anti-hyperglycemic potential of *Sonneratia caseolaris* (L.) Engl. fruits. Clinical Phytoscience, 7(1), 69.

Dey, D., Quispe, C., Hossain, R., Jain, D., Ahmed Khan, R., Janmeda, P., et al. (2021). Ethnomedicinal use, phytochemistry, and pharmacology of *xylocarpus granatum* J. Koenig. Evidence-based Complementary and Alternative Medicine, 2021(1), 8922196.

Dhawi, F. (2025). Mastering Resilience: *Avicennia marina*'s Survival in Hypersaline Arid Zones. Frontiers in Sustainable Food Systems, 9, 1598548.

Di Ferdinando, M., Brunetti, C., Fini, A., & Tattini, M. (2011). Flavonoids as antioxidants in plants under abiotic stresses. Abiotic stress responses in plants: metabolism, productivity and sustainability, 159-179.

Dodd, R. S., Blasco, F., Rafii, Z. A., & Torquebiau, E. (1999). Mangroves of the United Arab Emirates: ecotypic diversity in cuticular waxes at the bioclimatic extreme. Aquatic Botany, 63(3-4), 291-304.

Duke, N. C. (1992). Mangrove floristics and biogeography. Tropical mangrove ecosystems, 41, 63-100.

El-Tarabily, K. A., Sham, A., Elbadawi, A. A., Hassan, A. H., Alhosani, B. K., El-Esawi, M. A., et al. (2021). A consortium of rhizosphere-competent actinobacteria exhibiting multiple plant growth-promoting traits improves the growth of *Avicennia marina* in the United Arab Emirates. Frontiers in Marine Science, 8, 715123.

ElDohaji, L. M., Hamoda, A. M., Hamdy, R., & Soliman, S. S. (2020). *Avicennia marina* a natural reservoir of phytopharmaceuticals: Curative power and platform of medicines. Journal of ethnopharmacology, 263, 113179.

Eong, O. J. (1993). Mangroves-a carbon source and sink. Chemosphere, 27(6), 1097-1107.

Falahi, H., Sharifi, M., Maivan, H. Z., & Chashmi, N. A. (2018). Phenylethanoid glycosides accumulation in roots of *Scrophularia striata* as a response to water stress. Environmental and Experimental Botany, 147, 13-21.

Farahmandfar, R., Esmaeilzadeh Kenari, R., Asnaashari, M., Shahrampour, D., & Bakhshandeh, T. (2019). Bioactive compounds, antioxidant and antimicrobial activities of *Arum maculatum* leaves extracts as affected by various solvents and extraction methods. Food science & nutrition, 7(2), 465-475.

Farhath, A. A., Nandan, S. B., Sreelekshmi, S., Rifga, M., Naeem, I., Hershey, N. R., & Ntakirutimana, R. (2025). Mangrove Ecosystems in the Maldives: A Nationwide Assessment of Diversity, Habitat Typology and Conservation Priorities. Earth, 6(3), 66.

Fisher, R., O'Leary, R. A., Low-Choy, S., Mengersen, K., Knowlton, N., Brainard, R. E., & Caley, M. J. (2015). Species richness on coral reefs and the pursuit of convergent global estimates. Current Biology, 25(4), 500-505.

Franzoni, G., Trivellini, A., Bulgari, R., Cocetta, G., & Ferrante, A. (2019). Bioactive molecules as regulatory signals in plant responses to abiotic stresses. In Plant signaling molecules (pp. 169-182). Woodhead Publishing.

Friis, G., & Killilea, M. E. (2023). Mangrove Ecosystems of the United Arab Emirates. In A natural history of the emirates (pp. 217-240). Cham: Springer Nature Switzerland.

Ganamé, H. T., Karanga, Y., Tapsoba, I., Dicato, M., Diederich, M. F., Cerella, C., & Sawadogo, R. W. (2021). Phytochemical screening and antioxidant and cytotoxic effects of *Acacia macrostachya*. Plants, 10(7), 1353.

Genilar, L. A., Kurniawaty, E., Mokhtar, R. A. M., & Audah, K. A. (2021). Mangroves and their medicinal benefit: A mini review. Annals of the Romanian Society for Cell Biology, 25(4), 695-709.

Govindasamy, C., & Kannan, R. (2012). Pharmacognosy of mangrove plants in the system of unani medicine. Asian Pacific Journal of Tropical Disease, 2, S38-S41.

Grover, M., Behl, T., & Virmani, T. (2021). Phytochemical screening, antioxidant assay and cytotoxic profile for different extracts of *Chrysopogon zizanioides* roots. Chemistry & Biodiversity, 18(8), e2100012.

Grzegorczyk-Karolak, I., Krzemińska, M., Kiss, A. K., Olszewska, M. A., & Owczarek, A. (2020). Phytochemical profile and antioxidant activity of aerial and underground parts of *Salvia bulleyana* Diels. plants. Metabolites, 10(12), 497.

Guannel, G., Arkema, K., Ruggiero, P., & Verutes, G. (2016). The power of three: coral reefs, seagrasses and mangroves protect coastal regions and increase their resilience. PloS one, 11(7), e0158094.

Guo, Z., Wei, M., Xu, C., Wang, L., Li, J., Liu, J., et al. (2024). Genome-wide identification of *Avicennia marina* aquaporins reveals their role in adaptation to intertidal habitats and their relevance to salt secretion and vivipary. Plant, Cell & Environment, 47(3), 832-853.

Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine, 27(1), 1-93.

Habshi, A. A., Youssef, T., Aizpuru, M., & Blasco, F. (2007). New mangrove ecosystem data along the UAE coast using remote sensing. Aquatic Ecosystem Health & Management, 10(3), 309-319.

Hao, S., Su, W., & Li, Q. Q. (2021). Adaptive roots of mangrove *Avicennia marina*: Structure and gene expressions analyses of pneumatophores. Science of the Total Environment, 757, 143994.

Huamán-Castilla, N. L., Díaz Huamaní, K. S., Palomino Villegas, Y. C., Allcca-Alca, E. E., León-Calvo, N. C., Colque Ayma, E. J., et al. (2024). Exploring a sustainable process for polyphenol extraction from olive leaves. Foods, 13(2), 265.

Huang, C., Lu, C. K., Tu, M. C., Chang, J. H., Chen, Y. J., Tu, Y. H., & Huang, H. C. (2016). Polyphenol-rich *Avicennia marina* leaf extracts induce apoptosis in human breast and liver cancer cells and in a nude mouse xenograft model. Oncotarget, 7(24), 35874.

Island Development and Environmental Awareness Society (2017) Of famine and food security: a story from Kelaa's kandoofaa (mangrove). Ideaskelaa in mangroves, Mangroves of Maldives. Available at https://ideaskelaa.wordpress.com/2017/08/24/of-famine-and-food-security/ (Accessed 23 August 2025).

IUCN (International Union for Conservation of Nature), 2024. Red List of Ecosystems, More Than Half of All Mangrove Ecosystems at Risk of Collapse by 2050, First Global Assessment Finds. Available at https://www.iucn.org/press-release/202405/more-half-all-mangrove-ecosystems-risk-collapse-2050-first-global-assessment (Accessed 10 August 2025).

Jabbar, A. A. (2021). *Onosma mutabilis*: Phytochemical composition, antioxidant, cytotoxicity, and acute oral toxicity. Food Science & Nutrition, 9(10), 5755-5764.

Jin, J., Koroleva, O. A., Gibson, T., Swanston, J., Magan, J., Zhang, Y. A. N., et al. (2009). Analysis of phytochemical composition and chemoprotective capacity of rocket (*Eruca sativa* and *Diplotaxis tenuifolia*) leafy salad following cultivation in different environments. Journal of agricultural and food chemistry, 57(12), 5227-5234.

Kallscheuer, N., Classen, T., Drepper, T., & Marienhagen, J. (2019). Production of plant metabolites with applications in the food industry using engineered microorganisms. Current opinion in biotechnology, 56, 7-17.

Kathiresan, K. (2020). Bioprospecting potential of mangrove resources. In Biotechnological utilization of mangrove resources (pp. 225-241). Academic Press.

Kathiresan, K., & Bingham, B. L. (2001). Biology of mangroves and mangrove ecosystems.

Kerry, R. G., Pradhan, P., Das, G., Gouda, S., Swamy, M. K., & Patra, J. K. (2018). Anticancer potential of mangrove plants: Neglected plant species of the marine ecosystem. In Anticancer plants: Properties and Application: Volume 1 (pp. 303-325). Singapore: Springer Singapore.

khanh Tran, T., Ha, P. T. T., Henry, R. J., Nguyen, D. N. T., Tuyen, P. T., & Liem, N. T. (2023). Polyphenol Contents, Gas Chromatography-Mass Spectrometry (GC–; MS) and Antibacterial Activity of Methanol Extract and Fractions of *Sonneratia Caseolaris* Fruits from Ben Tre Province in Vietnam. Journal of Microbiology and Biotechnology, 34(1), 94.

Khazir, J., Mir, B. A., Mir, S. A., & Cowan, D. (2013). Natural products as lead compounds in drug discovery.

Kodikara, C., Netticadan, T., Bandara, N., Wijekoon, C., & Sura, S. (2024). A new UHPLC-HRMS metabolomics approach for the rapid and comprehensive analysis of phenolic compounds in blueberry, raspberry, blackberry, cranberry and cherry fruits. Food Chemistry, 445, 138778.

Lee, I. Y., Lee, D. H., Park, J. H., & Joo, N. (2025). UHPLC-HRMS/MS–Based Metabolic Profiling and Quantification of Phytochemicals in Different Parts of *Coccinia grandis* (L.) Voigt. Food Science & Nutrition, 13(2), e70004.

Lezoul, N. E. H., Belkadi, M., Habibi, F., & Guillén, F. (2020). Extraction processes with several solvents on total bioactive compounds in different organs of three medicinal plants. Molecules, 25(20), 4672.

Liebezeit, G., & Rau, M. T. (2006). New Guinean mangroves—Traditional usage and chemistry of natural products. Senckenbergiana maritima, 36(1), 1-10.

Li, J. W. H., & Vederas, J. C. (2009). Drug discovery and natural products: end of an era or an endless frontier?. Science, 325(5937), 161-165.

Lim, K. J. A., Cabajar, A. A., Lobarbio, C. F. Y., Taboada, E. B., & Lacks, D. J. (2019). Extraction of bioactive compounds from mango (*Mangifera indica* L. var. Carabao) seed kernel with ethanol–water binary solvent systems. Journal of Food Science and Technology, 56(5), 2536-2544.

Liu, C., Zhang, L., Shi, X., Tang, Y., Wang, M., & Wang, W. (2025). Element contents changes during the propagule development of two *Sonneratia* species. Frontiers in Marine Science, 11, 1430782.

Ma, D., Ding, Q., Guo, Z., Xu, C., Liang, P., Zhao, Z., et al. (2022). The genome of a mangrove plant, *Avicennia marina*, provides insights into adaptation to coastal intertidal habitats. Planta, 256(1), 6.

Mahmud, I., Islam, M. K., Saha, S., Barman, A. K., Rahman, M. M., Anisuzzman, M., et al. (2014). Pharmacological and ethnomedicinal overview of *Heritiera fomes*: future prospects. International Scholarly Research Notices, 2014(1), 938543.

Marrelli, M. (2021). Medicinal plants. Plants, 10(7), 1355.

Martínez-Díaz, M. G., & Reef, R. (2023). A biogeographical approach to characterizing the climatic, physical and geomorphic niche of the most widely distributed mangrove species, *Avicennia marina*. Diversity and Distributions, 29(1), 89-108.

Milke, L., Aschenbrenner, J., Marienhagen, J., & Kallscheuer, N. (2018). Production of plant-derived polyphenols in microorganisms: current state and perspectives. Applied Microbiology and Biotechnology, 102(4), 1575-1585.

Miranda, J. J. M. (2021). Medicinal plants and their traditional uses in different locations. In Phytomedicine (pp. 207-223). Academic Press.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends in plant science, 7(9), 405-410.

Mittler, R., Vanderauwera, S., Gollery, M., & Van Breusegem, F. (2004). Reactive oxygen gene network of plants. Trends in plant science, 9(10), 490-498.

Mohammed, H. A. (2022). Phytochemical analysis, antioxidant potential, and cytotoxicity evaluation of traditionally used *Artemisia absinthium* L.(Wormwood) growing in the central region of Saudi Arabia. Plants, 11(8), 1028.

Moore, G. E., Grizzle, R. E., Ward, K. M., & Alshihi, R. M. (2015). Distribution, pore-water chemistry, and stand characteristics of the mangroves of the United Arab Emirates. Journal of Coastal Research, 31(4), 957-963.

Muhlemann, J. K., Klempien, A., & Dudareva, N. (2014). Floral volatiles: from biosynthesis to function. Plant, cell & environment, 37(8), 1936-1949.

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. International journal of molecular sciences, 22(7), 3380.

Murthy, H. N., Yadav, G. G., Paek, K. Y., & Park, S. Y. (2025). Novel Secondary Metabolites from Mangrove Flora: Chemistry and Bioactivity. Bioactive Compounds in Mangroves and their Associates, 1-17.

Nabeelah Bibi, S., Fawzi, M. M., Gokhan, Z., Rajesh, J., Nadeem, N., RR, R. K., et al. (2019). Ethnopharmacology, phytochemistry, and global distribution of mangroves—A comprehensive review. Marine drugs, 17(4), 231.

Nebula, M., Harisankar, H. S., & Chandramohanakumar, N. (2013). Metabolites and bioactivities of Rhizophoraceae mangroves. Natural products and bioprospecting, 3(5), 207-232.

Neugart, S., Krumbein, A., & Zrenner, R. (2016). Influence of light and temperature on gene expression leading to accumulation of specific flavonol glycosides and hydroxycinnamic acid derivatives in kale (Brassica oleracea var. sabellica). Frontiers in plant science, 7, 326.

Newman, D. J., & Cragg, G. M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of natural products, 75(3), 311-335.

Nguyen, L. T. T., Nguyen, T. T., Nguyen, H. N., & Bui, Q. T. P. (2024). Analysis of active compounds and bioactivity of leaves extracts of *Sonneratia* species. Engineering Reports, 6(10), e12870.

Nugroho, R. Y., Hendri, M., Putri, W. A. E., & Agussalim, A. (2022). Phytochemical profile and toxicity of extracts from the leaf of *Avicennia marina* (Forssk.) Vierh. collected in mangrove areas affected by port activities. South African Journal of Botany, 150, 903-919.

Ochoa-Villarreal, M., Howat, S., Hong, S., Jang, M. O., Jin, Y. W., Lee, E. K., & Loake, G. J. (2016). Plant cell culture strategies for the production of natural products. BMB reports, 49(3), 149.

Olszowy, M. (2019). What is responsible for antioxidant properties of polyphenolic compounds from plants?. Plant Physiology and Biochemistry, 144, 135-143.

Paddon, C. J., Westfall, P. J., Pitera, D. J., Benjamin, K., Fisher, K., McPhee, D., ... & Newman, J. D. (2013). High-level semi-synthetic production of the potent antimalarial artemisinin. Nature, 496(7446), 528-532.

Palaiogiannis, D., Chatzimitakos, T., Athanasiadis, V., Bozinou, E., Makris, D. P., & Lalas, S. I. (2023). Successive solvent extraction of polyphenols and flavonoids from *Cistus creticus* L. leaves. Oxygen, 3(3), 274-286.

Parani, M., Lakshmi, M., Senthilkumar, P., Ram, N., & Parida, A. (1998). Molecular phylogeny of mangroves V. Analysis of genome relationships in mangrove species using RAPD and RFLP markers. Theoretical and Applied Genetics, 97(4), 617-625.

Parthiban, A., Sivasankar, R., Sachithanandam, V., Khan, S. A., Jayshree, A., Murugan, K., & Sridhar, R. (2022). An integrative review on bioactive compounds from Indian mangroves for future drug discovery. South African Journal of Botany, 149, 899-915.

Patlakas, P., Stathopoulos, C., Flocas, H., Kalogeri, C., & Kallos, G. (2019). Regional climatic features of the Arabian Peninsula. Atmosphere, 10(4), 220.

Patra, J. K., & Thatoi, H. N. (2011). Metabolic diversity and bioactivity screening of mangrove plants: a review. Acta Physiologiae Plantarum, 33(4), 1051-1061.

Patridge, E., Gareiss, P., Kinch, M. S., & Hoyer, D. (2016). An analysis of FDA-approved drugs: natural products and their derivatives. Drug discovery today, 21(2), 204-207.

Plaskova, A., & Mlcek, J. (2023). New insights of the application of water or ethanol-water plant extract rich in active compounds in food. Frontiers in Nutrition, 10, 1118761.

Platzer, M., Kiese, S., Herfellner, T., Schweiggert-Weisz, U., Miesbauer, O., & Eisner, P. (2021). Common trends and differences in antioxidant activity analysis of phenolic substances using single electron transfer based assays. Molecules, 26(5), 1244.

Polidoro, B. A., Carpenter, K. E., Collins, L., Duke, N. C., Ellison, A. M., Ellison, J. C., et al. (2010). The loss of species: mangrove extinction risk and geographic areas of global concern. PloS one, 5(4), e10095.

Primavera, J. H., Sadaba, R. B., Lebata, M. J. H., & Altamirano, J. P. (2004). Handbook of mangroves in the Philippines.

Quattrocchi, U. (2006). CRC world dictionary of grasses: common names, scientific names, eponyms, synonyms, and etymology-3 volume set. CRC Press.

Rahim, A. C., & Bakar, M. F. A. (2018). Pidada—Sonneratia caseolaris. In Exotic Fruits (pp. 327-332). Academic Press.

Raihan, A., Ali, T., Mortula, M., & Gawai, R. (2023). Spatiotemporal analysis of the impacts of climate change on mangroves located in the United Arab Emirates. Journal of Sustain Development of Energy, Water and Environment Systems, 11(3), 1-19.

Rozirwan, R., Hananda, H., Nugroho, R. Y., Apri, R., Khotimah, N. N., Fauziyah, F., et al. (2023). Antioxidant Activity, Total Phenolic, Phytochemical Content, and HPLC Profile of Selected Mangrove Species from Tanjung Api-Api Port Area, South Sumatra, Indonesia. Tropical Journal of Natural Product Research, 7(7).

Sadhu, S. K., Ahmed, F., Ohtsuki, T., & Ishibashi, M. (2006). Flavonoids from *Sonneratia caseolaris*. Journal of Natural Medicines, 60(3), 264-265.

Saranraj, P., & Sujitha, D. (2015). Mangrove medicinal plants: a review. American-Eurasian Journal of Toxicological Sciences, 7(3), 146-156.

Saroyo, H., & Saputri, N. F. M. (2021). Cytotoxicity of mangrove leaves (*Rhizophora*) ethanolic extract on cancer cells. Journal of Nutraceuticals and Herbal Medicine, 4(1), 43-52.

Sarri, E., Termentzi, A., Abraham, E. M., Papadopoulos, G. K., Baira, E., Machera, K., et al. (2021). Salinity stress alters the secondary metabolic profile of *M. sativa*, *M. arborea* and their hybrid (Alborea). International Journal of Molecular Sciences, 22(9), 4882.

Scholander, P. F. (1968). How mangroves desalinate seawater. Physiologia plantarum, 21(1), 251-261.

Selvam, V. (2007). Trees and shrubs of the Maldives. FAO Regional Office for Asia and the Pacific, Bangkok.

Ser, H. L., Tan, L. T. H., Law, J. W. F., Chan, K. G., Duangjai, A., Saokaew, S., et al. (2017). Focused review: cytotoxic and antioxidant potentials of mangrove-derived Streptomyces. Frontiers in Microbiology, 8, 2065.

Setiawati, A., Candrasari, D. S., Setyajati, F. E., Prasetyo, V. K., Setyaningsih, D., & Hartini, Y. S. (2022). Anticancer drug screening of natural products: In vitro: cytotoxicity assays, techniques, and challenges. Asian Pacific Journal of Tropical Biomedicine, 12(7), 279-289.

Shadiya, F., Jauharee, A. R., & Shazly, A. (2016). Environmental valuation of K. Huraa mangrove: a case study of ecological, social and economic perspectives.

Shi, S., Huang, Y., Zeng, K., Tan, F., He, H., Huang, J., & Fu, Y. (2005). Molecular phylogenetic analysis of mangroves: independent evolutionary origins of vivipary and salt secretion. Molecular Phylogenetics and Evolution, 34(1), 159-166.

Simlai, A., & Roy, A. (2013). Biological activities and chemical constituents of some mangrove species from Sundarban estuary: An overview. Pharmacognosy reviews, 7(14), 170.

Singh, A., & Choudhary, K. K. (2025). Utilizing UHPLC-HRMS-metabolomic profiling to uncover enhanced bioactive potential and health benefits in chili (*Capsicum annum* L.) under salinity stress. Food Chemistry, 483, 144255.

Spalding, M., & Parrett, C. L. (2019). Global patterns in mangrove recreation and tourism. Marine Policy, 110, 103540.

Srikanth, S., Lum, S. K. Y., & Chen, Z. (2016). Mangrove root: adaptations and ecological importance. Trees, 30(2), 451-465.

Stagos, D. (2019). Antioxidant activity of polyphenolic plant extracts. Antioxidants, 9(1), 19.

Sudhir, S., Arunprasath, A., & Vel, V. S. (2022). A critical review on adaptations, and biological activities of the mangroves. Journal of Natural Pesticide Research, 1, 100006.

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 71(3), 209-249.

Tatongjai, S., Kraichak, E., & Kermanee, P. (2021). Comparative anatomy and salt management of *Sonneratia caseolaris* (L.) Engl.(Lythraceae) grown in saltwater and freshwater. PeerJ, 9, e10962.

Teoh, E. S. (2016). Secondary metabolites of plants. In Medicinal orchids of Asia (pp. 59-73). Cham: Springer International Publishing.

Thao, N. P., Luyen, B. T. T., Diep, C. N., Tai, B. H., Kim, E. J., Kang, H. K., et al. (2015). In vitro evaluation of the antioxidant and cytotoxic activities of constituents of the mangrove *Lumnitzera racemosa* Willd. Archives of pharmacal research, 38(4), 446-455.

Thatoi, H. N., Patra, J. K., & Das, S. K. (2014). Free radical scavenging and antioxidant potential of mangrove plants: a review. Acta physiologiae plantarum, 36(3), 561-579.

Thatoi, H., Samantaray, D., & Das, S. K. (2016). The genus *Avicennia*, a pioneer group of dominant mangrove plant species with potential medicinal values: a review. Frontiers in Life Science, 9(4), 267-291.

The Angiosperm Phylogeny Group, Chase, M. W., Christenhusz, M. J. M., Fay, M. F., Byng, J. W., Judd, W. S., Soltis, D. E., Mabberley, D. J., Sennikov, A. N., Soltis, P. S., Stevens, P. F. (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV, Botanical Journal of the Linnean Society, 181(1), 1–20.

Tian, M., Dai, H., Li, X., & Wang, B. (2009). Chemical constituents of marine medicinal mangrove plant *Sonneratia caseolaris*. Chinese journal of oceanology and limnology, 27(2), 288-296.

Tomlinson, P. B. (2016). The botany of mangroves. Cambridge University Press.

Toscano, S., Trivellini, A., Cocetta, G., Bulgari, R., Francini, A., Romano, D., & Ferrante, A. (2019). Effect of preharvest abiotic stresses on the accumulation of bioactive compounds in horticultural produce. Frontiers in plant science, 10, 1212.

van Wyk, A. S., & Prinsloo, G. (2020). Health, safety and quality concerns of plant-based traditional medicines and herbal remedies. South African Journal of Botany, 133, 54-62.

Wang, L., Mu, M., Li, X., Lin, P., & Wang, W. (2011). Differentiation between true mangroves and mangrove associates based on leaf traits and salt contents. Journal of Plant Ecology, 4(4), 292-301.

Wang, S., Dong, G., & Sheng, C. (2019). Structural simplification of natural products. Chemical Reviews, 119(6), 4180-4220.

Wang, Y., Zhu, H., & Tam, N. F. Y. (2014). Polyphenols, tannins and antioxidant activities of eight true mangrove plant species in South China. Plant and soil, 374(1), 549-563.

Wetwitayaklung, P., Limmatvapirat, C., & Phaechamud, T. (2013). Antioxidant and anticholinesterase activities in various parts of *Sonneratia caseolaris* (L.). Indian journal of pharmaceutical sciences, 75(6), 649.

Yang, X. W., Dai, Z., Wang, B., Liu, Y. P., Zhao, X. D., & Luo, X. D. (2018). Antitumor triterpenoid saponin from the fruits of *Avicennia marina*. Natural products and bioprospecting, 8(5), 347-353.

Yang, Y., Duke, N. C., Peng, F., Li, J., Yang, S., Zhong, C., et al. (2016). Ancient geographical barriers drive differentiation among *Sonneratia caseolaris* populations and recent divergence from *S. lanceolata*. Frontiers in Plant Science, 7, 1618.

Youssef, A. M., Maaty, D. A., & Al-Saraireh, Y. M. (2022). Phytochemistry and anticancer effects of mangrove (*Rhizophora mucronata* Lam.) leaves and stems extract against different cancer cell lines. Pharmaceuticals, 16(1), 4.

Yu, M., Gouvinhas, I., Rocha, J., & Barros, A. I. (2021). Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. Scientific reports, 11(1), 10041.

Zanatta, A. C., Vilegas, W., & Edrada-Ebel, R. (2021). UHPLC-(ESI)-HRMS and NMR-based metabolomics approach to access the seasonality of *Byrsonima intermedia* and *Serjania marginata* From Brazilian Cerrado Flora diversity. Frontiers in chemistry, 9, 710025.

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M. H., & Bahadar, K. (2018). Role of secondary metabolites in plant defense against pathogens. Microbial pathogenesis, 124, 198-202.

Zhang, H., Wang, T., Liu, M., Jia, M., Lin, H., Chu, L. M., & Devlin, A. T. (2018). Potential of combining optical and dual polarimetric SAR data for improving mangrove species discrimination using rotation forest. Remote Sensing, 10(3), 467.

Zhou, P., Hu, H., Wu, X., Feng, Z., Li, X., Tavakoli, S., et al. (2025). Botany, traditional uses, phytochemistry, pharmacological activities, and toxicity of the mangrove plant *Avicennia marina*: a comprehensive review. Phytochemistry Reviews, 1-36.

Zhu, X., Liu, X., Liu, T., Wang, Y., Ahmed, N., Li, Z., & Jiang, H. (2021). Synthetic biology of plant natural products: From pathway elucidation to engineered biosynthesis in plant cells. Plant communications, 2(5).

CHAPTER 2

Literature review: phytochemistry, antioxidant, and anticancer potential of *Avicennia marina*

This chapter has been adapted, modified, and implemented from the following published work:

Cerri, F., Giustra, M., Anadol, Y., Tomaino, G., Galli, P., Labra, M., Campone, L., Colombo, M. (2022). Natural products from mangroves: an overview of the anticancer potential of *Avicennia marina*. *Pharmaceutics*, 14(12), 2793.

2.1. ABSTRACT

Natural products derived from plants are increasingly recognized as valuable sources of therapeutic agents due to their sustainability, wide availability, and diverse biological activity. *Avicennia marina* has been recently recognized as a potential source of natural substances with therapeutic activities. Phytochemical investigations have revealed a broad range of compounds, including naphthalene derivatives, flavonoids, iridoid glycosides, phenolic glycosides, terpenoids, phenolic acids and derivatives, fatty acids, and steroids, that contribute to its multiple biological effects. In particular, the antioxidant activity of both extracts and isolated compounds have been extensively documented. More importantly, numerous *in vitro* and *in vivo* studies have demonstrated the anticancer potential of *A. marina* extracts and purified molecules. Moreover, recent advances in nanoparticle-based formulations have enhanced the therapeutic efficacy of these compounds by improving their selectivity toward cancer cells and reducing systemic toxicity. This chapter reviews the chemical composition of *A. marina*, highlights its antioxidant properties, and emphasizes the growing evidence supporting its anticancer potential.

2.2 INTRODUCTION

Avicennia marina, commonly known as the grey mangrove, is a mangrove species that is highly resistant to extreme environmental conditions, such as high salinity, high temperature, strong winds, and anaerobic soil (Das et al., 2016; Eldohaji et al., 2020). These extraordinary capacities for resistance prompted researchers to investigate the potential of this plant for pharmaceutical uses and the development of new drugs.

A. marina exhibits diverse biological activities, including antimalarial, antibacterial, analgesic, antioxidant, antifouling, antidiabetic, anti-inflammatory, anti-rheumatoid arthritis, and anticancer effects (Babuselvam et al., 2013; Gandomani et al., 2014; Esau et al., 2015; Huang et al., 2016). Among these, *A. marina* has been particularly investigated for anticancer therapy. Cancer remains a leading global health challenge, with an estimated 19.3 million new cases and nearly 10 million cancer deaths worldwide in 2020 (Sung et al., 2020)

Based on this background, this chapter focuses on the anticancer potential of *A. marina*, as well as its antioxidant activity, reviewing the bioactive compounds isolated from different plant parts and summarizing studies that evaluated their cytotoxic, antiproliferative, and antioxidant properties.

2.3. CHEMICAL COMPOSITION

Extensive phytochemical investigations have revealed a wide range of chemical constituents in *A. marina*, including aliphatic alcohols, amino acids, carbohydrates, alkaloids, carotenoids, fatty acids, hydrocarbons, iridoid glycosides, abietane diterpenoid glycosides, carboxylic acids, steroids, tannins, triterpenes, naphthoquinones, flavones, flavonoids, phorbol esters, phenolic and related compounds, pheromones, inorganic salts, minerals, phytoalexins, and vitamins (**Figure 2.1**) (Sun et al., 2008; Esau et al., 2015; Eldohaji et al., 2021; Ibrahim et al., 2022). Among these, the most abundant and biologically significant secondary metabolites are naphthalene derivatives, flavonoids, iridoid glycosides, terpenoids, and phenolic glycosides.

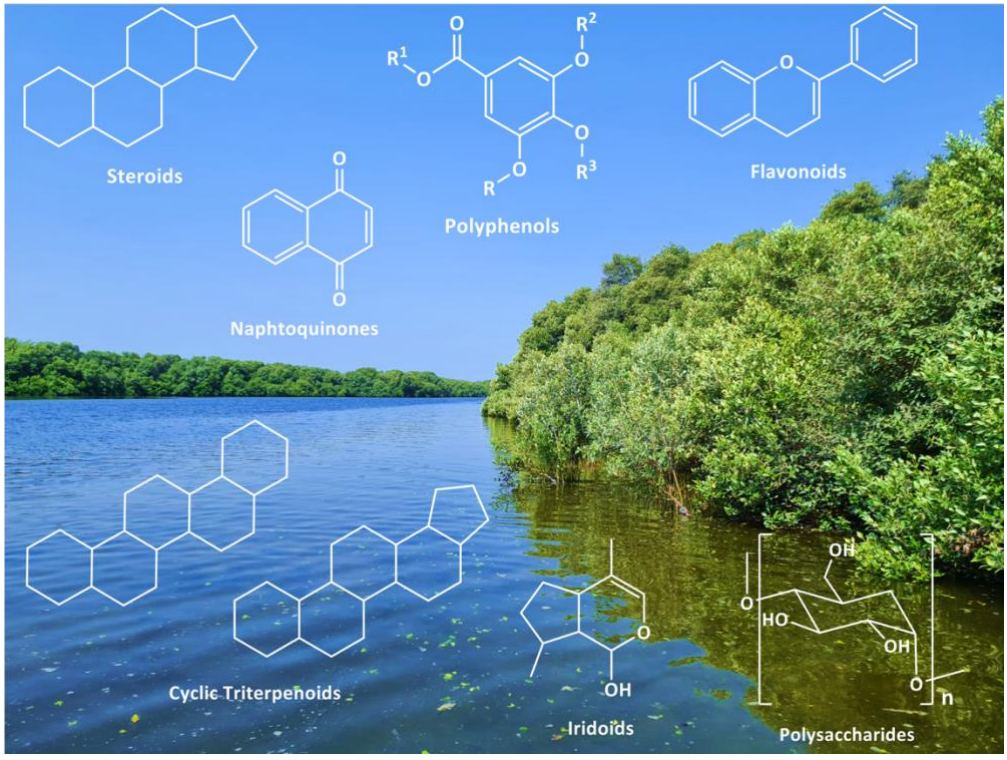

Figure 2.1. Main classes of compounds extractable from *Avicennia marina*: cyclic triterpenoids, flavonoids, iridoids, naphthoquinones, polyphenols, polysaccharides, and steroids. The picture was taken in the Al Zorah mangrove lagoon (Ajman, UAE).

2.3.1. Naphthalene derivatives

Han et al. (2007) reported the occurrence of seven unusual naphthalene derivatives isolated from the methanol extracts of twigs of *A. marina* collected in Xiamen (China), called avicennone A–G (**1–7**), together with 5 known compounds, stenocarproquinone B (**8**), avicequinone A (**9**), avicequinone C (**10**), avicenol A (**11**), and avicenol C (**12**). Compounds **8–10,** containing a 4,9-dione group as the main substituent, and a mixture of compounds **4** and **5**, showed high antiproliferative and moderate cytotoxic effects, as well as antibacterial activities.

Avicequinone C (**10)** was successfully employed in the treatment of androgenic alopecia due to its 5α-R1 inhibitory activity (Jain et al., 2014). Furthermore, naphtho[1,2-b]furan-4,5-dione (**13**), 3-hydroxy-naphtho[1,2-b]furan-4,5-dione (**14**), and 2-[2'-(2'-hydroxy)propyl]-naphtho[1,2-b]furan-4,5-dione (**15**) has been detected in seedlings (Sutton et al., 1985), and avicequinone B (**16**) in leaves (Jia et al., 2004).

The structures of naphthalene derivatives **1–12** are displayed in **Figure 2.2**.

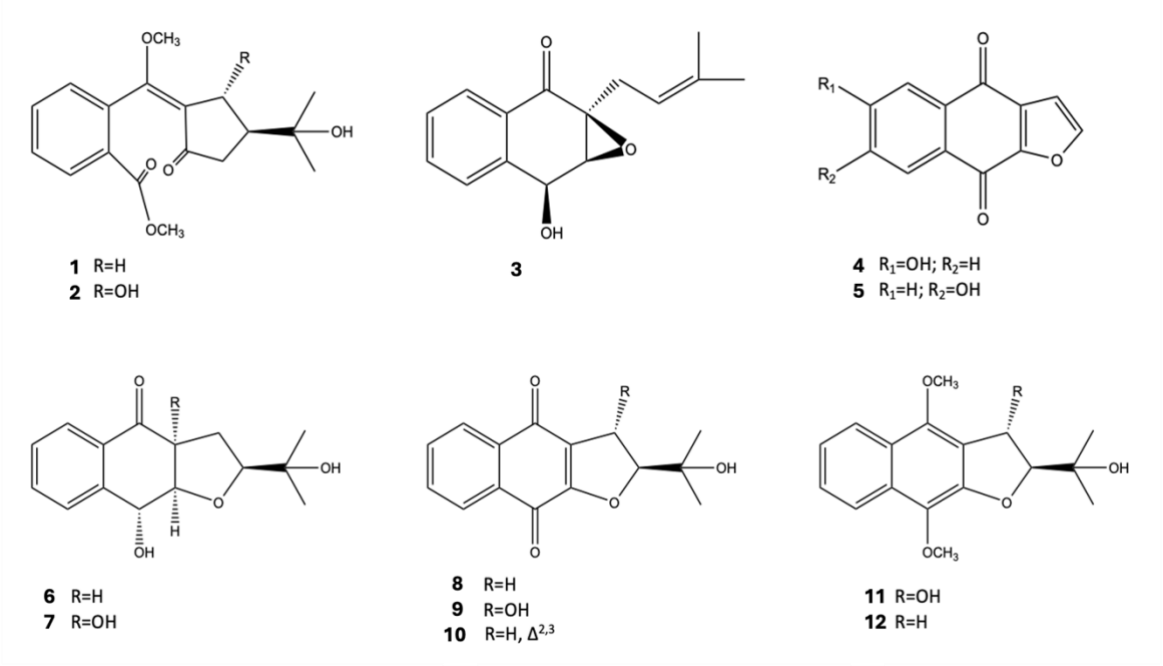

Figure 2.2. Naphthalene derivatives from *Avicennia marina*.

2.3.2. Flavonoids

Sharaf et al. (2000) isolated two flavonoids, luteolin 7-*O*-methylether 3′-O-β-D-glucoside (**17**) and its galactoside analogue (**18**), from aerial parts of A. *marina* collected in Hurghada (Egypt) and extracted with 80% methanol. Jia et al. (2004) identified a new flavone from the leaves, 5-hydroxy-4′,7-dimethoxyflavone (**19**), along with two common flavonoids, quercetin (**20**) and kaempferol (**21**). Feng et al. (2006) isolated four hydroxylated flavones from aerial parts of *A. marina* harvested in Hainan Island (China) and extracted with CHCl3/MeOH 1:1 (v/v), including 4′,5-dihydroxy-3′,7-dimethoxyflavone (**22**), 4′,5-dihydroxy-3′,5′,7-trimethoxyflavone (**23**), 4′,5,7- trihydroxyflavone (**24**), and 3′,4′,5-trihydroxy-7-methoxyflavone (**25**). Another methoxylated flavone, 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone (**26**), was isolated by the same researchers in 2007 (Feng et al., 2007). Furthermore, other flavonoids have been reported, including chrysoeriol 7-O-glucoside (**27**) and isorhamnetin 3-O-rutinoside (**28**) from the methanolic extract of the aerial parts; isoquercitrin (**29**) and luteolin

(**30**) from methanolic leaf extract collected in Iran and India, respectively; and flavoyadorinin B (**31**) from a ethanol extract of fruits collected in China (Sharaf et al., 2000; Momtazi-borojeni et al., 2013; Arumugam et al., 2017; Yang et al., 2018).

The chemical structures of flavonoids **17–26** are shown in **Figure 2.3**.

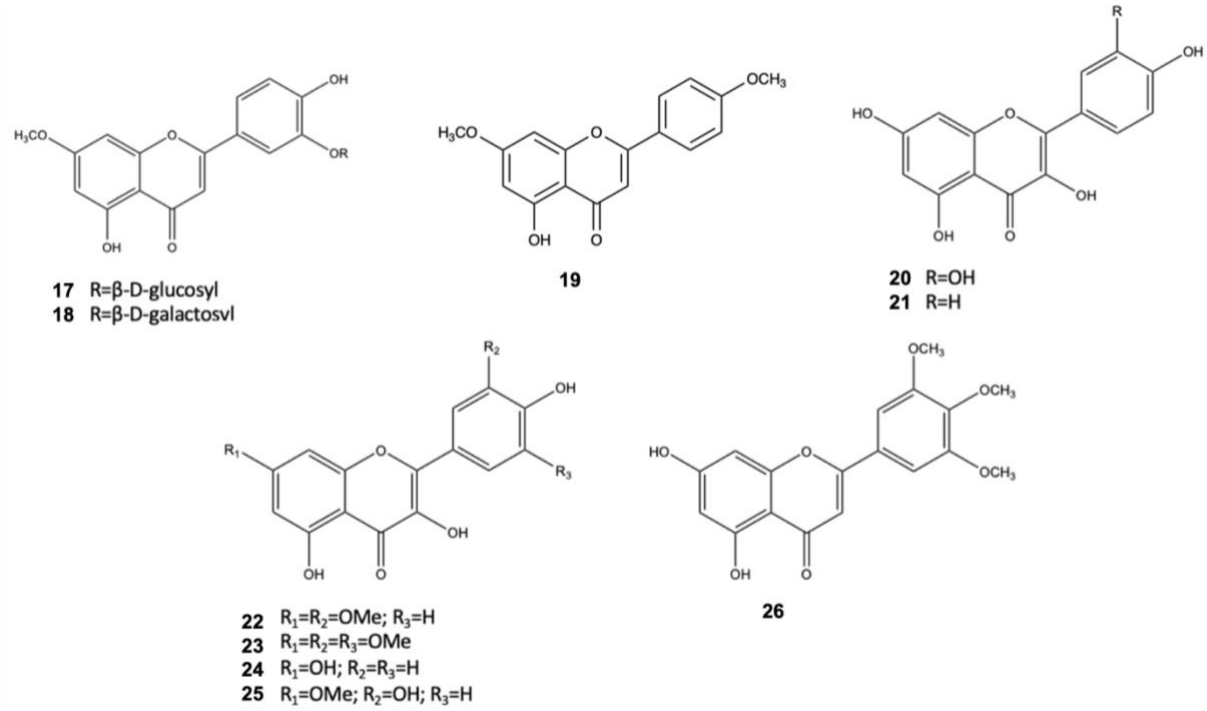

Figure 2.3. Flavones from *Avicennia marina*.

2.3.3. Iridoid glycosides

In 1985, König and Rimpler (1985) obtained seven iridoids from methylated extract of the leaves of *A. marina*. The isolated iridoid compounds were geniposidic acid (**32**), 2′-cinnamoyl-mussaenosidic acid (**33**), geniposide (**34**), mussaenoside (**35**), 2′-cinnamoyl- mussaenoside (**36**), 10-O-(5-phenyl—2,4-pentadienoyl)-geniposide (**37**), and 7-O-(5-phenyl-2,4-pentadienoyl)-8-epiloganin (**38**). This study also showed that iridoids occur as free acids in the plant. Shaker et al. (2001) described the isolation of three new geniposidic acid esters, namely 10-O-[(E)-cinnamoyl]-geniposidic acid (**39**), 10-O-[(E)-p-coumaroyl]-geniposidic acid (**40**), and 10-O-[(E)-caffeoyl]-geniposidic acid (**41**), from butanol extract of *A. marina,* which was collected in Hurghada, Egypt. To obtain the pure iridoid compounds, the authors used a combination of three purification steps: silica gel column, reverse phase C18, and Sephadex LH-20, respectively. Feng et al. (2006) reported, for the first time, the purification of two new iridoid glycosides, namely 2′-O-[5-phenylpenta-(2E,4E)-dienoyl] mussaenosidic acid (**42**) and

2′-O-(4-methoxycinnamoyl) mussaenosidic acid (**43**), and one known iridoid glycoside, 2′-O-coumaroylmussaenosidic acid (**44**).

Through a chemical investigation of the ethanol extract of the leaves of *A. marina* harvested on the coast of the Xiamen region (China), Sun et al. (2008) described, for the first time, the isolation and characterization of five iridoid glycosides, marinoids A-E (**45–49**). Furthermore, 10-O-[(E)-feruloyl]-geniposidic acid (**50**) were detected from the fruits (Yang et al., 2018).

The structures of iridoid glycosides **32–49** are shown in **Figure 2.4**.

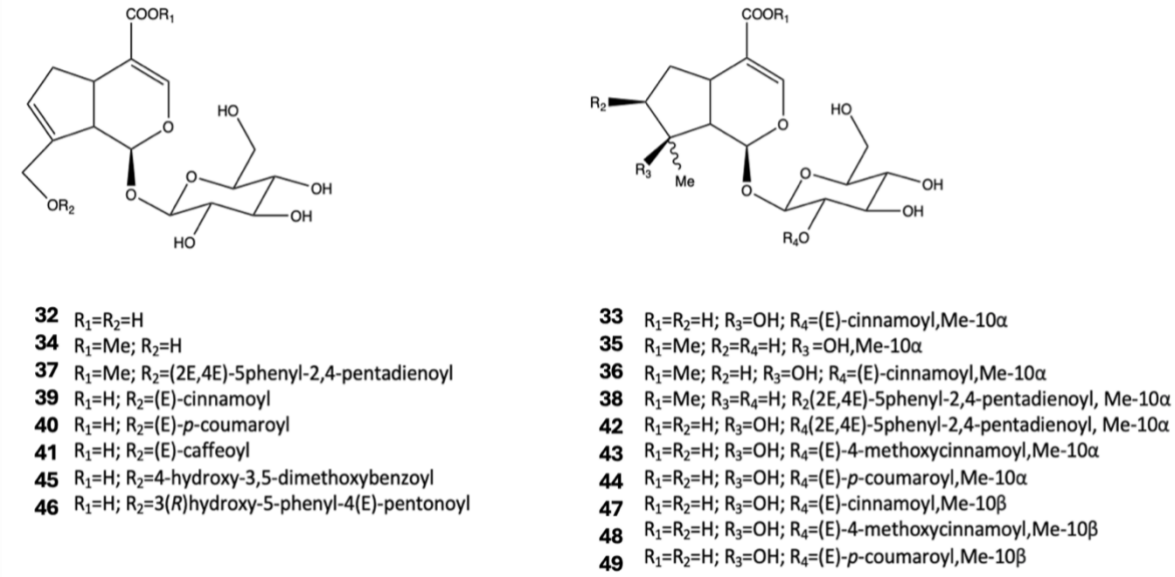

Figure 2.4. Iridoid glycosides from *Avicennia marina*.

2.3.4. Terpenoids

The presence of triterpenoids in *A. marina* bark (betulic acid (**51**) 0.3%, taraxerol (**52**) 0.06%, and taraxerone (**53**) 0.05%) was reported by Bell and Duewell (1961). Jia et al. (2004) isolated lupeol (**54**) and betulin (**55**) from the leaves of *A. marina,* which were collected in Beihai (China). Two new abietane diterpenoids, 6H-11,12,16-trihydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial 11,6-hemiacetal (**56**) and 6,11,12,16-tetrahydroxy-5,8,11,13-abitetetraen-7-one (**57**), were found in *A. marina* twigs collected Xiamen (China) and extracted with methanol by Han et al. (2008). Furthermore, several terpenoids have been identified, including ilekudinoside B (**58**), ilekudinoside C (**59**), and C 6′-O-(n-butanol) ilekudinoside B ester (**60**) from the ethanol extract of fruits collected in China (Yang et al., 2018); 11-hydroxy-8,11,13-abietatriene 12-O-β-xylopyranoside (**61**) from the methanolic extract twigs collected in China (Han et al., 2008); and ursolic acid (**62**) and α-amyrin (**63**) from the methanolic extract of pneumatophores collected in Pakistan (Mahera et al., 2013).

The structures of terpenoids **52–58** are shown in **Figure 2.5**.

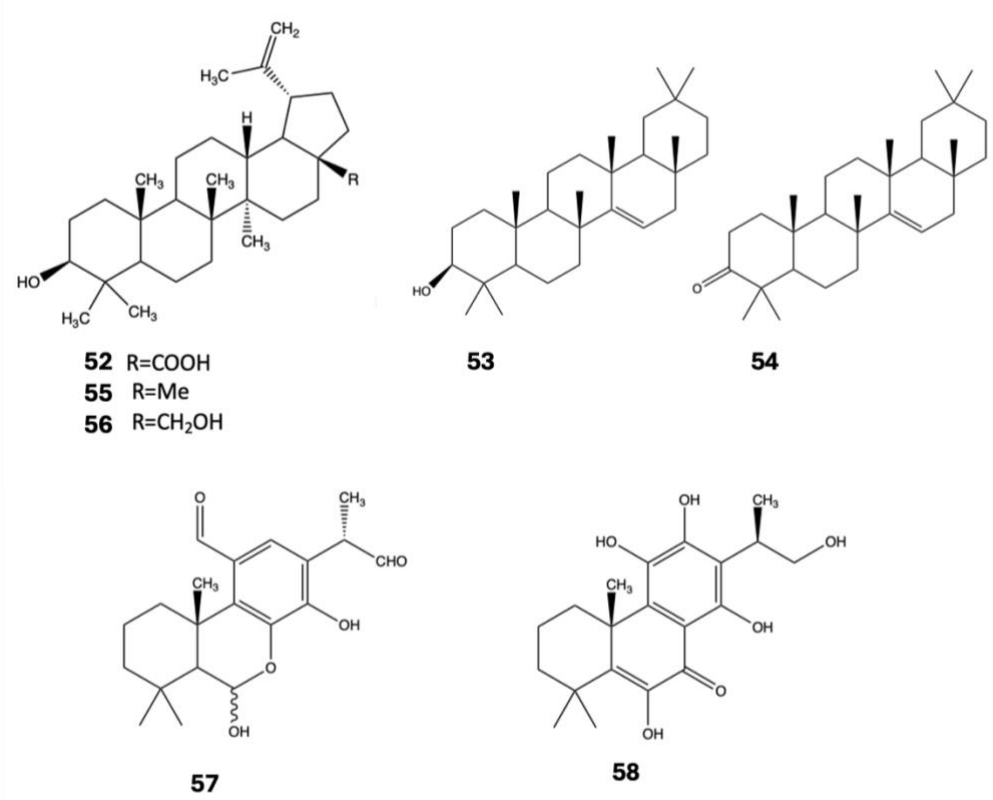

Figure 2.5. Terpenoids from *Avicennia marina*.

2.3.5. Phenolic glycosides

Fauvel et al. (1993) isolated three new phenylethanoid glycosides, verbascoside (**64**), isoverbascoside (**65**), and derhamnosylverbascoside (**66**) from the leaves of *A. marina* collected in Jakarta (Indonesia). Han et al. (2008) identified diacetylmartynoside (**67**) from the twigs of *A. marina* collected in China and extracted with methanol. Four undescribed phenylethanoid glycosides, marinoids J-M (**68-71**), were isolated by Gao et al. (2014) from *A. marina* fruits collected in China and extracted with EtOH–CH_2Cl_2 (2:1). Furthermore, six phenolic glycosides, rhyncoside A (**72**), coniferin (**73**), 1-(4-hydorxybenzoyl)-glucose (**74**), (Z)-4-coumaric acid 4-O-β-D-glucopyranoside (**75**), vanillic acid 4-O-β-D-glucopyranoside (**76**), and phenyl-β-D-glucopyranoside (**77**), have been identified from the fruits of *A. marina* (Xie et al., 2014), while Yang et al. (2018) isolated four phenylethanoid glycosides, 6''-O-acetylacteoside (**78**), crenatoside (**79**), decaffeoylacteoside (**80**), and campneoside I (**81**) from the from the ethanol extract of fruits collected in China.

The structures of phenolic glycosides **64–71** are shown in **Figure 2.6**.

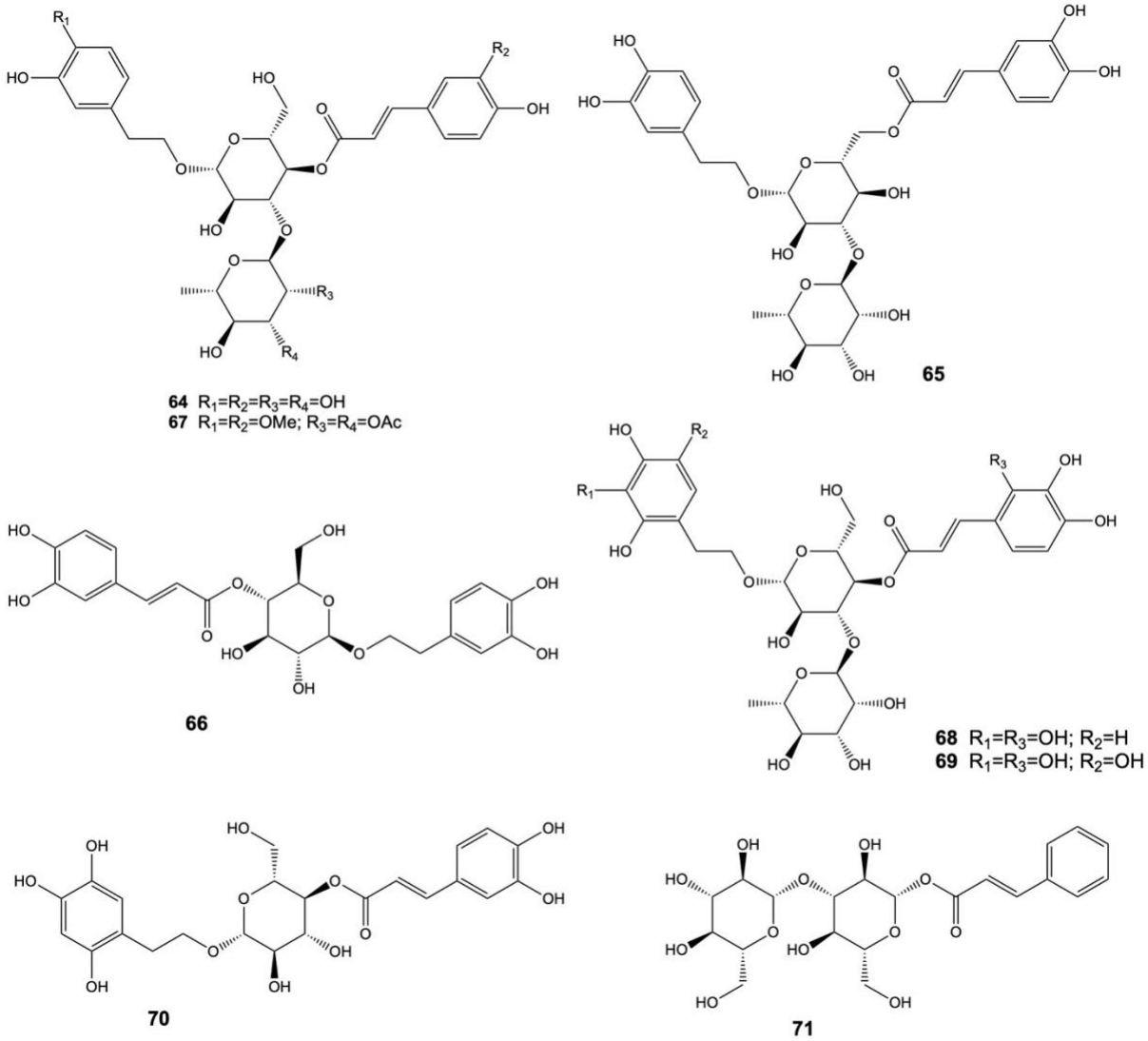

Figure 2.6. Phenolic glycosides from *Avicennia marina*.

2.3.6. Other classes of compounds

Manilal et al. (2009) isolated six fatty acids, alpha linolenic acid (**82**), palmitic acid (**83**), stearic acid (**84**), lauric acid (**85**), myristic acid (**86**), and oleic acid (**87**), from *A. marina* collected from the southwest coast of India and extracted with methanol.

Seven compounds belonging to the chemical class of phenolic acids and derivatives, caffeic acid (**88**), ferulic acid (**89**), 4-hydroxycinnamic acid (**90**), p-hydroxybenzoic acid (**91**), 3,4,5-trimethoxybenzoic acid (**92**), chlorogenic acid (**93**), and neochlorogenic acid (**94**), were isolated from the ethanol extract of fruits collected in China (Yang et al., 2018). Additionally, three more organic acids have been reported: 3(R)-hydroxy-5-phenyl-4(E)-pentenoic acid (**95**) from the ethanolic extract of leaves of *A. marina* collected in China (Sun et al., 2008); indolyl-3-

carboxylic acid (**96**) from the aerial parts (Feng et al., 2007); and p-methoxy cinnamic acid (**97**) from the leaves (Jia et al., 2004).

Five steroids, stigmasterol-3-O-β-D glucopyranoside (**98**), stigmasterol-3-O-β-D galactopyranoside (**99**), and stigmasterol (**100**) from pneumatophores of *A. marina* collected in Pakistan and extracted with methanol (Mahera et al., 2011; Mahera et al., 2013), together with β-Sitosterol (**101**), and ergost-6,22-diene-5,8-epidioxy-3β-ol (**102**) from the leaves of *A. marina* collected in China (Jia et al., 2004), were identified.

Three lignans were isolated from the twigs, including (7′S∗,8′R∗)-4,4′,9′-trihydroxy-3,3′,5,5′-tetramethoxy-7,8-dehydro-9-al-2,7′-cyclolignan (**103**), lyoniresinol (**104**), lyoniresinol 9′-O-β-D-glucopyranoside (**105**) (Han et al., 2008).

From the fruits of *A. marina* collected China, several other compounds have been identified, including marinoids F–I (**106–108**), following extraction with EtOH-CH_2Cl_2; maricaffeolylide A (**109**) and maricyclohexene A (**110**) also obtained from EtOH-CH_2Cl_2 extract; and 4-ethylcatechol (**111**), glechomol C (**112**), *trans*-1,3-bis(3',4'-dihydroxyphenyl)-1-butene (**113**), and cleroindicin E (**114**) from the ethanolic extract (Yi et al., 2014; Yan et al., 2015; Yang et al., 2018).

2.4. ANTIOXIDANT ACTIVITY

Feng et al. (2006) reported that flavones **24** and **25**, demonstrated moderated antioxidant capacity, with DPPH radical scavenging IC_{50} values of 52.0 and 37.0 μg/mL.

Beula et al. (2012) assessed the antioxidant activity of *A. marina* leaves collected along the Southeast coast of India extract using various *in vitro* assays. The extract showed notable free radical scavenging properties, with IC_{50} values 12.80 ± 0.93 (SOD), 142.06 ± 17.93 (DPPH), 19.91 ± 3.93 (NO), and 640.06 ± 34.93 μg/mL (LPO).

Gao et al. (2014) investigated the phenolic glycosides **68–71**, which demonstrated antioxidant potential in the cellular antioxidant assay (CAA), with EC_{50} values ranging from 23.0 ± 0.71 to 247.8 ± 2.47 μM.

The ethanolic extract of *A. marina* leaves collected from the Red Sea coast of Egypt exhibited a DPPH IC_{50} of 193.9 ± 1.03 μg/mL, ABTS activity of 326.8 ± 6.14 μM TE/mg, and FRAP of 340.29 ± 8.16 μM TE/mg (Yassien et al. 2021). In another investigation on Red Sea cost samples from Sudi Arabia, the methanolic extracts demonstrated efficient DPPH radical

scavenging activity. The leaf extract showed an IC_{50} of 51.7 µg/mL, closely matching Trolox (56.8 µg/mL), whereas the root extract recorded about half this activity (23.7 µg/mL) (Al-Mur 2021). Similarly, Audah et al. (2022) demonstrated that the aqueous extract of *A. marina* leaves (collected in Indonesia) displayed DPPH scavenging activity (IC_{50} = 8.1852 ppm), which was comparable to ascorbic acid (IC_{50} = 5.2456 ppm).

Additionally, Ibrahim et al. (2022) reported that ethyl acetate leaf extract from Egyptian *A. marina* exhibited antioxidant activity in the DPPH assay, with an IC_{50} of 50.3 µg/mL, lower than the value of Trolox (56.8 µg/mL).

2.5. ANTICANCER POTENTIAL

A. marina has emerged as a promising source of natural compounds with potential anticancer activity. Various parts of the plant, including leaves, stems, twigs, fruits, and roots, contain flavonoids, naphthalene derivatives, polyisoprenoids, and triterpenoids, many of which have demonstrated cytotoxic and antiproliferative effects against a range of human and animal cancer cell lines. The observed effects include inhibition of cell growth, induction of apoptosis, generation of reactive oxygen species (ROS), and modulation of key signalling pathways associated with cell cycle regulation and tumor progression. Although the number of studies is still limited, the evidence collected to date suggests that *A. marina* may provide valuable bioactive compounds for the development of novel anticancer therapies. This section reviews the experimental findings, highlighting the bioactive compounds and extracts that have shown cytotoxicity, as well as discussing strategies aimed at enhancing their therapeutic potential.

2.5.1. Experimental studies on anticancer activity

Luteolin 7-O-methylether 3′-O-β-d-glucoside (**17**) and its galactoside analogue (**18**), obtained from the methanol extract of the aerial parts of *A. marina,* proved to be cytotoxic against the human breast cancer cell line BT-20 showing ED_{50} values of 16 and 18 µg/mL, respectively (Sharaf et al., 2000).

Avicennone A (**1**), stenocarpoquinone B (**9**), avicequinone C (**10**), avicenol A (**11**), avicenol C (**12**), and a mixture of avicennone D (**4**) and E (**5**) were tested against L-929 mouse fibroblasts and K562 human chronic myeloid leukaemia cells for their antiproliferative effects, as well as against the human cervix carcinoma cell line HeLa for their cytotoxic activity.

Stenocarpoquinone B (**9**), avicequinone C (**10**), and the mixture of avicennone D (**4**) and F (**5**) exhibited antiproliferative effects against K562 (GI_{50} values of 0.2, 1.1, and 7.5 μg/mL, respectively) but also against L-929 (GI50 values of 1.2, 0.8, and 4.4 μg/mL, respectively); these values were higher than those of the standard paclitaxel (GI_{50} values of 0.1 and 0.01 μg/mL, respectively). These compounds also proved to be cytotoxic toward the HeLa cell line, with CC_{50} values of 4.3, 3.2, and 13.1 μg/mL, respectively, which were higher than those of the positive control (CC_{50} value of 0.01 μg/mL). An interesting point is that all the active compounds share the *p*-dione of the naphthoquinone core as a structural element. In contrast, the other compounds showed little activity against the aforementioned cancer cell lines (Han et al., 2007).

The methanolic extract of *A. marina* leaves collected in Iran exhibited cytotoxic activity on human breast MDA-MB 231 cancer cells, with an IC_{50} value of 480 μg/mL, while it had no significant effect against the normal cell line L929. In addition, the extract induced apoptosis in a dose-dependent manner and showed a time-dependent growth inhibition effect of 40%, 44%, and 59% after 24, 48, and 72 h of treatment, respectively (Behbahani et al., 2010).

An ethanol extract from *A. marina* leaves collected in Iran was found to be cytotoxic against human promyelocytic leukaemia HL-60 cells, with IC_{50} values of 600, 400, and 280 μg/mL after 24, 48, and 72 h, respectively, in a concentration- and time-dependent manner. Treated cells, compared to the control cells, appeared smaller, less refracted, showed membrane blebbing, and contained more granular material. Moreover, flow cytometric analysis confirmed that apoptosis was the mechanism of cell death induced by the extract and revealed that a concentration of 600 μg/mL induced 62% apoptosis at 24 h (Karami et al., 2012).

The methanolic and aqueous extracts of *A. marina* leaves collected in India were found to be cytotoxic against human promyelocytic leukaemia HL-60 cells (IC_{50} values of 277.129 and 291.773 μg/mL, respectively) and the human non-small cell lung cancer NCI-H23 cell line (IC_{50} values of 221.173 and 237.179 μg/mL, respectively) (Sukhramani et al., 2013).

The cytotoxic effects of the crude methanolic extract and fourteen fractions derived from *A. marina* leaves (collected in Iran) were assessed against MDA-MB 231 breast cancer cells and the non-cancerous HEK 293 cell line. Among them, the crude extract and fraction 10 (**luteolin**), showed notable activity, reducing the viability of MDA-MB 231 cells with CC_{50} values of 250 μg/mL and 28 μg/mL, respectively. Further investigation revealed that luteolin acted as an

apoptotic inducer in MDA-MB 231 cells, leading to DNA fragmentation (Momtazi-Borojeni et al., 2013).

The methanolic extract of *A. marina* stem bark exhibited cytotoxicity against HL-60 and NCI-H23 (IC_{50} values of 297.934 and 210.987 µg/mL, respectively), and the aqueous extract was found to be cytotoxic against HL-60 and NCI-H23 (IC_{50} values of 281.175 and 220.127 µg/mL, respectively). The extracts displayed comparable cytotoxic IC_{50} values with the standard doxorubicin and showed negligible toxicity against the human embryonic kidney (HEK-293T) normal cell line (Prakash et al., 2013).

The ethyl acetate extract of *A. marina* leaves and stems collected along the Red Sea coast of Saudi Arabia displayed, after 48 h, 65% and 75% growth inhibition of the breast adenocarcinoma cell line MCF-7, at, 100 µg/mL and 200 µg/mL, respectively. Further, 100 µg/mL of the extract showed 10% apoptosis at 24 h, while no increase value in apoptosis was found at 48 h or at 72 h. Nevertheless, increasing the extract concentration to 200 µg/mL displayed 25% apoptosis at 24 h, with an increase to 55% and 75% at 48 h and 72 h, respectively (Esau et al., 2015). Due to the lack of literature regarding the molecular mechanisms of cell death induced by *A. marina* extracts, Esau et al. decided to focus on the intracellular pathways involved in the apoptotic effect of the ethyl acetate extract against the MCF-7 cell line. Their study proved that the 200 µg/mL extract concentration triggered ROS-mediated autophagy, as well as caspase-independent apoptosis.

Huang et al. (2016) focused on the potential association between the phenol and flavonoid contents of water, ethanol, methanol, and ethyl acetate extracts of *A. marina* leaves (collected in the Xinfeng mangrove conservation area in Taiwan) with their anticancer activities. *In vitro* experiments were performed on three human breast cancer cell lines (AU565, MDA-MB-231, and BT483), two human liver cancer cell lines (HepG2 and Huh7), and one normal cell line (NIH3T3). The outcomes revealed that the ethyl acetate extract of *A. marina* was the one carrying the highest concentrations of flavonoids and phenolic compounds, and proved, at the same time, to have the most effective anticancer activities. Furthermore, the ethyl acetate extract was found to be unable to inhibit the proliferation of NIH 3T3 cells at 40–80-µg/mL, but significantly inhibited proliferation in AU565, BT483, HepG2, and Huh7 cancer cells. Therefore, subsequent analyses were performed at a concentration range of 40–80 µg/mL following treatments with the ethyl acetate extract. In addition, the colony formation in soft agar of AU565, BT483, HepG2, and Huh7 cancer cell lines was reduced after 14–21 days of treatment with the extract. Furthermore, F2-5, F3-2-9, and F3-2-10 ethyl acetate fractions were

all obtained by performing column chromatography, and they showed higher cytotoxic activity compared to other fractions. F2-5 was the most cytotoxic fraction with IC_{50} values of 0.75, 0.85, 0.79, and 15.6 μg/mL, against AU565, BT483, HepG2, and Huh7 cell lines, respectively. The ^{1}H-NMR and ^{13}C-NMR profiles demonstrated that the F3-2-10 fraction contained avicennones D (**4**) and E (**5**). Furthermore, the chemotherapeutic potential was evaluated in a xenograft mouse model and the suppression of MDA-MB231 by the ethyl acetate extract of leaves elicited the suggestion that this extract may be useful in the treatment of breast cancer.

The polyisoprenoids extract from *A. marina* leaves collected in Indonesia displayed weak cytotoxicity (IC_{50} value of 154.987 μg/mL) against WiDr colon cancer cells when compared with the standard doxorubicin (IC_{50} value of 5.445 μg/mL) (Illian et al., 2018). In addition, it was found that the mechanism underlying the cytotoxic effect of the extract was due to cell cycle inhibition and induction of apoptosis.

Ilekudinoside B ester (**60**), isolated from the ethanol extract of *A. marina* fruits was found to be cytotoxic against two human glioma stem cell lines, GSC-3# and GSC-18#, with IC_{50} values of 12.21 and 5.53 μg/mL, respectively (Yang et al., 2018).

Qurrohman et al. (2020) extracted polyisoprenoids from the n-hexane extract of *A. marina* leaves collected in North Sumatra (Indonesia). Polyisoprenoids exhibited cytotoxic activity against WiDr cells, with an IC_{50} value of 295.25 μg/mL, while the standard 5-FU had an IC_{50} value of 17.43 μg/mL. Cell cycle analysis revealed that the cell cycle inhibition of polyisoprenoids occurred in the G0-G1 phase. Furthermore, RT-PCR revealed that polyisoprenoids downregulated the PI3k, Akt1, mTOR, and EGFR gene expression; however, they upregulated p53 gene expression.

The hexane extract of *A. marina* leaves (collected along the Red Sea coast of Saudi Arabia) showed cytotoxic activity (after 72 h of treatment) against human colon HCT-116, human liver HepG2, and human breast MCF-7 cancer cell lines, with IC_{50} values of 23.7 ± 0.7, 44.9 ± 0.93, and 79.55 ± 0.57 μg/mL, respectively, which were lower than those of the standard doxorubicin (IC_{50} values of 0.45 ± 0.052, 0.42 ± 0.10, and 0.6 ± 0.022 μg/mL, respectively). The study also revealed that the hexane extract had a weak ability to induce apoptosis, although the cells showed membrane blebbings in addition to apoptotic bodies. Furthermore, it displayed inhibition of the cell cycle in the G0/G1 phase for HCT-116 cancer cells, and in the S phase for HepG2 and MCF-7 cell lines (Albinhassan et al., 2021).

The different acetone extracts of *A. marina* leaves (collected in India) were prepared in a concentration range of 40–160 mg/mL, and the maximum cytotoxic activity against the liver HepG2 cancer cell line was observed at 120 mg/mL (Azhagu Madhavan, 2021)

Eldohaji et al. (2021) isolated lupeol (**44**), a pentacyclic triterpenoid, from the hexane extract of the stem of *A. marina* collected in the United Arab Emirates, clarifying its mechanism of anticancer action, since the data reported on lupeol were approximate and contradictory. The results indicated that lupeol caused considerable ($p < 0.001$) growth inhibitory activity against breast MCF-7 (45%), resistant MCF-7 (46%), liver Hep3B (72%), and resistant Hep3B (35%) cancer cell lines, with slight toxic effects on normal fibroblast cells (F180). The mechanism of action of this triterpenoid was investigated by detecting its influence on key actors in cancer development and progression: BCL-2 anti-apoptotic and BAX pro-apoptotic proteins. They found that lupeol significantly ($p < 0.01$) downregulated BCL-2 gene expression in parental and resistant Hep3B cells by 33 and 3.5 times, respectively, contributing to the induction of apoptosis in Hep3B cells, while no effect on BAX was found. Proteins extracted from lupeol-treated Hep3B cells were analyzed by Western blot, which indicated the presence of activated caspase-3 cleaved by lupeol. Furthermore, as an indication of the absence of immune/inflammatory responses, the compound exhibited a negligible effect on the proliferation of monocytes, but caused an increase in the sub-G1 population and a reduction in the apoptosis rates of monocytes at 48 and 72 h.

Ethanolic extracts (400 µg/mL) of the lower half of pneumatophores, leaves, the upper half of pneumatophores, and shoots of *A. marina* (collected in the Arabian Gulf coast of Saudi Arabia) induced, after 48 h of treatment, cell growth inhibition of 50% or more of liver HepG2 cancer cells. Additionally, the leaf extract proved to be the most cytotoxic (Sohaib et al., 2022).

2.5.2. Formulation and delivery strategies

The poor efficacy of many cancer treatments is often associated with the low targeting ratio of drugs, and to the side effects on healthy tissues, due to their nonspecific distribution into other organs and tissues. Therefore, much attention has been dedicated to the study of strategies to obtain a site-specific accumulation of therapeutic agents in the tumor region, avoiding side effects and toxicity (Kok-Yong et al., 2015)

In this scenario, nanotechnology-based formulations are one of the most promising approaches exploited to overcome the bottlenecks of nonspecific biodistribution, side effects, and low tumor accumulation (Rosemblum et al., 2018; Mitchell et al., 2021). Nanoparticles (NPs) for

drug delivery are carriers in the 1–1000 nm range, composed of different materials, including biocompatible and biodegradable natural/synthetic molecules, polymers, lipids, or metals. NPs are designed and developed to be loaded or covalently linked with bioactive molecules, such as proteins, peptides, antibodies, and nucleic acids, with the aim of: (1) overcome the problems associated with molecules' solubility and *in vivo* bioavailability; (2) avoid the molecules' degradation in the bloodstream; (3) improve the molecules' targeting, internalization, and accumulation in the desired cells and tissues; (4) potentiate the drug's effect (Patra et al., 2018). For these reasons, NPs are associated with plants and natural compounds to obtain a therapeutic, synergistic effect. Some papers have reported the use of NPs to overcome the problems associated with the low tumor accumulation of *A. marina,* aiming to improve its therapeutic effect.

Biogenic engineered silver nanoparticles (AgNPs) were synthesized from the aqueous extract of *A. marina* leaves by Varunkumar et al. (2020). The resulting AgNPs displayed dose-dependent cytotoxic activity in the A549 lung cancer cell line (IC_{50} = 50 µg/mL), inducing ROS-mediated apoptosis, which was also confirmed by RT-PCR and Western blotting analysis. Both the p53-dependent and -independent caspase-mediated signalling pathways were demonstrated to be involved in the process. Tian et al. (2020) confirmed the anticancer properties of synthesized AgNPs in A549 lung cancer cells; they observed a dose-dependent effect based on ROS activity, with an inhibition of 54% at the concentration of 50 µg/mL and 94% inhibition at 80 µg/mL.

2.6. MATERIALS AND METHODS

A comprehensive literature review was conducted to gather information on the phytochemistry, antioxidant activity, and anticancer potential of *A. marina*. Scientific articles and books were retrieved primarily from online databases such as Google Scholar, using combination of keywords including: '*Avicennia*, '*Avicennia marina*, 'phytochemical analysis', 'secondary metabolites', 'natural products', 'bioactive compounds', 'biological activities', 'antioxidant activity', 'cytotoxic activity', 'anticancer potential'.

2.7. CONCLUSIONS

Research on *A. marina* has demonstrated that the species is a prolific source of structurally diverse secondary metabolites. The main compound classes identified include naphthalene derivatives (mainly from twigs and wood), flavonoids and iridoid glycosides (mainly from leaves and aerial parts), terpenoids, phenolic glycosides (predominantly from fruits, but also leaves and twigs), phenolic acids and derivatives (fruits), fatty acids, and steroids (pneumatophores and leaves). These findings establish this species as chemically versatile, yet they also reveal a bias toward aerial parts, with very limited studies on pneumatophores. Furthermore, a pronounced geographical gap is evident: most studies focused on *A. marina* samples collected in China, with no comprehensive phytochemical investigations conducted in the Arabian Gulf, despite *A. marina* being the dominant mangrove species in this region, where extreme environmental condition may drive unique metabolic adaptations. Antioxidant activity has been reported for extracts and isolated compounds, including flavonoids and phenolic acids, but again almost exclusively from leaves, confirming *A. marina* as a promising source of radical-scavenging metabolites. The anticancer potential of *A. marina* has been more extensively investigated, with cytotoxic and antiproliferative effects reported for both crude extracts and isolated metabolites (e.g., avicennones, avicequinones, flavonoids, triterpenoids). Most studies have relied on leaf material, while roots and other parts remain largely neglected. Notably, repeated activity has been documented against breast cancer, leukaemia, colon cancer, and liver cancer cell lines. Importantly, several studies confirmed that *A. marina* extracts exhibited lower cytotoxicity against normal cells. In addition, nanoformulation strategies have recently been explored to improve solubility, stability, and tumor-specific accumulation of *A. marina* metabolites, opening new avenues for therapeutic application.

Overall, the current state of knowledge underscores both the chemical richness and antioxidant and anticancer potential of *A. marina*. At the same time, it highlights critical gaps: a plant-part bias with studies largely confined to leaves; a geographical bias with Arabian Gulf populations understudied; and a lack of integrated approaches simultaneously assessing phytochemistry, antioxidant activity, and cytotoxicity across multiple plant parts, which is essential for linking bioactivities to tissue-specific metabolites. Addressing these gaps requires systematic, region-specific studies that can identify the most promising extracts, clarify the molecule-activity relationships, and prioritize candidates for fractionation and mechanistic investigations. Such an approach would provide a stronger foundation for the pharmacological development of *A. marina* and help translate its bioactive potential into future therapeutic applications.

2.8. REFERENCES

Afshar, A., Khoradmehr, A., Zare, M., Baghban, N., Mohebbi, G., Barmak, A., et al. (2022). Anticancer activity of ethanol and ethyl acetate extracts of *Avicennia marina* leaves on breast, ovarian and cervical cancer cell lines.

Albinhassan, T. H., Saleh, K. A., Barhoumi, Z., Alshehri, M. A., & Al-Ghazzawi, A. M. (2021). Anticancer, anti-proliferative activity of *Avicennia marina* plant extracts. Journal of Cancer Research and Therapeutics, 17(4), 879-886.

Arumugam, S., Bandil, K., Proksch, P., Murugiyan, K., & Bharadwaj, M. (2017). Effects of *A. marina*-derived isoquercitrin on TNF-related apoptosis-inducing ligand receptor (TRAIL-R) expression and apoptosis induction in cervical cancer cells. Applied Biochemistry and Biotechnology, 182(2), 697-707.

Audah, K. A., Ettin, J., Darmadi, J., Azizah, N. N., Anisa, A. S., Hermawan, T. D. F., et al. (2022). Indonesian mangrove *Sonneratia caseolaris* leaves ethanol extract is a potential super antioxidant and anti methicillin-resistant *Staphylococcus aureus* drug. Molecules, 27(23), 8369.

Azhagu Madhavan, S. (2021). Evaluation of potential and in-vitro antioxidant activity of mangrove leaves *Avicennia marina* ethanolic extract. Int. J. Sci. Res. Chem, 6, 32-37.

Babuselvam, M., Abideen, S., Gunasekaran, T., Beula, J. M., & Dhinakarraj, M. (2013). Bioactivity of *Avicennia marina* and *Rhizophora mucronata* for the management of diabetes mellitus. World Journal of Pharmaceutical Research, 3(1), 11-18.

Bell, K. H., & Duewell, H. (1961). Triterpenoids from the bark of *Avicennia marina*.

Beula, J. M., Gnanadesigan, M., Rajkumar, P. B., Ravikumar, S., & Anand, M. (2012). Antiviral, antioxidant and toxicological evaluation of mangrove plant from South East coast of India. Asian Pacific journal of Tropical biomedicine, 2(1), S352-S357.

Das, S. K., Patra, J. K., & Thatoi, H. (2016). Antioxidative response to abiotic and biotic stresses in mangrove plants: A review. International Review of Hydrobiology, 101(1-2), 3-19.

Eldohaji, L. M., Fayed, B., Hamoda, A. M., Ershaid, M., Abdin, S., Alhamidi, T. B., et al. (2021). Potential targeting of Hep3B liver cancer cells by lupeol isolated from *Avicennia marina*. Archiv der Pharmazie, 354(9), 2100120.

ElDohaji, L. M., Hamoda, A. M., Hamdy, R., & Soliman, S. S. (2020). *Avicennia marina* a natural reservoir of phytopharmaceuticals: Curative power and platform of medicines. Journal of ethnopharmacology, 263, 113179.

Esau, L., Sagar, S., Bajic, V. B., & Kaur, M. (2015). Autophagy inhibition enhances the mitochondrial-mediated apoptosis induced by mangrove (*Avicennia marina*) extract in human breast cancer cells.

Fauvel, M. T., Taoubi, K., Gleye, J., & Fouraste, I. (1993). Phenylpropanoid glycosides from *Avicennia marina*. Planta medica, 59(04), 387-387.

Feng, Y., Li, X. M., Duan, X. J., & Wang, B. G. (2006). A new acylated iridoid glucoside from *Avicennia marina*. Chinese Chemical Letters, 17(9), 1201.

Feng, Y., Li, X. M., Duan, X. J., & Wang, B. G. (2006). Iridoid glucosides and flavones from the aerial parts of *Avicennia marina*. Chemistry & biodiversity, 3(7), 799-806.

FENG, Y., LI, X., & WANG, B. (1994). Chemical constituents in aerial parts of mangrove plant *Avicennia marina*. Zhongcaoyao.

Gandomani, M. Z., & Malati, E. F. (2014). Evaluation of protective efficacy of *Avicennia marina* (Forssk.) vierh leaves against complete freund' s adjuvant-induced arthritis in wistar. Iranian Journal of Pharmaceutical Research: IJPR, 13(3), 945.

Gao, C. H., Yi, X. X., Xie, W. P., Chen, Y. N., Xu, M. B., Su, Z. W., et al. (2014). New antioxidative secondary metabolites from the fruits of a Beibu Gulf mangrove, *Avicennia marina*. Marine Drugs, 12(8), 4353-4360.

Han, L., Huang, X., Dahse, H. M., Moellmann, U., Fu, H., Grabley, S., et al. (2007). Unusual naphthoquinone derivatives from the twigs of *Avicennia marina*. Journal of natural products, 70(6), 923-927.

Han, L., Huang, X., Dahse, H. M., Moellmann, U., Grabley, S., Lin, W., & Sattler, I. (2008). New abietane diterpenoids from the mangrove *Avicennia marina*. Planta medica, 74(04), 432-437.

Huang, C., Lu, C. K., Tu, M. C., Chang, J. H., Chen, Y. J., Tu, Y. H., & Huang, H. C. (2016). Polyphenol-rich *Avicennia marina* leaf extracts induce apoptosis in human breast and liver cancer cells and in a nude mouse xenograft model. Oncotarget, 7(24), 35874.

Ibrahim, H. A., Abdel-Latif, H. H., & Zaghloul, E. H. (2022). Phytochemical composition of *Avicennia marina* leaf extract, its antioxidant, antimicrobial potentials and inhibitory properties on *Pseudomonas fluorescens* biofilm. Egyptian Journal of Aquatic Research, 48(1), 29-35.

Illian, D. N., Basyuni, M., Wati, R., & Hasibuan, P. A. Z. (2018). Polyisoprenoids from *Avicennia marina* and *Avicennia lanata* inhibit WiDr cells proliferation. Pharmacognosy Magazine, 14(58).

Jain, R., Monthakantirat, O., Tengamnuay, P., & De-Eknamkul, W. (2014). Avicequinone C isolated from *Avicennia marina* exhibits 5α-reductase-type 1 inhibitory activity using an androgenic alopecia relevant cell-based assay system. Molecules, 19(5), 6809-6821.

Jia, R., Guo, Y. W., & Hou, H. X. (2004). Studies on the chemical constituents from leaves of *Avicennia marina*. Chin. J. Nat. Med, 2, 16-19.

Karami, L., Majd, A., Mehrabian, S., Nabiuni, M., Salehi, M., & Irian, S. (2012). Antimutagenic and anticancer effects of *Avicennia marina* leaf extract on *Salmonella typhimurium* TA100 bacterium and human promyelocytic leukaemia HL-60 cells. Sci. Asia, 38(4), 349-355.

Kok-Yong, S., Lawrence, L., & Ahmed, T. (2015). Drug distribution and drug elimination. Basic pharmacokinetic concepts and some clinical applications, 99-116.

König, G., & Rimpler, H. (1985). Iridoid glucosides in *Avicennia marina*. *Phytochemistry*, *24*(6), 1245-1248.

Mahera, S. A., Ahmad, V. U., Saifullah, S. M., Mohammad, F. V., & Ambreen, K. (2011). Steroids and triterpenoids from grey mangrove *Avicennia marina*. Pakistan Journal of Botany, 43(2), 1417-1422.

Mahera, S. A., Saifullah, S. M., Ahmad, V. U., & Mohammad, F. V. (2013). Phytochemical studies on mangrove *Avicennia marina*. Pak. J. Bot, 45(6), 2093-2094.

Manilal, A., Sujith, S., Kiran, G. S., Selvin, J., & Shakir, C. (2009). Biopotentials of mangroves collected from the southwest coast of India. Global Journal of Biotechnology and Biochemistry, 4(1), 59-65.

Mitchell, M. J., Billingsley, M. M., Haley, R. M., Wechsler, M. E., Peppas, N. A., & Langer, R. (2021). Engineering precision nanoparticles for drug delivery. Nature reviews drug discovery, 20(2), 101-124.

Momtazi-Borojeni, A., Behbahani, M., & Sadeghi-Aliabadi, H. (2010). Evalution of cytotoxic effect of some extracts of *Avicennia marina* against MDA-MB 231 human breast cancer cell line.

Patra, J. K., Das, G., Fraceto, L. F., Campos, E. V. R., Rodriguez-Torres, M. D. P., Acosta-Torres, L. S., et al. (2018). Nano based drug delivery systems: recent developments and future prospects. Journal of nanobiotechnology, 16(1), 71.

Qurrohman, T., Basyuni, M., & Hasibuan, P. A. Z. (2020). Polyisoprenoids from *Avicennia marina* induces on P13k, Akt1, Mammalian target of rapamycin, Egfr, and P53 gene expression using

reverse transcription-polymerase chain reaction. Open Access Macedonian Journal of Medical Sciences, 8(A), 146-152.

Rosenblum, D., Joshi, N., Tao, W., Karp, J. M., & Peer, D. (2018). Progress and challenges towards targeted delivery of cancer therapeutics. Nature communications, 9(1), 1410.

Shaker, K. H., Elgamal, M. H. A., & Seifert, K. (2001). Iridoids from *Avicennia marina*. *Zeitschrift für Naturforschung C*, *56*(11-12), 965-968.

Sharaf, M., El-Ansari, M. A., & Saleh, N. A. M. (2000). New flavonoids from *Avicennia marina*. Fitoterapia, 71(3), 274-277.

Sohaib, M., Al-Barakah, F. N., Migdadi, H. M., & Husain, F. M. (2022). Comparative study among *Avicennia marina*, *Phragmites australis*, and *Moringa oleifera* based ethanolic-extracts for their antimicrobial, antioxidant, and cytotoxic activities. Saudi journal of biological sciences, 29(1), 111-122.

Sukhramani, P. S., & Patel, P. M. (2013). In-vitro biological screening of *Avicennia marina* for Anticancer activity. Research Journal of Pharmacology and Pharmacodynamics, 5(5), 297-301.

Sukhramani, P. S., Vidyasagar, G., & Patel, P. M. (2013). Biological screening of *Avicennia marina* for anticancer activity.

Sun, Y., Ouyang, J., Deng, Z., Li, Q., & Lin, W. (2008). Structure elucidation of five new iridoid glucosides from the leaves of Avicennia marina. Magnetic Resonance in Chemistry, 46(7), 638-642.

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 71(3), 209-249.

Sutton, D. C., Gillan, F. T., & Susic, M. (1985). Naphthofuranone phytoalexins from the grey mangrove, Avicennia marina. Phytochemistry, 24(12), 2877-2879.

Tian, S., Saravanan, K., Mothana, R. A., Ramachandran, G., Rajivgandhi, G., & Manoharan, N. (2020). Anti-cancer activity of biosynthesized silver nanoparticles using *Avicennia marina* against A549 lung cancer cells through ROS/mitochondrial damages. Saudi Journal of Biological Sciences, 27(11), 3018-3024.

Varunkumar, K., Anusha, C., Saranya, T., Ramalingam, V., Raja, S., & Ravikumar, V. (2020). *Avicennia marina* engineered nanoparticles induce apoptosis in adenocarcinoma lung cancer cell line through p53 mediated signaling pathways. Process Biochemistry, 94, 349-358.

Xie WenPei, X. W., Gao ChengHai, G. C., Yi XiangXi, Y. X., Yi Wei, Y. W., He BiJuan, H. B., & Chen Bo, C. B. (2014). Study on phenolic glycosides from fruit of the mangrove plant *Avicennia marina*.

Yan, D. M., Gao, C. H., Yi, X. X., Xie, W. P., Xu, M. B., & Huang, R. M. (2015). Two new secondary metabolites from the fruits of mangrove *Avicennia marina*. Zeitschrift für Naturforschung B, 70(9), 691-694.

Yang, X. W., Dai, Z., Wang, B., Liu, Y. P., Zhao, X. D., & Luo, X. D. (2018). Antitumor triterpenoid saponin from the fruits of *Avicennia marina*. Natural products and bioprospecting, 8(5), 347-353.

Yassien, E. E., Hamed, M. M., Abdelmohsen, U. R., Hassan, H. M., & Gazwi, H. S. (2021). In vitro antioxidant, antibacterial, and antihyperlipidemic potential of ethanolic *Avicennia marina* leaves extract supported by metabolic profiling. Environmental Science and Pollution Research, 28(21), 27207-27217.

Yi, X. X., Chen, Y., Xie, W. P., Xu, M. B., Chen, Y. N., Gao, C. H., & Huang, R. M. (2014). Four new jacaranone analogs from the fruits of a Beibu Gulf mangrove *Avicennia marina*. Marine drugs, 12(5), 2515-2525.

CHAPTER 3

Experimental investigation of the phytochemical profile, antioxidant activity, and *in vitro* cytotoxic potential of *Avicennia marina*

This chapter has been adapted and modified from the following published work:

Cerri, F., De Santes, B., Spena, F., Salvioni, L., Forcella, M., Fusi, P., Pagliari, S., Stahl, H., Galli, P., Colombo, M., Giustra, M., Campone, L. (2025). Phytochemical profiling, antioxidant activity, and *in vitro* cytotoxic potential of Mangrove *Avicennia marina*. *Pharmaceuticals*, *18*(9), 1308.

3.1. ABSTRACT

Avicennia marina (Forsk.) Vierh., a widely distributed mangrove species, is known for its diverse secondary metabolites with potential pharmacological applications. Despite its dominance in the Arabian Gulf, where *A. marina* may have adapted to extreme environmental conditions with a distinct set of bioactive molecules, research in this region remains limited. This study investigates the phytochemical composition, antioxidant activity, and *in vitro* cytotoxicity of extracts from different plant parts, including roots, leaves, propagules, pericarps, and cotyledons, collected in the United Arab Emirates (UAE). Extracts were analyzed using ultra-pressure liquid chromatography coupled with high-resolution mass spectrometry (UPLC-HRMS). Antioxidant activity was assessed using DPPH and ABTS assays, while cytotoxicity was evaluated against human cancer and normal cell lines. Analysis revealed 49 compounds, including iridoid glycosides, hydroxycinnamic acids, phenylethanoid glycosides, flavonoid glycosides, and triterpene saponins, several reported for the first time in *A. marina* and mangroves. The pericarp and root extracts exhibited the highest scavenging activity (DPPH: 187.14 ± 2.87 and 128.25 ± 1.12; ABTS: 217.16 ± 2.67 and 147.21 ± 2.42 µmol TE/g, respectively), correlating with phenylethanoid content. The root extract also displayed the highest cytotoxicity, with IC_{50} values of 58.46, 81.98, and 108.10 µg/mL against MDA-MB-231, SW480, and E705, respectively. *In silico* analysis identified triterpene saponins as potential contributors. These findings highlight the root extract of *A. marina* as a promising source of bioactive compounds with potential antioxidant and anticancer applications, supporting further exploration for novel therapeutic candidates.

3.2. INTRODUCTION

Extracts and isolated compounds from *Avicennia marina* have been reported to exert a wide spectrum of biological activities, with particular emphasis on their *in vitro* anticancer potential. However, research in this field remains relatively limited, especially in the context of the Arabian Gulf, where *A. marina* is the dominant mangrove species along the coasts of the United Arab Emirates (UAE), Saudi Arabia, Bahrain, Qatar, and Iran (El-Tarabily et al., 2021). Ethnobotanical records for *A. marina* in the Arabian Gulf are scarce but include traditional uses in Iran for treatments of ulcers, rheumatism, and burns (Bibi et al., 2019), and in the UAE for its use as an aphrodisiac, antifertility agent, and treatment for scabies and toothache (https://www.medicinalplants.doh.gov.ae). *A. marina* remains largely unexplored here in terms

of bioprospecting, with most studies focusing instead on its distribution, ecological significance, ecosystem services, and management and conservation (Friis et al., 2023; Haseeba et al., 2025). Phytochemical investigations have only examined populations from China, India, Pakistan, Egypt, other Indo-Pacific locations, and the Red Sea coasts of Saudi Arabia (Khattab et al., 2023; ElDohaji et al., 2020; Al-Mur et al., 2021; Mitra et al., 2023; Zhou et al., 2025).

Previous phytochemical studies on *A. marina* have generally examined only a few plant parts, typically aerial parts and primarily leaves, lacking a comprehensive analysis of plant-part-specific secondary metabolites. Biological activity also presents limitations, often focusing on few plant parts and, in the case of cytotoxicity, testing only a small number of cancer cell lines (Bibi et al., 2019; ElDohaji et al., 2020; Zhou et al., 2025). A major gap in existing research of *A. marina* is the absence of integrated studies combining detailed phytochemical profiling with antioxidant and cytotoxic assays across multiple parts of the plant, which is essential for linking bioactivities to tissue-specific metabolites. Such combined strategies are well established in plant research (Alzandi et al., 2021; Mohamed, 2022) and have been applied to mangroves (Youssef et al., 2022) as they facilitate the prioritization of promising extracts and compounds, thereby enhancing efficiency in the early discovery of bioactive molecules. Moreover, the majority of chemical studies have relied on GC-MS, which biases detection towards volatile constituents (ElDohaji et al., 2020; Cerri et al., 2022; Mitra et al., 2023). Although GC-MS can also characterize phenolic compounds, abundant in mangroves (Botosa et al., 2025), it requires a derivatization step that is both complex and time-consuming (Wang et al., 2025). In contrast, ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry (UPLC-HRMS) has emerged as a powerful platform for untargeted metabolomic profiling of complex plant matrices, offering superior sensitivity, selectivity, and mass accuracy, and enabling comprehensive detection and identification of secondary metabolites, especially phenolics (Zanatta et al., 2021; Kodikara et al., 2024; Singh et al., 2025; Lee et al., 2025).

In addition to these methodological gaps, the limited geographical scope of previous studies represents a crucial limitation in fully understanding the phytochemical diversity and bioactivity of *A. marina*. The Arabian Gulf is characterized by extreme environmental conditions, including elevated seawater temperatures, hypersalinity, and high turbidity, driven by its arid climate and shallow basin (Mateos-Molina et al., 2021), and summer air temperatures can reach 50 °C (https://climateknowledgeportal.worldbank.org), further stressing local ecosystems. Since the chemical composition of plants is influenced by geographical,

environmental, and climate factors (Jin et al., 2009; Abd-Elgawad et al., 2019; Rozirwan et al., 2022) , plants exposed to such stresses often respond by increasing the accumulation of secondary metabolites, such as flavonoids, iridoid glycosides, and phenylethanoid glycosides, which enhance their tolerance to adverse conditions and also possess bioactivities of pharmacological interest, including antioxidant and anticancer (Wang et al., 2010; Ferdinando et al., 2012; Neugart et al., 2016; Falahi et al., 2018; Toscano et al., 2019; Franzoni et al., 2019; Sarri et al., 2021).

It is plausible that *A. marina* in the Arabian Gulf may have adapted to extreme conditions through metabolic changes and the induction of antioxidant defense systems (Das et al., 2015), potentially resulting in a distinct set of secondary metabolites with unique biological activities. Consequently, the objective of this study is to conduct a comprehensive investigation of *A. marina* grown in the UAE by characterizing the secondary metabolite composition of multiple parts of the plant, including roots, leaves, propagules (pericarps and internal tissues), and cotyledons, and evaluating their potential health benefits through antioxidant and cytotoxic activity assays *in vitro*.

Unlike previous studies, this work employs UPLC-HRMS to present a detailed phytochemical profile of each tissue type, allowing for the identification of specific compounds of *A. marina* and their localization within the plant. Furthermore, while the antioxidant and anticancer potentials of *A. marina* extracts have been previously reported (ElDohaji et al., 2020; Cerri et al., 2022), this study provides a comprehensive evaluation of antioxidant and cytotoxic activities of all plant parts, along with expanded cytotoxic screening in multiple cancer cell lines. In addition, *in silico* predictions of biological activities were applied to compounds identified in the extracts. This multi-level approach addresses existing regional and methodological gaps and lays the groundwork for the discovery of bioactive compounds from plants adapted to extreme environmental conditions.

3.3. MATERIALS AND METHODS

3.3.1. Chemicals

Ethanol absolute, analytical-grade methanol, 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$), and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$) reagents were obtained from Sigma-Aldrich (Milan, Italy), while methanol and formic acid of LC-MS grade were sourced from

Romil (Cambridge, UK). Ultrapure water (18 MΩ) was prepared by a Milli-Q purification system (Millipore, Bedford, MA, USA).

3.3.2. Plant material

Samples were collected from different parts of *A. marina*, including leaves, roots, propagules, and cotyledons. The cotyledons were obtained from seedlings at an early growth stage, when the propagules had already opened and developed roots. All samples were harvested in September 2022 from multiple individual plants within the mangrove forest of Ajman Emirate, UAE. Although no herbarium voucher specimen was deposited, the plant material was identified based on morphological characteristics following established taxonomic keys (Duke, 1991). This identification is supported by the fact that *A. marina* is the only mangrove species forming the evergreen coastal forests of the UAE (Friis and Killea, 2023), and its presence in the region has been validated by previous molecular analyses (Friis et al., 2021; Friis et al., 2024).

For more precise phytochemical characterization, propagules were separated into the pericarp, representing the external protective tissue, and the internal tissues. Thus, in the manuscript, the term pericarp (and pericarp extract) refers exclusively to the external part, while the generic term propagule (and propagule extract) refers to the internal tissues. Each type of plant material (e.g., all collected leaves, roots, pericarps, propagules, and cotyledons) was pooled by type and immediately freeze-dried after collection. The dried samples were homogenized using a Grindomix GM 200 knife mill (Retsch, Haan, Germany) and then sieved through a test sieve (Retsch AS 200, Haan, Germany) with a mesh size range of 300–600 µm to obtain powders with uniform particle size distribution.

3.3.3. Sample preparation and extraction

Root, leaf, cotyledon, pericarp, and propagule samples of *A. marina* underwent exhaustive ultrasound-assisted extraction using a thermostatically controlled ultrasonic bath (Sonorex TK 52; Bandelin electronic, Berlin, Germany). Each sample was extracted under controlled conditions (25 °C, 15 min) with 50% aqueous ethanol (*v*/*v*) at a solid-to-solvent ratio of 1:10 (*w*/*v*), which is commonly applied in metabolite profiling studies (Che Sulaiman et al., 2017); specifically, 1 g of powdered sample was mixed with 10 mL of solvent in a 50-mL polypropylene tube. A 50% aqueous ethanol solution was selected as the extraction solvent due to its effectiveness as a green, low-toxicity system, making it well-suited for bioactivity

screening (Lim et al., 2019; Plaskova et al., 2023). Ethanol–water mixtures offer a balanced polarity and are widely recognized for their ability to efficiently extract a broad range of bioactive compounds, particularly polyphenolic metabolites, which are well known for their antioxidant properties (Palaiogiannis et al., 2023; Huamàn-Castilla et al., 2024). Moreover, this solvent was selected to ensure low toxicity in downstream biological assays, in case traces of solvent remain after evaporation, and because non-polar solvents or higher ethanol concentrations could reduce solubility in aqueous assay media, potentially compromising the suitability of the extracts for biological testing.

To ensure complete extraction, the process was repeated three times with fresh solvent. Following each extraction, the mixtures were centrifuged (13,000× *g*, 10 min), and the supernatants underwent filtration through Whatman No. 1 filter paper. The combined extracts were concentrated under pressure at 40 °C using a rotary evaporator to remove ethanol and subsequently lyophilized (Alpha 1-2 LD freeze dryer, Christ, Germany) to obtain dry residues for further analysis.

The extraction yields of *A. marina* were determined by calculating the ratio of the weight of dried extract obtained to the initial weight of dried plant material powder and expressed as a percentage. The yields were 34.23% for roots, 65.02% for cotyledons, 61.76% for pericarps, 38.55% for propagules, and 33.72% for leaves.

3.3.4. Characterization of extracts

The chemical characterization of extracts was performed in negative mode using liquid chromatography coupled with electrospray ionization (ESI) and high-resolution mass spectrometry (UPLC-ESI/HRMS). A Waters ACQUITY UPLC system coupled with a Waters Xevo G2-XS QTof Mass Spectrometer (Waters Corp., Milford, MA, USA) was used. The extracts were dissolved in ultrapure water at a concentration of 100 µg/mL, and then 5 µL of each sample was injected into a Biphenyl column (100 mm × 2.1 mm, 2.6 µm; Phenomenex, Torrance, CA, USA). The chromatographic gradient was conducted with solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol), starting with 95% A for 1 min, followed by a linear gradient to 95% B over 10 min, and 4 min of column washing at 95% B. The flow rate was maintained at 0.4 mL/min. The ESI source was operated under the following conditions: electrospray capillary voltage of 1.5 kV, source temperature of 140 °C, and desolvation temperature of 600 °C. MS spectra were acquired in full range mode, covering a mass range of 100–1000 *m*/*z*. MS/HRMS analysis was performed using data-dependent scan

(DDA), selecting the two most intense ions from the HRMS scan for collision-induced dissociation (CID) with the following conditions: a minimum signal threshold of 500,000, isolation width at 2.0, and normalized collision energy of 30%. Metabolite identification followed the Metabolomics Standards Initiative (MSI) guidelines, which define three confidence levels indicated in the "IL" column of **Table 1**: Level 1 (IL1): compounds were unequivocally identified by comparison with authentic reference standards (retention time, MS/MS spectrum, and exact mass); Level 2 (IL2): tentative identifications were assigned based on matches between experimental MS/MS spectra and literature data or spectral libraries (e.g., GNPS, MassBank); Level 3 (IL3): compounds were classified by spectral similarity to known chemical families and supported by taxonomic evidence.

Novelty verification was performed using general literature databases (e.g., Google Scholar) and the chemical database SciFinder^{n}. In SciFinder^{n}, each tentatively identified compound was queried by chemical name, and all related references were investigated using keywords such as "*Avicennia marina*", "*Avicennia*", and "mangroves". This process allowed determination of whether a compound had been previously reported in *A. marina*, other species within the genus *Avicennia*, or other mangrove species, or if it represents a first identification in mangroves.

3.3.5. Determination of antioxidant activity

The antioxidant capacities (AOCs) of the exhaustive extracts of *A. marina* (leaves, roots, pericarps, propagules, and cotyledons) were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) and 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS$^{\bullet+}$) assays according to Cannavacciuolo et al. (2023). The extracts were dissolved in ultrapure water and analyzed at a concentration of 0.5 mg/mL, with Trolox (0–500 µM) serving as a standard. The antioxidant activity was expressed as µmol Trolox equivalents per gram of sample matrix (TE/g MTX), representing the µmol of a standard Trolox solution exerting the same antioxidant capacity as 1 mg/mL of the tested extracts.

In the DPPH assay the stock solution of DPPH (5 mM) was prepared by dissolving 3.9 mg of DPPH in 100 mL of methanol and subsequently diluted to 100 µM to obtain the operating solution. This solution was prepared just before use and protected from light due to the photosensitivity of the reagent. The assay was set up in an Eppendorf by mixing 50 µL of sample with 950 µL of operative DPPH, and the mixture was incubated in the dark for 30 min.

Subsequently, 200 µL of the solution was transferred to an absorbance reading plate at the 515 nm wavelength.

For the ABTS assay, the stock solution of ABTS (7 mM) was diluted with phosphate-buffered saline (PBS; 5 mM, pH 7.4) to achieve working concentrations. The assay was performed by combining 5 µL of diluted sample (or PBS control) and 500 µL of ABTS radical cation solution (0.1 mM in PBS). The reaction mixtures were protected from light and incubated at 30 °C for 60 min to allow complete radical scavenging. Absorbance measurements were then recorded at 734 nm using a microplate reader.

3.3.6. Cytotoxic evaluation

3.3.6.1. Cell lines and culture conditions

Normal human fibroblasts (MRC-5), human glioblastoma (U-87), human triple-negative breast cancer (MDA-MB-231), human colorectal cancer (SW480), and human healthy mucosa (CCD841) cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA). The human cervical cancer cell line (HeLa) was acquired from System Biosciences, and the human colon cancer cell line (E705) was provided by the Fondazione IRCCS Istituto Nazionale dei Tumori (Milan, Italy). The E705 cell line represents epithelial tissue cells of colorectal adenocarcinoma derived from a patient at the National Cancer Institute in Milan.

MRC-5, U-87, and HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin/streptomycin (P/S), and 2 mM L-glutamine. MDA-MB-231 cells were maintained in Minimum Essential Medium (MEM) with Earl's Salts supplemented with 10% heat-inactivated FBS, 1% P/S, 2 mM L-glutamine, and 0.1 mM MEM Non-Essential Amino Acids (MEM NEAA). E705 and SW480 cell lines were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. The CCD 841 cell line was grown in EMEM medium supplemented with 10% heat-inactivated FBS, 1% P/S, 2 mM L-glutamine, and 0.1 mM non-essential amino acids. All cell lines were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. Cell culture media and reagents were purchased from EuroClone (Pero, Italy).

3.3.6.2. Viability assay

The cytotoxicity of *A. marina* extracts was evaluated using the MTT assay (CellTiter96®Non-Radioactive Cell Proliferation Assay, Promega, Madison, WI, USA) following the

manufacturer's protocol. The extract powders were solubilized in Milli-Q water, and four extract concentrations (20, 60, 180, and 540 µg/mL) were tested on all cell lines. Additionally, for the root extract, an extended dose-response analysis using ten concentrations (2, 10, 20, 40, 60, 100, 140, 180, 360, and 540 µg/mL) was performed on selected cell lines to enable IC_{50} determination. IC_{50} values were calculated only for extract–cell line combinations tested with this ten-point dilution series.

No positive control drugs were included in this preliminary screening, as the primary objective was to assess and compare the relative cytotoxicity of different *A. marina* extracts. Comparative analysis with standard anticancer agents will be incorporated in subsequent studies on purified fractions or isolated compounds.

Briefly, HeLa, MRC-5, U-87, and MDA-MB-231 cells were seeded into 96-well plates (from Euroclone, Pero, Italy) at a density of 5×10^3 cells/well in 100 µL of growth medium, while E-705 and SW-480 cells were seeded at a density of 8×10^3 cells/well. After 24 h of incubation at 37 °C in 5% CO_2, the medium was replaced, and cells were treated with various concentrations of *A. marina* extracts. Following 48 h of treatment, the medium was replaced, and 15 µL of MTT solution was added to each well. After 3 h of incubation at 37 °C, formazan crystals were solubilized using 100 µL of stop solution and incubated under stirring for 1 h. Reduced MTT was quantified using a UV–vis plate reader (EnSight Multimode Microplate Reader, PerkinElmer, Waltham, MA, USA) at 570 nm with a reference wavelength of 630 nm.

Cell viability was expressed as a percentage relative to untreated cells (negative control), and medium with MilliQ water at equivalent concentrations (10% *v*/*v*) was used as a blank. Dose-response curves and the IC_{50} values, representing the extract concentration required to inhibit 50% of cell viability relative to untreated control cells, were generated using GraphPad Prism v10.5.0 software.

3.3.7. *In silico* prediction for anticancer activity

To identify potential bioactive compounds responsible for the cytotoxic effect, an in silico prediction of biological activity was performed using PASS Online software (https://www.way2drug.com/PASSOnline/index.php; accessed on 15 July 2025), a predictive tool from Way2Drug Services. The reliability of PASS for predicting *in vitro* cytotoxic activity has been demonstrated in previous studies (Verbanac et al., 2012; Filimonov et al., 2014), including those focusing on triterpene saponins (Desai et al., 2019).

The canonical Simplified Molecular Input Line Entry System (SMILES) of each compound was gathered from SciFinder$_n$ and was used to run the software. The program independently calculates the estimated predictive biological activities based on structure–activity relationships, providing Pa (probability of activity) and Pi (probability of inactivity) values for each activity. Only activities with Pa > 0.7 were considered, as this threshold indicates a high likelihood that the substance will exhibit the predicted activity in experimental settings, although the probability of the compound being an analogue of a known pharmaceutical agent remains high (Lagunin et al., 2000). Notably, when Pa > 0.9, as frequently observed in our study, the likelihood of false-positive predictions is insignificant (Verbenac et al., 2012).

The predicted anticancer-related activities included antineoplastic activity, apoptosis-related effects (apoptosis agonist, caspase 3/8 stimulation), TP53 expression enhancement, NF-kB modulation, cytostatic activity, lipid peroxidase inhibition, and ICAM-1 expression inhibition. These results were analyzed in relation to the cytotoxicity data of the MTT assay to establish potential correlations between the phytochemical composition and the observed cytotoxicity against cancer cells.

3.3.8. Statistical analysis

Statistical analyses were conducted on data generated from three replicates. Cytotoxic activity results are presented as mean ± standard error of the mean (SEM). Antioxidant activity results are reported as mean ± standard deviation (SD). Before statistical analysis, the assumption of normality was assessed using the Shapiro–Wilk test. The homogeneity of the variances was evaluated using Levene's test. When normality and homogeneity assumptions were met, a one-way analysis of variance (ANOVA) was performed, followed by Tukey's honest significant difference (HSD) post hoc test to assess pairwise differences between the means of the group. In cases where the assumption of homogeneity of the variances was not met, Welch's ANOVA was applied, followed by the Games–Howell post hoc test. Statistical significance was considered when $p < 0.05$.

The correlation between phytochemical composition and antioxidant activity was assessed using Spearman's rank correlation coefficient (two-tailed). For each plant-part extract (n = 5), the number of tentatively identified compounds in each major chemical class (phenylethanoid glycosides, flavonoid glycosides, iridoid glycosides, hydroxycinnamic acids and derivatives, and triterpene saponins) was correlated with antioxidant activity (DPPH and ABTS, mean values). Statistical significance was set at $p < 0.05$.

All analyses were conducted with IBM SPSS Statistics v29.0.2.0.

3.4. RESULTS

3.4.1. Characterization of *Avicennia marina* extracts

Roots, leaves, cotyledons, pericarps, and propagules of *A. marina* displayed distinct metabolite profiles. The chromatographic profiles of the extracts are provided in Supplementary data section **(Figures S3.1–S3.5)**, and the list of tentatively identified compounds is shown in **Table 3.1**, along with their corresponding identification level (IL), which reflects the confidence of compound annotation based on MSI guidelines. For compounds assigned to IL2, the identification relied on comparisons of MS/MS fragmentation data with published spectra from the literature or spectral databases. The specific references used to support each IL2 assignment are included directly in the table.

Table 3.1. UHPLC-ESI/HRMS data of compounds detected in *Avicennia marina* extracts. The main fragment ion for each compound is indicated in bold.

No.	RT (min)	$[M-H]^-$	Formula	Δ ppm	MS/MS	Name	Class	Part	IL	Ref.
1	0.58	701.1893 $[M+Cl]^-$	$C_{24}H_{42}O_{21}$	−2.1677	665.2134, 485.1499, 443.1393, **383.1182**, 341.1075, 179.0549	Stachyose	Tetrasaccharides	Cotyledons/pericarps/propagules/roots	IL2	Galasso et al., 2014
2	0.99	191.0188	$C_6H_8O_7$	6.3850	111.0073	Citric acid	Tricarboxylic acids	Cotyledons/pericarps	IL2	Fiori et al., 2018
3	3.87	373.1139	$C_{16}H_{22}O_{10}$	0.3221	211.0605, 167.0700, 149.0597, **123.0440**, 105.0333	Geniposidic acid	Iridoid glycosides	Leaves/cotyledons/pericarps/propagules/roots	IL2	Wang et al., 2014
4	4.03	353.0875	$C_{16}H_{18}O_9$	0.8633	**191.0551**, 179.0339, 161.0233, 135.0439	Caffeoylquinic acid isomer	Hydroxycinnamic acids and derivatives	Roots	IL2	Seo et al., 2012
5	4.05	375.1291	$C_{16}H_{24}O_{10}$	1.5167	213.0756, 169.0857, **151.0753**, 133.0644, 125.0595, 107.0490	Mussaenosidic acid	Iridoid glycosides	Leaves/cotyledons/pericarps/propagules	IL2	Amessis-Ouchemoukh et al., 2014
6	4.33	375.1285	$C_{16}H_{24}O_{10}$	3.1119	213.0747, 169.0854, **151.0748**, 133.0644, 125.0591, 113.0230, 107.0484	(Epi)loganic acid	Iridoid glycosides	Leaves	IL2	Amessis-Ouchemoukh et al., 2014
7	4.53	487.1451	$C_{21}H_{28}O_{13}$	1.2588	**179.0334**, 161.0228, 135.0435	Cistanoside F	Phenylethanoid glycosides	Pericarps	IL2	Xie et al., 2019
8	4.80	327.0715	$C_{14}H_{16}O_9$	1.9983	179.0335, 165.0389, 147.0283, **135.0434**, 105.0178	Unidentified	-	Leaves		
9	5.09	353.0871	$C_{16}H_{18}O_9$	−3.0904	**191.0550**, 179.0337, 173.0442, 161.0232, 135.0439**,**	Caffeoylquinic acid isomer	Hydroxycinnamic acids and derivatives	Leaves/cotyledons/pericarps/propagules/roots	IL2	Seo et al., 2012
10	5.47	371.0982	$C_{15}H_{16}O_{11}$	−8.7858	**209.0635**, 179.0337, 161.0228, 135.0435, 129.0178	Caffeoyl hexaric acid	Hydroxycinnamic acids and derivatives	Leaves	IL2	Kiss et al., 2020
11	5.60	443.0655	$C_{18}H_{20}O_{11}S$	−0.3243	275.0218, **167.0338**, **152.0105**, 123.0440, 108.0204	Unidentified	-	Roots		

12	5.65	415.1603	$C_{19}H_{28}O_{10}$	1.6114	235.0963, **191.1062**, 173.0958, 149.0953, 137.0590, 101.0226	Icariside D1	Flavonoid glycosides	Leaves	IL2	Garcìa-Villegas et al., 2024
13	5.93	639.1964	$C_{29}H_{36}O_{16}$	−5.2193	621.1807, 529.1554, 459.1488, 251.0549, 179.0337, **161.0232**, 151.0387	Suspensaside isomer	Phenylethanoid glycosides	Pericarps/roots	IL2	Han et al., 2007; Zhou et al., 2017
14	5.95	639.1964	$C_{29}H_{36}O_{16}$	−5.2193	621.1807, 529.1554, 459.1488, 251.0549, 179.0337, **161.0232**, 151.0387	Suspensaside isomer	Phenylethanoid glycosides	Pericarps/roots	IL2	Han et al., 2007; Zhou et al., 2017
15	6.14	537.1628	$C_{25}H_{30}O_{13}$	−2.6672	493.1708, 375.1275, 323.0758, 213.0752, 179.0334, 169.0854, **161.0230**, 151.0750, 135.0435, 125.0593, 107.0486	Grandiflorosid e	Hydroxycinnamic acid and derivatives	Leaves	IL2	Zhang et al., 2015
16	6.33	619.1644	$C_{29}H_{32}O_{15}$	3.9407	383.0758, **311.0549**, 267.0646	Unidentified	-	Pericarps/roots		
17	6.41	639.1929	$C_{29}H_{36}O_{16}$	0.2477	**621.1817**, 529.1554, 459.1493, 251.0549 **179.0338**, 161.0236, 151.0385	Suspensaside isomer	Phenylethanoid glycosides	Roots	IL2	Han et al., 2007; Zhou et al., 2017
18	6.65	521.1658	$C_{25}H_{30}O_{12}$	1.2446	357.1176, 169.0854, 163.0385, 151.0749, 145.0280, 125.0591, 119.0486, 117.0329, 107.0486	Marinoid C	Iridoid glycosides	Leaves/cotyledons/pericarps	IL3	Sun et al., 2008
19	6.65	653.2091	$C_{29}H_{34}O_{17}$	9.5533	621.1822, 459.1499, 179.0338, **161.0234**, 151.0388, 135.0437	Suspensaside methyl ether	Phenylethanoid glycosides	Roots	IL2	Han et al., 2007; Zhou et al., 2017
20	6.79	623.1981	$C_{29}H_{36}O_{15}$	0.0705	461.1657, **161.0233**, 113.0283	Verbascoside (acteoside) isomer	Phenylethanoid glycosides	Leaves/pericarps/roots	IL2	Petreska et al., 2011
21	6.9	463.0874	$C_{21}H_{20}O_{12}$	1.7229	301.0324, **300.0264,** 271.0235, 255.0285	Quercetin 3-*O*-hexoside	Flavonoid glycosides	Roots	IL2	Hvattum et al., 2002; Kammerer et al., 2004; Gu et al., 2013
22	7.02	667.2239	$C_{31}H_{40}O_{16}$	0.6864	621.1824, 459.1499, **179.0338**, **161.0235**, 151.0386, 135.0436	β-ethyl-OH-verbascoside	Phenylethanoid glycosides	Pericarps	IL2	Innocenti et al., 2006; Matos et al., 2018
23	7.11	623.2001	$C_{29}H_{36}O_{15}$	−3.1336	461.1661, **161.0235**,	Verbascoside (acteoside) isomer	Phenylethanoid glycosides	Leaves/pericarps/roots	IL2	Petreska et al., 2011
24	7.21	621.1838	$C_{29}H_{34}O_{15}$	−2.0992	461.1652, 179.0337, **161.0233**, 151.0387	Suspensaside A	Phenylethanoid glycosides	Roots	IL2	Han et al., 2007; Zhou et al., 2017
25	7.21	461.0718	$C_{21}H_{18}O_{12}$	1.6222	**285.0391**	Kaempferol-3-*O*-glucuronide	Flavonoid glycosides	Leaves	IL2	Seeram et al., 2006
26	7.21	681.2063	$C_{31}H_{38}O_{17}$	−3.9236	519.1708, 490.1321, 181.0129, 179.0334, **161.0230**	Unidentified	-	Pericarps		
27	7.3	447.0926	$C_{21}H_{20}O_{11}$	1.5287	327.0494, 285.0648, **284.0315**, 255.0288, 227.0338, 151.0013	Kaempferol 3-*O*-glucoside	Flavonoid glycosides	Roots	IL2	Zhang et al., 2007; Zhang et al., 2010; Gu et al., 2013; Nijat et al., 2020
28	7.40	623.1658	$C_{28}H_{32}O_{16}$	−6.4750	**315.0494**, 314.0421, 300.0258, 299.0187, 271.0234	Isorhamnetin-3-*O*-rutinoside	Flavonoid glycosides	Leaves/pericarps	IL2	Parejo et al., 2004
29	7.40	491.0828	$C_{22}H_{20}O_{13}$	0.6385	**315.0499**, 300.0264	Isorhamnetin glucuronide	Flavonoid glycosides	Leaves	IL2	Ibrahim et al., 2024
30	7.51	535.1477	$C_{25}H_{28}O_{13}$	−3.7032	329.1021, **179.0338**, 161.0232, 149.0595, 135.0438	Unidentified	-	Leaves/Pericarps		
31	7.51	477.1036	$C_{22}H_{22}O_{12}$	−5.1250	315.0467, **314.0420**, 285.0392, 271.0236, 257.0441, 243.0286,	Isorhamnetin 7-glucoside	Flavonoid glycosides	Leaves	IL2	Ibrahim et al., 2024

32	7.61	471.1874	$C_{22}H_{32}O_{11}$	−0.4545	287.1273, **263.1278**, 219.1379, 201.1273, 186.1036, 147.1166	Unidentified	-	Pericarps		
33	7.86	519.1143	$C_{24}H_{24}O_{13}$	0.2199	315.0472, **314.0423**, 299.0186, 285.0383, 271.0236, 257.0443, 243.0286	Unidentified	-	Leaves		
34	7.90	553.1556	$C_{25}H_{30}O_{14}$	1.2256	329.1021, **197.0445**, 182.0206, 153.0454, 149.0596, 131.0489,	Marinoid D	Iridoid glycosides	Cotyledons/pericarps/propagules/roots	IL3	Sun et al., 2008
35	7.95	505.1757	$C_{25}H_{29}O_{11}$	0.2000	357.1184, 213.0757, 195.0650, 169.0857, 151.0753, **147.0439**, 125.0596, 113.0230, 107.0487, 103.0539	Marinoid A	Iridoid glycosides	Leaves	IL3	Sun et al., 2008
36	8.03	519.1505	$C_{25}H_{28}O_{12}$	0.5766	313.1072, 295.0961, **163.0388**, 149.0596, 145.0282, 131.0490, 119.0487	Unidentified	-	Leaves/cotyledons/pericarps/roots		
37	8.03	475.0887	$C_{22}H_{20}O_{12}$	−1.0510	300.0589, **299.0554**, 285.0358, **284.0318.**	Diosmetin 7-glucuronide	Flavonoid glycosides	Leaves	IL2	Wang et al., 2024
38	8.24	549.1616	$C_{26}H_{30}O_{13}$	−0.4279	343.1176, 325.1064, **193.0495**, 175.0387, 149.0595, 134.0360, 131.0489	Unidentified	-	Leaves/cotyledons/pericarps/roots		
39	8.36	591.2119	$C_{29}H_{36}O_{13}$	−6.0539	179.0333, **161.0234**, 133.0282, 113.0228	Jionoside C	Phenylethanoid glycosides	Pericarps	IL2	Lei et al., 2023
40	8.62	825.4276	$C_{44}H_{66}O_{16}$	0.2536	**663.3744**, 601.3735	Unknown triterpene saponin	Triterpene saponins	Roots	IL3	Human et al., 2002; Schliemann et al., 2008
41	8.70	539.2152	$C_{26}H_{36}O_{12}$	−3.3318	193.0485, 183.1010, 175.0382, 149.0591, 131.0485, 121.0642	Unidentified	-	Leaves		
42	8.80	541.2285	$C_{26}H_{38}O_{12}$	1.0147	193.0485, 185.1166, 175.0382, 149.0591, 131.0485, 121.0642	Unidentified	-	Leaves		
43	8.88	825.4285	$C_{42}H_{66}O_{16}$	−0.8354	**663.3744**, 601.3735, 487.3421	Unknown triterpene saponin	Triterpene saponins	Roots	IL3	Human et al., 2002; Schliemann et al., 2008
44	8.97	299.0546	$C_{16}H_{12}O_6$	5.0379	285.0345, **284.0313**, 256.0363, 227.0334	Trihydroxy-methoxyflavone	Flavones	Leaves	IL2	Zhao et al., 2023
45	8.99	825.4273	$C_{42}H_{66}O_{16}$	0.6166	**663.3744**, 601.3735,	Medicoside G (medicagenic acid 3,28-di-glucoside)	Triterpene saponins	Roots	IL2	Human et al., 2002; Schliemann et al., 2008
46	9.08	809.4316	$C_{42}H_{66}O_{15}$	1.5979	689.3884, **647.3788**, 629.3680, 585.3786	Esculentoside C (phycolaccoside D)	Triterpene saponins	Cotyledons/pericarps/propagules/roots	IL2	Saleri et al., 2017; Tavares-Silva et al., 2019
47	9.30	505.1711	$C_{25}H_{30}O_{11}$	0.8600	281.1170, 195.0649, 151.0750, **147.0438**, 133.0645, 107.0486	Unidentified	-	Leaves		
48	9.38	503.1572	$C_{25}H_{28}O_{11}$	−2.6077	279.1010, 253,0854, 209.0954, 195.0647, **147.0437**, 131.0486, 103.0536	Unidentified	-	Leaves/pericarps		
49	9.54	809.4342	$C_{42}H_{66}O_{15}$	−1.6102	**647.3797**, 471.3469	Azukisaponin III	Triterpene saponins	Roots	IL2	Liu et al., 2017

Analysis detected a total of 49 compounds across all plant parts. Triterpene saponins are a heterogeneous secondary metabolite consisting of a terpene-based aglycone linked to one or more sugar chains, commonly glucose (−162 Da), glucuronic acid (−176 Da), and pentoses

(−146 Da) (Pham et al., 2022). For example, compound 49, which has an m/z of 809.4342 and a molecular formula of $C_{42}H_{66}O_{15}$, displayed characteristic MS/MS fragments at *m*/*z* 647.3797 [M-H-162] and 471.3469 [M-H-162-176], corresponding to sequential losses of sugar moieties. Based on this fragmentation and the molecular formula, it was identified as Azukisaponin III. Using similar fragmentation patterns, compounds 40, 43, 45, and 46 were also assigned as triterpene saponins (Huhman et al., 2002; Schliemann et al., 2008; Saleri et al., 2017; Liu et al., 2017; Tavares-Silva et al., 2019).

Phenylethanoid glycosides are often based on β-D-glucosides of 2-phenylethanol, often with α-L-rhamnose (Rha) substitution at C-3′ of the glucose, resembling variants of verbascoside (Kite et al., 2020). Simple phenylethanoid glycosides such as acteoside, isoacteoside, and plantamajoside exhibit similar fragmentation patterns in MS/MS experiments. These are characterized by neutral losses of 162, 152, or 146 *m*/*z*, which are associated with the presence of caffeic acid, glucose, rhamnose, and the phenethanol aglycone. Diagnostic fragment ions at *m*/*z* 179, 161, and 135 indicate the presence of caffeoyl, anhydroglucose, and anhydrophenethanol. Additionally, losses of water (−18 Da) or CO_2 (−44 Da) are frequently observed (Qi et al., 2012). Based on this information, the compounds 7, 13, 14, 17, 20, 22, 23, 24, and 39 belonged to the phenylethanoid glycosides group.

Flavonoid glycosides are a group of secondary metabolites that are widely distributed throughout the plant kingdom. Depending on the bond of the sugar portion, they are divided into *O*-glycosides or C-glycosides and can be distinguished by their unique MS/MS fragmentation spectra, which depend on the nature of the sugar fraction. Generally, C-glycosides exhibit neutral losses of 30, 90, and 120 Da for hexose sugars; 74 and 104 Da for deoxyhexose sugars; and 60 Da for pentose sugars. In contrast, O-glycosides exhibit neutral losses of 162 Da (hexose sugars), 176 Da (glucuronic acid), 146 Da (deoxyhexose sugars), and 132 Da (pentose sugars) (Pagliari et al., 2024). Based on this information, compounds 12, 21, 25, 27, 28, 29, 31, and 37 were identified as O-glycosides.

Iridoid glycosides exhibit distinct fragmentation patterns that depend on the structure of the aglycone ring, the presence of functional groups, and the degree of unsaturation. Typically, a neutral loss of 162 Da is observed, corresponding to the breakage of the bond with the glucoside fraction. Subsequently, the formation of fragments due to the loss of water (18 Da) and the carboxyl group (44 Da) is also observed, together with characteristic fragments resulting from aglycone ring cleavage. Peak 3 with *m*/*z* 373.1139 and molecular formula $C_{16}H_{22}O_{10}$ was identified as geniposidic acid based on its MS/MS spectrum. Fragment *m*/*z* 211.0605

corresponded to the loss of hexose sugar (162 Da), followed by the presence of fragments *m*/*z* 167.0700 and 149.0597, reflecting subsequent losses of H2O (−18 Da) and CO2 (−44 Da), respectively (Fu et al., 2014). Furthermore, fragment 123.0440 is characteristic of the genistein ring. Based on the different fragmentation patterns, compounds 5, 6, 18, 34, and 35 were identified as iridoid glycosides.

Tissue-specific profiling revealed clear metabolic differentiation among parts, with the leaves containing the highest number of secondary metabolites (26), followed by the pericarps (23), roots (23), cotyledons (10), and propagules (6). Notably distinct distribution patterns were observed for specific classes of compounds across the different *A. marina* extracts. The results show that triterpene saponins occur almost exclusively in the root extract (five in roots and only one each in cotyledons, pericarps, and propagules; none in leaves). Phenylethanoid glycosides were predominantly found in root and pericarp extracts (seven in each), with only two detected in leaves and none in cotyledons and propagules. Flavonoid glycosides were mainly associated with leaf extract (six in leaves, two in roots, one in pericarps, and absent in cotyledons and propagules). In contrast, iridoid glycosides and hydroxycinnamic acid and derivatives showed a more uniform distribution across all the extracts.

Analysis confirmed several compounds previously reported in *A. marina*, including caffeoylquinic acid, geniposidic acid, marinoid A, C, and D, acteoside, quercetin 3-*O*-hexoside, kaempferol 3-*O*-glucuronide, isorhamnetin-3-*O*-rutinoside, diosmetin 7-glucuronide, and jionoside C (Sharaf et al., 2000; Sun et al., 2008; Sun et al., 2009; Mohamed et al., 2024; Zhang et al., 2024; Kartikaningsih et al., 2024). Additionally, cistanoside F and kaempferol 3-*O*-glucoside were also detected, previously reported in other mangrove species but not in *A. marina* (Wu et al., 2004; Vinh et al., 2019). To our knowledge, several compounds such as mussaenosidic acid, (epi)loganic acid, caffeoylglucaric acid, icariside D1, suspensaside, grandifloroside, suspensaside methyl ether, suspensaside A, isorhamnetin glucuronide, isorhamnetin 7-glucoside, medicoside G, esculentoside C, and azukisaponin III have been newly reported in mangrove species.

3.4.2. Antioxidant activity

3.4.2.1. DPPH and ABTS Assays

The antioxidant potential of *A. marina* extracts was evaluated using two spectrophotometric assays, ABTS and DPPH, which are widely used to assess the free radical scavenging activity of natural compounds. The results are shown in **Figure 3.1**.

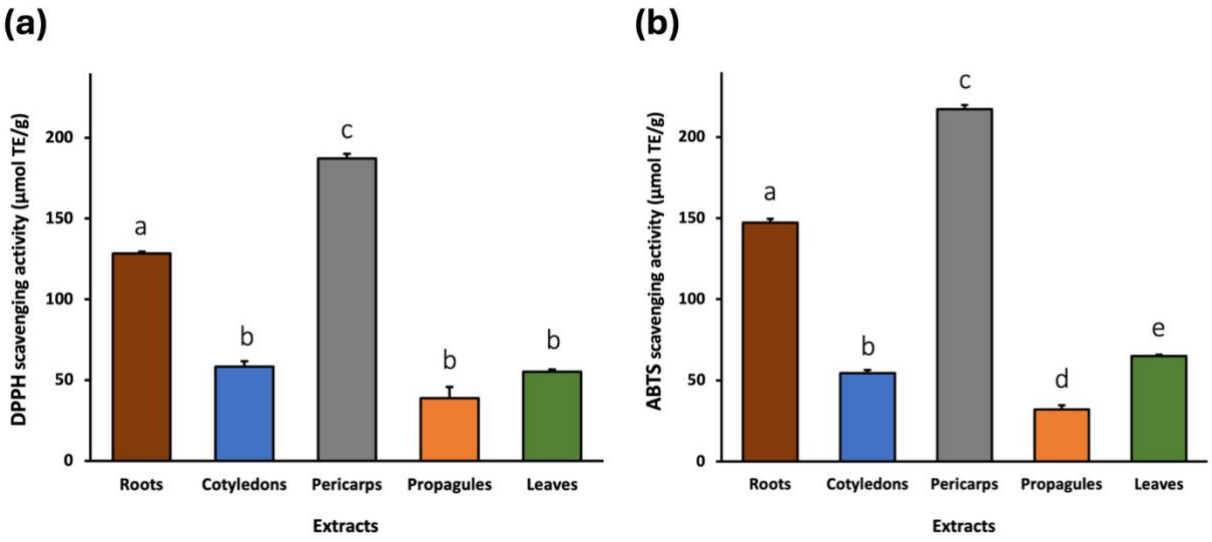

Figure 3.1. DPPH (**a**) and ABTS (**b**) radical scavenging activity of *Avicennia marina* extracts expressed as µmol Trolox equivalents per gram of sample matrix (µmol TE/g). The bars represent the mean ± standard deviation (SD) from n = 3 independent experiments. Different lowercase letters indicate statistically significant differences between extracts ($p < 0.05$).

The DPPH assay showed that the pericarp extract exhibited the highest radical scavenging activity, with a Trolox equivalent antioxidant capacity (TEAC) value of 187.14 ± 2.87 µmol TE/g. This was followed by the extracts of root (128.25 ± 1.12 µmol TE/g), cotyledon (58.23 ± 3.49 µmol TE/g), leaf (55.12 ± 1.52 µmol TE/g), and propagule (38.72 ± 6.96 µmol TE/g).

Similarly, the ABTS assay confirmed that the root and pericarp extracts exhibit high antioxidant activity compared to the other parts of the plant. In fact, the pericarp extracts again displayed the highest TEAC value (217.16 ± 2.67 µmol TE/g), followed by the root (147.21 ± 2.42 µmol TE/g), leaf (64.98 ± 0.84 µmol TE/g), cotyledon (54.46 ± 1.95 µmol TE/g), and propagule (32.23 ± 2.53 µmol TE/g) extracts.

3.4.2.2. Correlation between compound classes and antioxidant activity

The pericarp and root extracts, which exhibited the highest antioxidant activity, are also the ones that contain a high number of phenylethanoid glycosides, compared to the other extracts, which may explain their higher activity. To explore the potential associations between the phytochemical composition of each extract and their antioxidant capacity, a Spearman correlation analysis was performed between the number of compounds in each major chemical class and the measured antioxidant activities (DPPH and ABTS assay) across the five plant-part extracts (n = 5) (**Table 3.2**). The analysis revealed a significant positive correlation between the number of phenylethanoid glycosides in the extracts and DPPH activity ($\rho = 0.949$; $p = 0.014$). ABTS activity showed a similar trend, though the correlation did not reach statistical

significance (ρ = 0.791; p = 0.111). Antioxidant activity showed no statistically significant correlations with the number of other classes of compounds, including flavonoid glycosides, iridoid glycosides, hydroxycinnamic acids and derivatives, and triterpene saponins (all p > 0.05). Given the small sample size and the use of compound counts (not concentrations), these associations should be considered exploratory.

Table 3.2. Spearman correlation coefficients (ρ) between the number of compounds per chemical class and the antioxidant activity (DPPH and ABTS assays). Statistically significant correlations are indicated in bold (p value < 0.05).

Compound Class	DPPH		ABTS	
	ρ-Value	*p*-Value	*p*-Value	*p*-Value
Iridoid glycosides	−0.103	0.870	0.510	0.935
Hydroxycinnamic acid and derivatives	−0.112	0.858	0.224	0.718
Phenylethanoid glycosides	0.791	0.111	0.949	**0.014**
Flavonoid glycosides	0.205	0.741	0.574	0.322
Triterpene saponins	0.447	0.450	0.224	0.718

3.4.3. *In vitro* cytotoxic activity

The cytotoxic effects of *A. marina* extracts (leaf, cotyledon, pericarp, propagule, and root) were evaluated against a panel of human cancer cell lines using the MTT assay. Four concentrations (20, 60, 180, and 540 µg/mL) were tested on two colorectal cancer cell lines (SW480 and E705) (**Figure 3.2**) and three additional cancer cell lines: MDA-MB-231 (triple-negative breast cancer), U-87 (glioblastoma), and HeLa (cervical cancer) (**Figure 3.3**). Furthermore, two non-cancerous cell lines, MRC-5 (normal human fibroblasts) and CCD 841 (healthy human mucosa), served as controls to assess extract selectivity (**Figure 3.4**). The complete data for all concentrations and cell lines are reported in **Table S3.1**.

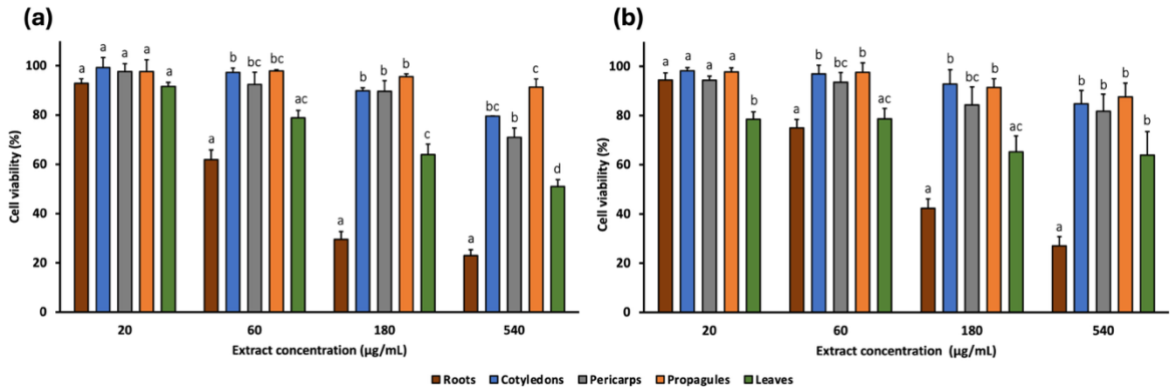

Figure 3.2. Cell viability (%) of SW480 (**a**) and E705 (**b**) human colorectal cancer cell lines treated with *Avicennia marina* extracts (20–540 µg/mL) for 48 h. Bars represent mean ± standard error of the mean (SEM) from n = 3 independent experiments. Different lowercase letters indicate statistically significant differences between extracts ($p < 0.05$) and were assigned independently for each concentration.

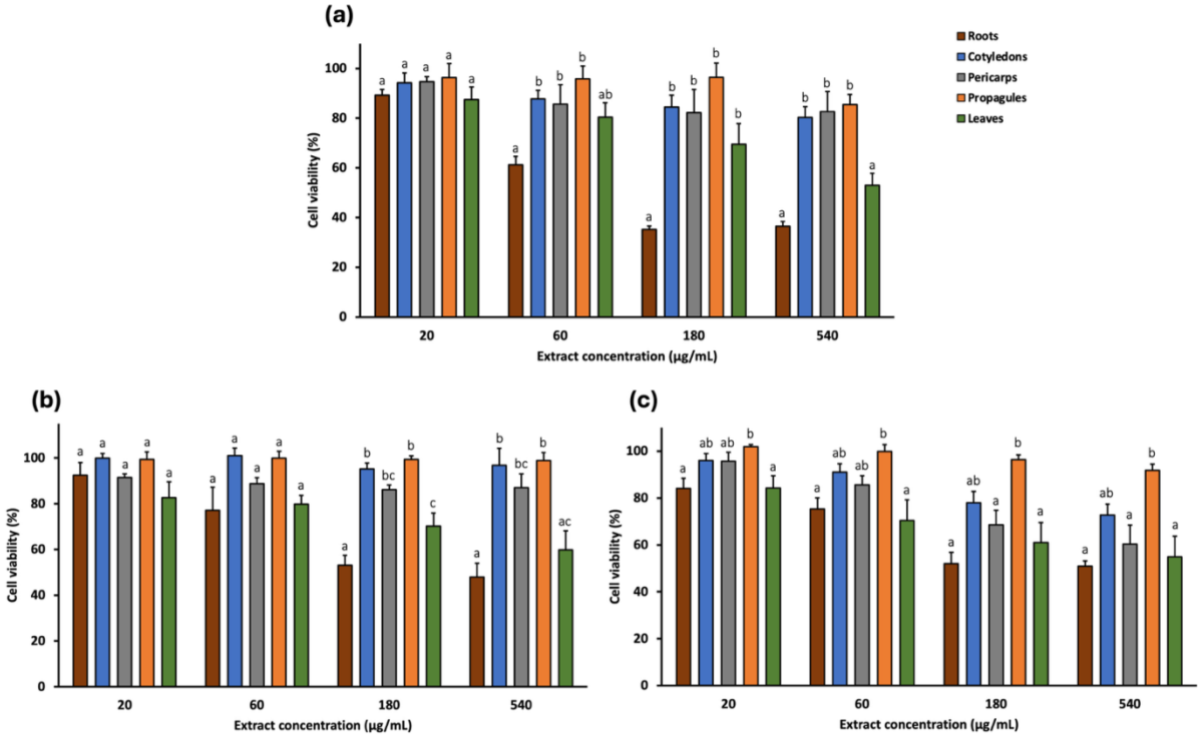

Figure 3.3. Cell viability (%) of MDA-MB-231 (**a**), U-87 (**b**), and HeLa (**c**) human cancer cell lines treated with *Avicennia marina* extracts (20–540 µg/mL) for 48 h. Bars represent mean ± standard error of the mean (SEM) from n = 3 independent experiments. Different lowercase letters indicate statistically significant differences between extracts ($p < 0.05$) and were assigned independently for each concentration.

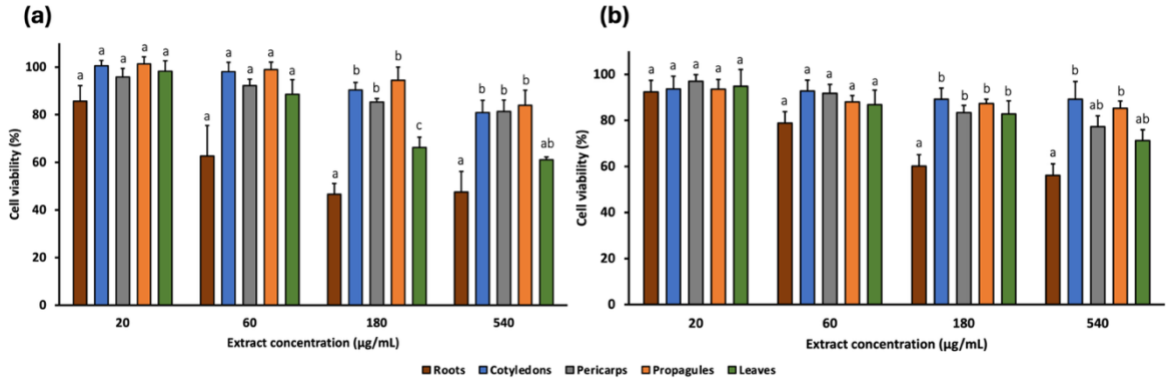

Figure 3.4. Cell viability (%) of CCD 841 (**a**) and MRC-5 (**b**) healthy human cell lines treated with *Avicennia marina* extracts (20–540 µg/mL) for 48 h. Bars represent mean ± standard error of the

mean (SEM) from n = 3 independent experiments. Different lowercase letters indicate statistically significant differences between extracts ($p < 0.05$) and were assigned independently for each concentration.

Among the extracts, cotyledon, pericarp, and propagule generally exhibited the lowest cytotoxic activity, reducing cell viability by no more than 70% at the highest concentration (540 µg/mL) across all cell lines. The exception was the pericarp extract, which reduced viability of HeLa cells to 60.30%.

The leaf extract showed low cytotoxicity at lower concentrations. At 60 µg/mL, cell viability remains near 80% for SW480, E705, and MDA-MB-231 and was 70.44% for HeLa. In the non-cancerous cell lines, viability was even higher: 88.59% and 86.86% for CCD 841 and MRC-5, respectively. However, at 540 µg/mL, the extract reduced viability to 50–60% in most cell lines, particularly 50.98% for SW480, 63.91% for E705, 53.00% for MDA-MB-231, 59.77% for U-87, 54.96% for HeLa, and 61.01% for CCD 841, while the least reduction occurred in MRC-5 (71.26%).

Among all extracts, the root extract exhibited the highest cytotoxic activity. At 180 µg/mL, it reduced cell viability to 29.47% (SW480), 42.40% (E705), 35.26% (MDA-MB-231), 53.06% (U87), 52.00% (HeLa), 46.60% (CCD 841), and 60.15% (MRC-5). These reductions were statistically significant compared to all other extracts, except for E705, where differences with the leaf extract were not significant, and for HeLa. At 540 µg/mL, cytotoxicity remained similar across most lines, although viability dropped further in SW480 and E705 (22.93% and 27.03%, respectively).

These findings highlight the notable activity of the root extract, particularly at the highest concentrations, against SW480, E705, and MDA-MB-231 cell lines (**Figure 3.5**). Notably, at 180 µg/mL, the cytotoxic activity of the root extract in these cell lines was significantly greater than in non-cancerous MRC-5 cells, while no significant difference was observed compared to the healthy mucosa cell line CCD 841. Additionally, at 540 µg/mL, viability of SW480 cells was significantly lower than that of CCD 841.

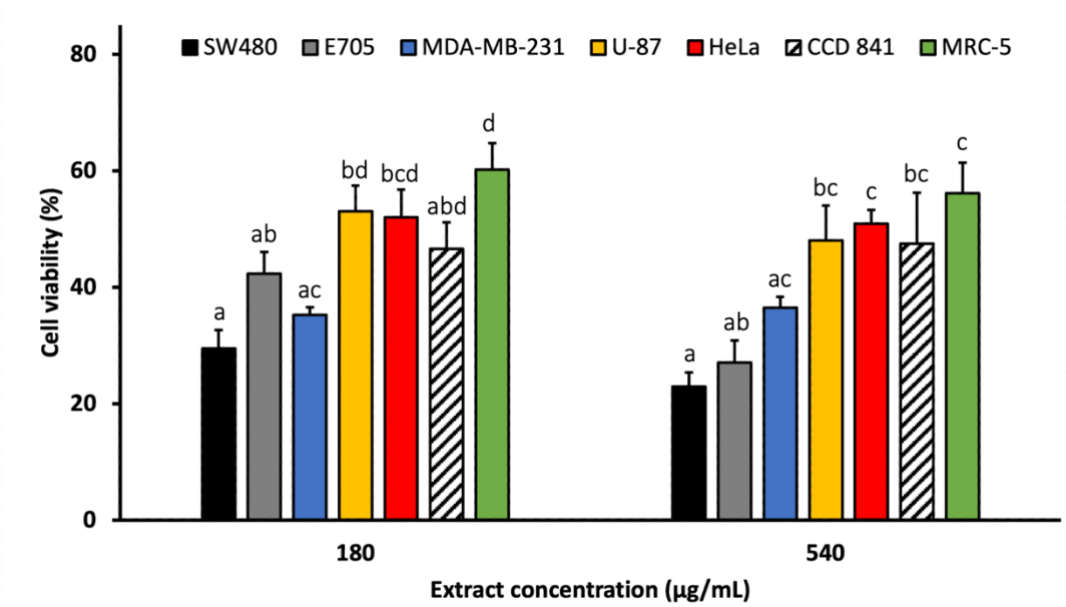

Figure 3.5. Cell viability (%) of the cancer cell lines treated with *Avicennia marina* root extract (180 and 540 µg/mL) for 48 h. Bars represent mean ± standard error of the mean (SEM) from n = 3 independent experiments. Different lowercase letters indicate statistically significant differences between extracts ($p < 0.05$) and were assigned independently for each concentration.

Given this pronounced response, the analysis focused on dose–response effects in SW480, E705, and MDA-MB-231. Each cell line was treated with ten increasing concentrations of root extract (2 to 540 µg/mL; **Figure S3.6**), and the IC_{50} values were 81.98 µg/mL for SW480, 108.10 µg/mL for E705, and 57.93 µg/mL for MDA-MB-231.

3.4.4. *In silico* analysis

Given the strong cytotoxic activity observed for the *A. marina* root extract *in vitro*, an *in silico* analysis was conducted to evaluate the predicted biological activities of the compounds found exclusively or predominantly in this extract. PASS Online predicted potential cytotoxicity-related effects, including antineoplastic activity, apoptosis induction (caspase 3/8 stimulation and apoptosis agonism), TP53 expression enhancement, NF-κB stimulation, cytostatic activity, lipid peroxidase inhibition, and inhibition of ICAM-1 expression (Clemente et al., 2020; Kang et al., 2024). The complete list of predicted biological activities for compounds exclusive of the root extract is provided in **Table S3.2** in the Supplementary data section.

Among the primary peaks detected in the root extract, the triterpene saponins medicoside G and azukisaponin III were found exclusively in the root extract, while esculentoside C was detected at high levels in the roots and in trace amounts in other extracts. These compounds showed high predicted probabilities (Pa) for antineoplastic activity (0.870, 0.905, and 0.908 for medicoside

G, esculentoside C, and azukisaponin III, respectively), caspase 3/8 stimulation (0.994/0.984, 0.989/0.986, and 0.964/0.934), apoptosis agonism (0.901, 0.862, and 0.883), and NF-κB stimulation (0.965, 0.917, and 0.904). They were also predicted to inhibit ICAM-1 expression (0.908, 0.961, and 0.987) and lipid peroxidase activity (0.927, 0.952, and 0.991).

Additional compounds found exclusively in the root extract, including I, suspensaside A, kaempferol 3-O-glucoside, and quercetin 3-O-hexoside, were also predicted to exhibit antineoplastic activity with Pa values of 0.804, 0.863, 0.834, and 0.833, respectively. Kaempferol 3-O-glucoside and quercetin 3-O-hexoside also showed cytostatic activity (Pa: 0.811 and 0.825), enhancement of TP53 expression (Pa: 0.952 and 0.959) and lipid peroxidase inhibition (0.960 and 0.976, resp.).

3.5. DISCUSSION

UHPLC-ESI/HRMS analyses enabled the characterization of *A. marina* extracts, revealing a total of 49 compounds, unevenly distributed across plant parts. The leaf extract contained the highest number of secondary metabolites, followed by pericarps, roots, cotyledons, and propagules. Notably, this study distinguishes between the external tissue of the propagule (here consistently called pericarp) and the internal tissues of the propagule (here consistently called simply propagule) (Tomlinson, 2016), which are often analyzed as a single fruit unit in other studies. The propagule, which consists mainly of the embryo, contained few compounds, likely due to the focus on primary metabolites essential for germination (Rosental et al., 2014). In contrast, the pericarp, which serves a protective function, was significantly richer in secondary metabolites (Sharif et al., 2022), particularly phenylethanoid glycosides. Similarly, cotyledons had low metabolite diversity and a profile similar to propagules but showed some additional peaks. These may reflect early biosynthesis of stress-related compounds in developing seedlings.

The compounds identified, including phenylethanoid glycosides, flavonoid glycosides, iridoid glycosides, hydroxycinnamic acid and derivatives, and triterpene saponins, are well known for their ecological roles in protecting plants from abiotic stressors such as drought, high salinity, intense sunlight, and elevated temperatures (Wang et al., 2010; Ferdinando et al., 2012; Neugart et al., 2016; Falahi et al., 2018; Sarri et al., 2021). These classes are widely reported in *A. marina* from other regions, and some metabolites identified here have been documented

previously, suggesting a shared core phytochemical profile with global populations (Zhou et al., 2025).

However, differences from previous studies were observed. Firstly, a distinctive feature of *A. marina* elsewhere is the presence of naphthalene derivatives (Zhou et al., 2025), which were not detected in our samples. This absence may reflect tissue specificity, as most of these compounds were extracted from branches, or differences in extraction methods (Han et al., 2008). More importantly, several compounds detected in this study have never been reported before in *A. marina*. These include one kaempferol-glycosides and two isorhamnetin glycosides. Notably, monohydroxy B-ring-substituted flavonoid glycosides (e.g., kaempferol-, diosmetin-, and isorhamnetin-glycosides) were more abundant than dihydroxy types, a pattern opposite to that expected under UV stress, where dihydroxy forms typically dominate due to their antioxidant potential (Ferdinando et al., 2012; Neugart at al., 2016; Zietz et al., 2010). This shift may reflect Gulf-specific regulation of flavonoid biosynthesis rather than species-specific traits, as it is not evident in previous reports (Zhou et al., 2025). The study identified novel compounds among the iridoid glycosides and hydroxycinnamic acids, but the most notable findings were within phenylethanoid glycosides and triterpene saponins, the latter all newly reported in *A. marina*. The high abundance of phenylethanoid glycosides in roots is consistent with their reported accumulation under water stress (Falahi et al., 2018), while the accumulation of triterpene saponins is associated with osmotic stress response (Wu et al., 2005; Sarri et al., 2021). These trends suggest that UAE-grown *A. marina* may possess a distinctive phytochemical profile shaped by the extreme environmental conditions of the Arabian Gulf (Oku et al., 2003; Das et al., 2015). Nonetheless, inter-regional comparisons are limited due to methodological differences. Future comparative studies using standardized LC-MS protocols would enhance our understanding of mangrove chemical ecology and support bioprospecting efforts.

The identified metabolite classes are associated with various biological activities, including antioxidant, anticancer, antimicrobial, and anti-inflammatory (Sparg et al., 2004; Xue et al., 2016; Yang et al., 2018; Wang et al., 2020; Sova et al., 2020). In particular, phenylethanoid glycosides are well-documented antioxidants, showing both DPPH and ABTS radical scavenging activity (Xue et al., 2016; De Marino et al., 2012; Budzianowska et al., 2024). In this study, extracts of the pericarp and root were rich in these compounds and showed the highest antioxidant activity (187.14 ± 2.87 and 217.16 ± 2.67 μmol TE/g for the pericarps; 128.25 ± 1.12 and 147.21 ± 2.42 μmol TE/g for the roots). The strong positive Spearman

correlation between the number of phenylethanoid glycosides and DPPH activity (ρ = 0.949; p = 0.014) supports the hypothesis that these compounds contribute to radical scavenging in the extracts. Key phenylethanoid glycosides identified in this study, such as cistanoside F, acteoside, and jionoside C, are known for their potent antioxidant activity (Ji et al., 2019; Wei et al., 2019; Lu et al., 2023), while less-studied molecules such as suspensaside and suspensaside A warrant further exploration. Our correlation findings are exploratory and do not prove causation. Definitive attribution will require targeted quantification of candidate phenylethanoid glycosides and subsequent activity testing.

In terms of cytotoxic activity, the cotyledon, pericarp, and propagule extracts showed negligible effects on cancer cell lines, even at the highest concentrations. In contrast, the leaf and root extracts displayed cytotoxicity at higher doses. The leaf extract showed limited cytotoxicity at lower concentrations but reduced viability (50–60%) at 540 µg/mL in several cancer cell lines. This agrees with Momtazi-Borojeni et al. (2013), who reported no toxicity at low concentrations but moderate effects at higher doses (250 µg/mL). The root extract had the most promising cytotoxic profile, particularly against SW480, E705, and MDA-MB-231 cancer cell lines. At 540 µg/mL, it reduced cell viability below 40% but showed lower toxicity against normal cell lines (MRC-5 and CCD 841). IC_{50} values for SW480, E705, and MDA-MB-231 were 81.98, 108.10, and 57.93 µg/mL, respectively. Based on the criteria established by the National Cancer Institute (NCI, USA) and the Geran protocol, which classified cytotoxicity as high when IC_{50} values are $\leq$ 20 µg/mL, moderate between 21 and 200 µg/mL, weak between 201 and 500 µg/mL, and absent above 500 µg/mL (Geran et al., 1972; Niksic et al., 2021; Addy et al., 2024), this corresponds to moderate cytotoxic activity. While these IC50 values were not compared with a standard drug, they suggest the presence of active compounds. The values reported here are for crude extracts; further fractionation and isolation of active constituents are expected to yield more potent compounds, for which *in vivo* and clinical potential could be more realistically assessed through comparison with standard anticancer drugs. These are potentially triterpene saponins, which were only detected in the roots.

Triterpene saponins are gaining attention in cancer research due to their ability to target tumor-related pathways while maintaining low toxicity (Du et al., 2014). Although widespread in medicinal plants (Yang et al., 2018; da Silva et al., 2020), their occurrence in mangroves is less documented, with only a few studies published on this topic (Yang et al., 2018; Vinh et al., 2019), *in silico* prediction using PASS software indicated a strong cytotoxic potential for several saponins identified in the root extract, including medicoside G, esculentoside C, and

azukisaponin III. Additionally, two unidentified saponins suggest the presence of a potentially novel structure that merits further isolation and structural characterization. Other compounds specific to the root extract, such as suspensaside A and kaempferol 3-*O*-glucoside, showed high predicted probabilities for antineoplastic effects.

Given that the root extract exhibited the highest cytotoxicity, further work will focus on bioactivity-guided fractionation of this extract, with particular emphasis on isolating the triterpene saponin-rich fraction. These purified fractions will be tested for cytotoxicity alongside a standard anticancer drug (e.g., doxorubicin) to identify the compounds responsible for the observed activity. Mechanistic studies, including apoptosis assays, cell cycle analysis, and molecular pathway investigations, will be conducted to elucidate the modes of action. Such comprehensive analyses, together with the targeted quantification of candidate constituents, will clarify structure–activity correlations and enhance both therapeutic efficacy and selectivity. Importantly, these efforts, combined with further purification and structural elucidation, could identify promising novel lead compounds suitable for subsequent *in vivo* evaluation and development as potential anticancer agents.

3.6. CONCLUSIONS

The findings of this study highlight *A. marina* as a valuable source of bioactive compounds with promising therapeutic applications. The pericarp and root extracts exhibited the highest antioxidant activity, possibly due to the presence of phenylethanoid glycosides, which are known for their antioxidant activities. Among all extracts tested, the root extract displayed the strongest cytotoxicity, in particular against the triple-negative breast cancer cell line MDA-MB-231 and two colorectal cancer cell lines, SW480 and E705, with IC_{50} values of 58.46, 81.98, and 108.10 µg/mL, respectively. *In silico* predictions identified triterpene saponins, including medicoside G, esculentoside C, and azukisaponin III, as likely contributors to these effects.

The detection of triterpene saponins not previously reported from mangroves, together with several phenylethanoid glycosides and other compounds not earlier described in *A. marina*, is noteworthy. Plants adapted to extreme environments can accumulate distinctive secondary metabolites, and our plant-part-specific UPLC-HRMS analysis of UAE-grown *A. marina* provides region-specific evidence that complements existing phytochemical surveys. Moreover, combining this untargeted phytochemical investigation with biological activity

screening and *in silico* analysis/statistical correlation offers a practicable approach to rapidly link observed activities to plant-part-specific compounds.

To advance these observations towards pharmacological relevance, future work should focus on bioactivity-guided fractionation of the extracts, structural elucidation, and targeted quantification of key compounds, along with mechanistic *in vitro* assays. These efforts will help clarify structure–activity correlations and potentially lead to the identification of a novel therapeutic candidate from this stress-adapted mangrove species.

3.7. REFERENCES

Abbas Momtazi-borojeni, A., Behbahani, M., & Sadeghi-Aliabadi, H. (2013). Antiproliferative activity and apoptosis induction of crude extract and fractions of *Avicennia marina*. Iranian journal of basic medical sciences, 16(11), 1203.

Abd-ElGawad, A. M., Elshamy, A. I., Al-Rowaily, S. L., & El-Amier, Y. A. (2019). Habitat affects the chemical profile, allelopathy, and antioxidant properties of essential oils and phenolic enriched extracts of the invasive plant *Heliotropium curassavicum*. Plants, 8(11), 482.

Addy, B. S., Firempong, C. K., Komlaga, G., Addo-Fordjour, P., Domfeh, S. A., Afolayan, O., & Emikpe, B. O. (2024). In vitro antiproliferative activities of some Ghanaian medicinal plants. Clinical Phytoscience, 10(1), 19.

Al-Mur, B. A. (2021). Biological activities of *Avicennia marina* roots and leaves regarding their chemical constituents. Arabian Journal for Science and Engineering, 46(6), 5407-5419.

Alzandi, A. A., Taher, E. A., Al-Sagheer, N. A., Al-Khulaidi, A. W., Azizi, M., & Naguib, D. M. (2021). Phytochemical components, antioxidant and anticancer activity of 18 major medicinal plants in Albaha region, Saudi Arabia. Biocatalysis and Agricultural Biotechnology, 34, 102020.

Amessis-Ouchemoukh, N., Abu-Reidah, I. M., Quirantes-Piné, R., Rodríguez-Pérez, C., Madani, K., Fernández-Gutiérrez, A., & Segura-Carretero, A. (2014). Tentative characterisation of iridoids, phenylethanoid glycosides and flavonoid derivatives from *Globularia alypum* L.(Globulariaceae) leaves by LC-ESI-QTOF-MS. Phytochemical Analysis, 25(5), 389-398.

Botosoa, E. P., & Shahidi, F. (2025). Phenolics and polyphenolics in mangrove plants: antioxidant activity and recent trends in food application–a review. Critical Reviews in Food Science and Nutrition, 1-35.

Budzianowska, A., Kikowska, M., & Budzianowski, J. (2024). Phenylethanoid glycosides accumulation and antiradical activity of fractionated extracts of *Plantago ovata* Forssk. callus cultures lines. Plant Cell, Tissue and Organ Culture (PCTOC), 156(2), 54.

Cannavacciuolo, C., Pagliari, S., Giustra, C. M., Carabetta, S., Guidi Nissim, W., Russo, M., et al. (2023). LC-MS and GC-MS data fusion metabolomics profiling coupled with multivariate analysis for the discrimination of different parts of faustrime fruit and evaluation of their antioxidant activity. Antioxidants, 12(3), 565.

Cerri, F., Giustra, M., Anadol, Y., Tomaino, G., Galli, P., Labra, M., et al. (2022). Natural products from mangroves: an overview of the anticancer potential of *Avicennia marina*. Pharmaceutics, 14(12), 2793.

Che Sulaiman, I. S., Basri, M., Fard Masoumi, H. R., Chee, W. J., Ashari, S. E., & Ismail, M. (2017). Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the

anti-radical activity of *Clinacanthus nutans* Lindau leaves by response surface methodology. Chemistry Central Journal, 11(1), 54.

Clemente, S. M., Martínez-Costa, O. H., Monsalve, M., & Samhan-Arias, A. K. (2020). Targeting lipid peroxidation for cancer treatment. Molecules, 25(21), 5144.

da Silva Magedans, Y. V., Phillips, M. A., & Fett-Neto, A. G. (2021). Production of plant bioactive triterpenoid saponins: from metabolites to genes and back. Phytochemistry Reviews, 20(2), 461-482.

Das, S. K., Patra, J. K., & Thatoi, H. (2016). Antioxidative response to abiotic and biotic stresses in mangrove plants: A review. International Review of Hydrobiology, 101(1-2), 3-19.

De Marino, S., Festa, C., Zollo, F., Incollingo, F., Raimo, G., Evangelista, G., & Iorizzi, M. (2012). Antioxidant activity of phenolic and phenylethanoid glycosides from *Teucrium polium* L. Food Chemistry, 133(1), 21-28.

Desai, T. H., & Joshi, S. V. (2019). In silico evaluation of apoptogenic potential and toxicological profile of triterpenoids. Indian Journal of Pharmacology, 51(3), 181-207.

Di Ferdinando, M., Brunetti, C., Fini, A., & Tattini, M. (2011). Flavonoids as antioxidants in plants under abiotic stresses. Abiotic stress responses in plants: metabolism, productivity and sustainability, 159-179.

Du, J. R., Long, F. Y., & Chen, C. (2014). Research progress on natural triterpenoid saponins in the chemoprevention and chemotherapy of cancer. The enzymes, 36, 95-130.

Duke, N. C. (1991). A systematic revision of the mangrove genus *Avicennia* (Avicenniaceae) in Australasia. Australian Systematic Botany, 4(2), 299-324.

El-Tarabily, K. A., Sham, A., Elbadawi, A. A., Hassan, A. H., Alhosani, B. K., El-Esawi, M. A., et al. (2021). A consortium of rhizosphere-competent actinobacteria exhibiting multiple plant growth-promoting traits improves the growth of *Avicennia marina* in the United Arab Emirates. Frontiers in Marine Science, 8, 715123.

ElDohaji, L. M., Hamoda, A. M., Hamdy, R., & Soliman, S. S. (2020). *Avicennia marina* a natural reservoir of phytopharmaceuticals: Curative power and platform of medicines. Journal of ethnopharmacology, 263, 113179.

Falahi, H., Sharifi, M., Maivan, H. Z., & Chashmi, N. A. (2018). Phenylethanoid glycosides accumulation in roots of *Scrophularia striata* as a response to water stress. Environmental and Experimental Botany, 147, 13-21.

Filimonov, D. A., Lagunin, A. A., Gloriozova, T. A., Rudik, A. V., Druzhilovskii, D. S., Pogodin, P. V., & Poroikov, V. V. (2014). Prediction of the biological activity spectra of organic compounds using the PASS online web resource. Chemistry of Heterocyclic Compounds, 50(3), 444-457.

Fiori, J., Amadesi, E., Fanelli, F., Tropeano, C. V., Rugolo, M., & Gotti, R. (2018). Cellular and mitochondrial determination of low molecular mass organic acids by LC–MS/MS. Journal of Pharmaceutical and Biomedical Analysis, 150, 33-38.

Franzoni, G., Trivellini, A., Bulgari, R., Cocetta, G., & Ferrante, A. (2019). Bioactive molecules as regulatory signals in plant responses to abiotic stresses. In Plant signaling molecules (pp. 169-182). Woodhead Publishing.

Friis, G., & Killilea, M. E. (2023). Mangrove Ecosystems of the United Arab Emirates. In A natural history of the emirates (pp. 217-240). Cham: Springer Nature Switzerland.

Friis, G., Smith, E. G., Lovelock, C. E., Ortega, A., Marshell, A., Duarte, C. M., & Burt, J. A. (2024). Rapid diversification of grey mangroves (*Avicennia marina*) driven by geographic isolation and extreme environmental conditions in the Arabian Peninsula. Molecular ecology, 33(4), e17260.

Friis, G., Vizueta, J., Smith, E. G., Nelson, D. R., Khraiwesh, B., Qudeimat, E., et al. (2021). A high-quality genome assembly and annotation of the gray mangrove, *Avicennia marina*. G3, 11(1), jkaa025.

Fu, Z., Xue, R., Li, Z., Chen, M., Sun, Z., Hu, Y., & Huang, C. (2014). Fragmentation patterns study of iridoid glycosides in Fructus Gardeniae by HPLC-Q/TOF-MS/MS. Biomedical Chromatography, 28(12), 1795-1807.

Galasso, S., Pacifico, S., Kretschmer, N., Pan, S. P., Marciano, S., Piccolella, S., et al. (2014). Influence of seasonal variation on *Thymus longicaulis* C. Presl chemical composition and its antioxidant and anti-inflammatory properties. Phytochemistry, 107, 80-90.

García-Villegas, A., Fernández-Ochoa, Á., Alañón, M. E., Rojas-García, A., Arráez-Román, D., Cádiz-Gurrea, M. D. L. L., & Segura-Carretero, A. (2024). Bioactive compounds and potential health benefits through cosmetic applications of cherry stem extract. International Journal of Molecular Sciences, 25(7), 3723.

Geran, R. I., Greenberg, N. H., & Mac Donald, M. M. (1972). Protocols for screening chemical agents and natural products against animal tumors and other biological systems.

Gu, D., Yang, Y., Bakri, M., Chen, Q., Xin, X., & Aisa, H. A. (2013). A LC/QTOF–MS/MS application to investigate chemical compositions in a fraction with protein tyrosine phosphatase 1B inhibitory activity from *Rosa rugosa* flowers. Phytochemical Analysis, 24(6), 661-670.

Han, J., Ye, M., Guo, H., Yang, M., Wang, B. R., & Guo, D. A. (2007). Analysis of multiple constituents in a Chinese herbal preparation Shuang-Huang-Lian oral liquid by HPLC-DAD-ESI-MSn. Journal of Pharmaceutical and Biomedical Analysis, 44(2), 430-438.

Haseeba, K. P., Aboobacker, V. M., Vethamony, P., & Al-Khayat, J. A. (2025). Significance of *Avicennia Marina* in the Arabian Gulf environment: A review. Wetlands, 45(1), 16.

Huamán-Castilla, N. L., Díaz Huamaní, K. S., Palomino Villegas, Y. C., Allcca-Alca, E. E., León-Calvo, N. C., Colque Ayma, E. J., et al. (2024). Exploring a sustainable process for polyphenol extraction from olive leaves. Foods, 13(2), 265.

Huhman, D. V., & Sumner, L. W. (2002). Metabolic profiling of saponins in *Medicago sativa* and *Medicago truncatula* using HPLC coupled to an electrospray ion-trap mass spectrometer. Phytochemistry, 59(3), 347-360.

Hvattum, E. (2002). Determination of phenolic compounds in rose hip (*Rosa canina*) using liquid chromatography coupled to electrospray ionisation tandem mass spectrometry and diode-array detection. Rapid Communications in Mass Spectrometry, 16(7), 655-662.

Ibrahim, R. M., Fayez, S., Eltanany, B. M., Abu-Elghait, M., El-Demerdash, A., Badawy, M. S. E., et al. (2024). Agro-byproduct valorization of radish and turnip leaves and roots as new sources of antibacterial and antivirulence agents through metabolomics and molecular networking. Scientia Horticulturae, 328, 112924.

Innocenti, M., Marca, G. L., Malvagia, S., Giaccherini, C., Vincieri, F. F., & Mulinacci, N. (2006). Electrospray ionisation tandem mass spectrometric investigation of phenylpropanoids and secoiridoids from solid olive residue. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry, 20(13), 2013-2022.

Ji, S. L., Cao, K. K., Zhao, X. X., Kang, N. X., Zhang, Y., Xu, Q. M., et al. (2019). Antioxidant activity of phenylethanoid glycosides on glutamate-induced neurotoxicity. Bioscience, Biotechnology, and Biochemistry, 83(11), 2016-2026.

Jin, J., Koroleva, O. A., Gibson, T., Swanston, J., Magan, J., Zhang, Y. A. N., et al. (2009). Analysis of phytochemical composition and chemoprotective capacity of rocket (*Eruca sativa* and *Diplotaxis tenuifolia*) leafy salad following cultivation in different environments. Journal of agricultural and food chemistry, 57(12), 5227-5234.

Kammerer, D., Carle, R., & Schieber, A. (2004). Characterization of phenolic acids in black carrots (Daucus carota ssp. sativus var. atrorubens Alef.) by high-performance liquid

chromatography/electrospray ionization mass spectrometry. Rapid Communications in Mass Spectrometry, 18(12), 1331-1340.

Kang, J. H., Uddin, N., Kim, S., Zhao, Y., Yoo, K. C., Kim, M. J., et al. (2024). Tumor-intrinsic role of ICAM-1 in driving metastatic progression of triple-negative breast cancer through direct interaction with EGFR. Molecular Cancer, 23(1), 230.

Kartikaningsih, H., Djamaludin, H., Fauziyah, J. N., Audina, N., Noviyanti, L., & Saputra, D. (2024). Green extraction of *Avicennia marina* leaves by natural deep eutectic solvents: phytochemical profile, antioxidant activity, molecular docking and admet analysis. Rasay J Chem, 17, 1123-1133.

Kiss, A. K., Michalak, B., Patyra, A., & Majdan, M. (2020). UHPLC-DAD-ESI-MS/MS and HPTLC profiling of ash leaf samples from different commercial and natural sources and their in vitro effects on mediators of inflammation. Phytochemical Analysis, 31(1), 57-67.

Kite, G. C. (2020). Characterisation of phenylethanoid glycosides by multiple-stage mass spectrometry. Rapid communications in mass spectrometry, 34, e8563.

Kodikara, C., Netticadan, T., Bandara, N., Wijekoon, C., & Sura, S. (2024). A new UHPLC-HRMS metabolomics approach for the rapid and comprehensive analysis of phenolic compounds in blueberry, raspberry, blackberry, cranberry and cherry fruits. Food Chemistry, 445, 138778.

Lagunin, A., Stepanchikova, A., Filimonov, D., & Poroikov, V. (2000). PASS: prediction of activity spectra for biologically active substances. Bioinformatics, 16(8), 747-748.

Lee, I. Y., Lee, D. H., Park, J. H., & Joo, N. (2025). UHPLC-HRMS/MS–Based Metabolic Profiling and Quantification of Phytochemicals in Different Parts of *Coccinia grandis* (L.) Voigt. Food Science & Nutrition, 13(2), e70004.

Lei, H., Xin, J., Lv, Y., Chen, W., Xu, X., Wang, J., et al. (2023). Effects of processing on the efficacy and metabolites of *Cistanche tubulosa* using ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Biomedical Chromatography, 37(6), e5621.

Lim, K. J. A., Cabajar, A. A., Lobarbio, C. F. Y., Taboada, E. B., & Lacks, D. J. (2019). Extraction of bioactive compounds from mango (*Mangifera indica* L. var. Carabao) seed kernel with ethanol–water binary solvent systems. Journal of Food Science and Technology, 56(5), 2536-2544.

Liu, R., Cai, Z., & Xu, B. (2017). Characterization and quantification of flavonoids and saponins in adzuki bean (*Vigna angularis* L.) by HPLC–DAD–ESI–MSn analysis. Chemistry Central Journal, 11(1), 93.

Lu, S. H., Zuo, H. J., Huang, J., Li, W. N., Huang, J. L., & Li, X. X. (2023). Chemical constituents from the leaves of *Ligustrum robustum* and their bioactivities. Molecules, 28(1), 362.

Mateos-Molina, D., Lamine, E. B., Antonopoulou, M., Burt, J. A., Das, H. S., Javed, S., et al. (2021). Synthesis and evaluation of coastal and marine biodiversity spatial information in the United Arab Emirates for ecosystem-based management. Marine Pollution Bulletin, 167, 112319.

Matos, P., Figueirinha, A., Paranhos, A., Nunes, F., Cruz, P., Geraldes, C. F. G. C., et al. (2018). Bioactivity of *Acanthus mollis*–contribution of benzoxazinoids and phenylpropanoids. Journal of ethnopharmacology, 227, 198-205.

Mitra, S., Naskar, N., Lahiri, S., & Chaudhuri, P. (2023). A study on phytochemical profiling of *Avicennia marina* mangrove leaves collected from Indian Sundarbans. Sustainable Chemistry for the Environment, 4, 100041.

Mohamed, A. S., Elmi, A., Spina, R., Kordofani, M. A., Laurain-Mattar, D., Nour, H., et al. (2024). In vitro and in silico analysis for elucidation of antioxidant potential of Djiboutian *Avicennia Marina* (Forsk.) Vierh. phytochemicals. Journal of Biomolecular Structure and Dynamics, 42(7), 3410-3425.

Mohammed, H. A. (2022). Phytochemical analysis, antioxidant potential, and cytotoxicity evaluation of traditionally used *Artemisia absinthium* L.(Wormwood) growing in the central region of Saudi Arabia. Plants, 11(8), 1028.

Nabeelah Bibi, S., Fawzi, M. M., Gokhan, Z., Rajesh, J., Nadeem, N., RR, R. K., et al. (2019). Ethnopharmacology, phytochemistry, and global distribution of mangroves—A comprehensive review. Marine drugs, 17(4), 231.

Neugart, S., Krumbein, A., & Zrenner, R. (2016). Influence of light and temperature on gene expression leading to accumulation of specific flavonol glycosides and hydroxycinnamic acid derivatives in kale (*Brassica oleracea* var. *sabellica*). Frontiers in plant science, 7, 326.

Nijat, D., Abdulla, R., Liu, G. Y., Luo, Y. Q., & Aisa, H. A. (2020). Identification and quantification of Meiguihua oral solution using liquid chromatography combined with hybrid quadrupole-orbitrap and triple quadrupole mass spectrometers. Journal of Chromatography B, 1139, 121992.

Niksic, H., Becic, F., Koric, E., Gusic, I., Omeragic, E., Muratovic, S., et al. (2021). Cytotoxicity screening of *Thymus vulgaris* L. essential oil in brine shrimp nauplii and cancer cell lines. Scientific Reports, 11(1), 13178.

Nugroho, R. Y., Hendri, M., Putri, W. A. E., & Agussalim, A. (2022). Phytochemical profile and toxicity of extracts from the leaf of *Avicennia marina* (Forssk.) Vierh. collected in mangrove areas affected by port activities. South African Journal of Botany, 150, 903-919.

Oku, H., Baba, S., Koga, H., Takara, K., & Iwasaki, H. (2003). Lipid composition of mangrove and its relevance to salt tolerance. Journal of plant research, 116(1), 37-45.

Pagliari, S., Sicari, M., Pansera, L., Guidi Nissim, W., Mhalhel, K., Rastegar, S., et al. (2024). A comparative metabolomic investigation of different sections of Sicilian *Citrus* x *limon* (L.) Osbeck, characterization of bioactive metabolites, and evaluation of in vivo toxicity on zebrafish embryo. Journal of Food Science, 89(6), 3729-3744.

Palaiogiannis, D., Chatzimitakos, T., Athanasiadis, V., Bozinou, E., Makris, D. P., & Lalas, S. I. (2023). Successive solvent extraction of polyphenols and flavonoids from *Cistus creticus* L. leaves. Oxygen, 3(3), 274-286.

Parejo, I., Jauregui, O., Sánchez-Rabaneda, F., Viladomat, F., Bastida, J., & Codina, C. (2004). Separation and characterization of phenolic compounds in fennel (*Foeniculum vulgare*) using liquid chromatography− negative electrospray ionization tandem mass spectrometry. Journal of agricultural and food chemistry, 52(12), 3679-3687.

Petreska, J., Stefova, M., Ferreres, F., Moreno, D. A., Tomás-Barberán, F. A., Stefkov, G., et al. (2011). Potential bioactive phenolics of *Macedonian sideritis* species used for medicinal "Mountain Tea". Food Chemistry, 125(1), 13-20.

Pham, H. N., Tran, C. A., Trinh, T. D., Nguyen Thi, N. L., Tran Phan, H. N., Le, V. N., et al. (2022). UHPLC-Q-TOF-MS/MS dereplication to identify chemical constituents of *Hedera helix* leaves in Vietnam. Journal of Analytical Methods in Chemistry, 2022(1), 1167265.

Plaskova, A., & Mlcek, J. (2023). New insights of the application of water or ethanol-water plant extract rich in active compounds in food. Frontiers in Nutrition, 10, 1118761.

Qi, M., Xiong, A., Geng, F., Yang, L., & Wang, Z. (2012). A novel strategy for target profiling analysis of bioactive phenylethanoid glycosides in *P lantago* medicinal plants using ultra-performance liquid chromatography coupled with tandem quadrupole mass spectrometry. Journal of separation science, 35(12), 1470-1478.

Rosental, L., Nonogaki, H., & Fait, A. (2014). Activation and regulation of primary metabolism during seed germination. Seed science research, 24(1), 1-15.

Saleri, F. D., Chen, G., Li, X., & Guo, M. (2017). Comparative analysis of saponins from different Phytolaccaceae species and their antiproliferative activities. Molecules, 22(7), 1077.

Sarri, E., Termentzi, A., Abraham, E. M., Papadopoulos, G. K., Baira, E., Machera, K., et al. (2021). Salinity stress alters the secondary metabolic profile of *M. sativa*, *M. arborea* and their hybrid (Alborea). International Journal of Molecular Sciences, 22(9), 4882.

Schliemann, W., Ammer, C., & Strack, D. (2008). Metabolite profiling of mycorrhizal roots of *Medicago truncatula*. Phytochemistry, 69(1), 112-146.

Seeram, N. P., Lee, R., Scheuller, H. S., & Heber, D. (2006). Identification of phenolic compounds in strawberries by liquid chromatography electrospray ionization mass spectroscopy. Food chemistry, 97(1), 1-11.

Seo, O. N., Kim, G. S., Park, S., Lee, J. H., Kim, Y. H., Lee, W. S., et al. (2012). Determination of polyphenol components of *Lonicera japonica* Thunb. using liquid chromatography–tandem mass spectrometry: contribution to the overall antioxidant activity. Food Chemistry, 134(1), 572-577.

Sharaf, M., El-Ansari, M. A., & Saleh, N. A. M. (2000). New flavonoids from *Avicennia marina*. *Fitoterapia*, *71*(3), 274-277.

Sharif, Y., Chen, H., Deng, Y., Ali, N., Khan, S. A., Zhang, C., et al. (2022). Cloning and functional characterization of a pericarp abundant expression promoter (AhGLP17-1P) from peanut (*Arachis hypogaea* l.). Frontiers in Genetics, 12, 821281.

Singh, A., & Choudhary, K. K. (2025). Utilizing UHPLC-HRMS-metabolomic profiling to uncover enhanced bioactive potential and health benefits in chili (*Capsicum annum* L.) under salinity stress. Food Chemistry, 483, 144255.

Sova, M., & Saso, L. (2020). Natural sources, pharmacokinetics, biological activities and health benefits of hydroxycinnamic acids and their metabolites. Nutrients, 12(8), 2190.

Sparg, S., Light, M. E., & Van Staden, J. (2004). Biological activities and distribution of plant saponins. Journal of ethnopharmacology, 94(2-3), 219-243.

Sun, Y., Ding, Y., & Lin, W. H. (2009). Isolation and identification of compounds from marine mangrove plant *Avicennia marina*. Journal of Peking University. Health Sciences, 41(2), 221-225.

Sun, Y., Ouyang, J., Deng, Z., Li, Q., & Lin, W. (2008). Structure elucidation of five new iridoid glucosides from the leaves of *Avicennia marina*. Magnetic Resonance in Chemistry, 46(7), 638-642.

Tavares-Silva, C., Holandino, C., Homsani, F., Luiz, R. R., Prodestino, J., Farah, A., et al. (2019). Homeopathic medicine of Melissa officinalis combined or not with *Phytolacca decandra* in the treatment of possible sleep bruxism in children: A crossover randomized triple-blinded controlled clinical trial. Phytomedicine, 58, 152869.

Temraz, T., & Khattaba, R. (2017). Mangrove *Avicennia marina* of Yanbu, Saudi Arabia: GC-MS Constituents and Mosquito Repellent Activities.

Tomlinson, P. B. (2016). The botany of mangroves. Cambridge University Press.

Toscano, S., Trivellini, A., Cocetta, G., Bulgari, R., Francini, A., Romano, D., & Ferrante, A. (2019). Effect of preharvest abiotic stresses on the accumulation of bioactive compounds in horticultural produce. Frontiers in plant science, 10, 1212.

Verbanac, D., Jain, S. C., Jain, N., Chand, M., Paljetak, H. Č., Matijašić, M., et al. (2012). An efficient and convenient microwave-assisted chemical synthesis of (thio) xanthones with additional in vitro and in silico characterization. Bioorganic & medicinal chemistry, 20(10), 3180-3185.

Vinh, L. B., Nguyet, N. T. M., Yang, S. Y., Kim, J. H., Thanh, N. V., Cuong, N. X., et al. (2019). Cytotoxic triterpene saponins from the mangrove *Aegiceras corniculatum*. Natural product research, 33(5), 628-634.

Wang, C., Gong, X., Bo, A., Zhang, L., Zhang, M., Zang, E., et al. (2020). Iridoids: research advances in their phytochemistry, biological activities, and pharmacokinetics. Molecules, 25(2), 287.

Wang, D. D., Liang, J., Yang, W. Z., Hou, J. J., Yang, M., Da, J., et al. (2014). HPLC/qTOF-MS-oriented characteristic components data set and chemometric analysis for the holistic quality control of complex TCM preparations: Niuhuang Shangqing pill as an example. Journal of pharmaceutical and biomedical analysis, 89, 130-141.

Wang, D. H., Du, F., Liu, H. Y., & Liang, Z. S. (2010). Drought stress increases iridoid glycosides biosynthesis in the roots of *Scrophularia ningpoensis* seedlings. J Med Plants Res, 4(24), 2691-2699.

Wang, Y., Xing, L., Zhang, J., Chen, Y., & Lu, S. (2025). Simultaneous Determination of 32 Polyphenolic Compounds in Berries via HPLC–MS/MS. Molecules, 30(9), 2008.

Wang, Z., Yao, M., Ouyang, H., He, M., Zhao, W., Wei, W., et al. (2024). Characterization of chemical constituents and metabolites in rat plasma after oral administration of *Ainsliaea fragrans* Champ by using UHPLC-QTOF-MS/MS. Journal of Chromatography B, 1244, 124259.

Wei, W., Lan, X. B., Liu, N., Yang, J. M., Du, J., Ma, L., et al. (2019). Echinacoside alleviates hypoxic-ischemic brain injury in neonatal rat by enhancing antioxidant capacity and inhibiting apoptosis. Neurochemical Research, 44(7), 1582-1592.

Wu, J. Y., Wong, K., Ho, K. P., & Zhou, L. G. (2005). Enhancement of saponin production in Panax ginseng cell culture by osmotic stress and nutrient feeding. Enzyme and Microbial Technology, 36(1), 133-138.

Wu, J., Huang, J., Xiao, Q., Zhang, S., Xiao, Z., Li, Q., et al. (2004). Complete assignments of 1H and 13C NMR data for 10 phenylethanoid glycosides. Magnetic Resonance in Chemistry, 42(7), 659-662.

Xie, G., Xu, Q., Li, R., Shi, L., Han, Y., Zhu, Y., et al. (2019). Chemical profiles and quality evaluation of Buddleja officinalis flowers by HPLC-DAD and HPLC-Q-TOF-MS/MS. Journal of Pharmaceutical and Biomedical Analysis, 164, 283-295.

Xue, Z., & Yang, B. (2016). Phenylethanoid glycosides: Research advances in their phytochemistry, pharmacological activity and pharmacokinetics. Molecules, 21(8), 991.

Yang, B., Liu, H., Yang, J., Gupta, V. K., & Jiang, Y. (2018). New insights on bioactivities and biosynthesis of flavonoid glycosides. Trends in food science & technology, 79, 116-124.

Yang, X. W., Dai, Z., Wang, B., Liu, Y. P., Zhao, X. D., & Luo, X. D. (2018). Antitumor triterpenoid saponin from the fruits of *Avicennia marina*. Natural products and bioprospecting, 8(5), 347-353.

Youssef, A. M., Maaty, D. A., & Al-Saraireh, Y. M. (2022). Phytochemistry and anticancer effects of mangrove (*Rhizophora mucronata* Lam.) leaves and stems extract against different cancer cell lines. Pharmaceuticals, 16(1), 4.

Zanatta, A. C., Vilegas, W., & Edrada-Ebel, R. (2021). UHPLC-(ESI)-HRMS and NMR-based metabolomics approach to access the seasonality of *Byrsonima intermedia* and *Serjania marginata* From Brazilian Cerrado Flora diversity. Frontiers in chemistry, 9, 710025.

Zhang, Y. C., Zhuang, L. H., Zhou, J. J., Song, S. W., Li, J., Huang, H. Z., et al. (2024). Combined metabolome and transcriptome analysis reveals a critical role of lignin biosynthesis and lignification in stem-like pneumatophore development of the mangrove *Avicennia marina*. Planta, 259(1), 12.

Zhang, Y. D., Huang, X., Zhao, F. L., Tang, Y. L., & Yin, L. (2015). Study on the chemical markers of Caulis *Lonicerae japonicae* for quality control by HPLC-QTOF/MS/MS and chromatographic fingerprints combined with chemometrics methods. Analytical Methods, 7(5), 2064-2076.

Zhang, Y., Liu, C., Zhang, Z., Wang, J., Wu, G., & Li, S. (2010). Comprehensive separation and identification of chemical constituents from *Apocynum venetum* leaves by high-performance counter-current chromatography and high performance liquid chromatography coupled with mass spectrometry. Journal of Chromatography B, 878(30), 3149-3155.

Zhang, Y., Shi, P., Qu, H., & Cheng, Y. (2007). Characterization of phenolic compounds in *Erigeron breviscapus* by liquid chromatography coupled to electrospray ionization mass spectrometry. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry, 21(18), 2971-2984.

Zhao, D., Chen, X., Wang, R., Pang, H., Wang, J., & Liu, L. (2023). Determining the chemical profile of *Caragana jubata* (Pall.) Poir. by UPLC–QTOF–MS analysis and evaluating its anti-ischemic stroke effects. Journal of Ethnopharmacology, 309, 116275.

Zhou, F., Peng, J., Zhao, Y., Huang, W., Jiang, Y., Li, M., et al. (2017). Varietal classification and antioxidant activity prediction of *Osmanthus fragrans* Lour. flowers using UPLC–PDA/QTOF–MS and multivariable analysis. Food chemistry, 217, 490-497.

Zhou, P., Hu, H., Wu, X., Feng, Z., Li, X., Tavakoli, S., et al. (2025). Botany, traditional uses, phytochemistry, pharmacological activities, and toxicity of the mangrove plant *Avicennia marina*: a comprehensive review. Phytochemistry Reviews, 1-36.

Zietz, M., Weckmüller, A., Schmidt, S., Rohn, S., Schreiner, M., Krumbein, A., & Kroh, L. W. (2010). Genotypic and climatic influence on the antioxidant activity of flavonoids in kale (*Brassica oleracea* var. *sabellica*). Journal of Agricultural and Food Chemistry, 58(4), 2123-2130.

3.8. SUPPLEMENTARY DATA

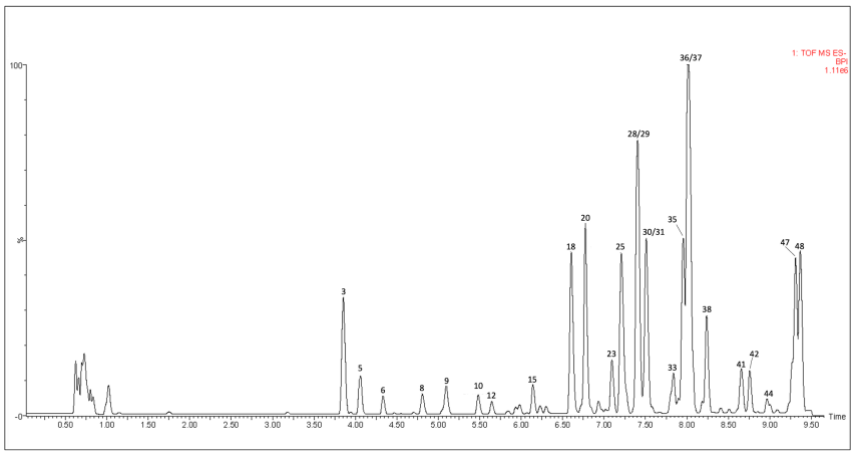

Figure S3.1. Representative chromatogram of the leaf extract of *A. marina.*

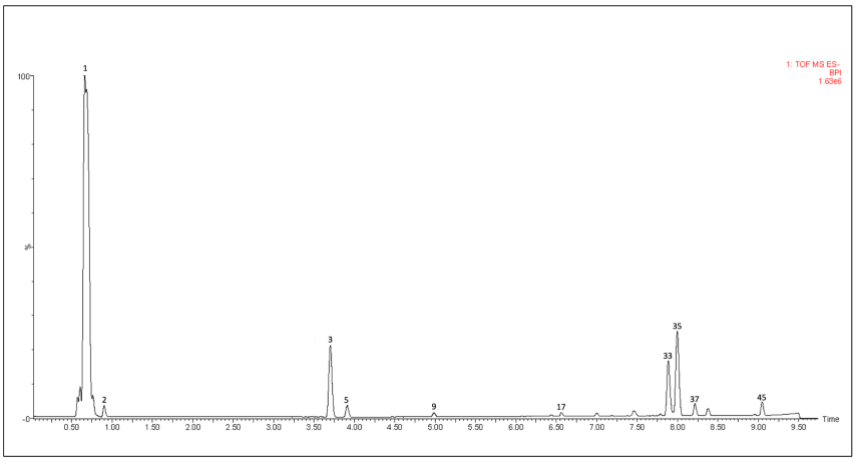

Figure S3.2. Representative chromatogram of the cotyledon extract of *A. marina.*

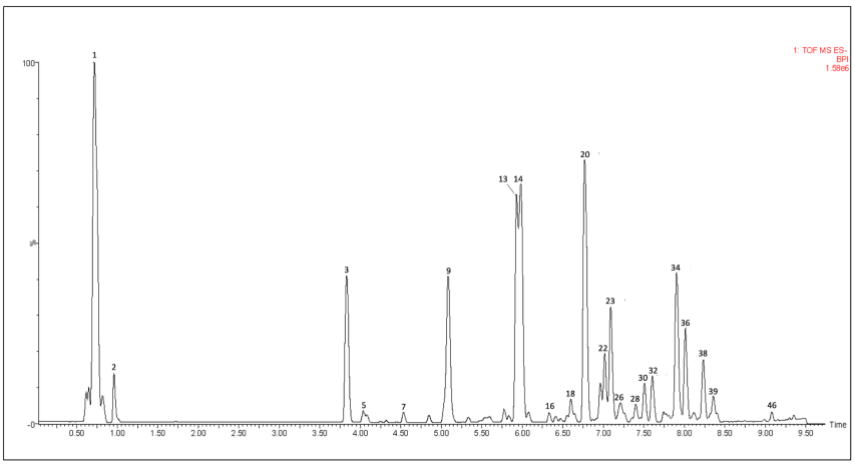

Figure S3.3. Representative chromatogram of the pericarp extract of *A. marina.*

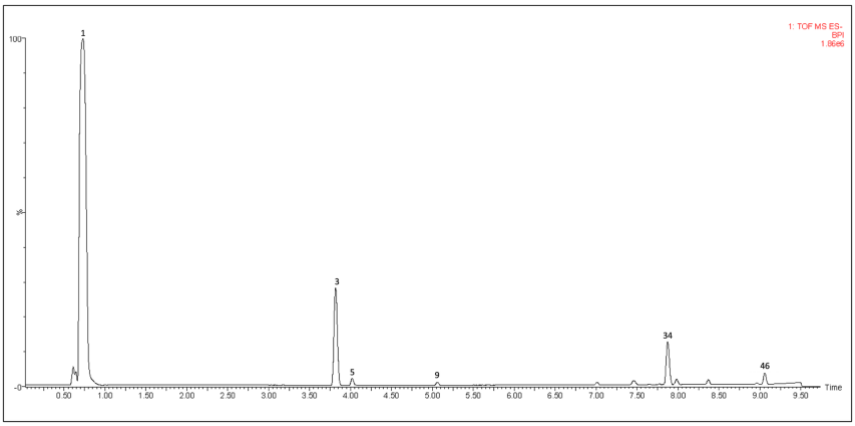

Figure S3.4. Representative chromatogram of the propagule extract of *A. marina.*

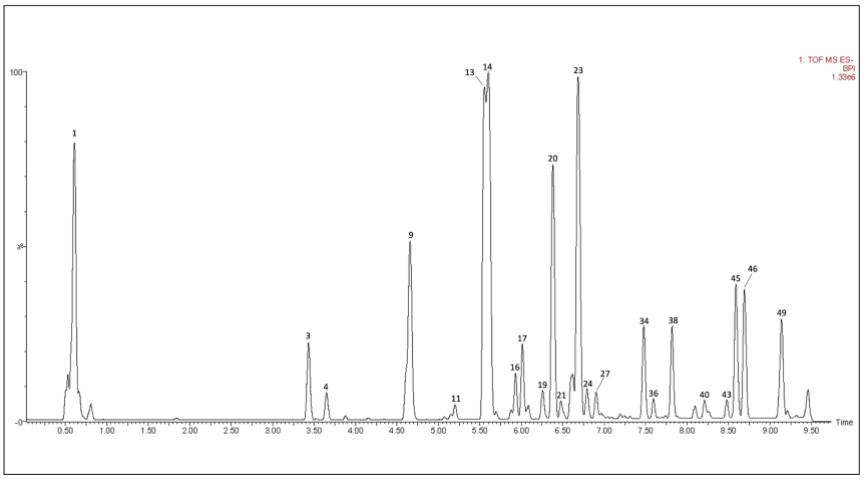

Figure S3.5. Representative chromatogram of the root extracts of *A. marina.*

Table S3.1. Cytotoxicity of *Avicennia marina* extracts on the tested cell lines at four concentrations (20–540µg/mL). Cell viability is expressed as a percentage relative to untreated cells (negative control). Standard error of the mean (SEM) is also reported.

Cell line	Extract conc. (µg/mL)	Roots		Cotyledons		Pericarps		Propagules		Leaves	
		Cell viability (%)	SEM	Cell viability (%)	SEM	Cell viability (%)	SEM	Cell viability (%)	SEM	Cell viability (%)	SEM
SW480	**20**	92.84	1.83	99.21	4.09	97.64	3.19	97.62	4.84	91.61	1.55
	60	61.92	4.00	97.25	1.85	92.42	4.93	97.89	0.49	78.80	3.02
	180	29.47	3.20	89.80	1.27	89.63	4.27	95.61	1.07	63.95	4.28
	540	22.93	2.42	79.51	0.15	70.95	3.80	91.30	3.32	50.98	2.82
E705	**20**	94.40	2.85	98.20	1.24	94.33	1.71	97.74	1.64	78.50	2.93
	60	74.94	3.42	96.94	3.50	93.48	4.00	97.59	3.86	78.67	4.26
	180	42.30	3.77	92.77	5.92	84.34	7.33	91.45	3.48	65.22	6.57
	540	27.03	3.81	84.76	5.44	81.71	6.96	87.57	5.58	63.91	9.56
MDA-MB-231	**20**	89.26	2.25	94.24	3.95	94.68	2.07	96.37	5.59	87.46	5.05
	60	61.27	3.37	87.76	3.46	85.62	7.82	95.83	5.16	80.37	5.79
	180	35.26	1.30	84.44	4.81	82.19	9.32	96.41	5.75	69.46	8.36
	540	36.47	1.90	80.29	4.39	82.62	8.10	85.50	4.03	53.00	4.84
U-87	**20**	92.49	5.55	99.97	2.02	91.47	1.55	99.38	3.26	82.61	6.94
	60	77.08	10.16	101.03	3.29	88.72	2.65	99.89	3.09	79.86	3.78
	180	53.06	4.39	95.16	2.69	86.18	2.02	99.32	1.63	70.18	5.75
	540	47.99	6.03	96.81	7.42	86.97	6.13	98.85	3.52	59.77	8.42
HeLa	**20**	84.11	4.33	95.98	2.94	95.72	3.81	101.93	0.85	84.28	5.20
	60	75.36	4.73	91.05	3.59	85.63	3.88	99.86	2.90	70.44	8.82
	180	52.00	4.79	77.94	4.91	68.55	6.14	96.37	1.97	61.00	8.50
	540	50.91	2.31	72.71	4.68	60.30	8.12	91.82	2.64	54.96	8.68
CCD 841	**20**	85.66	6.48	100.50	2.29	95.77	3.68	101.34	2.95	98.25	4.32
	60	62.58	12.88	98.06	3.92	92.16	2.76	98.97	3.07	88.59	6.13
	180	46.60	4.54	90.40	3.16	85.26	1.53	94.45	5.48	66.22	4.37
	540	47.50	8.69	80.90	5.14	81.34	4.76	83.91	6.35	61.01	1.22
MRC-5	**20**	92.36	1.25	93.64	5.58	96.99	2.88	93.50	4.33	94.79	7.38
	60	78.79	5.45	92.72	4.79	91.75	3.82	88.01	2.78	86.86	6.28
	180	60.15	4.56	89.19	4.81	83.34	3.16	87.30	1.93	82.82	5.68
	540	56.13	5.27	89.19	7.67	77.21	4.81	85.23	3.21	71.26	4.66

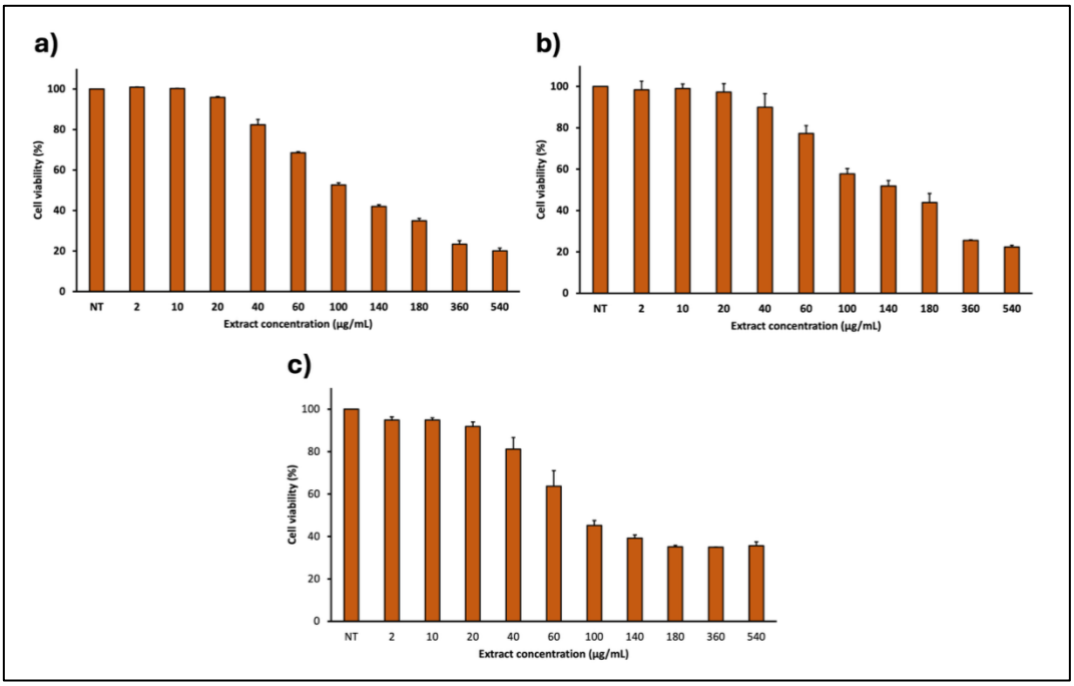

Figure S3.6. Cell viability of SW480 (a), E705 (b), and MDA-MB-231 (c) human cancer cell lines treated with root extract (2–540 µg/mL) for 48 h. Average values ± SEM are shown.

Table S3.2. Probable cytotoxicity-related biological activities of 6 compounds tentatively identified in the root extract of *A. marina* by PASS (Prediction of Activity Spectra for Substances). Pa = probable biological activity of compound; only activities with Pa > 0.7 are shown.

Compound	Class	Cytotoxicity-related activities (Pa)
Quercetin 3-*O*-hexoside	Flavonoid glycosides	Lipid peroxidase inhibitor (0.976) TP53 expression enhancer (0.959) Antineoplastic (0.833) Cytostatic (0.825) Caspase 3 stimulant (0.801) Apotosis agonist (0.792)
Suspensaside A	Phenylethanoid glycosides	Antineoplastic (0.863) Caspase 8 stimulant (0.743)
Kaempferol 3-*O*-glucoside	Flavonoid glycosides	Lipid peroxidase inhibitor (0.960) TP53 expression enhancer (0.952) Antineoplastic (0.834) Cytostatic (0.811) Caspase 3 stimulant (0.772) Apoptosis agonist (0.772)
Medicoside G	Triterpene saponins	Caspase 3 stimulant (0.994) Caspase 8 stimulant (0.984) Trascription factor NF-kB stimulant (0.965) Lipid peroxidase inhibitor (0.927) ICAM1 expression inhibitor (0.908) Apoptosis agonist (0.901) Antineoplastic (0.870)

Esculentoside C	Triterpene saponins	Caspase 3 stimulant (0.989) Caspase 8 stimulant (0.986) ICAM1 expression inhibitor (0.961) Lipid peroxidase inhibitor (0.952) Trascription factor NF-kB stimulant (0.917) Antineoplastic (0.905) Apoptosis agonist (0.862) Antineoplastic (lung cancer) (0.807)
Azukisaponin III	Triterpene saponins	Lipid peroxidase inhibitor (0.991) ICAM1 expression inhibitor (0.987) Caspase 3 stimulant (0.964) Caspase 8 stimulant (0.934) Antineoplastic (0.908) Trascription factor NF-kB stimulant (0.904) Apoptosis agonist (0.883) Antineoplastic (lung cancer) (0.791)

CHAPTER 4

Literature review: phytochemistry, antioxidant, and anticancer potential of *Sonneratia caseolaris*

This chapter has been adapted and modified from the following published work:

Cerri, F., Galli, P. (2025). Phytochemistry and pharmacological potential of the Mangrove plant *Sonneratia caseolaris*: a comprehensive review. *Marine Drugs*, 23(10), 378.

4.1. ABSTRACT

Mangroves represent a promising yet underexplored source of natural products. *Sonneratia caseolaris* (mangrove apple) is a widely distributed species with a long history of use in traditional medicine and increasing recognition for its bioactive secondary metabolites. Research has expanded in recent decades, but findings remain dispersed across diverse sources, complicating interpretation of its chemistry and pharmacological potential. This review consolidates four decades of investigations, documenting 141 identified compounds from studies largely restricted to India, Bangladesh, Indonesia and China, and focusing on leaves, fruits, bark, stem and twigs, with roots notably unexplored. The phytochemical profile is dominated by phenolic acids, flavonoids and tannins, alongside terpenoids, steroids, fatty acids, fatty alcohols, aldehydes, hydrocarbons, and polysaccharides. Antioxidant activity is among the most extensively studied activities, with extracts consistently exhibiting strong free-radical scavenging capacity. In contrast, anticancer investigations remain scarce, despite promising outcomes reported for related mangrove taxa. By consolidating and critically evaluating the existence evidence, this review highlights the pharmacological potential of *S. caseolaris* and identifies key knowledge gaps to guide future marine drug discovery.

4.2. INTRODUCTION

Among mangrove genera, *Sonneratia* which belongs to the Sonneratiacea family (Kathiresan and Bingham, 2001), has attracted significant attention in this field (Nguyen et al., 2024). In particular, *Sonneratia caseolaris* (L.) Engl. has been of interest as it has been widely used in folk medicine to treat several ailments such as sprains, piles, cuts and bruises, hemorrhages, intestinal parasites, diarrhea, coughs, hematuria, smallpox, and hepatitis (Bandaranayake, 1998; Ghalib et al., 2011; Dev et al., 2021) and is known to produce a wide range of polyphenols with various biological activities (Nguyen et al., 2024; Asha et al., 2012; Kundu et al., 2022; Tran et al., 2023). Commonly known as mangrove apple (Cerri et al., 2024), *S. caseolaris* is distributed across various regions including the Maldives, Sri Lanka, China, Bangladesh, the Malay Peninsula, Indonesia, Borneo, the Philippines, Timor, New Guinea, the Solomon Islands, and northern Australia (Sadhu et al., 2006; Tian et al., 2009; Yang et al., 2016; Dev et al., 2021; Cerri et al., 2024).

Despite its traditional and pharmacological relevance, *S. caseolaris* has received far less systematic attention compared with other mangrove specie. For instance, Bibi et al. (2019)

reported 27 mangrove species validated for pharmacological activity yet *S. caseolaris* was not included. Similarly, other reviews on the anticancer potential of man-grove-derived phytochemicals (Chowdhury et al., 2024) and on phytochemistry and bioactivities of mangrove plants (Patra et al., 2011) did not report this species. These omissions underline the significance and prospects of studying *S. caseolaris*, a promising but historically underexplored mangrove species.

In a context where specific mangrove plants are now being extensively reviewed, including *Avicennia* spp. (Beniwal et al., 2024; Zhou et al., 2025) and *Aegiceras corniculatum* (Sarkar et al., 2024), the phytochemical and pharmacological data available for *S. caseolaris* remain limited and highly scattered across different sources. This comprehensive review therefore aims to systematically consolidate four decades of research on *S. caseolaris,* with particular emphasis on its bioactive compounds and pharmacological activities. The objectives are to systematically analyze all available studies, highlight the most findings, and identify key research gaps that warrant further investigation. By clarifying what is known and where uncertainties persist, this review, which to our knowledge, is the first systematic consolidation of four decades of research on *S. caseolaris* phytochemistry and pharmacological activities, provides a timely and comprehensive foundation for advancing the exploration of *S. caseolaris* as a promising candidate for drug discovery and therapeutic development.

4.3. CHEMICAL COMPOSITION

The chemical composition of *S. caseolaris* has been extensively investigated, revealing a diverse array of secondary metabolites. These include phenolic compounds, flavonoids, tannins, terpenoids, steroids, fatty acids, alcohols and aldehydes, hydrocarbons, polysaccharides, and various other constituents. **Table 4.1** provides a comprehensive overview of the chemical composition of *S. caseolaris,* including the plant part in which each molecule was identified, the solvent used for extraction, the identification method, the region of sample collection, and the corresponding reference. Regarding the extraction methods, the majority of studies employed maceration, in which plant material, typically dried and powdered (with the exception of Tiwari et al. (2010), who explicitly reported using fresh material), was soaked in solvent at room temperature for varying durations, generally 24 h or longer. One exception is Jha et al. (2023), who used hot water extraction.

Table 4.1. Compounds identified in *Sonneratia caseolaris* extracts.

Molecule	Plant part	Solvent	Method	Region	Ref.
Phenolic acids and derivatives					
Gallic acid (**1**)	Leaf	Methanol	LC-MS	India	Dahibhate et al., 2018
			HPLC-DAD	Vietnam	Nguyen et al., 2024
		Ethanol	LC-MS	India	Dahibhate et al., 2018
			UHPLC-HRMS	Indonesia	Audah et al., 2022
			HPLC-DAD	Vietnam	Nguyen et al., 2024
		70% aqueous acetone	RP-HPLC	China	Fang et al., 2019
Ellagic acid (**2**)	Fruits	Ethanol	HPLC-DAD	Bangladesh	Dev et al., 2021
	Leaves	70% aqueous acetone	RP-HPLC	China	Fang et al., 2019
Vanillic acid (**3**)	Fruits	Ethanol	HPLC-DAD	Bangladesh	Dev et al., 2021
Chlorogenic acid (**4**)	Leaves	Ethyl acetate	UPLC-ESI-MS/MS	India	Dahibhate et al., 2021
Caffeic acid (**5**)					
p-Coumaric acid (**6**)					
Ferulic acid (**7**)					
Flavonoids					
Luteolin (**8**)	Leaves	Methanol	HPLC-DAD	Vietnam	Nguyen et al., 2024
		Ethanol	MS; 1D- and 2D-NMR	Bangladesh	Sadhu et al., 2006
	Fruits	Methanol	TLC, IR, ^{1}H NMR	India	Tiwari et al., 2020
		Ethanol	^{1}H and ^{13}C NMR	China	Wu et al., 2009
	Stem and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
Quercetin (**9**)	Leaves	Methanol	LC-MS	India	Dahibhate et al., 2018
		Ethanol	LC-MS	India	Dahibhate et al., 2018
		Ethyl acetate	UPLC-ESI-MS/MS	India	Dahibhate et al., 2021
Apigenin (**10**)	Leaves	Ethyl acetate	UPLC-ESI-MS/MS	India	Dahibhate et al., 2021
Myricetin (**11**)	Fruits	Ethanol	HPLC-DAD	Bangladesh	Dev et al., 2021
	Leaves	Ethyl acetate	UPLC-ESI-MS/MS	India	Dahibhate et al., 2021
Luteolin-7-*O*-glucoside (**12**)	Leaves	Ethanol	MS; 1D- and 2D-NMR	Bangladesh	Sadhu et al., 2006
			^{1}H and ^{13}C NMR	Indonesia	Arung et al., 2015
		Methanol	HPLC-DAD	Vietnam	Nguyen et al., 2024
	Fruits	Ethanol	^{1}H and ^{13}C NMR	China	Wu et al., 2009
Kaempferol glucoside (**13**)	Bark	Methanol	GC-MS	India	Simlai et al., 2014
Quercetin-3-O-β-L-arabinopyranoside (**14**)	Stem and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
Isovitexin (**15**)	Leaves	Ethanol	UHPLC-HRMS	Indonesia	Audah et al., 2022
Quercitrin (**16**)					
(+)-Dihydrokaempferol (**17**)	Stems and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
Riccionidin A (**18**)	Leaves	Ethanol	UHPLC-HRMS	Indonesia	Audah et al., 2022
Cyanidin 3-O-[β-D-xylosyl-(1-2)- β-D-galactoside] (**19**)					
Epigallochatechin gallate (**20**)	Leaves	Ethyl acetate	UPLC-ESI-MS/MS	India	Dahibhate et al., 2021
Naringenin (**21**)					
Tannins					
Tannic acid (**22**)	Leaves	Ethanol	LC-MS	India	Dahibhate et al., 2018
		Methanol	LC-MS	India	Dahibhate et al., 2018
Methyl gallate (**23**)	Stems and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
3,3′-Di-*O*-methyl ether ellagic acid (**24**)					
3,3′,4-*O*-Tri-*O*-methyl ether ellagic acid (**25**)					
Other phenolic compounds					
Estragole (**26**)	Fruits	Methanol	GC-MS	Vietnam	Tran et al., 2023
Aspirin (**27**)	Leaves	Ethanol	UHPLC-HRMS	Indonesia	Audah et al., 2022

2-Phenylehtyl (**28**)					
Vanillin (**29**)	Leaves	Ethanol	LC-MS	India	Dahibhate et al., 2018
		Methanol	LC-MS	India	Dahibhate et al., 2018
	Bark	Hexane	GC-TOFMS	Malay Peninsula	Ghalib et al., 2011
Piperonal (**30**)	Wood and Bark	Hexane	GC-TOFMS	Malay Peninsula	Ghalib et al., 2011
2,4-Bis(1,1-dimethylethyl)-phenol (**31**)					
3,7,8-Trihydroxy-5,10-dioxo-5,10-dihydrochromeno[5,4,3-cde]chromen-2-olate (**32**)	Fruits	Ethanol	UHPLC-HRMS	Indonesia	Audah et al., 2022
3,5-Di-tert-Butyl-4-hydroxybenzaldehyde (**33**)	Leaves	Ethanol Water	LC-HRMS	Indonesia	Kartikaningsih et al., 2025
[(-)-(R)-Nyasol (**34**)	Fruits	Ethanol	^{1}H and ^{13}C NMR	China	Wu et al., 2009
(-)-(R)-4′-*O*-Methylnyasol (**35**)					
3,8-Dihydroxy-6H-benzo[b,d]pyran-6-one (**36**)					
3-Hydroxy-6H-benzo[b,d]pyran-6-one (**37**)					
Benzyl-*O*-β- glucopyranoside (**38**)					
Terpenoids					
β-Curcumene (**39**)	Fruits	Methanol	GC-MS	Vietnam	Tran et al., 2023
Cubedol (**40**)					
α-Santonin (**41**)					
Oleanolic acid (**42**)	Fruits	Methanol	TLC, IR, ^{1}H NMR	India	Tiwari et al., 2020
		Ethanol	^{1}H and ^{13}C NMR	China	Wu et al., 2009
	Stems and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
	Bark	Methanol	GC-MS	India	Simlai et al., 2014
Maslinic acid (**43**)	Fruits	Ethanol	^{1}H and ^{13}C NMR	China	Wu et al., 2009
Lupeol (**44**)	Stems and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
	Bark	Methanol	GC-MS	India	Simlai et al., 2014
Ursolic acid (**45**)	Stems and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
	Bark	Methanol	GC-MS	India	Simlai et al., 2014
Abietin (**46**)	Leaves	Ethanol	UHPLC-HRMS	Indonesia	Audah et al., 2022
Squalene (**47**)	Leaves	Chloroform-methanol (1:1)	GC	Australia	Hogg and Gillan, 1984
Rhodopin (**48**)	Fruits	Methanol	GC-MS	Vietnam	Tran et al., 2023
Lup-20(29)-en-3β,24-diol (**49**)	Stems and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
3β-*O*-(E)-Cumaroyl-alphitolinsaeure (**50**)					
3β-Acetyl-oleanolic acid (**51**)					
3β,13β-Dihydroxy-urs-11-en-28-oic acid-13-lactone (**52**)					
3β-Hydroxy-20(29)-lupen-24-oic acid (**53**)					
Betulin (**54**)					
1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1ar (1aà,4á,4aá,7à,7aá,7bà)]- (**55**)	Wood	Hexane	GC-TOFMS	Malay peninsula	Ghalib et al., 2011
Steroids					
Campesterol (**56**)	Leaves	Chloroform-methanol (1:1)	GC	Australia	Hogg and Gillan, 1984
28-Isofucosterol (**57**)					
Sitosterol (**58**)	Bark	Methanol	GC-MS	India	Simali et al., 2014
	Stem and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
Cholesterol (**59**)	Leaves	Chloroform-methanol (1:1)	GC	Australia	Hogg and Gillan, 1984

	Stem and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
Stigmasterol (**60**)	Leaves	Chloroform-methanol (1:1)	GC	Australia	Hogg and Gillan, 1984
	Stem and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
	Leaves	Acetone	UV-Vis, FTIR, ^{1}H and ^{13}C NMR, 2D-NMR	Indonesia	Latief et al., 2019
Cholest-5-ene-diol (**61**)	Bark	Methanol	GC-MS	India	Simlai et al., 2014
	Stem and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
β-Sistosterol-β-D-glucopyranoside (**62**)	Fruits	Methanol	TLC, IR, ^{1}H NMR	India	Tiwari et al., 2020
Prednisone (**63**)	Fruits	Methanol	GC-MS	Vietnam	Tran et al., 2023
β-Sitosterol palmitate (**64**)	Stem and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
Stigmast-5-en-3β-O-(6-O-hexadecanoyl-β-D-glucopyranoside) (**65**)					
Daucosterol (**66**)					
6′-*O*-Acetyl-β-daucosterol (**67**)					
Fatty acid and derivatives					
13S-Hydroxyoctadecadienoic acid (**68**)	Leaves	Ethanol	UHPLC-HRMS	Indonesia	Audah et al., 2022
9-Hydroperoxy-11-(3-pentyl-2-oxiranyl)-10-undecenoate (**69**)					
9,12,13-Trihydroxy-10-octadecenoate (**70**)					
Octanoid acid (**71**)	Bark	Hexane	GC-TOFMS	Malay peninsula	Ghalib et al., 2011
Butanoic acid (**72**)	Leaves	Methanol	GC-MS	Vietnam	Tran et al., 2023
Dodecanamide (**73**)	Wood and Bark	Hexane	GC-TOFMS	Malay peninsula	Ghalib et al., 2011
Myristynoyl pantetheine (**74**)	Leaves	Methanol	GC-MS	Vietnam	Tran et al., 2023
Fatty aldehydes					
2-Heptenal (**75**)	Wood and bark	Hexane	GC-TOFMS	Malay peninsula	Ghalib et al., 2011
2-Octenal (**76**)					
Nonanal (**77**)					
2,4-Decadienal (**78**)					
2-Undecenal (**79**)					
Hexadecanal (**80**)					
Tetradecanal (**81**)	Wood				
Octadecanal (**82**)	Bark				
2-Nonenal (**83**)					
Decanal (**84**)					
Tridecanedial (**85**)	Leaves	Methanol	GC-MS	Vietnam	Tran et al., 2023
Fatty alcohols					
13-Heptadecyn-1-ol (**86**)	Leaves	Methanol	GC-MS	Vietnam	Tran et al., 2023
2-Hexadecanol (**87**)					
1-Octanol (**88**)					
Falcarinol (**89**)					
Trans-9-hexadecen-1-ol (**90**)	Wood	Hexane	GC-TOFMS	Malay peninsula	Ghalib et al., 2011
Other lipid-derived compounds					
4,8,12,16-Tetramethylheptadecan-4-olide (**91**)	Wood	Hexane	GC-TOFMS	Malay peninsula	Ghalib et al., 2011
Oxacycloheptadec-8-en-2-one (**92**)					
13-Methyl-oxacyclotetradecane-2,11-dione (**93**)					
Tert-hexadecanethiol (**94**)	Leaves	Methanol	GC-MS	Vietnam	Tran et al., 2023
Triacetin (**95**)					
Azelaic acid (**96**)	Leaves	Ethanol	UHPLC-HRMS	Indonesia	Audah et al., 2022
Hydrocarbons					
Pentadecane (**97**)	Wood and Bark	Hexane	GC-TOFMS	Malay peninsula	Ghalib et al., 2011
1-Hexadecene (**98**)					
1-Docosene (**99**)					
Octacosane (**100**)					
Hentriacontane (**101**)					

Heptadecane (**102**)	Wood				
2-Methyl-nonadecane (**103**)					
Octadecane (**104**)					
Tetracosane (**105**)					
Heptacosane (**106**)					
Eicosane (**107**)	Bark				
17-Pentatriacontene (**108**)					
Isobutane (**109**)					
3-Methyl-hexane (**110**)					
1-Chloro-heptacosane (**111**)					
Polysaccharides					
Hexose (**112**)	Leaves	Ethanol	UHPLC-HRMS	Indonesia	Audah et al., 2022
Sorbitol (**113**)					
Rhamnose (**114**)		Water (low molecular weight polysaccharide fraction)	RP-HPLC	India	Jha et al., 2023
Xylose (**115**)					
Mannose (**116**)					
Galactose (**117**)					
Other compounds					
Diisobutyl phthalate (**118**)	Leaves	Water	LC-HRMS	Indonesia	Kartikaningsih et al., 2025
Bis(3,5,5 trimethylhexyl)phthalate (**119**)					
Monobutyl phthalate (**120**)					
Bis(2 ethylhexyl)phthalate (**121**)					
Betaine (**122**)					
Choline (**123**)					
Hexamethylenetetramine (**124**)					
2,2,6,6 tetramethyl 1 piperidinol (TEMPO) (**125**)					
Caprolactam (**126**)					
2-[(2-chlorobenzyl)sulfanyl]-4,6-dimethylnicotinonitrile (**127**)					
Zearalenone (**128**)					
Tributyl phosphate (**129**)					
Bis(4-ethylbenzylidene)sorbitol (**130**)					
DL-arginine (**131**)					
Bis(2-ethylhexyl)benzene-1,2-dicarboxylate (**132**)	Stems and twigs	Methanol	HR-ESI-MS, ^{1}H and ^{13}C NMR	China	Tian et al., 2009
Safrole (**133**)	Fruits	Methanol	GC-MS	Vietnam	Tran et al., 2023
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (**134**)	Wood and bark	Hexane	GC-TOFMS	Malay peninsula	Ghalib et al., 2011
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (**135**)					
Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl)ethyl]pentyl esterv (**136**)					
Trimethylamine (**137**)	Wood				
1,2-Benzenedicarboxylic acid, diisooctyl ester (**138**)					
Propiolactone (**139**)	Bark				
Diethyl phthalate (**140**)					
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (**141**)					

4.3.1. Phenolic compounds

Phenolic compounds are abundant in *S. caseolaris*, with their concentration varying significantly depending on the plant part and extraction solvent used, as reflected by the total phenolic content (TPC) values reported in multiple studies (see **Table 4.2**). Methanol end ethanol extractions generally yield the highest phenolic contents, consistent with the polarity-

dependent solubility of these compounds. Leaves consistently exhibit higher phenolic content than fruits and bark. For example, ethanol extracts of leaves typically range from 50.03 to 219.53 mg GAE/g (Barman et al., 2021; Audah et al., 2022; Pagarra et al., 2022), compared to 12.21–122 mg GAE/g in fruits (Yoong et al., 2021; Kundu et al., 2022) and 50.70–63.00 mg GAE/g in bark (Simlai et al., 2014; Munira et al., 2019). Notably, the ethanolic leaf extracts of *S. caseolaris* contain higher TPC than those of other mangrove species such as *Avicennia marina*, *Rhizophora mucronata* and *Rhizophora apiculata* (Audah et al., 2022). Using the classification by Audah et al. (2022), where the phenolic content is categorized as high (>70 mg GAE/g), moderate (10–70 mg GAE/g), and low (<10 mg GAE/g), most methanol and ethanol extracts from *S. caseolaris* leaves and fruits fall within the high phenolic concentration category. However, notable exceptions exist, such as the very low TPC (<2 mg GAE/g) reported by Kartikaningsih et al. (2025) for methanol and water extracts, illustrating the significant impact of plant material, solvent composition and extraction protocols on phenolic recovery.

Table 4.2. Total phenolic content (TPC) and total flavonoid content (TFC) of *Sonneratia caseolaris*.

Plant part	Solvent	TPC (mg GAE/g)	TFC (mg QE/g)	Ref.
Leaves	Methanol	200	-	Nguyen et al., 2024
		1.52 ± 0.02	1.98 ± 0.08	Kartikaningsih et al., 2025
	Ethanol	182.89 ± 1.76	22.70 ± 0.48	Audah et al., 2022
		50.03	-	Pagarra et al., 2022
		219.53	454.88	Barman et al., 2021
	Ethanol 70%	74.77	-	Pagarra et al., 2022
	Ethyl acetate	5.83	-	
	n-Hexane	4.67	-	
	Water	0.23-1.00	0.55-0.96	Kartikaningsih et al., 2025
Fruits	Ethanol	122	613	Kundu et al., 2022
		12.21 ± 1.31	26.06 ± 0.30	Yoong et al., 2021
	Methanol	82.27 ± 0.41	41.0 ± 0.34	Tran et al., 2023
	Methanol (n-butanol fraction)	82.67 ± 0.81	9.13 ± 0.34	
	Methanol (ethyl acetate fraction)	77.67 ± 0.32	26.28 ± 0.93	
	Methanol (aqueous fraction)	70.26 ± 0.35	1.81 ± 0.24	
	Methanol (Hexane fraction)	59.58 ± 2.70	16.54 ± 0.44	
Bark	Ethanol	63.00	-	Munira et al., 2019
		50.70 ± 0.74	90.04 ± 3.57	Simlai et al., 2014
	Ethyl acetate	60.25	-	Munira et al., 2019
	Chloroform	36.25	-	
	Petroleum ether	26.28	-	

4.3.1.1. Phenolic acids and derivatives

Phenolic acids are a major class of phenolic compounds broadly divided into hydroxycinnamic and hydroxybenzoic acid groups (Taofiq et al., 2017). Hydroxybenzoic acids such as gallic acid (1), ellagic acid (2) and vanillic acid (3) have been identified in S. caseolaris leaves and fruits (Dahibhate et al., 2018; Fang et al., 2019; Dev et al., 2021; Audah et al., 2022; Nguyen et al., 2024). Hydroxycinnamic acids including chlorogenic acid (4), caffeic acid (5), p-coumaric acid (6), and ferulic acid (7) were also found in the leaves (Dahibhate et al., 2021). The structures of phenolic acids and derivatives 1–7 are displayed in **Figure 4.1**.

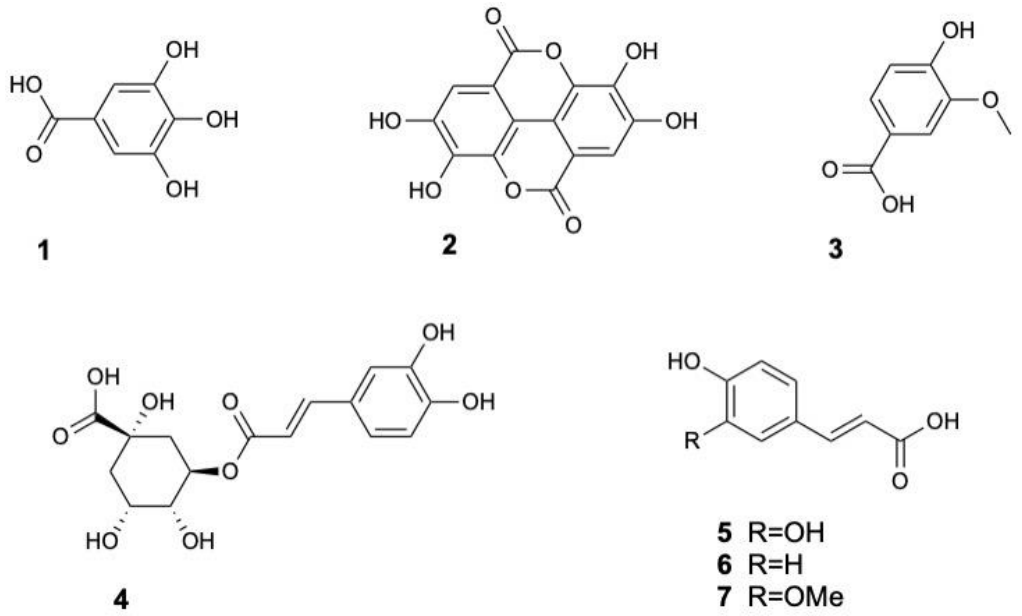

Figure 4.1. Phenolic acids and derivatives from *Sonneratia caseolaris*.

4.3.1.2. Flavonoids

As shown in **Table 4.2**, total flavonoid content (TFC) is also high and varies with plant part and extraction solvent. Methanol end ethanol extractions typically yield the highest TFC across all tissues, though values vary widely among studies. For instance, leaf extracts range from 22.70 to 454.88 mg QE/g (Barman et al., 2021; Audah et al., 2022), fruit extracts from 26.06 to 613 mg GAE/g (Yoong et al., 2021; Kundu et al., 2022) and bark extracts around 90.04 ± 3.57 (Simlai et al., 2014). Importantly, *S. caseolaris* leaves consistently show higher TFC than leaves of other mangrove species such as *A. marina*, *R. mucronata* and *R. apiculata* when extracted with ethanol (Audah et al., 2022).

Several flavonoid aglycones have been consistently identified in the leaves and fruits of *S. caseolaris*, including luteolin (**8**), quercetin (**9**), apigenin (**10**) and myricetin (**11**) (Sadhu et al., 2006; Wu et al., 2009; Tiwari et al., 2010; Dahibhate et al., 2018; Dahibhate et al., 2021; Dev et al., 2021; Nguyen et al., 2024). Flavonoid glycosides such as luteolin-7-O-glucoside (**12**) (Sadhu et al., 2006; Wu et al., 2009; Arung et al., 2015; Nguyen et al., 2024), kaempferol glucoside (**13**) (Simlai et al., 2014), quercetin-3-O-β-L-arabinopyranoside (**14**) (Tian et al., 2009), isovitexin (**15**) and quercitrin (**16**) (Audah et al., 2022) have also been detected. Flavonoid (+)-dihydrokaempferol (**17**) were found in stems and twigs (Tian et al., 2009), while anthocyanin derivatives such as riccionidin A (**18**) and cyanidin 3-O-[β-D-xylosyl-(1-2)- β-D-galactoside] (**19**) were identified in leaves (Audah et al., 2022). Catechins, a subclass of flavan-3-ols with well-known antioxidant properties (Bernatoniene et al., 2018), were also found, including epigallocatechin gallate (**20**) (Dahibhate et al., 2021). Moreover, the flavanone naringenin (**21**) was detected (Dahibhate et al., 2021), further enriching the flavonoid diversity of the species. The structures of flavonoids **8–21** are displayed in **Figure 4.2**.

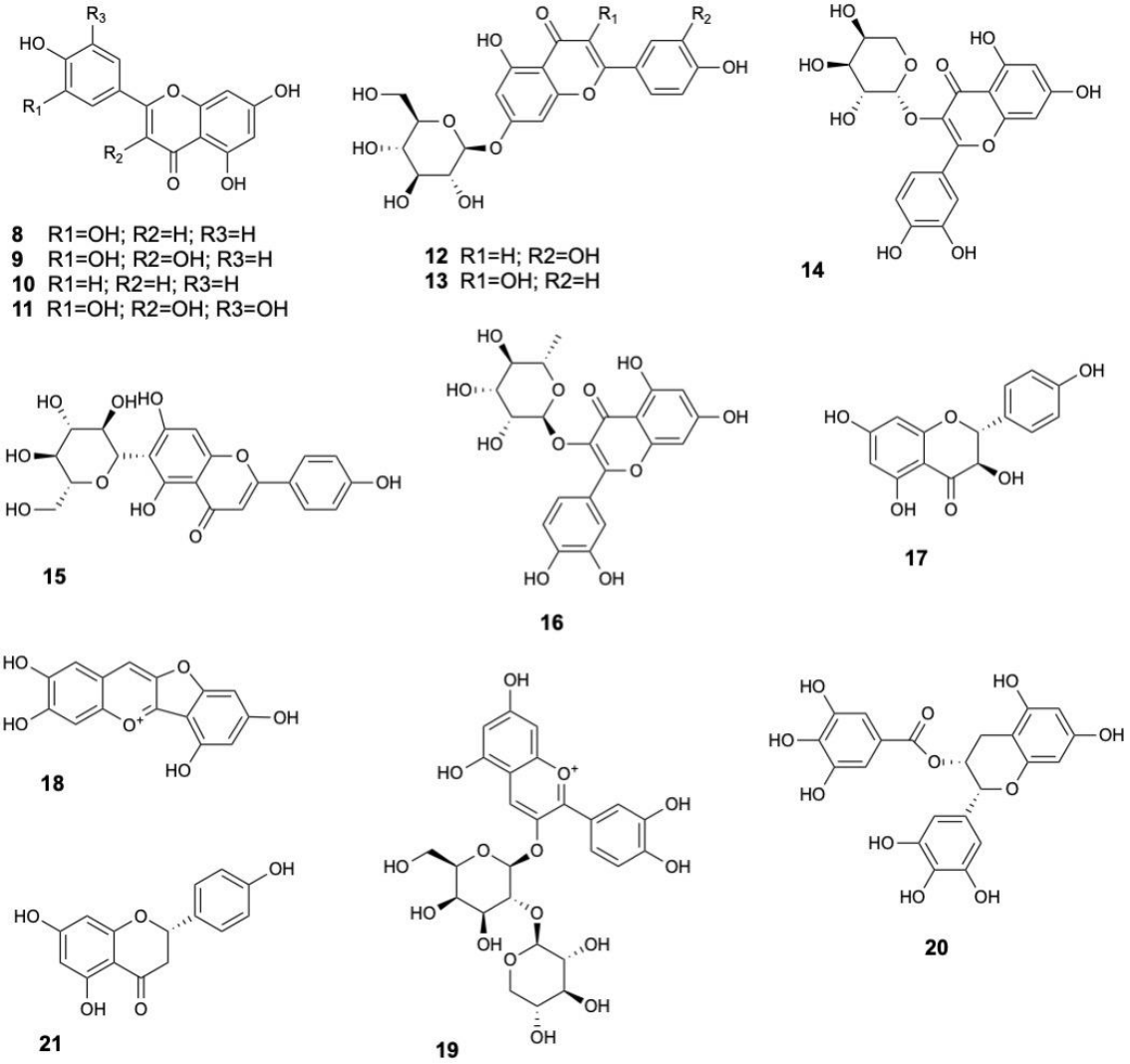

Figure 4.2. Flavonoids from *Sonneratia caseolaris*.

4.3.1.3. Tannins

Tannins, a significant subclass of polyphenols known for their ability to complex with proteins, cellulose, and minerals, are broadly divided into hydrolysable and condensed forms. In *S. caseolaris*, the bark is a particularly rich source, with tannin content reported at 48.04 ± 0.91 mg TAE/g (Simlai et al., 2014). Fruits also contribute significantly, with the ethanol extracts yielding 30 mg GAE/g (Kundu et al., 2022). Chemical analyses have identified hydrolyzable tannins like tannic acid (**22**), methyl gallate (**23**) and two ellagic acid derivatives, namely 3,3′-di-O-methyl ether ellagic acid (**24**) and 3,3′,4-O-tri-O-methyl ether ellagic acid (**25**) (Tian et al., 2009; Dahibhate et al., 2018). The structures of tannins **22–25** are displayed in **Figure 4.3.**

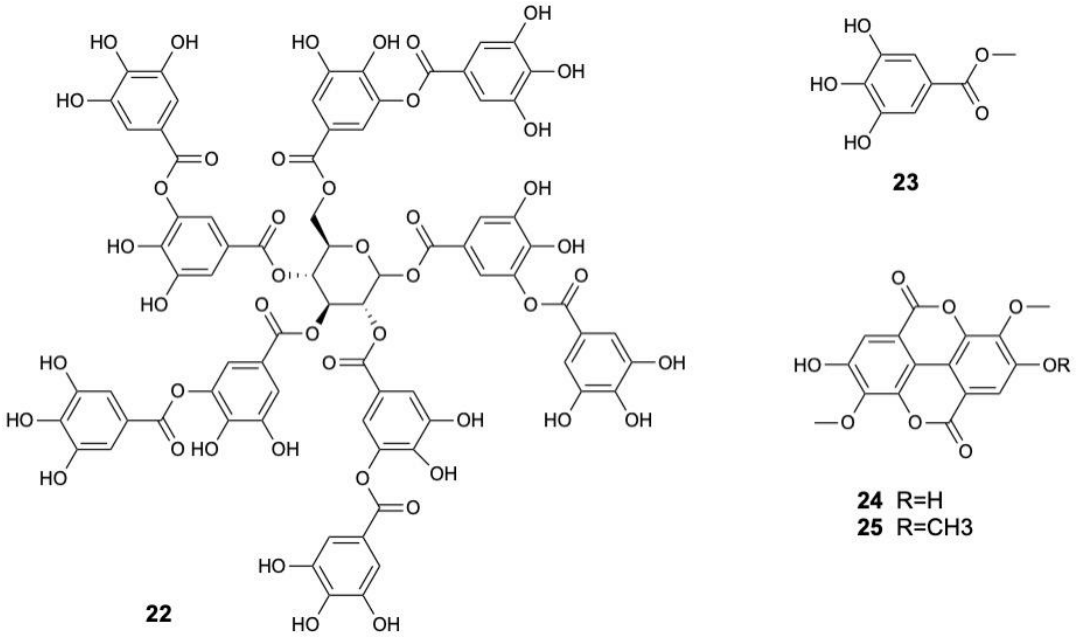

Figure 4.3. Tannins from *Sonneratia caseolaris*.

4.3.1.4. Other phenolic compounds

Besides phenolic acids, flavonoids, and tannins, *S. caseolaris* was also found to contain various other phenolic compounds (**26–38**) extracted from its fruits, leaves, bark, and wood. These include simple phenolics, phenolic aldehydes, benzopyrans, lignans, and glycosylated derivatives. Their structural diversity highlights the rich and complex phenolic profile of *S. caseolaris* beyond the major phenolic classes (Wu et al., 2009; Ghalib et al., 2011; Dahibhate et al., 2018; Audah et al., 2022; Kartikaningsih et al., 2025).

4.3.2. Terpenoids and steroids

S. caseolaris contains a wide range of terpenoids. Sesquiterpenes such as β-curcumene (**39**), cubebol (**40**), and α-santonin (**41**), have been identified in fruits (Tran et al., 2023). Triterpenoids are particularly prominent, including oleanolic acid (**42**) detected in fruits, stem, twigs, and bark (Tian et al., 2009; Wu et al., 2009; Tiwari et al., 2010; Simlai et al., 2014), maslinic acid (**43**) in fruits (Wu et al., 2009; Tiwari et al., 2010), and lupeol (**44**) and ursolic acid (**45**) in stems, twigs and bark (Tian et al., 2009; Simlai et al., 2014). Diterpenoids, such as abietin (**46**), have been found in leaves (Audah et al., 2022). Other terpenoids identified include squalene (**47**) in leaves (Hogg and Gillan, 1984) and rhodopin (**48**) in fruits (Tran et al., 2023) and other compounds (**49–55**) in stems and twigs (Tian et al., 2009). The structures of terpenoids **39–48** are displayed in **Figure 4.4.**

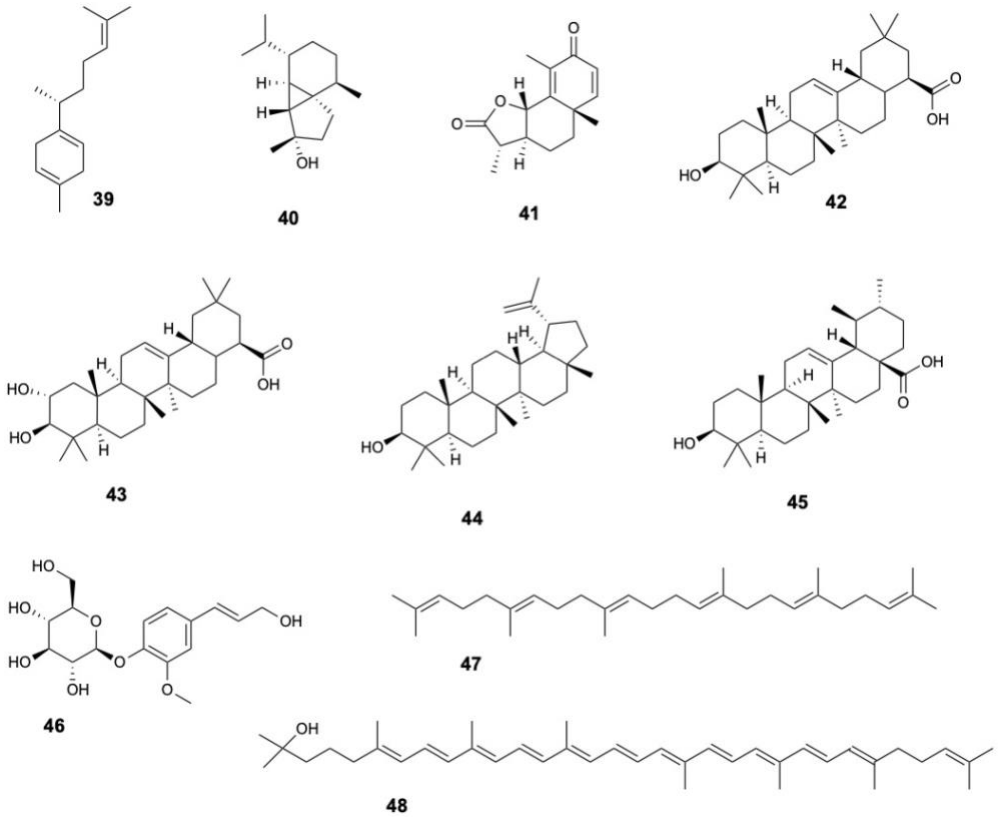

Figure 4.4. Terpenoids from *Sonneratia caseolaris*.

Phytosterols present in the leaves and bark include campesterol (**56**), 28-isofucosterol (**57**), sitosterol (**58**), cholesterol (**59**), stigmasterol (**60**) (Hogg and Gillan, 1984; Tian et al., 2009). Additionally, cholest-5-ene-diol (**61**) was found in bark, stems and twigs (Tian et al., 2009; Simlai et al., 2014), and the sterol glycoside β-sistosterol-β-D-glucopyranoside (**62**) in fruits (Tiwari et al., 2010). The steroid prednisone (**63**) was identified in fruits (Tran et al., 2023), while stems and twigs contain various steroids and sterol derivatives including β-sitosterol

palmitate (**64**), stigmast-5-en-3β-O-(6-O-hexadecanoyl-β-D-glucopyranoside) (**65**), and daucosterol (**66**), 6′-O-acetyl-β-daucosterol (**67**) (Tian et al., 2009). The structures of steroids **56–67** are displayed in **Figure 4.5**.

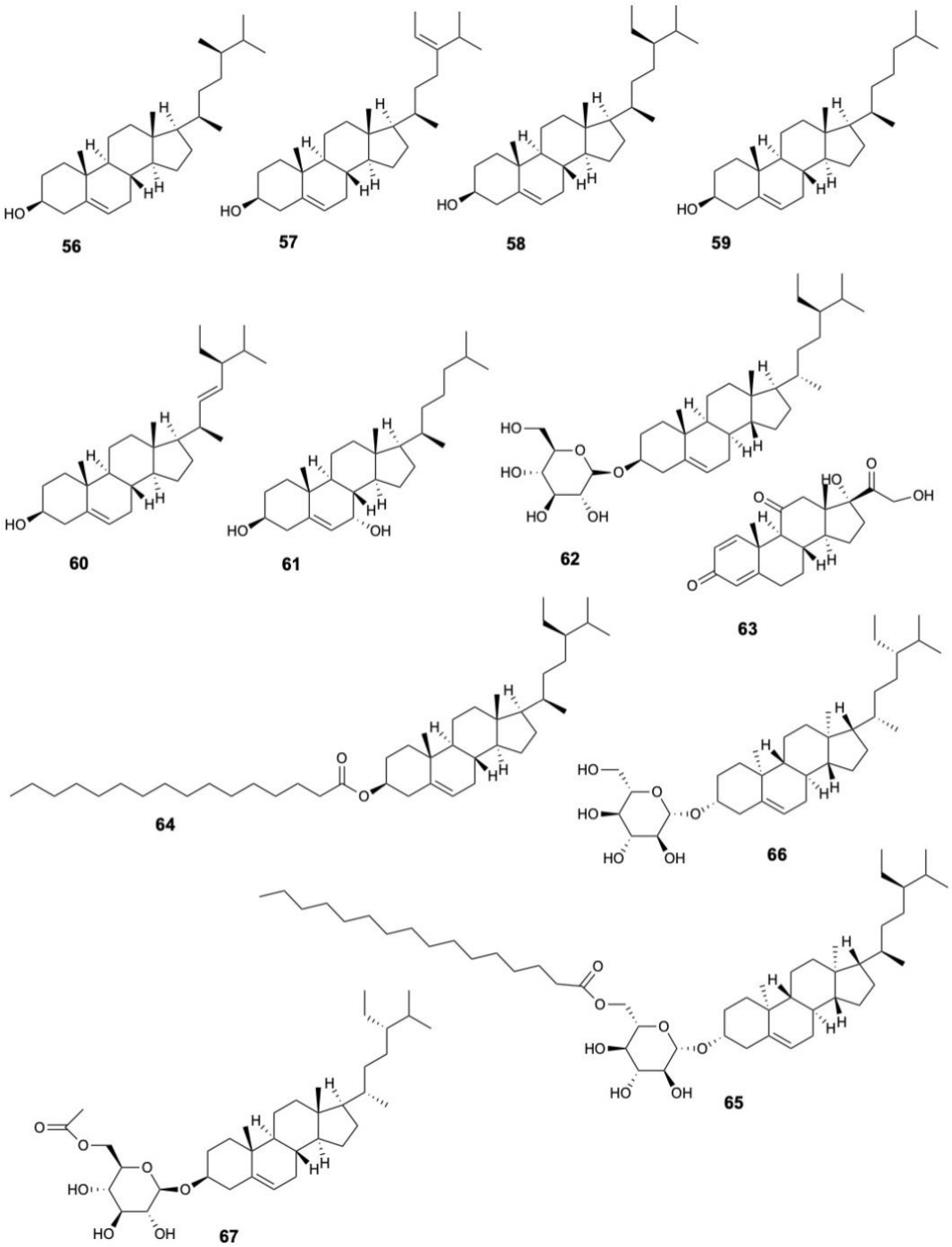

Figure 4.5. Steroids from *Sonneratia caseolaris*.

4.3.3. Fatty acids and derivatives, fatty alcohols, and fatty aldehydes

S. caseolaris contains several notable long-chain fatty acids, such as 13S-hydroxyoctadecadienoic acid (**68**), 9-hydroperoxy-11-(3-pentyl-2-oxiranyl)-10-undecenoate

(**69**), 9,12,13-trihydroxy-10-octadecenoate (**70**) (Audah et al., 2022). Other fatty acids include octanoic acid (**71**) and butanoic acid (**72**), as well as derivatives like dodecanamide (**73**), and myristynoyl pantetheine (**74**) (Ghalib et al., 2011; Tran et al., 2023).

Fatty aldehydes identified include 2-heptenal (**75**), 2-octenal (**76**), nonanal (**77**), 2,4-decadienal (**78**), 2-undecenal (**79**), hexadecanal (**80**), tetradecanal (**81**), octadecanal (**82**), 2-nonenal (**83**), decanal (**84**) (Ghalib et al., 2011). Additionally, tridecanedial (**85**) was detected in leaves (Tran et al., 2023).

Fatty alcohols reported in the leaves include 13-heptadecyn-1-ol (**86**), 2-hexadecanol (**87**), 1-octanol (**88**), and falcarinol (**89**) (Tran et al., 2023), while trans-9-hexadecen-1-ol (**90**) was found in the wood (Ghalib et al., 2011). Furthermore, other lipid-derived compounds (**91–96**) were identified in wood and leaves of *S. caseolaris* (Ghalib et al., 2011; Audah et al., 2022; Tran et al., 2023). The structures of fatty acids and derivatives, fatty alcohols, and fatty aldehydes **68–90** are displayed in **Figure 4.6.**

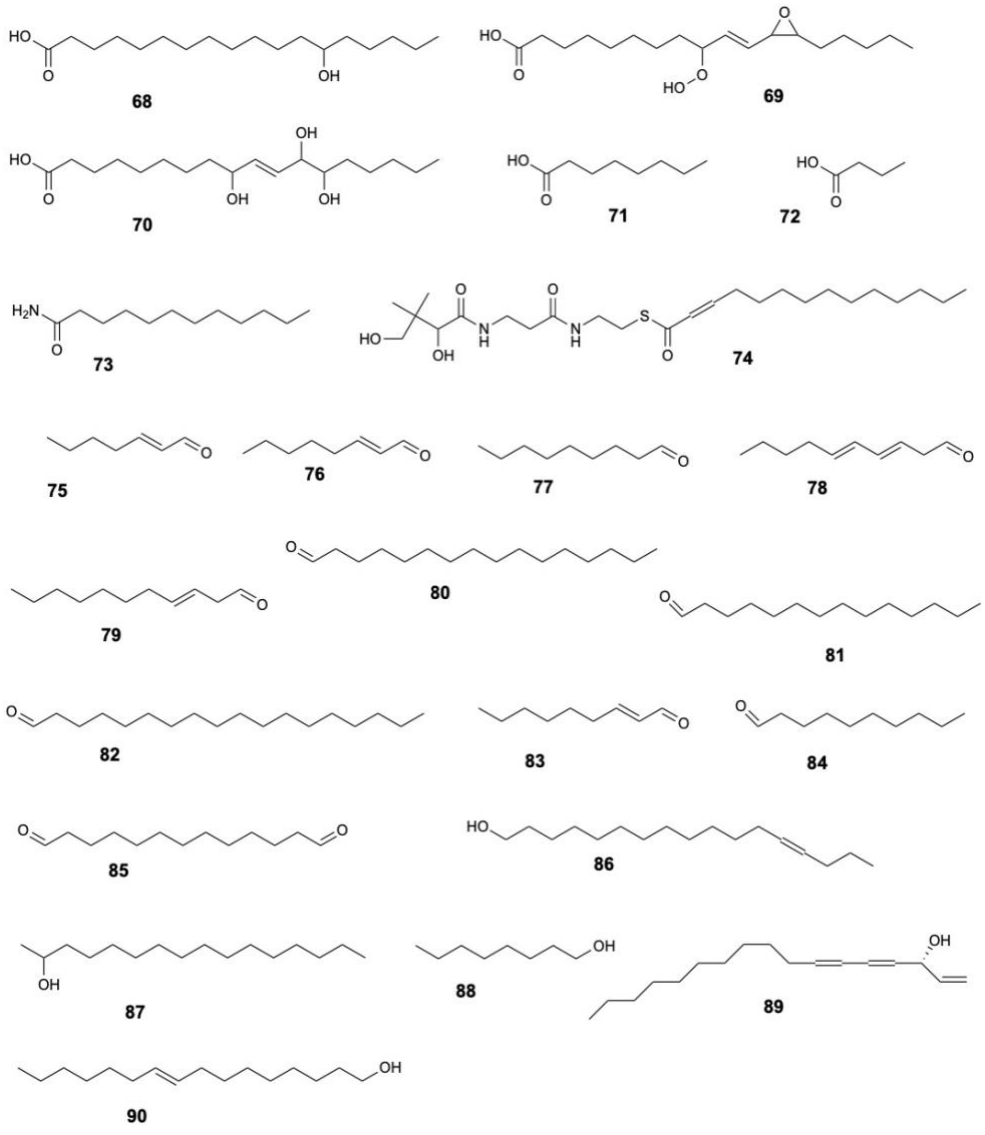

Figure 4.6. Fatty acids and derivatives, fatty alcohols, and fatty aldehydes from *Sonneratia caseolaris*.

4.3.4. Hydrocarbons, polysaccharides, and other constituents

Several hydrocarbons have been detected in wood and bark extracts, including pentadecane (**97**), 1-hexadecene (**98**), 1-docosene (**99**), octacosane (**100**), and hentriacontane (**101**). Wood also contains heptadecane (**102**), 2-methyl-nonadecane (**103**), octadecane (**104**), tetracosane (**105**), and heptacosane (**106**), while bark includes eicosane (**107**), 17-pentatriacontene (**108**), isobutane (**109**) and 3-methyl-exane (**110**), and the halogenated hydrocarbon 1-chloro-heptacosane (**111**) (Ghalib et al., 2011).

Leaves of *S. caseolaris* are a source of carbohydrates, both simple and polymeric. Ethanol leaf extracts contain the free sugar hexose (**112**) and the sugar alcohol sorbitol (**113**) (Audah et al., 2022). Low-molecular-weight leaf polysaccharide fraction were found to comprise rhamnose (**114**) (28.25%), xylose (**115**) (27.17%), mannose (**116**) (18.90%), and galactose (**117**) (17.17%), indicated branched heteropolysaccharide structures (Jha et al., 2023).

Kartikaningsih et al. (2025) reported the presence of anthropogenic contaminants in leaves, such as diisobutyl phthalate (**118**), bis(3,5,5 trimethylhexyl)phthalate (**119**), monobutyl phthalate (**120**), and bis(2 ethylhexyl)phthalate (**121**), along with naturally occurring nitrogenous and zwitterionic compounds including betaine (**122**), choline (**123**), hexamethylenetetramine (**124**), and the nitroxide 2,2,6,6 tetramethyl 1 piperidinol (TEMPO) (**125**). Additional constituents includes caprolactam (**126**), 2-[(2-chlorobenzyl)sulfanyl]-4,6-dimethylnicotinonitrile (**127**), the mycotoxin zearalenone (**128**), tributyl phosphate (**129**), bis(4-ethylbenzylidene)sorbitol (**130**), and the amino acid DL-arginine (**131**). Stems and twigs yielded benzenecarboxylate derivatives such as bis(2-ethylhexyl)benzene-1,2-dicarboxylate (**132**) (Tian et al., 2009), while fruits contains safrole (**133**) (Tran et al., 2023). Furthermore, Wood and bark contained phthalate esters, spiroketones, and amines (**134-141**) (Ghalib et al., 2011).

4.4. BIOLOGICAL ACTIVITIES

4.4.1. Safety and general toxicity

Dev et al. (2021) conducted both acute and sub-acute toxicity studies on male Swiss albino mice using the fruit ethanol extract of *S. caseolaris*. In the acute assay, doses up to 3000 mg/kg

body weight produced no mortality or behavioral abnormalities, indicating a high safety margin. For the sub-acute test, mice received 500 mg/kg daily for 14 days; there were no significant changes in biochemical markers (urea, creatinine, bilirubin, SGPT, SGOT), confirming the extract's safety as a crude herbal medicine. These findings align with Kundu et al. 2023, who observed no mortality in Swiss albino mice treated acutely with up to 5000 mg/kg of the same extract over 7 days.

Shamsuddin et al. (2013) assessed the toxicity of aqueous and ethanolic leaf extracts via *Artemia salina* lethality. The LC_{50} values were 0.8 µg/mL and 6.3 µg/mL, respectively, suggesting potential cytotoxic constituents. Bokshi et al. (2020) fractionated ethanol extracts of leaves and stems, identifying the ethyl acetate stem and CCl_4 leaf fractions as most toxic with LC50 values of 25.0 ± 0.07 and 25.0 ± 0.05 µg/ml, respectively, compared to vincristine sulfate (LC_{50} of 0.156 ± 0.09 µg/mL). Hosen et al. (2021) reported weak lethality for ethanol:methanol (1:1) fruit extract with a LC_{50} value of 444.3 µg/mL (vincristine sulphate: 0.45 µg/ml) while Kundu et al. (2023) found the fruit ethanol extract LC_{50} of 219.3 µg/mL (vincristine sulphate: 0.584 µg/mL).

4.4.2. Antioxidant activity

The antioxidant potential of S. caseolaris has been widely evaluated using a variety of *in vitro* assays, including radical scavenging activity (DPPH, ABTS, superoxide, hydrogen peroxide), reducing power (FRAP), and metal chelation methods. These approaches have consistently demonstrated notable antioxidant properties in different plant parts. To date, the available evidence is limited to *in vitro* evaluations, and no *in vivo* studies have been reported.

4.4.2.1. DPPH Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay has been widely applied to evaluate the free-radical scavenging capacity of *S. caseolaris* (**Table 4.3**) with leaf extracts being the most extensively studied and generally showing strong antioxidant activity.

Table 4.3. DPPH scavenging activity of *Sonneratia caseolaris* extracts.

Plant part	Solvent	Fraction	Results: IC_{50} (or %)	Standard: IC_{50} (or %)	Ref.
Leaves	Ethanol	-	1.92 ± 0.38 µg/ml	AA: 12 ± 1.29 µg/ml	Kaewpiboon et al., 2012
			4.25 ppm	AA: 5.25 ppm	Audah et al., 2022
			23.84 µg/mL	-	Nguyen et al., 2024
			26.30 µg/mL	AA: 3.16 µg/mL	Ahmed et al., 2006

			27.0 µg/mL	AA: 12.0 µg/mL	Barman et al., 2021
			171 ppm	AA: 20.5 ppm	Latief et al., 2019
			80.21% at 2.5 mg/mL	-	Pagarra et al., 2022
		Ethyl acetate	12.0 ± 0.12 µg/mL	AA: 8.0 ± 0.12 µg/mL	Bokshi et al., 2020
		chloroform	19.0 ± 0.07 µg/mL		
		Carbon tetrachloride	49.0 ± 0.05 µg/mL		
	Ethanol 70%	-	75.89% at 2.5 mg/mL	-	Pagarra et al., 2022
	Ethyl acetate	-	16.71% at 2.5 mg/mL		
	n-Hexane	-	22% at 2.5 mg/mL		
	Acetone	-	166.2 ppm	-	Latief et al., 2019
	Water	-	89 mg/mL	-	Kartikaningsih et a., 2025
	70% aqueous acetone	Ellagitannin-rich fraction	69.39 ± 0.29 µg/mL	BHA: 116.52 ± 0.95 µg/mL	Feng et al., 2019
	Water	Low molecular weight polysaccharide fraction	41.33 ± 0.82% at 3.2 mg/ml	AA: 85.26 ± 0.96% at 3.2 mg/mL	Jha et al., 2023
Stems	Ethanol	Ethyl acetate	138 ± 0.8 µg/mL	AA: 8.0 ± 0.12 µg/mL	Bokshi et al., 2020
		Chloroform	69.0 ± 0.21 µg/mL		
		Carbon tetrachloride	201.0 ± 0.15 µg/mL		
Fruits	Ethanol	-	1.16 ± 0.76 µg/mL	AA: 5.28±0.58 µg/mL	Yoong et al., 2021
		-	87 µg/ml	AA: 15 µg/ml	Kundu et al., 2022
		n-Hexane	191.31 ppm	AA: 3.70 ppm	
		Ethyl acetate	96.02 ppm		
		Butanol	371.16 ppm		
	Ethanol-methanol (1:1)	-	48.1% at 50 µg/mL	-	Hosen et al., 2020
	Water	-	7.3 mg/mL	-	Doan et al., 2018
Bark	Ethanol	-	4.57 µg/mL	BHT: 3.25 µg/mL	Munira et al., 2019
	Petroleum ether	-	12.32 µg/mL		
	Ethyl acetate	-	13.09 µg/mL		
	Chloroform		192.27 µg/mL		
	Methanol		21.74 µg/mL	Quercetin: 10.14 µg/mL.	Simlai et al., 2014

AA = ascorbic acid, BHA = butylated hydroxyanisole, BHT = butylated hydroxytoluene.

Ethanol has emerged as the most effective solvent for leaf extract, with Pagarra et al. (2019) reported up to 80.21% DPPH scavenging using 96% ethanol at 2.5 mg/mL. In a comparative screening of 53 medicinal plants, Kaewpiboon et al. (2012) identified *S. caseolaris* leaf ethanol extract as the most potent, with an EC_{50} values of 1.92 ± 0.38 µg/ml, substantially lower than the positive control, ascorbic acid (EC_{50}: 12 ± 1.29 µg/ml). However, other studies, reported higher IC_{50} values for the same extract, ranging from 4.25 to 171 µg/ml (Ahmed et al., 2006; Latief et al., 2019; Barman et al., 2021; Audah et al., 2022; Nguyen et al., 2024), likely reflecting differences in plant origin, extraction conditions and assay protocols.

Fractionation of ethanol leaf extracts using solvents of varying polarity revealed a clear polarity-dependent trend in antioxidant activity. Bokshi et al. (2020) reported IC_{50} values of 12.0 ± 0.12, 19.0 ± 0.07, 49.0 ± 0.05, for ethyl acetate, chloroform and carbon tetrachloride fractions, respectively. In contrast, stem fractions showed weaker activity, with the chloroform fraction being the most effective (IC_{50}: 69.0 ± 0.21 µg/mL; AA: 8.0 ± 0.12 µg/mL). Additionally, specialized fractions of the ethanol leaf extract also demonstrated antioxidant activity: an ellagitannin-rich leaf yielded an IC_{50} of 69.39 ± 0.29 µg/mL, surpassing the butylated hydroxyanisole BHA (IC_{50}:116.52 ± 0.95 µg/mL) (Fang et al., 2019), while a low molecular weight polysaccharide fraction achieved 41.33 ± 0.82% scavenging at 3.2 mg/ml (AA: 85.26 ± 0.96%) (Jha et al., 2023).

Fruit extracts have likewise been explored for DPPH scavenging activity. Ethanol extracts demonstrated IC_{50} values ranging from 1.16 to 87 µg/ml (Yoong et al., 2021; Kundu et al., 2022). Additionally, ethanol-methanol (1:1) extract revealed a 48.1% scavenging at 50 µg/mL (Hosen et al., 2020), while aqueous extract displayed an IC_{50} values of 7.3 mg/mL (Doan et al., 2018).

Bark extracts, though less frequently studied, have also demonstrated significant DPPH radical scavenging activity. Ethanol bark extract exhibited strong antioxidant activity with IC_{50} of 4.57 µg/mL, while petroleum ether, ethyl acetate, and chloroform extracts showed IC_{50} values of 12.32, 13.09, and 192.27 µg/mL, respectively (BHT: IC_{50} of 3.25 µg/mL) (Munira et al., 2019). Additionally, the methanol bark extract exhibited an IC_{50} of 21.74 µg/mL (Simlai et al., 2014).

4.4.2.2. ABTS scavenging activity

ABTS•+ scavenging assay complements DPPH by measuring both hydrophilic and lipophilic antioxidants. An ellagitannin-rich leaf fraction showed strong activity with an IC_{50} of 45.11 ± 0.49 µg/mL, outperforming BHA (IC_{50} of 88.46 ± 0.11 µg/mL) (Fang et al., 2019). Syamsul et al. (2022) reported very low IC_{50} values for leaf ethanol extracts (1.53 ppm; AA: 0.72 ppm), with fractionation yielding IC_{50} values of 19.89 (n-hexane), 0.50 (ethyl acetate), and 1.63 ppm (ehtnaol). Fruit ethanol extracts recorded IC_{50} of 97.32 ± 3.27 µg/mL (AA: 12.71 ± 1.14 µg/mL) (Yoong et al., 2021). In addition, the low molecular weight leaf polysaccharide fraction exhibited a scavenging effect of 87.13 ± 0.66% at 3.2 mg/ml, lower than that of ascorbic acid (Jha et al., 2023).

Wetwitayaklung et al. (2013) assessed ABTS•+ radical scavenging using the TEAC method and also reported IC_{50} values for various plant parts of *S. caseolaris* obtained through both

maceration and Soxhlet extraction. Methanolic macerates of different organs showed IC_{50} values ranging from 13.64 to 31.95 µg/50 µL, with the calyx of flowers demonstrating the strongest activity (TEAC: 0.69). Soxhlet extraction with methanol yielded IC_{50} values between 10.38 and 51.61 µg/50 µL, with seeds exhibiting the highest antioxidant potential (TEAC: 0.96). Ethyl acetate Soxhlet extracts showed IC_{50} values ranging from 8.85 to 37.65 µg/50 µL, where the stamen displayed the greatest activity (TEAC: 1.05). In contrast, Soxhlet extraction with dichloromethane resulted in significantly higher IC_{50} values (277.75–2293.79 µg/50 µL), indicating substantially weaker antioxidant performance.

4.4.2.3. Reducing power and ferric-reducing antioxidant power (FRAP)

Reducing power assays measure electron-donating capacity. A fruit ethanol–methanol (1:1) extract showed a reducing power (OD) of 0.54 at 0.3 mg/mL (Hosen et al., 2020). Leaf ethanol extracts recorded RC_{50} of 395.5 µg/mL (Barman et al., 2021), while fruit ethanol extracts displayed FeCl3 reducing power with RC_{50} value of 80 µg/mL (AA RC_{50}: 28 µg/mL) (Kundu et al., 2022) Syamsul et al. (2022) reported leaf ferric reducing antioxidant power (FRAP) values of 345.125 ± 4.196 mM/g, highlighting strong redox activity. Wetwitayaklung et al. (2013) also assessed FRAP activity using the GEAC method, expressing results as gallic acid equivalent antioxidant capacity. Extracts obtaining using methanol (maceration and Soxhlet extractions) and ethyl acetate (Soxhlet) of different plant parts exhibited GEAC values were in range of 0.06-0.23.

4.4.2.4. Hydrogen peroxide and superoxide scavenging

Fruit ethanol extract scavenged H_2O_2 with EC_{50} of 66 µg/mL and superoxide ($O_2^{\bullet-}$) with EC_{50} of 347 µg/mL, while ascorbic acid exhibited EC_{50} of 11 µg/mL and 111 µg/mL, respectively (Kundu et al., 2022). Jha et al. (2023) reported that low-molecular-weight polysaccharide fractions from leaves achieved 66.0±1.00% superoxide scavenging at 3.2 mg/mL (AA: 95.62 ± 1.05 at 3.2 mg/ml).

4.4.2.5. Metal-chelation assay

Heavy-metal chelation mitigates oxidative catalysis. Leaf polysaccharide fractions chelated of 69.25 ± 1.18% Fe^{2+} at 3.2 mg/mL, compared to EDTA-2Na (92.44 ± 0.37%) as the positive control (Jha et al., 2023).

4.4.3. Anticancer activity

The anticancer potential of *S. caseolaris* has been assessed *in vitro* using human cancer cell lines, with both crude extracts and isolated compounds evaluated for cytotoxic effects. Tian et al. (2009) tested crude methanol extracts from stems and twigs against the SMMC 7721 human hepatoma cell line using an MTT assay. While the crude extract showed weak cytotoxicity, the isolated compound luteolin (**8**) demonstrated potent anticancer activity with an IC_{50} of 2.8 µg/mL. However, this was still less effective than the standard drug mitomycin C, which had an IC_{50} of 1.1 µg/mL. In another study, Wu et al. (2009) evaluated nine compounds derived from fruits. Among them, (-)-(R)-nyasol (**34**), (-)-(R)-4′-O-methylnyasol (**35**), and maslinic acid (**43**) showed moderate cytotoxicity against cancer cells, with IC_{50} values ranging from 19.0 to 31.8 µg/mL. These activities were weaker compared to the reference drug 5-fluorouracil, which had an IC_{50} of 5.84 µg/mL.

4.5. CHALLENGES AND FUTURE PERSPECTIVES

Research on *S. caseolaris* has notably intensified only over the last decade, with scarce reports before 2010 and a solitary phytochemical analysis by Hogg and Gillan in 1984, underscoring the novelty and promise of this field. To date, investigations remain geographically confined to India, Vietnam, Indonesia, Bangladesh and China, even though it is known that geographical, environmental, and climate factors may influence metabolite profiles in plants (Jin et al., 2009; Abd-Elgawad, et al., 2019; Rozirwan et al., 2022). The focus of previous studies on only a few countries restricts the full understanding of the phytochemistry and bioactivity of *S. caseolaris*. Populations in unstudied regions may experience different environmental pressures, leading to metabolic adaptations that could result in unique secondary metabolites with potentially novel biological activities. Seasonal and geographic chemodiversity, while posing challenges to standardization and reproducibility, also offers unique opportunities for chemical ecology and natural product discovery. Expanding sampling to underexplored regions, particularly those characterized by abiotic stressors such as hypersalinity, high temperatures, or intense solar radiation, could unveil novel stress-induced metabolites, as already been documented in various plant species (Wang et al., 2010; Di Ferdinando et al., 2012; Neugart et al., 2016; Falahi et al., 2018; Franzoni et al., 2019; Sarri et al., 2021). Interestingly, as shown in **Table 4.1**, several compounds, including gallic acid, ellagic acid, luteolin, luteolin 7-*O*-glucoside, vanillin, oleanolic acid, lupeol, ursolic acid, sitosterol, cholesterol, and stigmasterol, have been identified

across studies from different regions, suggesting the existence of a potential core phytochemical profile conserved across global populations. Future comparative studies employing standardized LC-MS or other advanced analytical protocols would enhance our understanding of *S. caseolaris* chemical ecology and support targeted bioprospecting efforts.

In addition to the geographical limitations of existing studies, while leaves, fruits, bark, stems, and twigs have been studied, no phytochemical investigation of the roots has yet been conducted. This omission is significant, as mangrove roots often accumulate unique metabolites associated with stress adaptation and ecological interaction, many of which hold pharmacological potential (Cerri et al., 2025). Target exploration of this organ could therefore uncover novel classes of bioactive compounds with distinct therapeutic relevance properties.

The unique adaptability of mangroves for extreme environments has prompted extensive interest in their pharmaceutical potential, particularly for cancer therapy (Cerri et al., 2022). Mangrove-derived compounds have demonstrated a wide spectrum of anticancer activities in preclinical pharmacological systems (Patra et al., 2011). However, while antioxidant and antimicrobial properties of *S. caseolaris* are relatively well documented, studies exploring its anticancer effects remain extremely limited. Only a few investigations have assessed crude extracts or isolated compounds against cancer cell lines, leaving a substantial knowledge gap, especially given the considerable promise shown by other mangrove taxa. Systematic exploration of *S. caseolaris* for anticancer activity should therefore be a high priority and could involve comprehensive cytotoxic screening of crude extracts and fractions on a panel of cancer and normal cell lines to evaluate both efficacy and selectivity, with comparisons to a standard anticancer drug. Fractions demonstrating significant activity would be prioritized for further purification to enhance therapeutic efficacy and selectivity, as well as for mechanistic studies, including apoptosis induction, cell cycle analysis, and molecular pathway investigation, alongside quantification of bioactive compounds to establish structure-activity relationships. Subsequent structural elucidation of active fractions may lead to the identification of novel anticancer candidates suitable for comprehensive *in vivo* evaluation. This integrated approach would provide a clearer understanding of the anticancer potential of *S. caseolaris* and guide future preclinical and translational research.

Concurrently, *S. caseolaris* populations, like mangroves more broadly, are under significant ecological threat. Coastal development, aquaculture, pollution, and overexploitation have contributed to the degradation of mangrove ecosystems, and the IUCN warns that more than half of global mangrove habitats may collapse by 2050. Thus, bioprospecting initiatives must

be firmly grounded in conservation principles to ensure a reliable, renewable source of plant material. Equally important are frameworks for fair benefit-sharing and engagement with local communities to safeguard ethical and sustainable resource use.

Overall, long-term and comprehensive *in vivo* studies of both extracts and isolated compounds are essential to establish safety, efficacy, and mechanistic insights, thereby providing a solid foundation for eventual clinical evaluations. Moreover, the process of isolating and identifying individual bioactive compounds from the chemically complex matrices of mangrove extracts is often time-consuming and typically requires advanced analytical techniques and substantial quantities of plant material (Atanasov et al., 2021), while regulatory pathways for botanical drugs demand rigorous standards of purity, reproducibility and safety. Without dedicated funding, clear intellectual-property frameworks and industry partnerships, progress toward clinical translation will remain slow (Cerri et al., 2022).

Although natural compounds offer several advantages, such as lower toxicity, reduced side effects, and strong therapeutic potential, concerns about their biocompatibility and potential toxicity remain significant obstacles to their development as clinical medicines (Patra et al., 2018). As such, innovative drug delivery systems represent a critical direction for future research. Among these, nanotechnology-based approaches are particularly promising. Nanoparticles (NPs) can be engineered to encapsulate or conjugate bioactive molecules, thereby enhancing solubility, stability, and targeted delivery (Cerri et al., 2022; Varunkumar et al., 2020). In fact, NPs formulated with mangrove-derived extracts have already demonstrated improved anticancer activity in preclinical models, particularly through mechanisms such as ROS-mediated apoptosis (Varunkumar et al., 2020; Tian et al., 2020). Extending these nanotechnological strategies to *S. caseolaris* could significantly advance its therapeutic application and address current limitations in bioavailability and efficacy.

Thus, realizing the full pharmaceutical value of *S. caseolaris* will require a multifaceted and interdisciplinary approach. Priorities include (1) expanding geographical and seasonal sampling to capture chemodiversity, including populations exposed to extreme environmental conditions that may induce unique metabolites; (2) integrating conservation and sustainable resource management into bioprospecting pipelines; (3) conducting in-depth *in vitro* and *in vivo* studies, especially for anticancer potential but also for other pharmacological activities, with the need to increase the number of investigations and emphasis on mechanism-based investigations; (4) exploring under-investigated plant organs, such as roots, which may harbour unique metabolites with distinct pharmacological properties; (5) improving extraction, standardization, and

compound isolation techniques; (6) fostering cross-sector funding and research collaborations; and (7) leveraging drug delivery innovations, particularly nanotechnology, to overcome limitations related to bioavailability and targeting. Addressing these interconnected challenges will be crucial to transforming *S. caseolaris* from a largely exploratory subject of study into a practical source of novel bioactive agents with real-world therapeutic applications.

4.6. MATERIALS AND METHODS

A comprehensive literature review was conducted to gather information on the phytochemistry and pharmacological potential of *S. caseolaris*. Scientific articles and books were retrieved primarily from online databases such as Google Scholar, using combination of keywords including: '*Sonneratia'*, '*Sonneratia caseolaris'*, 'phytochemical analysis', 'secondary metabolites', 'natural products', 'bioactive compounds', 'biological activities', 'antioxidant activity', 'cytotoxic activity'. In addition, chemical constituents associated with *S. caseolaris* were identified and verified through searches in the CAS SciFinder chemical compound database. All studies and references mentioning *S. caseolaris* were critically assessed for inclusion.

4.7. CONCLUSIONS

S. caseolaris has emerged as a valuable source of structurally diverse secondary metabolites, including phenolic acids, flavonoids, tannins, terpenoids, steroids, fatty acids and their derivatives, fatty alcohols, aldehydes, and hydrocarbons. These phytochemicals form the basis of a wide array of pharmacological activities demonstrated by extracts prepared using various solvents. Notably, *S. caseolaris* exhibits potent antioxidant and free-radical scavenging properties, largely attributed to its high polyphenolic content. Collectively, these findings support the notion that *S. caseolaris* holds considerable promise as a multi-target therapeutic agent. Its antioxidant and anti-inflammatory properties, in particular, align with its traditional uses for treating infections and inflammatory ailments.

However, current evidence is primarily based on crude extracts and preliminary *in vitro*. A major challenge lies in the variability of extract composition and bioactivity, which can fluctuate depending on factors such as geographical origin, seasonal variation, and especially extraction method. Importantly, no phytochemical analyses of the roots have been reported to

date, representing a critical knowledge gap given the ecological and metabolic importance of this organ in mangroves. Future research should therefore prioritize systematic root investigations alongside the isolation, purification, and structural elucidation of the most bioactive constituents. These compounds should be evaluated not only through mechanistic *in vitro* studies but also in disease-relevant animal models to assess pharmacological efficacy and safety. In addition, expanding sampling across different geographic regions, integrating standardized extraction and analytical approaches, and applying innovative strategies such as nanotechnology-based delivery systems would provide valuable insights and enhance translational potential. By pursuing these directions, research groups can move beyond exploratory findings and accelerate the development of *S. caseolaris* as a practical source of novel bioactive agents with therapeutic applications.

4.8. REFERENCES

Abd-ElGawad, A. M., Elshamy, A. I., Al-Rowaily, S. L., & El-Amier, Y. A. (2019). Habitat affects the chemical profile, allelopathy, and antioxidant properties of essential oils and phenolic enriched extracts of the invasive plant *Heliotropium curassavicum*. Plants, 8(11), 482.

Ahmed, F., Shahid, I. Z., Razzak, M. A., Rahman, M. M., Hoque, T., Rahman, M. T., & Sadhu, S. K. (2006). Free radical scavenging activity of some Mangroves available in Bangladesh. Oriental Pharmacy and experimental medicine, 6(1), 58-64.

Arung, E. T., Kuspradini, H., Kusuma, I. W., Bang, T. H., Yamashita, S., Katakura, Y., & Shimizu, K. (2015). Effects of isolated compound from *Sonneratia caseolaris* leaf: a validation of traditional utilization by melanin biosynthesis and antioxidant assays. Journal of Applied Pharmaceutical Science, 5(10), 039-043.

Asha, K. K., Mathew, S., & Lakshmanan, P. T. (2012). Flavonoids and phenolic compounds in two mangrove species and their antioxidant property.

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: advances and opportunities. Nature reviews Drug discovery, 20(3), 200-216.

Audah, K. A., Ettin, J., Darmadi, J., Azizah, N. N., Anisa, A. S., Hermawan, T. D. F., et al. (2022). Indonesian mangrove *Sonneratia caseolaris* leaves ethanol extract is a potential super antioxidant and anti methicillin-resistant *Staphylococcus aureus* drug. Molecules, 27(23), 8369.

Bandaranayake, W. (1998). Traditional and medicinal uses of mangroves. Mangroves and salt marshes, 2(3), 133-148.

Barman, A. K., Ahmed, T., Das, H., Biswas, B., Ali, M. S., Acharyya, R. N., et al. (2021). Evaluation of antidiabetic potential of extract of *Sonneratia Caseolaris* (L.) engl. leaves against Alloxan-induced diabetes in mice.

Beniwal, D., Dhull, S. S., Gulia, V., & Rani, J. (2024). Avicennia: a mangrove genus unveiled through its phytochemistry, pharmacological, and ecological importance. Rendiconti Lincei. Scienze Fisiche e Naturali, 35(4), 907-929.

Bernatoniene, J., & Kopustinskiene, D. M. (2018). The role of catechins in cellular responses to oxidative stress. Molecules, 23(4), 965.

Bokshi, B., Zilani, M. N. H., Hossain, H., Ahmed, M. I., Anisuzzman, M., Biswas, N. N., & Sadhu, S. K. (2020). Bioactivities of *Sonneratia caseolaris* (Linn) leaf and stem using different solvent systems. Biomed J Sci & Tech Res, 31(5), 24578-24582.

Cerri, F., De Santes, B., Spena, F., Salvioni, L., Forcella, M., Fusi, P., et al. (2025). Phytochemical Profiling, Antioxidant Activity, and In Vitro Cytotoxic Potential of Mangrove *Avicennia marina*. Pharmaceuticals, 18(9), 1308.

Cerri, F., Giustra, M., Anadol, Y., Tomaino, G., Galli, P., Labra, M., et al. (2022). Natural products from mangroves: an overview of the anticancer potential of *Avicennia marina*. Pharmaceutics, 14(12), 2793.

Cerri, F., Louis, Y. D., Fallati, L., Siena, F., Mazumdar, A., Nicolai, R., et al. (2024). Mangroves of the Maldives: A review of their distribution, diversity, ecological importance and biodiversity of associated flora and fauna. Aquatic Sciences, 86(2), 44.

Cerri, F., Saliu, F., Maggioni, D., Montano, S., Seveso, D., Lavorano, S., et al. (2022). Cytotoxic compounds from Alcyoniidae: An overview of the last 30 years. Marine Drugs, 20(2), 134.

Chowdhury, R., Bhuia, M. S., Al Hasan, M. S., Hossain Snigdha, S., Afrin, S., Büsselberg, D., et al. T. (2024). Anticancer potential of phytochemicals derived from mangrove plants: Comprehensive mechanistic insights. Food Science & Nutrition, 12(9), 6174-6205.

Dahibhate, N. L., Roy, U., & Kumar, K. (2020). Phytochemical screening, antimicrobial and antioxidant activities of selected mangrove species. Current Bioactive Compounds, *16*(2), 152-163.

Dev, S., Acharyya, R. N., Akter, S., Al Bari, M. A., Asma, K., Hossain, H., et al. (2021). Toxicological screening and evaluation of anti-allergic and anti-hyperglycemic potential of *Sonneratia caseolaris* (L.) Engl. fruits. Clinical Phytoscience, 7(1), 69.

Di Ferdinando, M., Brunetti, C., Fini, A., & Tattini, M. (2011). Flavonoids as antioxidants in plants under abiotic stresses. Abiotic stress responses in plants: metabolism, productivity and sustainability, 159-179.

Falahi, H., Sharifi, M., Maivan, H. Z., & Chashmi, N. A. (2018). Phenylethanoid glycosides accumulation in roots of *Scrophularia striata* as a response to water stress. Environmental and Experimental Botany, 147, 13-21.

Franzoni, G., Trivellini, A., Bulgari, R., Cocetta, G., & Ferrante, A. (2019). Bioactive molecules as regulatory signals in plant responses to abiotic stresses. In Plant signaling molecules (pp. 169-182). Woodhead Publishing.

Ghalib, R. M., Hashim, R., Sulaiman, O., Awalludin, M. F. B., Mehdi, S. H., & Kawamura, F. (2011). Fingerprint chemotaxonomic GC–TOFMS profile of wood and bark of mangrove tree *Sonneratia caseolaris* (L.) Engl. Journal of Saudi Chemical Society, 15(3), 229-237.

Hogg, R. W., & Gillan, F. T. (1984). Fatty acids, sterols and hydrocarbons in the leaves from eleven species of mangrove. Phytochemistry, 23(1), 93-97.

Hosen, M. Z., Biswas, A., Islam, M. R., & Hossain, S. J. (2021). Anti-bacterial, anti-diarrheal, and cytotoxic activities of edible fruits in the Sundarbans mangrove forest of Bangladesh. Preventive nutrition and food science, 26(2), 192.

Hosen, M. Z., Biswas, A., Islam, M. R., Nazrul, M., Bhuiyan, I., & Hossain, S. J. (2020). Comparison of physicochemical and antioxidant properties of edible fruits in the Sundarbans' mangrove forest, Bangladesh. Bangladesh Journal of Botany, 49(3), 671-678.

Jha, N., Madasamy, S., Prasad, P., Lakra, A. K., Esakkiraj, P., Tilwani, Y. M., & Arul, V. (2023). Optimization and physicochemical characterization of polysaccharide purified from *Sonneratia caseolaris* mangrove leaves: a potential antioxidant and antibiofilm agent. Applied Biochemistry and Biotechnology, 195(12), 7832-7858.

Jin, J., Koroleva, O. A., Gibson, T., Swanston, J., Magan, J., Zhang, Y. A. N., et al. (2009). Analysis of phytochemical composition and chemoprotective capacity of rocket (*Eruca sativa* and *Diplotaxis*

tenuifolia) leafy salad following cultivation in different environments. Journal of agricultural and food chemistry, 57(12), 5227-5234.

Kaewpiboon, C., Lirdprapamongkol, K., Srisomsap, C., Winayanuwattikun, P., Yongvanich, T., Puwaprisirisan, P., et al. (2012). Studies of the in vitro cytotoxic, antioxidant, lipase inhibitory and antimicrobial activities of selected Thai medicinal plants. BMC complementary and alternative medicine, 12(1), 217.

Kartikaningsih, H., Fitriana, N., Anggraeni, I. L., Semedi, B., & Koentjoro, M. P. (2025). The potential of *Sonneratia caseolaris* mangrove leaves extract as a bioactive food ingredient using various water extract. *F1000Research, 13, 249*.

Kathiresan, K., & Bingham, B. L. (2001). Biology of mangroves and mangrove ecosystems.

khanh Tran, T., Ha, P. T. T., Henry, R. J., Nguyen, D. N. T., Tuyen, P. T., & Liem, N. T. (2023). Polyphenol Contents, Gas Chromatography-Mass Spectrometry (GC–; MS) and Antibacterial Activity of Methanol Extract and Fractions of *Sonneratia Caseolaris* Fruits from Ben Tre Province in Vietnam. Journal of Microbiology and Biotechnology, 34(1), 94.

Kundu, P., Debnath, S. L., Ahad, M. F., Devnath, H. S., Saha, L., Karmakar, U. K., & Sadhu, S. K. (2023). Exploration of in vivo and in vitro biological effects of *Sonneratia caseolaris* (L.) fruits supported by molecular docking and ADMET study. BioMed Research International, 2023(1), 4522446.

Kundu, P., Debnath, S. L., Devnath, H. S., Saha, L., & Sadhu, S. K. (2022). Analgesic, Anti-inflammatory, Antipyretic, and In Silico Measurements of *Sonneratia caseolaris* (L.) Fruits from Sundarbans, Bangladesh. BioMed Research International, 2022(1), 1405821.

Latief, M. (2019). The Characterization of Active Compound of Pedada Magrove Plants (*Sonneratia caseolaris*) Which HaveThePotential as Natural Antioxidants. Journal of Chemical Natural Resources, 1(1), 1-11.

More than Half of All Mangrove Ecosystems at Risk of Collapse by 2050, First Global Assessment Finds - Press Release | IUCN. Available at https://iucn.org/press-release/202405/more-half-all-mangrove-ecosystems-risk-collapse-2050-first-global-assessment (accessed 9 September 2025).

Munira, M. S., Islam, M. A., Islam, M. S., Koly, S. F., Nesa, M. L., & Muhit, M. A. (2019). Phytochemical screening and comparative antioxidant activities of fractions isolated from *Sonneratia caseolaris* (Linn.) Bark Extracts. Eur. J. Med. Plants, 28(4), 1-9.

Nabeelah Bibi, S., Fawzi, M. M., Gokhan, Z., Rajesh, J., Nadeem, N., RR, R. K., et al. (2019). Ethnopharmacology, phytochemistry, and global distribution of mangroves—A comprehensive review. Marine drugs, 17(4), 231.

Neugart, S., Krumbein, A., & Zrenner, R. (2016). Influence of light and temperature on gene expression leading to accumulation of specific flavonol glycosides and hydroxycinnamic acid derivatives in kale (*Brassica oleracea* var. *sabellica*). Frontiers in plant science, 7, 326.

Nguyen, L. T. T., Nguyen, T. T., Nguyen, H. N., & Bui, Q. T. P. (2024). Analysis of active compounds and bioactivity of leaves extracts of *Sonneratia* species. Engineering Reports, 6(10), e12870.

Pagarra, H., Rahman, R. A., Hala, Y., & Esivan, S. M. M. (2022). Phytochemical screening, antimicrobial and antioxidant activity from *Sonneratia caseolaris* leaves extract. Jurnal Teknologi (Sciences & Engineering), 84(5), 59-66.

Patra, J. K., & Thatoi, H. N. (2011). Metabolic diversity and bioactivity screening of mangrove plants: a review. Acta Physiologiae Plantarum, 33(4), 1051-1061.

Patra, J. K., Das, G., Fraceto, L. F., Campos, E. V. R., Rodriguez-Torres, M. D. P., Acosta-Torres, L., et al. (2018). Nano based drug delivery systems: recent developments and future prospects. Journal of nanobiotechnology, 16(1), 71.

Rozirwan, R. Y. N., Hendri, M., Putri, W. A. E., & Agussalim, A. (2022). Phytochemical profile and toxicity of extracts from the leaf of *Avicennia marina* (Forssk.) Vierh. collected in mangrove areas affected by port activities. South African Journal of Botany, 150, 903-919.

Sadhu, S. K., Ahmed, F., Ohtsuki, T., & Ishibashi, M. (2006). Flavonoids from *Sonneratia caseolaris*. Journal of Natural Medicines, 60(3), 264-265.

Sarkar, P., Ahnaf, T. R., Rouf, R., Shilpi, J. A., & Uddin, S. J. (2024). A review on bioactive phytochemical constituents and pharmacological activities of *Aegiceras corniculatum*: a pharmaceutically important mangrove plant. Journal of Chemistry, 2024(1), 9992568.

Sarri, E., Termentzi, A., Abraham, E. M., Papadopoulos, G. K., Baira, E., Machera, K., et al. (2021). Salinity stress alters the secondary metabolic profile of *M. sativa*, *M. arborea* and their hybrid (Alborea). International Journal of Molecular Sciences, 22(9), 4882.

Shamsuddin, A. A., Najiah, M., Suvik, A., Azariyah, M. N., Kamaruzzaman, B. Y., Effendy, A. W., & John, B. A. (2013). Antibacterial properties of selected mangrove plants against *Vibrio* species and its cytotoxicity against *Artemia salina*. World Applied Sciences Journal, 25(2), 333-340.

Simlai, A., Rai, A., Mishra, S., Mukherjee, K., & Roy, A. (2014). Antimicrobial and antioxidative activities in the bark extracts of *Sonneratia caseolaris*, a mangrove plant. Excli Journal, 13, 997.

Syamsul, E. S., Umar, S., Wahyuni, F. S., Martien, R., & Hamidi, D. (2022). Anti-aging activity, in silico modeling and molecular docking from *Sonneratia Caseolaris*. Open Access Macedonian Journal of Medical Sciences, 10(A), 1471-1477.

Taofiq, O., González-Paramás, A. M., Barreiro, M. F., & Ferreira, I. C. (2017). Hydroxycinnamic acids and their derivatives: Cosmeceutical significance, challenges and future perspectives, a review. Molecules, 22(2), 281.

Tian, M., Dai, H., Li, X., & Wang, B. (2009). Chemical constituents of marine medicinal mangrove plant *Sonneratia caseolaris*. Chinese journal of oceanology and limnology, 27(2), 288-296.

Tian, S., Saravanan, K., Mothana, R. A., Ramachandran, G., Rajivgandhi, G., & Manoharan, N. (2020). Anti-cancer activity of biosynthesized silver nanoparticles using *Avicennia marina* against A549 lung cancer cells through ROS/mitochondrial damages. Saudi Journal of Biological Sciences, 27(11), 3018-3024.

Tiwari, A. K., Viswanadh, V., Gowri, P. M., Ali, A. Z., Radhakrishnan, S. V. S., Agawane, S. B., et al. (2010). Oleanolic acid-an [alpha]-Glucosidase inhibitory and antihyperglycemic active compound from the fruits of *Sonneratia caseolaris*. Open Access Journal of Medicinal and Aromatic Plants, 1(1), 19.

Van Thuoc, D., Mai, N. T. N., Le Thi Viet Ha, L. D., Hung, D. H. T., Hung, N. K., & Hung, N. P. (2018). Evaluation of antibacterial, antioxidant and antiobese activities of the fruit juice of crabapple mangrove *Sonneratia caseolaris* (Linn.). International Journal of Agricultural Sciences and Natural Resources, 5(2), 25-29.

Varunkumar, K., Anusha, C., Saranya, T., Ramalingam, V., Raja, S., & Ravikumar, V. (2020). *Avicennia marina* engineered nanoparticles induce apoptosis in adenocarcinoma lung cancer cell line through p53 mediated signaling pathways. Process Biochemistry, 94, 349-358.

Wang, D. H., Du, F., Liu, H. Y., & Liang, Z. S. (2010). Drought stress increases iridoid glycosides biosynthesis in the roots of *Scrophularia ningpoensis* seedlings. J Med Plants Res, 4(24), 2691-2699.

Wetwitayaklung, P., Limmatvapirat, C., & Phaechamud, T. (2013). Antioxidant and anticholinesterase activities in various parts of *Sonneratia caseolaris* (L.). Indian journal of pharmaceutical sciences, 75(6), 649.

Wu, S. B., Wen, Y., Li, X. W., Zhao, Y., Zhao, Z., & Hu, J. F. (2009). Chemical constituents from the fruits of *Sonneratia caseolaris* and *Sonneratia ovata* (Sonneratiaceae). Biochemical Systematics and Ecology, 37(1), 1-5.

Yang, Y., Duke, N. C., Peng, F., Li, J., Yang, S., Zhong, C., et al. (2016). Ancient geographical barriers drive differentiation among *Sonneratia caseolaris* populations and recent divergence from *S. lanceolata*. Frontiers in Plant Science, 7, 1618.

Yoong, M. H., & Rozaina, T. T. (2021). Effects of mangrove apple (*Sonneratia caseolaris*) fruit extract on oxidative stability of palm olein under accelerated storage. Food Res, 5(1), 461-470.

Zhou, P., Hu, H., Wu, X., Feng, Z., Li, X., Tavakoli, S., et al. (2025). Botany, traditional uses, phytochemistry, pharmacological activities, and toxicity of the mangrove plant *Avicennia marina*: a comprehensive review. Phytochemistry Reviews, 1-36.

CHAPTER 5

Experimental investigation of the phytochemical composition and antioxidant activities of *Sonneratia caseolaris* leaves and roots

This chapter has been adapted and modified from following submitted work:

Cerri, F., Pagliari S., Mohamed, S., Labra, M., Campone L., Galli, P. (2025). Phytochemical composition and antioxidant activities of *Sonneratia caseolaris* (L.) Engl. leaves and roots: Insights into a promising mangrove species. *Food Science and Nutrition.*

5.1. ABSTRACT

Mangroves thrive in extreme environments and produce secondary metabolites with significant pharmacological potential, making them a rich reservoir of bioactive natural products for the development of new therapeutic agents. *Sonneratia caseolaris* (mangrove apple) has long been utilized in traditional medicine for its antioxidant, antimicrobial, and antifungal activities. However, comprehensive studies of its phytochemistry and biological activities remain limited. The present study provides the first comprehensive profiling of the chemical constituents and antioxidant capacity of *S. caseolaris* leaves and roots collected in the Maldives. Ethanol 50% extracts of leaves and roots were analyzed using ultraperformance liquid chromatography-electrospray ionization-high resolution mass spectrometry (UPLC-ESI/HRMS) operated in positive and negative ionization modes. Antioxidant potential was assessed using spectrophotometric ABTS, DPPH, and ORAC assays. A total of 45 molecules were detected, predominantly polyphenols, including phenolic acids, flavonoids, and tannins. Flavonoid glycosides and gallotannins were the major groups, with several compounds not previously documented in this species or among mangroves. Both extracts exhibited strong antioxidant responses, yielding IC_{50} values comparable to or lower than ascorbic acid. This study enhances the phytochemical understanding of *S. caseolaris*, underscores its antioxidant activity and highlights its pharmacological and nutraceutical potential.

5.2. INTRODUCTION

S. caseolaris has traditionally been employed to treat a variety of ailments, including infections, inflammation, gastrointestinal disorders, and skin conditions and is reputed for its antioxidant, antimicrobial, and antifungal properties (Bandaranayake, 1998; Dev et al., 2021). Scientific interest in this species has grown in recent years, with several studies identifying bioactive compounds. In particular, leaf extracts have been shown to represent a rich source of metabolites contributing to its biological activities, including phenolic acids such as gallic, ellagic, and vanillic acids, hydroxycinnamic acids, flavonoid aglycones (e.g., luteolin, quercetin, apigenin, myricetin) and flavonoid glycosides, catechins, phytosterols, fatty aldehydes, and fatty alcohols (Audah et al., 2022; Kundu et al., 2022; Tran et al., 2023). Nevertheless, the majority of this research has been geographically limited to countries such as India, Vietnam, Bangladesh, and China.

Given the influence of geographical and environmental conditions on plant phytochemistry (Jin et al., 2019; Tran et al., 2023), it is important to study this species in other less-studied regions. In this context, the present study examines the phytochemical constituents and antioxidant potential of *S. caseolaris* leaves and roots collected from the Maldives, a region with unique ecological characteristics that may affect metabolite production. The chemical profiles of both organs were characterized by coupling ultra-performance liquid chromatography with high-resolution mass spectrometry (UPLC-HRMS). Notably, this study constitutes the firs in-depth chemical analysis of the roots of *S. caseolaris*. Furthermore, various *in vitro* assays were conducted to evaluate the antioxidant efficacy of the extracts.

This study contributes valuable insights into the phytochemistry of *S. caseolaris* and reinforces the potential of mangrove species in the development of future therapeutic and nutraceutical applications.

5.3. MATERIALS AND METHODS

5.3.1. Plant sources and collection

Leaves and roots of *S. caseolaris* were gathered from at least ten adult, healthy individuals located in Baa Atoll, Maldives (5°17'50.0"N, 72°58'06.0"E) during the month of November 2024, at the end of the wet season (see **Figure 5.1**). The identification of the species was based on morphological traits as described by Primavera et al. (2004). Since no fruits were available at the time of collection, they were excluded from the current study. The sampling site corresponds to a closed-system, marsh-based inland mangrove (Cerri et al., 2024), occupying a low-lying depression with muddy substrate and shallow surface water. Water salinity was measured in situ at three points using a handheld multiparameter probe (Hanna Multiprobe Meter, model HI98494, Hanna Instruments Inc., USA), yielding a mean salinity value of 0.16 ± 0.07 PSU. After harvesting, the samples were rinsed thoroughly with distilled water to eliminate surface contaminants.

Figure 5.1. Leaves (a) and roots (b) of *Sonneratia caseolaris*.

5.3.2. Extraction procedures

The samples underwent lyophilization and the dried material was then ground to a fine powder using a Retsch Grindomix GM 200 knife mill (Retsch, Haan, Germany). Ultrasound assisted extraction (UAE) was carried out to obtain the plant extracts. For each extraction, 0.5 g of dry sample (DW) were transferred to 50 mL polypropylene tubes and combined with 10 mL of either 50% ethanol-water (v/v), pure ethanol, or pure water. Following manual mixing, the samples were sonicated in an ultrasonic bath (Sonorex Tk 52; Bandelin Electronic, Berlin, Germany) for 10 minutes at 25 °C. After sonication, the mixtures were subjected to centrifuge at 6000 rpm for 5 minutes and the resulting supernatants were carefully collected. This step was performed three times per sample and the collected supernatants were mixed and filtered using Whatman Grade 0965 filter paper to eliminate fine particulates. Solvent removal was achieved with a Heidolph Hei-Vap Core rotary evaportator (Heidolph Instruments, Schwabach, Germany) and the residues were subsequently lyophilized. The extracts were preserved at –20 °C for later analysis.

5.3.3. Phytochemical analysis by UPLC-DAD-HRMS/MS

Chemical analysis of the extracts was conducted using a UPLC/HRMS system (XEVO G2-XS QTOF, WATERS CORP., MILFORD, MA, USA) and electrospray ionization operated in positive and negative ionization modes. Chromatographic separation employed a binary mobile phase: solvent A consisted of 0.1% formic acid in water and solvent B was 0.1% formic acid in methanol. The elution protocol began with 5% B for the first 2 minutes, increased linearly to

95% B over the next 14 minutes, held at 95% for 5 minutes, then returned to starting conditions for a 5-minutes re-equilibration. A flow rate of 400 µL/min, a 5 µL injection volume, and a column temperature of 30 °C were used. UV detection ranged from 210 to 400 nm**.**

Mass spectrometric settings included a 2.0 kV capillary voltage, source temperature of 150°C, and desolvation temperature of 500 °C. The cone gas flow was set to 10 L/h and desolvation gas to 1000 L/h, with spectra acquired over *m/z* 50–1000. Data-dependent MS/MS targeted the two most intense precursors per scan at 30 V collision energy. The compound annotation relied on the exact mass, retention time, UV–Vis profiles, and fragmentation patterns, and was categorized using the Metabolomics Standards Initiative (MSI): Level 1: confirmed via comparison with authentic standards; Level 2: putative identification using MS^2 library or literature data; Level 3: tentative classification based on structural similarity and chemotaxonomic inference.

5.3.4. Assessment of antioxidant capacity

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay was conducted following the method of Pagliari et al. (2024) with some modifications. A 950 µL aliquot of 0.1 mM ABTS solution was mixed with 50 µL phosphate-buffered saline (PBS), standard or extracts (0.02–1.00 mg/mL). 300 µL of each mixture were pipetted into a 96-well microplate and placed in the dark for 30 minutes. Absorbance readings were taken at 734 nm with a microplate reader (Infinity M Nano+. Tecan Italia Srl). Ascorbic acid (AA) served as reference standard and IC_{50} values (µg/mL) were determined using GraphPad Prism v.8, with all tests performed in triplicate (n = 3).

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was conducted following the method of Pagliari et al. (2024). 50 µL of the extract or standard (2.5–1000 µg/mL) was mixed with 950 µL of 0.1 M DPPH• solution. Following a 30-minute incubation at ambient temperature, absorbance readings were taken at 515 nm. AA served as reference standard IC_{50} values (µg/mL) were based on triplicate measurements (n = 3).

The oxygen radical absorbance capacity (ORAC) assays were conducted in black 96-well plates following Pagliari et al. (2024). Each well received 100 µL extract (aqueous) at 0.02 mg/mL, 100 µL of 590 mM AAPH in PBS (pH 7.5), 25 µL fluorescein, and 100 µL PBS. Fluorescence (excitation at 485 nm and emission at 530 nm) was recorded every 5 minutes over 1 hour at 37 °C. AA served as reference standard. IC_{50} values (µg/mL) were based on triplicate measurements (n = 3)

5.3.5. Data analysis and statistics

Results are expressed as mean ± standard error of the mean (SEM) (n = 3). Antioxidant assay outcomes (ABTS, DPPH, ORAC) for leaves, roots, and controls were evaluated by one-way ANOVA with Tukey's post hoc test, with $p < 0.05$ regarded as statistically significant.

5.4. RESULTS AND DISCUSSION

5.4.1. Extraction

Mangroves are recognized for their abundant production of polyphenolic compounds, which are known for their strong antioxidant properties. These bioactive compounds can be efficiently extracted using green solvents such as ethanol-water mixtures (Palaiogiannis et al., 2023; Huamán-Castilla et al., 2024). Water and ethanol are commonly used to recover phenolic compounds as a result of their low toxicity and high extraction efficiency. Compared to other commonly used organic solvents for polyphenol extraction, such as methanol or acetone, ethanol is significantly less toxic and is generally recognised as safe (Generally Recognized As-Safe according to the US Food and Drug Administration), making it a preferable choice for use in the food, nutraceutical and pharmaceutical industries (Galanakis, 2012; Chaves, 2020). Furthermore, using aqueous ethanol reduces the environmental impact and risks associated with handling and disposing of solvents compared to using more aggressive organic solvents.

Combining these two solvents enhances the polarity range compared to those of using them individually, leading to better extraction of phenolic compounds. Since this class of secondary metabolites is highly diverse, their hydrophilic or lipophilic nature is governed by the count of hydroxyl substituents and the molecule's overall size. For this reason, hydroalcoholic mixtures provide a more effective and balanced extraction, accommodating a wider range of phenolic compounds.

Based on these findings, three solvent systems were tested for the extraction of secondary metabolites from the leaves and roots of S. caseolaris: pure ethanol, a 1:1 mixture of ethanol and water (v/v), and pure waterUAE. The extraction yields for leaves and roots were 26.20% (262.0 mg/g DW) and 21.92% (219.2 mg/g DW) for 100% ethanol, 41.24% (412.4 mg/g DW) and 18.80% (188.0 mg/g DW) for 50% ethanol-water, and 31.76% (317.6 mg/g DW) and 16.26% (162.6 mg/g DW) for 100% water. The 50% hydroalcoholic mixture produced a high extraction efficiency, particularly for S. caseolaris leaves, where the yield was higher than that

obtained using pure solvents. These results are consistent with those of other studies that have demonstrated improved extraction yields of phenolic compounds using UAE at low ethanol concentrations, with the greatest extraction capacity being achieved at around 50% ethanol (Paini et al., 2016). In addition, mixtures of these two solvents offer a better polarity range than pure solvent solutions, allowing a better recovery of phenolic compounds of varying hydrophilicity (Vijayalaxm et al., 2015). Therefore, the hydroalcoholic extract was selected for subsequent investigations.

5.4.2. Characterization of compounds

Analyses were performed in both ionization modes to gather complementary information to profile *S. caseolaris* leaves and roots. Among the solvents tested (ethanol, water and ethanol 50%), the 50% ethanol-water mixture provided the highest extraction yield and the most representative extract in terms of quality, making it the optimal choice for characterization. The chromatographic profiles of leaves and roots are presented in **Figures 5.2 and 5.3,** respectively.

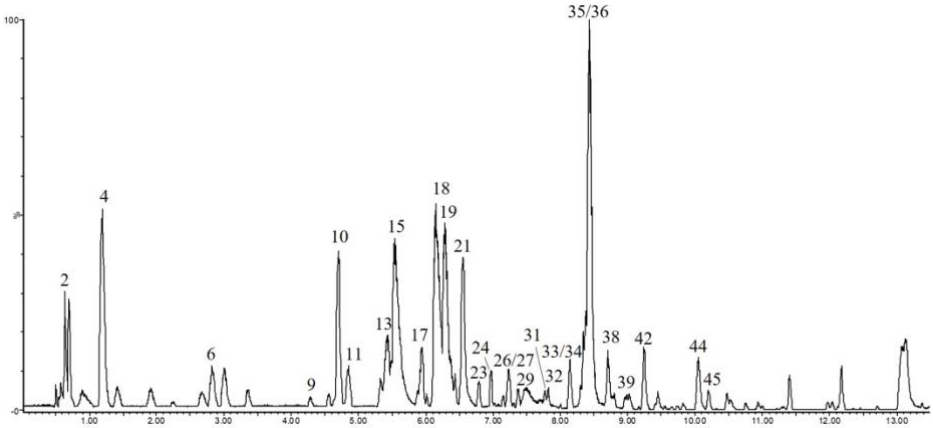

Figure 5.2. Chemical profile of *Sonneratia caseolaris* leaves: UPLC-HRMS chromatogram in negative ion mode (m/z 50–1200). The numbered peaks correspond to the compounds listed in Table 1.

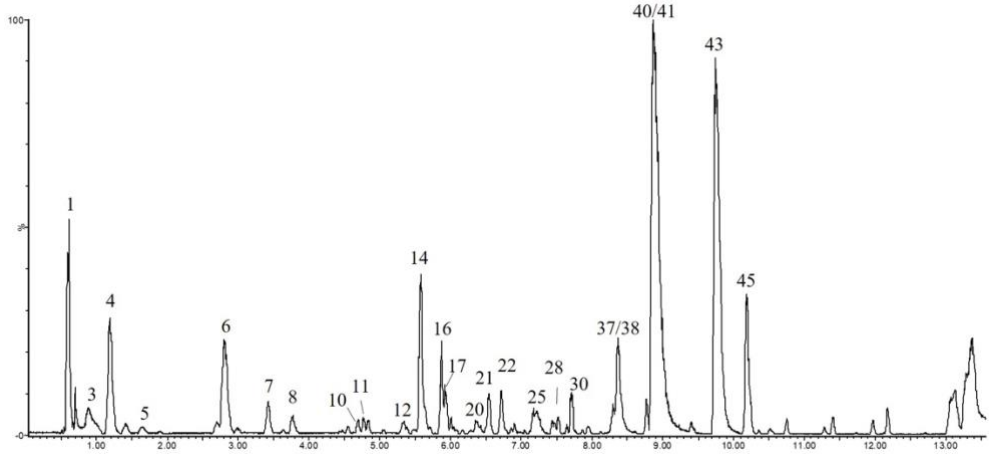

Figure 5.3. Chemical profile of *Sonneratia caseolaris* root: UPLC-HRMS chromatogram in negative ion mode (m/z 50–1200). The numbered peaks correspond to the compounds listed in Table 1.

The leaves were found to contain 30 compounds, while the roots 27, with 10 compounds common to both extracts, as summarized in **Table 5.1**. Most of the identified molecules belong to the polyphenols group. Polyphenols are typically categorized into four main groups: phenolic acids, flavonoids, stilbenes, and lignans. Flavonoids are further subdivided into six principal subclasses: flavan-3-ols, flavones, flavonols, flavanones, isoflavones, and anthocyanins. These molecules occur as free aglycones or as sugar-bound glycosides, the latter being the predominant form in plants (Dias et al., 2019). Additionally, certain flavonoids can undergo polymerization to yield tannins (Rauf et al., 2019).

Analytes were characterized by the combination of accurate mass measurements, retention times, UV–Vis absorbance profiles, and MS/MS fragmentation spectra, with reference to the literature databases and standard compounds where available.

Table 5.1. UPLC-HRMS data of compounds detected in *Sonneratia caseolaris* leaves and roots.

N°	RT (min)	$[M-H]^-$	$[M+H]^+$	Formula	Error (ppm)	Diagnostic products ion (m/z)	Name	Class	Leaves Roots	MSI Level[a]	Ref.
1	0.59	377.0851	n.d.	$C_{18}H_{18}O_9$	7.1561	341.1083, 215.0547, 179.0547, 161.0439, 119.0339, **89.0235**	Caffeic acid derivative	phenolic acid and derivatives	Roots	2	Riethmuller et al., 2013
2	0.66	191.0176	n.d.	$C_6H_8O_7$	9.0719	**111.0073**, 87.0076, 85.0286,	Citric acid	Tricarboxylic acids	Leaves	2	Bylund et al., 2007
3	0.88	481.0621	n.d.	$C_{20}H_{18}O_{14}$	0.5783	**300.9984**, 275.0191	Hexahydroxydiphenoyl-D-glucose (HHDP-glucose)	Ellagitannins	Roots	2	Álvarez-Fernándezet al., 2015
4	1.15	169.0129	n.d.	$C_7H_6O_5$	7.9214	**125.0228**, 97.0279, 81.0338, 79.0182, 69.0341	Gallic acid	Phenolic acids and derivatives	Leaves/ Roots	1	std
5	1.66	609.1242	n.d.	$C_{30}H_{26}O_{14}$	1.2769	441.0821, **423.0715**, 305.0652, 177.0175, 125.0228	Prodelphinidin B-4	Proanthocyanidins	Roots	2	Dou et al., 2007

6	2.82	305.0650	307.0818	$C_{15}H_{14}O_7$	5.4768	219.0626, 179.0329, 167.0331, 165.0176, 139.0392, 137.0240, **125.0230**, 111.0433, 109.0276	(Epi)gallocatechin	Catechins	Leaves/ Roots	2	Hofmann et al., 2016
7	3.44	439.0541	n.d			**241.0011**, 197.0446, 138.9694, 96.9588	Unidentified		Roots		
8	3.78	933.0654	n.d	$C_{41}H_{26}O_{26}$		915.0551, 889.0781, **631.0568**, 613.0564, 587.0659, 569.0590, 425.0163, 300.9985, 275.0190, 249.0405	Vescalagin/castalagin	Ellagitannins	Roots	2	Sanz et al., 2010
9	4.29	783.0682	n.d.	$C_{34}H_{24}O_{22}$	0.5688	481.0624, **300.9964**, 275.0209	Bis-HHDP-glucose (pedunculagin)	Ellagitannins	Leaves	2	Álvarez-Fernándezet al., 2015
10	4.67	289.0702	291.0868	$C_{15}H_{14}O_6$	5.3837	271.0594, 245.0807, 221.0800, 205.0490, 203.0700, 187.0383, 179.0334, 161.0589, 151.0387, 137.0226, 125.0232, 123.0435, 121.0278, **109.0280**, 97.0279, 81.0333	(Epi)catechin	Catechins	Leaves/ Roots	2	Hofmann et al., 2016
11	4.79	483.0779	n.d.	$C_{20}H_{20}O_{14}$	1.2992	331.0640, 313.0548, **271.0447**, 211.0234, 169.0122, 125.0216	Di-*O*-galloyl-β-D-glucose isomer	Galloannins	Leaves/ Roots	2	Hofmann et al., 2016
12	5.34	483.0779	n.d.	$C_{20}H_{20}O_{14}$	0.2663	439.0879, 31.0668, 313.0563, 287.0767, 271.0457, 211.0234, **169.0130**, 125.0231	Di-O-galloyl- β-D-glucose isomer	Gallotannins	Roots	2	Hofmann et al., 2016
13	5.46	635.0885	n.d.	$C_{27}H_{24}O_{18}$	0.7664	483.0744, 465.0660, 423.0563, 313.0553, 295.0457, **169.0125**, 125.0230	Tri-*O*-galloyl-β-D-glucose isomer	Gallotannins	Leaves	2	Hofmann et al., 2016
14	5.58	453.1028	n.d.	$C_{20}H_{22}O_{12}$	2.3115	327.0717, 313.0556, 297.0613, 285.0607, 255.0396, 183.0297, **169.0129**, 151.0025, 139.0389, 125.0230, 124.0152	Unidentified	Unidentified gallic acid derivative	Roots	3	-
15	5.69	785.0828	n.d.	$C_{34}H_{26}O_{22}$	1.9031	633.0743, 615.0633, 483.0741, 419.0607, **300.9970**, 275.0790, 249.0380, 169.0117	Di-*O*-galloyl-HHDP-glucose (tellimagrandin I) isomer	Ellagitannins	Leaves	2	Hofmann et al., 2016
16	5.89	483.1137	n.d.	$C_{21}H_{24}O_{13}$	1.4757	327.0713, 313.0548, 297.0615, 285.0594, 183.0294, **169.0134**, 155.0329, 140.0101, 125.0225, 124.0149	Unidentified	Unidentified gallic acid derivative	Roots	3	-
17	5.92	467.0834	n.d.	$C_{20}H_{20}O_{13}$	-0.6106	**423.0926**, 315.0708, 169.0120, 152.0095, 125.0223	Digalloyl deoxyhexoside	Gallotannins	Leaves/ Roots	2	Li and Seeram, 2018
18	6.24	785.0841	n.d.	$C_{34}H_{26}O_{22}$	0.2493	633.0734, 615.0637, 483.0774, 419.0612, **300.9973**, 275.0183, 249.0387, 169.0125	Di-*O*-galloyl-HHDP-glucose (tellimagrandin I) isomer	Ellagitannins	Leaves	2	Hofmann et al., 2016
19	6.37	635.0889	n.d.	$C_{27}H_{24}O_{18}$	0.1376	483.0771, **465.0732**, 313.0608, 169.0145	Tri-*O*-galloyl-β-D-glucose isomer	Gallotannins	Leaves	2	Hofmann et al., 2016
20	6.38	457.0773	n.d.	$C_{22}H_{18}O_{11}$	0.7311	305.0654, **169.0128**, 125.0230	(Epi)gallocatechin gallate	Catechins	Roots	2	Dou et al., 2007
21	6.54	197.0438	n.d.	$C_9H_{10}O_5$	8.8209	169.0127, 125.0223, **124.0150**	Ethyl gallate	Gallotannins	Leaves/ Roots	2	Sun et al., 2007
22	6.74	445.1339	n.d.	$C_{19}H_{26}O_{12}$	2.8013	**293.1236**, 271.0449, 169.0134, 131.0705	Unidentified	Unidentified gallic acid derivative	Roots	3	-

23	6.77	593.1505	595.1658	$C_{27}H_{30}O_{15}$	1.1677	503.1194, **473.1079**, 383.0765, 353.0651	Apigenin 6,8-di-*C*-D-glucoside (vicenin2)	Flavonoid glycosides	Leaves	2	Hofmann et al., 2016
24	6.97	523.1447	n.d.	$C_{24}H_{28}O_{13}$	1.9355	**313.0545**, 169.0122**,** 125.0233	Unidentified	Unidentified gallic acid derivative	Leaves	3	-
25	7.20	495.1506	n.d.	$C_{23}H_{28}O_{12}$	0.4029	**313.0541**, 181.0853, 169.0137	Unidentified	Unidentified gallic acid derivative	Roots	3	-
26	7.22	447.0922	449.1078	$C_{21}H_{20}O_{11}$	2.4213	429.0834, **357.0494**, 327.0494, **297.0392**, 285.0386,	Luteolin-6-*C*-glucoside (isorientin)	Flavonoid glycosides	Leaves	2	Sánchez-Rabaneda et al., 2007
27	7.39	463.0869	n.d.	$C_{21}H_{20}O_{12}$	2.8003	**301.0335**, 300.0256	Quercetin-3-*O*-galactoside (hyperoside)	Flavonoid-glycosides	Leaves	2	Sánchez-Rabaneda et al., 2007
28	7.55	457.1157				**260.0346**, 96.9588	Unidentified		Roots		-
29	7.65	441.0818	n.d.	$C_{22}H_{18}O_{10}$	2.0817	289.0699, 245.0821, **169.0125**, 125.0223	(Epi)catechin 3-*O*-gallate	Catechins	Leaves	2	Dou et al., 2007
30	7.74	537.1973	n.d.	$C_{26}H_{34}O_{12}$	0.8363	313.0548, 271.0446, 211.0238, **169.0129**, 151.0026, 124.0150	Unidentified	Unidentified gallic acid derivative	Roots	3	-
31	7.85	431.0967	n.d.	$C_{21}H_{20}O_{10}$	3.8657	341.0642, 323.0529, **311.0539**, 283.0589,	Apigenin 8-*C*-β-D-glucoside (vitexin) isomer	Flavonoid glycosides	Leaves	2	Sun et al., 2013
32	7.91	787.1000	n.d.	$C_{34}H_{28}O_{22}$	-0.0684	635.0889, **617.0764**, 465.0671, 447.0549, 295.0445, 169.0112	Tetra-*O*-galloyl-β-D-glucose	Gallotannins	Leaves	2	Hofmann et al., 2016
33	8.11	463.0869	n.d.	$C_{21}H_{20}O_{12}$	2.8003	301.0327, **300.0255**	Quercetin-3-*O*-glucoside (isoquercitrin),	Flavonoid-glycosides	Leaves	2	Sánchez-Rabaneda et al., 2007
34	8.11	431.0971	433.1135	$C_{21}H_{20}O_{10}$	2.9400	413.0868, 353.0652, 341.0647, 323.0551, **311.0543**, 283.0589, 269.0436	Apigenin 6-*C*-β-D-glucoside (isovitexin) isomer	Flavonoid glycosides	Leaves	2	Sun et al., 2013
35	8.34	593.1509	n.d.	$C_{27}H_{30}O_{15}$	0.4945	**285.0386**	kaempferol 3-O-rutinoside	Flavonoid-diglycosides	Leaves	2	Sánchez-Rabaneda et al., 2007
36	8.37	447.0924	449.1082	$C_{21}H_{20}O_{11}$	1.9750	**285.0394**	Luteolin 7-*O*-glucoside	Flavonoid-glycosides	Leaves	2	Sánchez-Rabaneda et al., 2007
37	8.40	300.9975	n.d.	$C_{14}H_6O_8$	4.9360	283.9957, 257.0080, **229.0130**, 185.0232	Ellagic acid	Phenolic acids and. derivatives	Roots	1	std
38	8.40	421.1135	n.d.	$C_{20}H_{22}O_{10}$	1.2331	313.0557**, 169.0124**, 151.0024, 125.0222	Benzyl-*O*-galloylglucose	Gallotannins	Leaves/ Roots	2	Ghareeb et al., 2019
39	8.75	447.0924	n.d.	$C_{21}H_{20}O_{11}$	1.9750	**301.0334**, 300.0261	Quercitrin (quercetin 3-O-rhamnoside)	Flavonoid-glycosides	Leaves	2	Sánchez-Rabaneda et al., 2007
40	8.92	394.9706	n.d.	$C_{15}H_8O_{11}S$	2.1607	**315.0128**, 299.9896	Isorhamnetin sulfate	Flavonoid derivatives	Roots	2	Su et al., 2010
41	8.92	315.0137	317.0291	$C_{15}H_8O_8$	3.2934	**299.9898**, 282.9861, 270.9882, 243.9994, 228.0048, 216.0054, 200.0098, 172.0157, 160.0153	3-O-methylellagic acid	Phenolic acid and. derivatives	Roots	2	Rosa et al., 2021
42	9.26	431.0982	n.d.	$C_{21}H_{20}O_{10}$	0.3943	269.0427, **268.0358**	Genistein 7-O-glucoside (genistin)	Isoflavonoid-glycosides	Leaves	2	Kachlicki et al., 2008
43	9.73	409.0199	n.d.	$C_{17}H_{14}O_{10}S$	-7.4813	329.0294, **314.0059**, 298.9823,	Quercetin dimethyl ether sulfate	Flavones derivatives	Roots	2	Taamalli et al., 2015
44	10.09	285.0385	n.d.	$C_{15}H_{10}O_6$	6.8576	257.0439, 243.0291, 241.0497, 217.0485, 199.0385, 175.0380, 151.0017, **133.0276**, 107.0117	Luteolin	Flavones	Leaves	1	std

45	10.24	461.0718	n.d.	$C_{21}H_{18}O_{12}$	1.6222	**315.0134**, 299.9897	Isorhamnetin pentoside	Flavonoid-glycosides	Leaves/ Roots	2	Hofmann et al., 2016

n.d. not detected; according to metabolomics standards initiative (MSI); std standard compound

5.4.2.1. Phenolic acid and derivatives

Four phenolic acid compounds were detected. These include two phenolic acids in their underivatized forms: gallic acid (**4**) and ellagic acid (**37**), with $[M–H]^-$ ions at *m/z* 169.0129 ($C_7H_6O_5$) and 300.9975 ($C_{14}H_6O_8$), respectively. These molecules were identified by a coincidence of the MS fragmentation pattern with previous literature data (Lee et al., 2005) and confirmed by reference standards. Peak **1** showed a $[M–H]^-$ ion at *m/z* 377.0851 ($C_{18}H_{18}O_9$) and was assigned to a caffeic acid derivative (Riethmüller et al., 2013). The product ions observed during fragmentation were *m/z* 215.0547 $[M–H–162]^-$ (loss of a hexose unit), 179.0547 [caffeic acid–H]$^-$, and 161.0439 [caffeic acid–H–H_2O]$^-$. Peak **41**, with $[M–H]^-$ ion at *m/z* 315.0137 ($C_{15}H_8O_8$), was assigned to 3-*O*-methylellagic acid based on the coincident MS fragmentation pattern with previous literature data (Rosa et al., 2021).

Among the tentatively identified phenolic acids, four were detected in roots, and among these, only gallic acid was also present in leaves. This pattern likely reflects their defensive role against soil pathogens (Mandal et al., 2010; Pratyusha, 2022; Saini et al., 2024). Both gallic acid (**4**) and ellagic acid (**37**) have been previously identified in *S. caseolaris*, with gallic acid detected in leaves and ellagic acid found in leaves and fruits, while 3-*O*-methylellagic acid (**41**) has only been previously identified before in the mangrove *Lumnitzera racemosa* (Phuong et al., 2017; Fang et al., 2019)

5.4.2.2. Flavonoids

Flavonoids glycosides

Most flavonoids are found in glycosylated form, as glycosylation improves their solubility, transport, and metabolism. In the present study, 11 flavonoid glycosides and derivatives were identified in *S. caseolaris* extracts, almost exclusively in leaf extract, in agreement with previous findings (Cerri et al., 2025a) and with their well-known function as antioxidants protecting against UV radiation and other abiotic stresses (Di Ferdinando et al., 2011). The most abundant glycosides are O-linked, in which the sugar moiety is bound via hydroxylic oxygen, although C-linked variants, characterized by direct carbon-carbon bonds between sugar and flavonoid core, are also common (Dias et al., 2021; Xie et al., 2022). The two types of glycosylation exhibit distinct fragmentation patterns. C-glycosides produce neutral MS/MS

losses of 30, 90 and 120 Da for hexose sugars, 74 and 104 Da for deoxyhexose sugars, 60 Da for pentose sugars, and 18 Da for water loss (Chiavaroli et al., 2020; Vinh et al., 2021). The peak **26**, with $[M–H]^-$ ion at *m/z* 447.0922 ($C_{21}H_{20}O_{11}$), was assigned to luteolin-6-C-glucoside (isorientin). Fragments ions were detected at *m/z* 429.0834 $[M–H–18]^-$ (loss of H_2O), 357.0494 $[M–H–90]^-$ and 327.0494 $[M–H–120]^-$ (neutral loss of C-hexoside), and 297.0392 $[M–H–150]^-$ (Sánchez-Rabaneda et al., 2003). The ion at *m/z* 285.0386 corresponds to luteolin. Following the same fragmentation pathway the peak **23**, **31**, **34** were attributed to apigenin 6,8-di-C-D-glucoside (vicenin 2), 8-C-glycosidic flavonoid (vitexin) and 6-C-glycosidic flavonoid (isovitexin), respectively. The O-glycosides typically involve neutral losses of 162 Da for hexose, 146 Da for deoxy-hexose, and 132 Da for pentose sugars. Therefore peak **36**, with a $[M–H]^-$ ion at *m/z* 447.0924 ($C_{21}H_2O_{11}$) and a *m/z* 285.0394 $[M–H–162]^-$ ion produced in the fragmentation spectrum by the neutral loss of an O-hexoside, corresponded to luteolin 7-*O*-glucoside (Hofmann et al., 2016). Based on this fragmentation pathway the peak **27**, **33**, **35**, **39**, **42**, **45** were tentatively identified as quercetin-3-*O*-galactoside (hyperoside), quercetin-3-*O*-glucoside (isoquercitrin), kaempferol 3-*O*-rutinoside, quercetin 3-*O*-rhamnoside (quercitrin), genistein 7-*O*-glucoside (genistin) and isorhamnetin-*O*-pentoside.

In addition, peak **44** was detected as the free aglycon luteolin with its a $[M–H]^-$ ion at *m/z* 285.0385 ($C_{15}H_{10}O_6$) by a coincident MS fragmentation pattern with data from previous literature (Sánchez-Rabaneda et al., 2003) and confirmed by analytical standard.

Regarding the flavonoid glycosides identified in the present study, only isovitexin (**34**), quercitrin (**39**), and luteolin 7-glucoside (**36**) have previously been reported in *S. caseolaris* (Audah et al., 2022; Kundu et al., 2023). Isorientin (**26**), hyperoside (**27**), vitexin (**31**), isoquercitrin (**33**), and kaempferol 3-*O*-rutinoside (**35**) have been reported in other mangrove species (Glasenapp et al., 2019; Chiavaroli et al., 2020; Vinh et al., 2021) but not in *S. caseolaris*. Furthermore, apigenin 6,8-d-C-D-glucoside (**23**), genistein 7-*O*-glucoside (**42**) and isorhamnetin-*O*-pentoside (**45**) have not been identified in mangroves prior to this study.

Flavonoids represented one of the major classes of secondary metabolites identified in this study. This finding is consistent with previous work showing that flavonoid glycosides are abundantly present in leaf extracts of *S. caseolaris* from other geographic regions (Cerri et al., 2025b). A similar pattern has been reported for other mangrove species growing in extreme habitats (Cerri et al., 2025a). Together, these observations highlight the prominent role of flavonoids in mangrove adaptation, as these compounds are widely associated with tolerance to abiotic stresses, that are characteristic of mangrove environments.

Flavonoid derivatives

Two sulphated flavonoids were detected in the root extract. Peak **40**, with a $[M–H]^-$ ion at *m/z* 394.9706 ($C_{15}H_8O_{11}S$), was classified as isorhamnetin sulphate, evidenced by its product ion at *m/z* 315.0128 corresponding to the loss of an 80 Da sulphate moiety, along with the ion at *m/z* 299.9896 (Rosa et al., 2021). Peak **43**, a $[M–H]^-$ ion at *m/z* 408.9863 ($C_{17}H_{14}O_{10}S$) and fragment ions at *m/z* 329.0294 (quercetin dimethyl ether ion), 314.0059, and 298.9823, was tentatively identified as quercetin dimethyl sulphate (Taamalli et al., 2015).

Sulfated flavonoids have been increasingly reported in plants adapted to saline, waterlogged, or mineral-rich environments, suggesting that sulfate conjugation may represent a relevant biochemical response to such conditions. Sulfation has been proposed to facilitate the detoxification or compartmentalization of excess inorganic sulfate, contributing to the maintenance of ionic balance and the mitigation of oxidative stress typically associated with high-salinity habitats. Usually, their occurrence have been reported in several halophytic and marine species such as (*Myriophyllum*, *Zostera* and *Halophila*), where sulfated flavonoids are attributed to the high concentration of sulfate ions in seawater provides valuable insights into their possible functions (Teles et al., 2018). The detection of two sulfated flavonoids in the roots of *S. caseolaris* reported here for the first time in this species suggests that sulfate conjugation may represent a habitat specific adaptive response to the saline and mineral rich conditions of Maldivian coastal environments. In addition to these ecological implications, sulphated flavonoids have attracted interest for their biological activities, as sulphation can modify the pharmacological profile of the parent flavonoid by altering solubility, stability, and, and interactions with molecular targets. Reported activities include anticoagulant, anti-inflammatory, antimicrobial, antiviral anticancer, antioxidant, and antidiabetic effects, in some cases differing from those of the corresponding non-sulphated analogues (Teles et al., 2018; Mohammed et al., 2025).

5.4.2.3. Catechins

Catechins, polyphenolic flavonoids classified as flavan-3-ols (or flavanols), possess potent antioxidant properties (Bedlack et al., 2015). Four catechins were detected in *S. caseolaris* extracts

Peak **10**, with a $[M–H]^-$ ion at *m/z* 289.0702 ($C_{15}H_{14}O_6$), corresponded to (epi)catechin based on its distinctive fragment ions at *m/z* 245.0807 $[M–H–CO_2]^-$, 205.0490 $[M–H–2C_2H_2O]^-$,

203.0700 [M–H–CO_2–C_2H_2O]$^-$, and 137.0226 [M–H–$C_8H_8O_3$]. The [M−H]$^-$ion resulted in several other fragment ions, some of which were reported in the MS fragmentation of previous studies, including ions at *m/z* 271.0594, 221.0800, 179.0334, 125.0232, 109.0280 (Hofmann et al., 2016).

Peak **6**, with a [M–H]$^-$ ion at *m/z* 305.0650 ($C_{15}H_{14}O_7$), was attributed to (epi)gallocatechin based on its MS fragmentation pattern, which matched previously reported data (Hofmann et al., 2016). The product ions observed during fragmentation were *m/z* 219.0262 [M–H–$C_4H_6O_2$], 179.0329 [M–H–$C_6H_6O_3$]$^-$, 167.0331 [M–H–$C_7H_6O_3$]$^-$, 165.0176 [M–H–$C_7H_8O_3$]$^-$. The fragment ion at *m/z* 125.0230 corresponds of the intact flavonoid A ring (Pacifico et al., 2014).

Peak **20**, with a [M–H]$^-$ ion at *m/z* 457.0773 ($C_{27}H_{18}O_{11}$), was assigned to (epi)gallocatechin gallate with characteristic fragments at *m/z* 305.0654 and 169.0128, distinctive deprotonated ions of (epi)gallogatechin and gallic acid, respectively (Pacifico et al., 2017).

Peak **29**, with a [M–H]$^-$ ion at *m/z* 441.0818 ($C_{22}H_{18}O_{10}$), was attributed to (epi)catechin-3-O-gallate (Dou et al., 2007). The detection of neutral loss of 152 Da, indicative of a galloyl unit, alongside the presence of a (epi)catechin moiety was confirmed by distinctive fragment ions at *m/z* 289.0699 [M–H–152]$^-$ and 245.0821 [M–H–152–44]$^-$ (loss of CO_2). Furthermore, the ions at *m/z* 169.0125 and 125.0223 are the diagnostic ions of a galloyl moiety

Among these, only epigallocatechin gallate (**20**) has been previously reported in *S. caseolaris.* **(**Huamán-Castilla et al., 2024) (Epi)catechin (**10**) and (Epi)gallocatechin (**6**) have been identified in various species of mangroves, including *Aegiceras corniculatum*, *Rhizophora* spp., and *L. racemosa* (Rahim et al., 2008; Wei et al., 2012; Glasenapp et al., 2019). However, to our knowledge, this is the first report of these flavonoids in *S. caseolaris*. Additionally, (epi)catechin-3-*O*-gallate (**29**) has previously been found in *A. corniculatum*, but not in *S. caseolaris* (Rahim et al., 2008; Wei et al., 2012).

5.4.2.4. Tannins

Tannis consist of multiple units with polyhydroxyphenolic groups or their derivatives, capable of forming complexes with other substances such as proteins, cellulose, and minerals. Tannins can be broadly categorized into hydrolysable and condensed tannins. Esters of gallic acid and polyols, primarily D-glucose, constitute the hydrolysable tannin group, which is further classified into gallotannins or ellagitannins depending on whether they yield gallic acid or ellagic acid upon hydrolysis. Condensed tannins, referred to as proanthocyanidins, are oligomeric structures composed of flavonoid subunits that vary in degree of polymerization

(Rauf et al., 2019). Tannins are well-documented antioxidant metabolites (Koleckar et al., 2008) and were similarly distributed between leaf and root extracts in this study. Among them, gallotannins were the most abundant and are recognized for their role in plant defense against herbivores and pathogens (He, 2022).

Gallotannins

Eight gallotannins were detected in the present study. Peaks **11** and **12** were assigned to two di-O-galloyl-D-glucose isomers, with a $[M–H]^–$ ion at *m/z* 483.0779 ($C_{20}H_{20}O_{14}$). The MS2 spectra for both isomers revealed distinctive fragments at *m/z* 331.0640, 313.0548, 271.0447, 211.0234, 169.0122 and 125.0216, consistent with data from the literature (Hofmann et al., 2016). Ions at *m/z* 331 and 313 were formed due to the elimination of units of galloyl (152 Da) and gallic acid (170 Da), respectively, and are indicative of the structural class of gallotannin. The successive loss of C_2H_2O, CH_2O and CH_2O from the ion at *m/z* 313 produced ions at *m/z* 313 produced ions 271, 241 and 211, respectively. Additional key diagnostic ions included *m/*z 169 [gallic acid–H]$^–$ and 125 [gallic acid–H–44]$^–$ (loss of CO_2). Gallotannins are often present with varying pattern of galloyl substitution. Repeated neutral loss events at *m/z* 152 corresponding to galloyl moieties**.** On this basis, peaks **13** and **19** with a $[M–H]^–$ ion at *m/z* 635.0885 ($C_{27}H_{24}O_{18}$) were assigned to tri-*O*-galloyl-D-glucose, while peak **32** with a $[M–H]^–$ ion at *m/z* 787.1000 ($C_{34}H_{28}O_{22}$) was attributed to tetra-*O*-galloyl-β-D-glucose (Hofmann et al., 2016) and peak **17**, with a $[M–H]^–$ ion at *m/z* 467.0834 ($C_{20}H_{20}O_{12}$), corresponded to luteolin digalloyl deoxyhexoside (Li et al., 2018), peak **21**, with a $[M–H]^–$ ion at *m/z* 197.0438 ($C_9H_{10}O_5$), was assigned to ethyl gallate, an ester of gallic acid and ethanol (Sun et al., 2007), and peak **38**, with a $[M–H]^–$ ion at *m/z* 421.1135 ($C_{20}H_{22}O_{10}$), corresponded to benzyl-*O*-galloylglucose (Ghareeb et al., 2019).

Tri-*O*-galloyl- β-D-glucose (**13**, **19**), ethyl gallate (**21**), and tetra-*O*-galloyl- β-D-glucose (**32**) have been previously found in other mangrove species (Kachlicki et al., 2008). However, to our knowledge, di-*O*-galloyl-D-glucose isomers (**11**, **12**) and benzyl-*O*-galloylglucose (**38**) have not previously been reported from mangroves. Furthermore, none of these compounds have been reported in *S. caseolaris*.

Ellagitannins

Five ellagitannins were identified in the present study. The diagnostic fragment ion of this class of compounds is *m/z* 301 [ellagic acid–H]$^–$, and the loss of the HHDP (hexahydroxydiphenoyl) groups, resulting in *m/z* $[M–H–302]^–$ fragments (Hofmann et al., 2016). On the basis of this

pattern of fragmentation, the peaks **3**, **8**, **9**, **15**, **18** were tentatively identified as hexahydroxydiphenoyl-D-glucose (HHDP-glucose), vescalagin/castalagin, bis-HHDP-glucose (pedunculagin), di-*O*-galloyl-HHDP-glucose (tellimagrandin I) isomers 1 and 2. HDPP-glucose (**3**), pedunculagin (**8**), and tellimagrandin I (**15**, **18**) have not previously been reported from mangrove species.

Proanthocyanidins (condensed tannins)

Peak **5**, with a $[M–H]^-$ ion at *m/z* 609.1242 ($C_{30}H_{26}O_{14}$), was identified as prodelphinidin B-4 [24], a (epi)gallocatechin-(4,8′)-(epi)gallocatechin dimer (Jaiswal et al., 2012), never before identified from mangroves. In fact, except the ion at *m/z* 305.0652, in accordance with the cleavage of epigallocatechin (or gallocatechin), the fragment ions at *m/z* 441.0821 $[M–H–168]^-$ and 423.0715 $[M–H–168–18]^-$ were produced via a retro–Diels–Alder (RDA) cleavage pattern involving the C-ring, particularly breaking the O1–C2 and C3–C4 bounds, followed by dehydration (Pacifico et al., 2014).

Notably, we detected a total of 14 tannins, making tannins one of the major chemical classes identified in this study. To date, only four tannins have been previously reported from *S. caseolaris* (Cerri et al., 2025b). This discrepancy likely reflects differences in tissue coverage, because earlier studies focused in other parts of *S. caseolaris*, even though mangrove roots are often particularly rich in tannins, where they may contribute to stress mitigation and microbial interactions in the rhizosphere (Kimura et al., 1989). Tannins are also abundant constituents of leaves, where they function primarily in defense against herbivores and pathogens (He, 2022; Constabel et al., 2014). Nevertheless, foliar tannins of *S. caseolaris* have likewise been only marginally explored to date (Cerri et al., 2025b). The comparatively high tannin diversity observed here may therefore reflect both previous under-sampling roots and leaves and ecological features of Maldivian habitats, including potential pressure from herbivores and soil pathogens. In addition, tannins may participate in tolerance to abiotic stress, although their precise roles in stress adaptation remain insufficiently resolved and warrant further investigation (Constabel et al., 2014; Iqbal et al., 2025).

5.4.2.5. Non-polyphenolic compounds

Peak **2**, with a $[M–H]^-$ ion at *m/z* 191.0176 ($C_6H_8O_7$), was assigned to citric acid. Identification was based on its coincident pattern of MS fragmentation with previous literature data (Bylund et al., 2007).

5.4.2.6. Unknown gallic compounds

Peak **14**, with a $[M–H]^-$ ion at *m/z* 453.1028 ($C_{20}H_{22}O_{12}$), was classified as an unidentified gallic acid derivative for the diagnostic fragment ions at *m/z* 169.0129 [gallic acid–H]$^-$, 151.0025 [galloyl–H]$^-$, and 125.0230 [gallic acid–H–44]$^-$. Although the fragmentation behavior resembles that reported for galloylquercetin (Hofmann et al., 2016), the absence of characteristic quercetin fragments (*m/z* 301, 300) and the presence of a fragment at *m/z* 285.0607 (luteolin or kaempferol) suggested an alternative structure.

Peak **16**, with a $[M–H]^-$ ion at *m/z* 483.1137 ($C_{21}H_{24}O_{13}$), exhibited a comparable MS/MS fragmentation profile to that of **14**. It was classified as an unidentified gallic acid derivative, with diagnostic fragment ions at *m/z* 169.0134 [gallic acid–H]$^-$ and 125.0225 [gallic acid–H–44]$^-$.

Peak **22**, with a $[M–H]^-$ ion at *m/z* 445.1339 ($C_{19}H_{26}O_{12}$), was classified as an unidentified gallic acid derivative for the characteristic product ions observed during fragmentation: *m/z* 293.1236 [M–H–galloyl]$^-$ and 169.0134 [gallic acid–H]$^-$.

Peak **24**, with a $[M–H]^-$ ion at *m/z* 523.1447 ($C_{24}H_{28}O_{13}$), peak **25**, with a $[M−H]^-$ ion at *m/z* 495.1506 ($C_{24}H_{28}O_{12}$), and peak **28**, with a $[M-H]^-$ ion at *m/z* 537.1973 ($C_{26}H_{34}O_{12}$), were similarly classified as unidentified gallic acid derivatives for the characteristic key ion *m/z* 169.0122 [gallic acid–H]$^-$ produced in the fragmentation spectrum. Furthermore, fragment ions at *m/z* 125.0233 [gallic acid–H–44]$^-$ and 151.0026 [galloyl−H]$^-$ were detected for peaks **24** and **28**, respectively.

Although the precise structures of these compounds could not be fully resolved, their occurrence may be biologically relevant because gallic acid-based metabolites are widely associated with bioactive potential. Gallic acid derivatives have been reported to antioxidant, antimicrobial, antiviral, anticancer, anti-inflammatory, and neuroprotective (Lu et al., 2006; Badhani et al., 2015).

5.4.3. Antioxidant activity

Because of the abundant presence of secondary metabolites known for their antioxidant power, such as phenolic compounds, the radical scavenging capacity was monitored using spectrophotometric assays. The activity of leaf and root extracts was investigated by DPPH, ABTS, and ORAC assays (**Figure 5.4**). The results of all assays showed a concentration-dependent antioxidant effect (**Figure 5.4a, c, e**). The antioxidant activity was expressed as IC_{50},

where a lower IC_{50} indicates a higher free radical scavenging activity. In detail, in the DPPH assay the IC_{50} values for leaf and root extracts were 79.55±1.32 µg/mL and 81.40±1.22 µg/mL, respectively, demonstrating slightly lower antioxidant activity than ascorbic acid (IC_{50}: 68.15±4.71 µg/mL) (**Figure 5.4b**). Instead, the ABTS test reported IC_{50} values of 53.53±0.79 µg/mL (leaf) and 49.95±0.90 µg/mL (root), significantly lower than those of ascorbic acid (IC_{50}: 72.40±2.34 µg/mL) (**Figure 5.4d**). The ORAC assay further validated the potent antioxidant properties of hydroalcoholic extracts with IC_{50} values of 1.267±0.088 (leaf) and 1.302±0.092 µg/mL (root), lower than the positive control (IC_{50} 1.975±0.134 µg/mL). No statistically significant differences between leaves and roots were found, indicating that they are equally effective in neutralizing free radicals.

The present results are consistent with literature values for the anti-scavenger effect of mangrove. Reported DPPH IC_{50} for *S. caseolaris* leaf extracts span a broad range (1.92–171 µg/mL), and our results fall within the middle of this interval (Cerri et al., 2025b). This emphasises the high anti-scavenger power of *S. caseolaris* plants grown in the Maldives, which is consistent with observations made on other plants of the same species. In addition, the ABTS IC_{50} values obtained here are comparable to those reported for ellagitannin-rich *S. caseolaris* leaf fractions (Fang et al., 2019). Furthermore, the antioxidant power observed in *S. caseolaris* is higher or comparable to that observed in other mangroves. A study of *Avicennia marina*, one of the best known and most studied mangroves, showed an antioxidant capacity in the ethanolic extract of the leaves with IC_{50} 257.04±3.30 µg/mL (DPPH) and 42.73±0.36 µg/mL (ABTS) (Nguyen et al., 2022). *S. caseolaris* has been shown to be very effective against various radicals. This demonstrates its promise as a natural antioxidant reservoir. However, it should be noted that, although the three spectrophotometric assays used here are widely applied for evaluating antioxidant activity in mangrove extracts (Thatoi et al., 2014), they provide only a limited scope, serving primarily as indicators of the plant's capacity to neutralize free radicals. Additional *in vitro* studies, as well as cell-based and *in vivo* oxidative stress models, will be required to fully clarify the functional relevance and mechanisms of action of the detected metabolites.

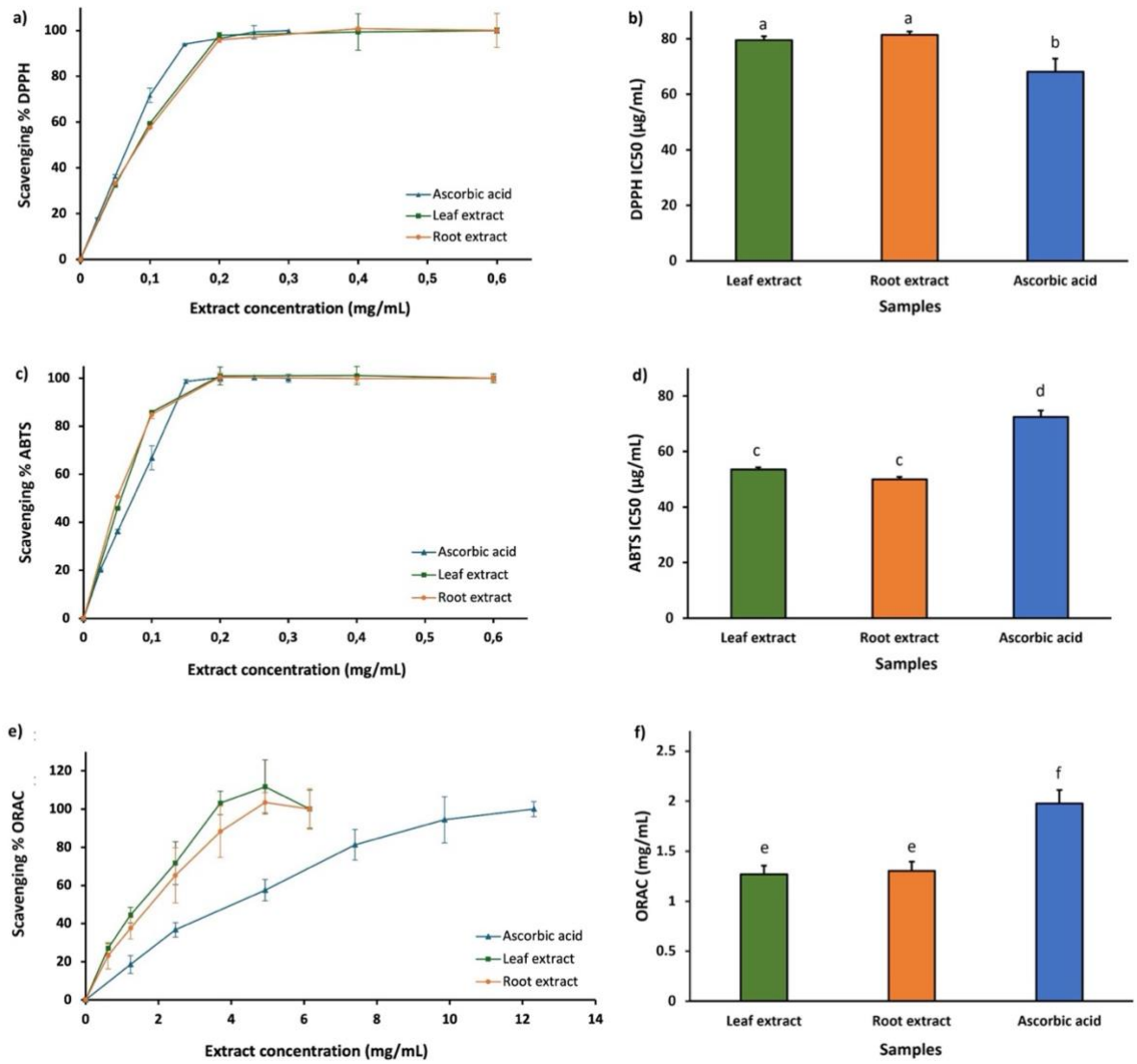

Figure 5.4. Antioxidant activities of *Sonneratia caseolaris* leaf (green) and root (orange) extract species determined by DPPH, ABTS and ORAC assays. Ascorbic acid was used as a positive control (blue). The graphic a), c) and d) show the dose-dependent response of samples and positive control in DPPH, ABTS and ORAC assay, respectively. The graphic b), d) and f) compare the IC50 of samples and positive control. Different letters in each graph indicate a statistical difference (p-value < 0.05).

5.5. CONCLUSIONS

This research constitutes the first in-depth investigation into the phytochemical composition and antioxidant behavior of *S. caseolaris* leaves and roots collected in the Maldives. Using UPLC-ESI/HRMS analyses, 45 compounds were detected. Among these, 36 were identified as polyphenolic compounds belonging to major categories such as phenolic acids and their derivatives, flavonoids, and tannins. The predominant groups were flavonoid glycosides (11

compounds) and gallotannins (8 compounds). Additionally, six unidentified compounds exhibited characteristic fragmentation patterns of gallic acid derivatives, while six others were classified as non-polyphenolic compounds. The total number of detected metabolites was comparable between and roots, with 30 compounds identified in roots, 27 in leaves, including 10 shared constituents. However, some differences emerged in the dominant chemical classes: flavonoid glycosides were more typical of the leaves, while phenolic acids were relatively more abundant in the roots. Notably, only six of the identified compounds had previously been reported from S. caseolaris. Importantly, our analysis also revealed several compounds not previously documented in this species, including two sulphated flavonoids, metabolites commonly associated with plants adapted to swampy and mineral-rich environments. These observations suggest that the unique Maldivian habitat of S. caseolaris may have influenced the biosynthesis of distinctive secondary metabolites.

Both leaf and root extracts demonstrated significant antioxidant capacities in three spectrophotometric assays, highlighting their potential as sources of bioactive compounds. This study offers important perspectives on the chemical and biological properties of *S. caseolaris* and lays the foundation for further investigations into its pharmacological and nutraceutical applications. Given their rich antioxidant profile, *S. caseolaris* extracts could be explored for the development of natural health supplements and cosmetic products with anti-ageing and skin-protection benefits. Furthermore, its bioactive compounds are promising for therapeutic applications, particularly in the management of disorders related to oxidative stress. Future studies should include preliminary cytotoxicity or acute toxicity evaluations to confirm the safety of these extracts and to further substantiate their nutraceutical and pharmacological potential, as well as in vivo and mechanistic investigations to better elucidate their antioxidant relevance and modes of action. Additionally, future work should also include collections from different locations and seasons to evaluate environmental and phenological influences on the metabolite profile of *S. caseolaris*. Furthermore, to improve the data on this species, fruits and other parts could be included in future sampling to obtain a more complete phytochemical picture of the species in this region. The findings of this study also emphasize the need for further bioprospecting research on underexplored mangrove species to unlock their full therapeutic and commercial potential.

5.6. REFERENCES

Álvarez-Fernández, M. A., Cerezo, A. B., Canete-Rodriguez, A. M., Troncoso, A. M., & García-Parrilla, M. C. (2015). Composition of nonanthocyanin polyphenols in alcoholic-fermented strawberry products using LC–MS (QTRAP), high-resolution MS (UHPLC-Orbitrap-MS), LC-DAD, and antioxidant activity. Journal of agricultural and food chemistry, 63(7), 2041-2051.

Audah, K. A., Ettin, J., Darmadi, J., Azizah, N. N., Anisa, A. S., Hermawan, T. D. F., et al. (2022). Indonesian mangrove *Sonneratia caseolaris* leaves ethanol extract is a potential super antioxidant and anti methicillin-resistant *Staphylococcus aureus* drug. Molecules, 27(23), 8369.

Badhani, B., Sharma, N., & Kakkar, R. (2015). Gallic acid: A versatile antioxidant with promising therapeutic and industrial applicationns. Rsc Advances, 5, 27540–27557.

Bandaranayake, W. (1998). Traditional and medicinal uses of mangroves. Mangroves and salt marshes, 2(3), 133-148.

Bedlack, R. S., Joyce, N., Carter, G. T., Pagononi, S., & Karam, C. (2015). Complementary and alternative therapies in ALS. Neurologic clinics, 33(4), 909.

Bui, N. T., Pham, T. L. T., Nguyen, K. T., Le, P. H., & Kim, K. H. (2021). Effect of extraction solvent on total phenol, flavonoid content, and antioxidant activity of *Avicennia officinalis*. Res. Appl. Chem, 12, 2678-2690.

Bylund, D., Norström, S. H., Essén, S. A., & Lundström, U. S. (2007). Analysis of low molecular mass organic acids in natural waters by ion exclusion chromatography tandem mass spectrometry. Journal of Chromatography A, 1176(1-2), 89-93.

Cerri, F., De Santes, B., Spena, F., et al. (2025a). Phytochemical Profiling, Antioxidant Activity, and In Vitro Cytotoxic Potential of Mangrove Avicennia marina. Pharmaceuticals, 18, 1308.

Cerri, F., & Galli, P. (2025b). Phytochemistry and Pharmacological Potential of the Mangrove Plant *Sonneratia caseolaris*: A Comprehensive Review. Marine Drugs, 23, 378.

Cerri, F., Louis, Y.D., Fallati, L., et al. (2024). Mangroves of the Maldives: a review of their distribution, diversity, ecological importance and biodiversity of associated flora and fauna. Aquatic Sciences, 86, 44.

Chaves, J.O., De Souza, M.C., Da Silva, L. C., et al. (2020). Extraction of flavonoids from natural sources using modern techniques. Frontiers in chemistry, 8, 507887.

Chiavaroli, A., Sinan, K. I., Zengin, G., Mahomoodally, M. F., Bibi Sadeer, N., Etienne, O. K., et al. (2020). Identification of chemical profiles and biological properties of *Rhizophora racemosa* G. Mey. Extracts obtained by different methods and solvents. Antioxidants, 9(6), 533.

Constabel, P.C., Yoshida, K., & Walker, V. (2014). Diverse ecological roles of plant tannins: plant defense and beyond. Recent advances in polyphenol research, 4, 115–142.

Dev, S., Acharyya, R. N., Akter, S., Al Bari, M. A., Asma, K., Hossain, H., et al. (2021). Toxicological screening and evaluation of anti-allergic and anti-hyperglycemic potential of *Sonneratia caseolaris* (L.) Engl. fruits. Clinical Phytoscience, 7(1), 69.

Di Ferdinando, M., Brunetti, C., Fini, A., & Tattini, M. (2011). Flavonoids as antioxidants in plants under abiotic stresses. Abiotic stress responses in plants: metabolism, productivity and sustainability, 159–179.

Dias, M. C., Pinto, D. C., & Silva, A. M. (2021). Plant flavonoids: Chemical characteristics and biological activity. Molecules, 26(17), 5377.

Dou, J., Lee, V. S., Tzen, J. T., & Lee, M. R. (2007). Identification and comparison of phenolic compounds in the preparation of oolong tea manufactured by semifermentation and drying processes. Journal of Agricultural and Food Chemistry, 55(18), 7462-7468.

Fang, Z., Sun, K., Liu, X., Zhou, H., Song, W., Chen, H., & Wei, S. (2019). Structural characterization of ellagitannin-rich fractions from leaves of three *Sonneratia* species, and their antioxidant

activity and α-amylase inhibitory effect and mechanism. International Journal of Food Properties, 22(1), 1760-1772.

Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. Trends in Food Science & Technology, 26, 68–87.

Ghareeb, M. A., Sobeh, M., El-Maadawy, W. H., Mohammed, H. S., Khalil, H., Botros, S., & Wink, M. (2019). Chemical profiling of polyphenolics in *Eucalyptus globulus* and evaluation of its hepato–renal protective potential against cyclophosphamide induced toxicity in mice. Antioxidants, 8(9), 415.

Glasenapp, Y., Cattò, C., Villa, F., Saracchi, M., Cappitelli, F., & Papenbrock, J. (2019). Promoting beneficial and inhibiting undesirable biofilm formation with mangrove extracts. International Journal of Molecular Sciences, 20(14), 3549.

He, H. F. (2022). Recognition of gallotannins and the physiological activities: from chemical view. Frontiers in Nutrition, 9, 888892.

Hofmann, T., Nebehaj, E., & Albert, L. (2016). Antioxidant properties and detailed polyphenol profiling of European hornbeam (*Carpinus betulus* L.) leaves by multiple antioxidant capacity assays and high-performance liquid chromatography/multistage electrospray mass spectrometry. Industrial Crops and Products, 87, 340-349.

Huamán-Castilla, N. L., Díaz Huamaní, K. S., Palomino Villegas, Y. C., Allcca-Alca, E. E., León-Calvo, N. C., Colque Ayma, E. J., et al. (2024). Exploring a sustainable process for polyphenol extraction from olive leaves. Foods, 13(2), 265.

Iqbal, N., & Poór, P. (2025). Plant protection by tannins depends on defence-related phytohormones. Journal of Plant Growth Regulation, 44, 22–39.

Jaiswal, R., Jayasinghe, L., & Kuhnert, N. (2012). Identification and characterization of proanthocyanidins of 16 members of the *Rhododendron* genus (Ericaceae) by tandem LC–MS. Journal of Mass Spectrometry, 47(4), 502-515.

Jin, J., Koroleva, O. A., Gibson, T., Swanston, J., Magan, J., Zhang, Y. A. N., et al. (2009). Analysis of phytochemical composition and chemoprotective capacity of rocket (*Eruca sativa* and *Diplotaxis tenuifolia*) leafy salad following cultivation in different environments. Journal of agricultural and food chemistry, 57(12), 5227-5234.

Kachlicki, P., Einhorn, J., Muth, D., Kerhoas, L., & Stobiecki, M. (2008). Evaluation of glycosylation and malonylation patterns in flavonoid glycosides during LC/MS/MS metabolite profiling. Journal of Mass Spectrometry, 43(5), 572-586.

khanh Tran, T., Ha, P. T. T., Henry, R. J., Nguyen, D. N. T., Tuyen, P. T., & Liem, N. T. (2023). Polyphenol Contents, Gas Chromatography-Mass Spectrometry (GC–; MS) and Antibacterial Activity of Methanol Extract and Fractions of *Sonneratia Caseolaris* Fruits from Ben Tre Province in Vietnam. Journal of Microbiology and Biotechnology, 34(1), 94.

Kimura, M., & Wada, H. (1989). Tannins in mangrove tree roots and their role in the root environment. Soil Science and Plant Nutrition, 35, 101–108.

Koleckar, V., Kubikova, K., Rehakova, Z., et al. (2008). Condensed and hydrolysable tannins as antioxidants influencing the health. Mini reviews in medicinal chemistry, 8, 436–447.

Kundu, P., Debnath, S. L., Ahad, M. F., Devnath, H. S., Saha, L., Karmakar, U. K., & Sadhu, S. K. (2023). Exploration of in vivo and in vitro biological effects of *Sonneratia caseolaris* (L.) fruits supported by molecular docking and ADMET study. BioMed Research International, 2023(1), 4522446.

Kundu, P., Debnath, S. L., Devnath, H. S., Saha, L., & Sadhu, S. K. (2022). Analgesic, Anti-inflammatory, Antipyretic, and In Silico Measurements of *Sonneratia caseolaris* (L.) Fruits from Sundarbans, Bangladesh. BioMed Research International, 2022(1), 1405821.

Lee, J. H., Johnson, J. V., & Talcott, S. T. (2005). Identification of ellagic acid conjugates and other polyphenolics in muscadine grapes by HPLC-ESI-MS. Journal of agricultural and food chemistry, 53(15), 6003-6010.

Li, C., & Seeram, N. P. (2018). Ultra-fast liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry for the rapid phenolic profiling of red maple (*Acer rubrum*) leaves. Journal of separation science, 41(11), 2331-2346.

Lu, Z., Nie, G., Belton, P.S., et al. (2006). Structure–activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. Neurochemistry international, 48, 263–274.

Mandal, S. M., Chakraborty, D., & Dey, S. (2010). Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant signaling & behavior, 5, 359–368.

Mohammed, H.A., Ragab, E.A., Dutta, A., et al. (2025). Sulfated flavonoids: An updated and comprehensive review of their chemistry and bioactivities. Phytochemistry Reviews, 24, 6244–6310.

Pacifico, S., Piccolella, S., Marciano, S., Galasso, S., Nocera, P., Piscopo, V., et al. (2014). LC-MS/MS profiling of a mastic leaf phenol enriched extract and its effects on H2O2 and Aβ (25–35) oxidative injury in SK-B-NE (C)-2 cells. Journal of agricultural and food chemistry, 62(49), 11957-11966.

Pagliari, S., Domínguez-Rodríguez, G., Cifuentes, A., Ibáñez, E., Labra, M., & Campone, L. (2024). Pressurized liquid extraction of glucosinolates from *Camelina sativa* (L.) Crantz by-products: Process optimization and biological activities of green extract. Food chemistry: X, 22, 101324.

Paini, M., Casazza, A. A., Aliakbarian, B., et al. (2016). Influence of ethanol/water ratio in ultrasound and high-pressure/high-temperature phenolic compound extraction from agri-food waste. International Journal of Food Science and Technology, 51, 349–358.

Palaiogiannis, D., Chatzimitakos, T., Athanasiadis, V., Bozinou, E., Makris, D. P., & Lalas, S. I. (2023). Successive solvent extraction of polyphenols and flavonoids from *Cistus creticus* L. leaves. Oxygen, 3(3), 274-286.

Pratyusha, S. (2022). Phenolic Compounds in the Plant Developmentand Defense: An Overview. Plant Stress Physiology-Perspectives in Agriculture, 125.

Phuong, N. H., Thuy, N. T., Duc, N. T., Tuyet, N. T., Mai, N. T., & Phung, N. K. (2017). A new glycoside and in vitro evalution of α-glucosidase inhibitory activity of constituents of the mangrove *Lumnitzera racemosa*. Natural Product Communications, 12(11), 1934578X1701201125.

Primavera, J. H., Sadaba, R. B., Lebata, M. J. H., Altamirano, J. P. (2004). Handbook of mangroves in the Philippines.

Rahim, A. A., Rocca, E., Steinmetz, J., Kassim, M. J., Ibrahim, M. S., & Osman, H. (2008). Antioxidant activities of mangrove *Rhizophora apiculata* bark extracts. Food chemistry, 107(1), 200-207.

Rauf, A., Imran, M., Abu-Izneid, T., Patel, S., Pan, X., Naz, S., et al. (2019). Proanthocyanidins: A comprehensive review. Biomedicine & Pharmacotherapy, 116, 108999.

Riethmüller, E., Alberti, Á., Tóth, G., Béni, S., Ortolano, F., & Kéry, Á. (2013). Characterisation of diarylheptanoid-and flavonoid-type phenolics in *Corylus avellana* L. leaves and bark by HPLC/DAD–ESI/MS. Phytochemical Analysis, 24(5), 493-50.

Rosa, W., da Silva Domingos, O., de Oliveira Salem, P. P., Caldas, I. S., Murgu, M., Lago, J. H. G., et al. (2021). In vivo anti-inflammatory activity of Fabaceae species extracts screened by a new ex vivo assay using human whole blood. Phytochemical Analysis, 32(5), 859-883.

Saini, N., Anmol, A., Kumar, S., et al. (2024). Exploring phenolic compounds as natural stress alleviators in plants-a comprehensive review. Physiological and Molecular Plant Pathology, 133, 102383.

Sánchez-Rabaneda, F., Jáuregui, O., Casals, I., Andrés-Lacueva, C., Izquierdo-Pulido, M., & Lamuela-Raventós, R. M. (2003). Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (*Theobroma cacao*). Journal of mass spectrometry, 38(1), 35-42.

Sanz, M., Cadahia, E., Esteruelas, E., Muñoz, Á. M., Fernandez de Simon, B., Hernandez, T., & Estrella, I. (2010). Phenolic compounds in chestnut (*Castanea sativa* Mill.) heartwood. Effect of toasting at cooperage. Journal of Agricultural and Food Chemistry, 58(17), 9631-9640.

Su, S., Guo, J., Duan, J. A., Wang, T., Qian, D., Shang, E., & Tang, Y. (2010). Ultra-performance liquid chromatography–tandem mass spectrometry analysis of the bioactive components and their metabolites of Shaofu Zhuyu decoction active extract in rat plasma. Journal of Chromatography B, 878(3-4), 355-362.

Sun, J., Liang, F., Bin, Y., Li, P., & Duan, C. (2007). Screening non-colored phenolics in red wines using liquid chromatography/ultraviolet and mass spectrometry/mass spectrometry libraries. Molecules, 12(3), 679-693.

Sun, Y., Li, H., Hu, J., Li, J., Fan, Y. W., Liu, X. R., & Deng, Z. Y. (2013). Qualitative and quantitative analysis of phenolics in *Tetrastigma hemsleyanum* and their antioxidant and antiproliferative activities. Journal of agricultural and food chemistry, 61(44), 10507-10515.

Taamalli, A., Arráez-Román, D., Abaza, L., Iswaldi, I., Fernández-Gutiérrez, A., Zarrouk, M., & Segura-Carretero, A. (2015). LC-MS-based metabolite profiling of methanolic extracts from the medicinal and aromatic species *Mentha pulegium* and *Origanum majorana*. Phytochemical analysis, 26(5), 320-330.

Teles, Y. C., Souza, M. S. R., & Souza, M. D. F. V. D. (2018). Sulphated flavonoids: biosynthesis, structures, and biological activities. Molecules, 23(2), 480.

Thatoi, H.N., Patra, J.K., & Das, S.K. (2014). Free radical scavenging and antioxidant potential of mangrove plants: a review. Acta physiologiae plantarum, 36, 561–579.

Vijayalaxmi, S., Jayalakshmi, S. K., & Sreeramulu, K. (2015). Polyphenols from different agricultural residues: extraction, identification and their antioxidant properties. Journal of Food Science and Technology, 52(5), 2761-2769.

Vinh, L. B., Nguyet, N. T. M., Thanh, C. D., Huong, T. T., Tram, L. H., Van Thong, N., et al. (2021). Chemical constituents of Vietnamese mangrove *Hibiscus tiliaceus* with antioxidant and alpha-glucosidase inhibitory activity. Natural Product Research, 35(17), 2899-2904.

Wei, S. D., Lin, Y. M., Liao, M. M., Zhou, H. C., & Li, Y. Y. (2012). Characterization and antioxidative properties of condensed tannins from the mangrove plant *Aegiceras corniculatum*. Journal of Applied Polymer Science, 124(3), 2463-2472.

Xie, L., Deng, Z., Zhang, J., Dong, H., Wang, W., Xing, B., & Liu, X. (2022). Comparison of flavonoid O-glycoside, C-glycoside and their aglycones on antioxidant capacity and metabolism during in vitro digestion and in vivo. Foods, 11(6), 882.

CHAPTER 6

General conclusions

6.1. GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

Mangroves are a specialized group of salt-tolerant plants inhabiting the intertidal zones of tropical and subtropical latitudes. They thrive at the land-sea interface, combining the bioprospecting potential of both terrestrial plants and marine ecosystems. Their ability to cope with extreme environmental conditions relies not only on morphological and physiological adaptation but also on unique metabolic pathways, which make them a promising source of structurally diverse secondary metabolites. Despite their ethnomedicinal importance, however, mangroves have historically been overlooked in drug discovery compared to other marine organisms such as sponges, soft corals, and algae. Although the number of publications has increased in recent years, research on mangrove phytochemistry and pharmacology remains limited, partly due to the logistical challenges of working in their habitats.

This thesis focused on two ecologically and ethnomedicinally relevant mangrove species from underexplored regions characterized by unique environmental conditions. The first, *Avicennia marina*, dominates the coasts of the Arabian Gulf, an area with extreme heat, salinity, solar radiation, and anoxic and nutrient-poor soils. While *A. marina* is known for its bioactive compounds, most research in the Arabian Gulf has cantered on its ecology and conservation, with little attention to its phytochemistry or pharmacology. The second species, *Sonneratia caseolaris*, was studied in the Maldives, where mangroves remain far less explored than coral reefs. We selected this species for its ethnomedicinal significance, its unique swampy habitat with brackish to low salinity, and its lower of bioprospecting investigation compared to other mangrove taxa.

The aim of this thesis was to expand the chemical and pharmacological knowledge of these species by generating phytochemical profiles of different plant parts, testing antioxidant capacity, and, in the case of *A. marina*, evaluating cytotoxic potential against human cancer cell lines: SW480 and E705 (colorectal cancer), MDA-MB-231 (triple-negative breast cancer), U-87 (glioblastoma), and HeLa (cervical cancer). This integrated approach, combining detailed phytochemical profiling with antioxidant and cytotoxic assays across multiple plant parts, is well-established in plant research and has been applied to some mangrove species, but not on these two. Such a strategy is essential to link bioactivities to tissue-specific metabolites and to identify promising extracts for drug discovery

Our literature review (chapter 2) confirmed that *A. marina* is chemically versatile, producing naphthoquinones, flavonoids, iridoid glycosides, terpenoids, phenolic glycosides, and steroids. Previous studies reported antioxidant activity, especially in leaves, and demonstrated anticancer potential of extracts and compounds against breast, leukaemia, colon, and liver cancer cell lines, generally with lower toxicity toward normal cells. However, most investigations focused almost exclusively on aerial parts, particularly leaves, leaving other organs understudied. Moreover, Arabian Gulf populations have been largely ignored, despite the possibility that extreme conditions may have shaped unique metabolic profile of *A. marina*, potentially resulting in a distinct set of bioactive compounds.

Our experimental work (chapter 3) addressed this gap by conducting the first detailed UPLC-HRMS phytochemical profiling of *A. marina* from the UAE, analyzing leaves, roots, cotyledons, and propagules. We identified compounds mainly belonging to phenylethanoid glycosides, flavonoid glycosides, iridoid glycosides, and triterpene saponins, which are chemical classes well known for their ecological role in protecting plants from abiotic stresses and which have been reported from *A. marina* collected in other regions. We identified previously reported metabolites, suggesting a core phytochemical profile conserved across global populations of *A. marina*. At the same time, numerous compounds, particularly phenylethanoid glycosides and triterpene saponins, were newly described for this species, suggesting metabolic adaptations to Gulf-specific environmental stresses. Furthermore, pericarp and root extracts, which contained high levels of phenylethanoid glycosides, exhibited the strongest antioxidant activity. This was supported by a significant Spearman correlation between phenylethanoid glycoside abundance and antioxidant activity, and by the presence of well-documented antioxidants such as cistanoside F, acteoside, and jionoside C. In particular, the DPPH scavenging activity of the leaf extract (55.12 ± 1.52 µmol TE/g) was approximately half that of the root extract (128.25 ± 1.12 µmol TE/g), in contrast to previous findings (Al-Mur 2021).

Cytotoxic assays revealed that pericarp, propagule, and cotyledon extracts were weakly active, while the hydroalcoholic leaf extract reduced cell viability to approximately 50–60% at 540 µg/mL (48 h), in line with literature data. For example, methanolic extracts of leaves showed an IC_{50} of 480 µg/mL against MDA-MB-231 (Karami et al., 2012), while stronger effects have been reported for ethyl acetate extract (Esau et al., 2015). Although tested on different cancer models, ethanolic extracts of leaves and roots from Saudi Arabian gulf populations inhibited

>50% of HepG2 growth at 400 µg/mL (Sohaib et al., 2022). Notably, that study, one of the few conducted in the Arabian Gulf, found higher cytotoxicity in leaves than in roots. In contrast, in our study the root extract consistently showed the strongest activity, particularly against SW480, E705, and MDA-MB-231 cells, reducing viability to 22.93%, 27.03%, and 36.47%, respectively, at 540 µg/mL. These effects allowed the calculation of IC_{50} values of 81.98, 108.10, and 57.93 µg/mL, corresponding to moderate cytotoxicity according to the National Cancer Institute (NCI, USA) criteria and the Geran protocol. Importantly, root extracts displayed weaker effect against two normal cell lines (CCD841 and MRC-5). The root extract was enriched in triterpene saponins, compounds increasingly recognized for anticancer activity with relatively low toxicity, and *in silico* analysis supported their potential role in the observed activity.

In contrast, *S. caseolaris* have received little attention compared to other mangrove species and was not even included in previous broad reviews (Patra et al., 2011; Bibi et al., 2019; Chowdhury et al., 2024). Our literature review (chapter 4) reported 18 studies identifying 141 molecules, dominated by flavonoids together with phenolic acids, tannins, terpenoids and various other constituents. Notably, 17 of these studies referred to the last two decades and 12 referring to the last 10 years, confirming that bioprospecting of *S. caseolaris* is a recent field of research. Reflecting the high prevalence of phenolic compounds, the antioxidant activity has been consistently investigated, while anticancer studies remain scarce and inconclusive. Importantly, as with *A. marina*, research remains geographically confined, in this case particularly to India, Vietnam, Indonesia, Bangladesh and China. In this context, we performed the first detailed phytochemical investigation of *S. caseolaris* roots and leaves from collected in the Maldives (chapter 5). Using UPLC-HRMS, we detected 45 compounds, mainly flavonoid glycosides, hydrolysable tannins, catechins, and phenolic acids. Only six of these have been previously reported, with epigallocatechin gallate being the only compound identified here from roots that had been identified previously in *S. caseolaris*. In contrast, several metabolites were newly described for this species, including two sulphated flavonoids, compounds typically found in plants inhabiting swampy environments, suggesting a distinctive phytochemical profile potentially shaped by the unique Maldivian mangrove habitat. Both roots and leaves exhibited similar antioxidant activity, comparable or higher than that of the positive control ascorbic acid across DPPH, ABTS, and ORAC assays, likely reflecting their high polyphenolic content. To our knowledge, no previous studies have investigated the antioxidant activity of *S. caseolaris* root extract, making direct comparison not possible. For leaves, however, several

reports are available: published values for DPPH activity of ethanol extracts range widely from 1.92 to 171 µg/ml. Our findings (IC_{50} of 79.55 ± 1.32 µg/mL for leaves and 81.40 ± 1.22 µg/mL) fall within the mid-range. The ABTS yielded IC_{50} values of 53.53 ± 0.79 µg/mL (leaf) and 49.95 ± 0.90 µg/mL (root), which are close to those of an ellagitannin-rich leaf fraction reported previously (Fang et al., 2019), though notably higher than those of another ethanol leaf extract (Syamsul et al., 2022). Finally, this is, to our knowledge, the first investigating the ORAC activity for *S. caseolaris* extracts, revealing IC_{50} values of 1.267±0.088 (leaf) and 1.302±0.092 µg/mL (root).

A comparative view highlights the distinct chemical profiles of the two species, reflecting both taxonomic differences and contrasting environmental contexts. *S. caseolaris* and *A. marina* belong to different botanical families with divergent dominant secondary metabolic pathways, and the sample were collected from geographically and ecologically distinct habitats. *A. marina*, from the Arabian Gulf, was enriched in iridoid glycosides, phenylethanoid glycosides, and triterpene saponins, metabolite classes often associated with osmotic and drought stress (Wu et al., 2005; Falahi et al., 2018; Sarri et al., 2021). This profile is consistent with adaptation to the extreme aridity, high salinity, and intense solar radiation characteristic of this region. In contrast, *S. caseolaris*, sampled in the Maldives from swampy, low-salinity environments with comparative milder abiotic pressures, was dominated not only by flavonoids but also by hydrolysable tannins, catechins, and phenolic acids. While these metabolites also play roles in abiotic stress responses, one of their most widely recognized ecological functions is defense against biotic stresses, such as pathogens and herbivores (Constabel et al., 2014; Marchiosi et al., 2020; Li et al., 2021; Ahammed et al., 2023; Iqbal and Poor, 2024). Despite these differences, the two species shared a conserved set of flavonoid glycosides, underlining their importance in mangrove adaptation to abiotic stresses (Di Ferdinando et al., 2011). For instance, both species contained quercetin 3-O-hexoside, and additional flavonoid glycosides with kaempferol or isorhamnetin aglycones were identified in both taxa.

Taken together, this thesis makes a significant contribution to the field of mangrove bioprospecting. Fist, it expands the phytochemical knowledge of two species from underexplored regions, providing the fist detailed analyses of tissues such as pneumatophores, which have rarely been studied before. Through this work, several compounds were tentatively identified for the first time in these species, with more than 20 compounds reported here for the first time from mangroves in general, substantially enriching the chemical diversity known for

this group of plants. Importantly, by integrating detailed phytochemical profile with antioxidant and cytotoxic investigation, this study not only identified the most promising extracts but also began to establish preliminary links between tissue-specific metabolite classes and observed bioactivities.

For *A. marina*, the root extract emerged as the most promising candidate, particularly for its cytotoxic effects, which are likely related to its enrichment in triterpene saponins. Building on these findings, future research will focus on bioactivity-guided fractionation, with an emphasis on isolating the triterpene saponin-rich fraction to enhance therapeutic efficacy and selectivity. These purified fractions will be tested alongside standard anticancer drugs (e.g., doxorubicin), while mechanistic studies, including apoptosis assays, cell cycle analysis, and molecular pathway investigations, will clarify their modes of action. Targeted quantification and structural elucidation will provide further insights into structure-activity relationships and may lead to the discovery of novel anticancer candidates for *in vivo* evaluation. In the case of *S. caseolaris*, both root and leaf extracts exhibited strong antioxidant activity, most likely attributable to their high polyphenolic content. Future work should focus on fractionation and bioassay-guided studies to identify the most active fractions, as well as expanding investigations into other pharmacological properties. Given the limited anticancer studies to date, cytotoxic assays will be particularly relevant but extracts and fractions should also be tested against other pharmacological targets of interest, such as antidiabetic, anticholinesterase, and antimicrobial activities. Such work will be crucial to fully realize the pharmaceutical potential of this species.

In conclusion, this work provides both new knowledge and a methodological framework for the integrative study of mangrove phytochemistry and pharmacology. By bridging underexplored ecosystems and species, it underscores the importance of mangroves as reservoirs of chemical diversity with significant promise for future drug discovery.

6.2. REFERENCES

Ahammed, G. J., Wu, Y., Wang, Y., Guo, T., Shamsy, R., & Li, X. (2023). Epigallocatechin-3-Gallate (EGCG): A unique secondary metabolite with diverse roles in plant-environment interaction. Environmental and Experimental Botany, 209, 105299.

Al-Mur, B. A. (2021). Biological activities of *Avicennia marina* roots and leaves regarding their chemical constituents. Arabian Journal for Science and Engineering, 46(6), 5407-5419.

Chowdhury, R., Bhuia, M. S., Al Hasan, M. S., Hossain Snigdha, S., Afrin, S., Büsselberg, D., et al. (2024). Anticancer potential of phytochemicals derived from mangrove plants: Comprehensive mechanistic insights. Food Science & Nutrition, 12(9), 6174-6205.

Di Ferdinando, M., Brunetti, C., Fini, A., & Tattini, M. (2011). Flavonoids as antioxidants in plants under abiotic stresses. Abiotic stress responses in plants: metabolism, productivity and sustainability, 159-179.

Esau, L., Sagar, S., Bajic, V. B., & Kaur, M. (2015). Autophagy inhibition enhances the mitochondrial-mediated apoptosis induced by mangrove (*Avicennia marina*) extract in human breast cancer cells.

Falahi, H., Sharifi, M., Maivan, H. Z., & Chashmi, N. A. (2018). Phenylethanoid glycosides accumulation in roots of Scrophularia striata as a response to water stress. Environmental and Experimental Botany, 147, 13-21.

Fang, Z., Sun, K., Liu, X., Zhou, H., Song, W., Chen, H., & Wei, S. (2019). Structural characterization of ellagitannin-rich fractions from leaves of three *Sonneratia* species, and their antioxidant activity and α-amylase inhibitory effect and mechanism. International Journal of Food Properties, 22(1), 1760-1772.

Karami, L., Majd, A., Mehrabian, S., Nabiuni, M., Salehi, M., & Irian, S. (2012). Antimutagenic and anticancer effects of *Avicennia marina* leaf extract on *Salmonella typhimurium* TA100 bacterium and human promyelocytic leukaemia HL-60 cells. Sci. Asia, 38(4), 349-355.

Li, X., Zhang, J., Lin, S., Xing, Y., Zhang, X., Ye, M., et al. (2022). (+)-Catechin, epicatechin and epigallocatechin gallate are important inducible defensive compounds against *Ectropis grisescens* in tea plants. Plant, Cell & Environment, 45(2), 496-511.

Marchiosi, R., dos Santos, W. D., Constantin, R. P., de Lima, R. B., Soares, A. R., Finger-Teixeira, A., et al. (2020). Biosynthesis and metabolic actions of simple phenolic acids in plants. Phytochemistry Reviews, 19(4), 865-906.

Nabeelah Bibi, S., Fawzi, M. M., Gokhan, Z., Rajesh, J., Nadeem, N., RR, R. K., et al. (2019). Ethnopharmacology, phytochemistry, and global distribution of mangroves—A comprehensive review. Marine drugs, 17(4), 231.

Patra, J. K., & Thatoi, H. N. (2011). Metabolic diversity and bioactivity screening of mangrove plants: a review. Acta Physiologiae Plantarum, 33(4), 1051-1061.

Peter Constabel, C., Yoshida, K., & Walker, V. (2014). Diverse ecological roles of plant tannins: plant defense and beyond. Recent advances in polyphenol research, 115-142.

Peter Constabel, C., Yoshida, K., & Walker, V. (2014). Diverse ecological roles of plant tannins: plant defense and beyond. Recent advances in polyphenol research, 115-142.

Sarri, E., Termentzi, A., Abraham, E. M., Papadopoulos, G. K., Baira, E., Machera, K., et al. (2021). Salinity stress alters the secondary metabolic profile of M. sativa, M. arborea and their hybrid (Alborea). International Journal of Molecular Sciences, 22(9), 4882.

Sohaib, M., Al-Barakah, F. N., Migdadi, H. M., & Husain, F. M. (2022). Comparative study among *Avicennia marina*, *Phragmites australis*, and *Moringa oleifera* based ethanolic-extracts for their antimicrobial, antioxidant, and cytotoxic activities. Saudi journal of biological sciences, 29(1), 111-122.

Syamsul, E. S., Umar, S., Wahyuni, F. S., Martien, R., & Hamidi, D. (2022). Anti-aging activity, in silico modeling and molecular docking from *Sonneratia Caseolaris*. Open Access Macedonian Journal of Medical Sciences, 10(A), 1471-1477.

Wu, J. Y., Wong, K., Ho, K. P., & Zhou, L. G. (2005). Enhancement of saponin production in Panax ginseng cell culture by osmotic stress and nutrient feeding. Enzyme and Microbial Technology, 36(1), 133-138.

APPENDIX

Peer-reviewed papers published during the PhD program

Phytochemistry and pharmacological potential of the mangrove plant *Sonneratia caseolaris*: a comprehensive review

Cerri, F. and Paolo, G.

Marine Drugs, 2025, 23, 378

ABSTRACT

Mangroves represent a promising yet underexplored source of natural products. *Sonneratia caseolaris* (mangrove apple) is a widely distributed species with a long history of use in traditional medicine, and it is receiving increasing recognition for its bioactive secondary metabolites. Research has expanded in recent decades, but findings remain dispersed across diverse sources, complicating interpretation of its chemistry and pharmacological potential. This review consolidates four decades of investigations, documenting 141 identified compounds from studies largely restricted to India, Bangladesh, Indonesia, and China and focusing on leaves, fruits, bark, stems, and twigs, with roots notably unexplored. The phytochemical profile is dominated by phenolic acids, flavonoids, and tannins, alongside terpenoids, steroids, fatty acids, fatty alcohols, aldehydes, hydrocarbons, and polysaccharides. The most extensively studied activities are antioxidant and antimicrobial, with extracts consistently exhibiting strong free-radical scavenging capacity and broad-spectrum antibacterial and antifungal effects, including efficacy against drug-resistant strains. Additional reports describe central nervous system depressant, antidiarrheal, metabolic, anti-inflammatory, analgesic, antipyretic, and anti-allergic activities. In contrast, anticancer investigations remain scarce, despite promising outcomes reported for related mangrove taxa. By consolidating and critically evaluating the existing evidence, this review highlights the pharmacological potential of *S. caseolaris* and identifies key knowledge gaps to guide future marine drug discovery.

Phytochemical profiling, antioxidant activity, and *in vitro* cytotoxic potential of mangrove *Avicennia marina*

Cerri, F., De Santes, B., Spena, F., Salvioni, l., Forcella, M., Fusi, P., Pagliari, S., Stahl, H., Galli, P., Colombo, M., Giustra, M., Campone, L.

Pharmaceuticals, 2025, 18, 1308

ABSTRACT

Background: *Avicennia marina* (Forsk.) Vierh., a widely distributed mangrove species, is known for its diverse secondary metabolites with potential pharmacological applications. Despite its dominance in the Arabian Gulf, where *A. marina* may have adapted to extreme environmental conditions with a distinct set of bioactive molecules, research in this region remains limited. Methods: This study investigates the phytochemical composition, antioxidant activity, and *in vitro* cytotoxicity of extracts from different plant parts, including roots, leaves, propagules, pericarps, and cotyledons, collected in the United Arab Emirates (UAE). Extracts were analyzed using ultra-pressure liquid chromatography coupled with high-resolution mass spectrometry (UPLC-HRMS). Antioxidant activity was assessed using DPPH and ABTS assays, while cytotoxicity was evaluated against human cancer and normal cell lines. Results: Analysis revealed 49 compounds, including iridoid glycosides, hydroxycinnamic acids, phenylethanoid glycosides, flavonoid glycosides, and triterpene saponins, several reported for the first time in *A. marina* and mangroves. The pericarp and root extracts exhibited the highest scavenging activity (DPPH: 187.14 ± 2.87 and 128.25 ± 1.12; ABTS: 217.16 ± 2.67 and 147.21 ± 2.42 μmol TE/g, respectively), correlating with phenylethanoid content. The root extract also displayed the highest cytotoxicity, with IC50 values of 58.46, 81.98, and 108.10 μg/mL against MDA-MB-231, SW480, and E705, respectively. *In silico* analysis identified triterpene saponins as potential contributors. Conclusions: These findings highlight the root extract of *A. marina* as a promising source of bioactive compounds with potential antioxidant and anticancer applications, supporting further exploration for novel therapeutic candidates.

The benthic marine algae of the Maldives: historical insights into their diversity and distribution

Nicolai, R., Zuccarello, G.C., Karsten, U., Louis, Y.D., Cerri, F., Senna, G., Mohamed, S., Galli, P.

Botanica Marina, 2025, 68, 101–111

ABSTRACT

In tropical ecosystems worldwide, benthic marine algae are important primary producers and habitat providers for many juvenile fish and invertebrate species. Calcified species are known to provide structural support to their respective communities, thus enhancing the overall system's productivity. In the Republic of the Maldives, algae are an important yet currently poorly studied biological resource. We reviewed the literature around algal diversity and distribution across Maldivian atolls and compiled an extensive and updated taxonomic list. The list contains 353 species, of which 31 are Cyanobacteria, 26 Phaeophyceae, 109 Chlorophyta, and 187 Rhodophyta. Algal collections have been reported from 12 out of 20 atolls, and these mostly occurred during 20th century expeditions. The taxonomic status of 110 species has changed since first reported. While several species have been documented from the country, identifications have thus far almost solely relied on morphological assessments. Many of the reported algal groups require molecular confirmation. This suggests that benthic algal diversity from the Maldives is likely an underestimate. Since anthropogenic activities can significantly alter algal community dynamics, a baseline understanding of algal diversity is necessary to determine how such shifts affect the ecosystem as a whole, thus underpinning future management and conservation efforts.

Mangrove forests as a natural trap for marine plastic litter: insights from the Maldives

Cerri, F., Mohamed, S., Galli, P.

Marine Pollution Bulletin, 2025, 213, 117677

ABSTRACT

Plastic pollution poses a significant threat to coastal ecosystems, including mangroves, which naturally trap debris due to their complex, three-dimensional structures. In the Maldives, inadequate plastic waste management exacerbates the accumulation of plastic in these critically endangered ecosystems, which are characterized by unique morphologies consisting of small patches with tide-influenced water bodies. Despite their ecological and socio-economic importance, mangroves in the archipelago have remained undocumented in terms of plastic pollution. This micro article presents the first evidence of plastic debris in Maldivian mangroves with accumulation observed on four islands dominated by species like *Ceriops tagal*, *Bruguiera cylindrica*, *Rhizophora mucronata*, and *Pemphis acidula*. The high tree density and the three-dimensional structure of these mangroves act as natural traps for marine litter, particularly single-use PET water bottles. These findings underscore the urgent need for conservation efforts and waste management policies to prevent further degradation and ensure their long-term sustainability.

Are coexisting mangrove-coral assemblages missing in the Maldives?

Louis, Y.D., Cerri, F., Galli, P.

Marine Biodiversity, 2025, 55, 3

DESCRIPTION

Mangrove-associated coral assemblages, where corals grow on or among mangrove roots, are recognized as potential climate refugia but remain understudied in the Maldives. This study surveyed eight embayment mangrove sites across four Maldivian atolls (Baa, Kaafu, Laamu, and Seenu) from June 2023 to April 2024 to assess the presence of coexisting mangrove-coral (CMC) assemblages. Rapid visual surveys along mangrove edges and within 2–5 m inland revealed a complete absence of corals and minimal occurrence of other fouling organisms, including barnacles, oysters, and sponges. Observed environmental conditions—including extreme tidal fluctuations, high water temperatures (31–38 °C), elevated pH (7.82–8.70), variable turbidity, and soft, unstable substrates—likely inhibit coral settlement and growth. These findings suggest that, unlike other global regions with documented CMC assemblages, Maldivian embayment mangroves do not currently support coral colonization. The study highlights a significant ecological pattern and underscores the need for future research to investigate permanently submerged mangrove habitats, long-term environmental monitoring, and potential antibiofouling mechanisms. Documenting the absence of CMC assemblages provides critical baseline data for understanding global coral-mangrove interactions and refining predictive models of coral refugia.

Mangroves of the Maldives: a review of their distribution, diversity, ecological importance and biodiversity of associated flora and fauna

Cerri, F., Louis, Y.D., Fallati, L., Siena, F., Mazumdar, A., Nicolai, R., Zitouni, M.S., Adam, A.S., Mohamed, S., Lavorano, S., Galli, P.

Aquatic Sciences, 2024, 86, 44

ABSTRACT

Mangrove forests are one of the most important biological, ecological and economic ecosystems in the world. In the Maldives, they play a crucial role in maintaining coastal biodiversity, providing ecosystem services, such as coastal protection, and supporting livelihoods by providing income and food. Overall, 23 Maldivian islands have at least 1 protected mangrove area. However, knowledge of the mangroves of the Maldives is scarce, scattered and sometimes conflicting. There is a lack of information on a national scale regarding their distribution, diversity, ecological importance and associated biodiversity. The aim of this review is to analyse scientific publications, reports, and online documents on mangroves for the entire Maldivian archipelago to provide the first comprehensive summary of the current state of knowledge of mangroves from a national perspective. This includes the geographical location of mangrove forests, the identity and distribution of mangrove species, ecosystem services, ecological importance and diversity of mangrove-associated flora and fauna. We analyzed available information from both the grey literature and scientific publications and found that 14 mangrove species have been documented on 108 islands (9% of all Maldivian islands). Mangroves are mainly concentrated in northern atolls and are associated with diverse flora and fauna. Furthermore, we identified inconsistencies and gaps in the literature and proposed future directions for research. This is crucial for informed decision-making, developing effective conservation strategies and long-term sustainability of mangrove ecosystems.

Natural products from mangroves: an overview of the anticancer potential of *Avicennia marina*

Cerri, F., Giustra, M., Yaprak, A., Tomaino, G., Galli, P., Labra, M., Campone, L., Colombo, M.

Pharmaceutics, 2022, 14, 2793

ABSTRACT

Exploring the potential of natural extracts for pharmaceutical applications in the treatment of different diseases is an emerging field of medical research, owing to the tremendous advantages that they can offer. These include compound sustainability due to the natural origin and virtually unlimited availability. In addition, they contribute to promoting the countries in which they are extracted and manufactured. For this reason, wild active compounds derived from plants are attracting increasing interest due to their beneficial properties. Among them, *Avicennia marina* has been recently recognized as a potential source of natural substances with therapeutic activities for anti-cancer treatment. *A. marina* beneficially supplies different chemical compounds, including cyclic triterpenoids, flavonoids, iridoids, naphtaquinones, polyphenols, polysaccharides, and steroids, most of them exhibiting potent antitumor activity. The *in vivo* and *in vitro* studies on different models of solid tumors demonstrated its dose-dependent activity. Moreover, the possibility to formulate the *A. marina* extracted molecules in nanoparticles allowed researchers to ameliorate the therapeutic outcome of treatments exploiting improved selectivity toward cancer cells, thus reducing the side effects due to nonspecific spread.

Cytotoxic compounds from Alcyoniidae: an overview of the last 30 years

Cerri, F., Saliu, F., Maggioni, D., Montano, S., Seveso, D., Lavorano, S., Zoia, L., Gosetti, F., Lasagni, M., Orlandi, M., Taglialatela-Scafati, O., Galli, P.

Marine Drugs, 2022, 20, 134

ABSTRACT

The octocoral family Alcyoniidae represents a rich source of bioactive substances with intriguing and unique structural features. This review aims to provide an updated overview of the compounds isolated from Alcyoniidae and displaying potential cytotoxic activity. In order to allow a better comparison among the bioactive compounds, we focused on molecules evaluated *in vitro* by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, by far the most widely used method to analyze cell proliferation and viability. Specifically, we surveyed the last thirty years of research, finding 153 papers reporting on 344 compounds with proven cytotoxicity. The data were organized in tables to provide a ranking of the most active compounds, to be exploited for the selection of the most promising candidates for further screening and pre-clinical evaluation as anti-cancer agents. Specifically, we found that (22S,24S)-24-methyl-22,25-epoxyfurost-5-ene-3β,20β-diol (16), 3β,11-dihydroxy-24-methylene-9,11-secocholestan-5-en-9-one (23), (24S)-ergostane-3β,5α,6β,25 tetraol (146), sinulerectadione (227), sinulerectol C (229), and cladieunicellin I (277) exhibited stronger cytotoxicity than their respective positive control and that their mechanism of action has not yet been further investigated.

AKNOWLEDGMENTS

I would like to express my deepest gratitude to my Tutor, Prof. Paolo Galli, who trusted in my abilities and gave me the opportunity to undertake this PhD journey. He has been a mentor and academic reference point since my bachelor's thesis, continuing through my master's and into this doctoral program. His guidance, marked by trust, thoughtful advice, encouragement, constructive criticism, and the freedom to develop independently, has shaped me both academically and personally. Moreover, his experience, network of collaborations, and research initiatives has bene instrumental in enabling me to complete this work.

I'm sincerely grateful to my Supervisor, Prof. Miriam Colombo, for her valuable contribution, advice, and continuous support to this project; to Prof. Luca Campone and Dr. Stefania Pagliari for their crucial expertise and guidance throughout the chemical analyses; and to Prof. Paola Fusi and Dr. Matilde Forcella for their essential support with the cellular experiments. Without their laboratories, materials, and instrumentation, this project would not have bene possible. I also wish to thank all the other collaborators involved in this work for their theoretical and experimental contributions.

My sincere thanks also go to all the MaRHE Center team and my colleagues for their valuable advice, insightful discussions, and, above all, for sharing much of the daily life at the university, helping to make the most intense periods lighter. A special acknowledgment to Yohan, Giulia, and Rossella, which whom I shared fieldwork during the challenging yet unbelievable and unforgettable expeditions to the Maldivian mangroves. Only those who have experienced such work firsthand can truly understand the physical and logistical effort it requires. Despite the challenges, our "mangrove team" always managed to organize effectively and overcome every difficulty, leading to extensive sampling and remarkable discoveries.

Outside the university, my deepest gratitude goes to my family, who have supported me constantly throughout these years. They have always believed in me, stood by every decision I made, and proudly celebrated every milestone I achieved. Their unconditional love, trust, and, above all, the sense of serenity they have given me have been fundamental throughout this journey.

To my partner, Ele, who, together with my family, has been my greatest supporter, thank you for being by my side through both the successes and the setbacks that inevitably accompany this journey. Your invaluable love, support, patience, and understanding have bene essential in

keeping me motivated through the most intense phases of this PhD. In the last year, as both my partner and colleague, you have also helped and inspired me in my work.

Finally, I would also like to thank my closest friends for their loyal presence and friendship over these years. They have always shown genuine interest in my journey, listening to my stories with their characteristic irony and humour.

La borsa di dottorato cofinanziata con risorse dell'Unione europea-*NextGeneration EU*
Piano Nazionale di Ripresa e Resilienza Missione 4 – Componente 1 – Riforma 4.1 Riforma dei Dottorati – Inv. 4.1
Borse PNRR patrimonio Culturale – CUP H41J22000160002