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Phytochemical analysis and antimicrobial properties of Rhizophora species: A Review

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Abstract:
Rhizophora are a type of plant that are vital in the marine ecosystem and may be found along the coasts of many different countries. Rhizophora are an important part of the pharmaceutical industry because of the abundance of several kinds of phytochemicals they contain. Only 5% of the world's forested land is covered by rhizophora forests, which are home to salt-tolerant vegetation confined to coastal areas. Extremely few plant species are adapted to these conditions, which include a highly variable tidal and salty regime. However, coastal communities benefit economically and ecologically from these plants. Many rhizophora species have been employed in folk medicine, and others have limited utility as insecticides and pesticides. Rhizophora produce a vast variety of natural chemicals with unusual bioactivity, making them biochemically distinct. Rhizophora contain a wide variety of chemical groups represented by their active metabolites, including alkaloids, phenols, steroids, terpenoids, tannins, etc. This article summarizes recent research on the antimicrobial, and phytochemicals identified from rhizophora and their associates.

***Key words**: Rhizophora, phytochemical analysis, antimicrobial studies, plant species.*

1. Introduction

Rhizophora have, for some time now, captured the interest of members of the general public as well as those in the scientific community on account of the breadth of their application in fields such as industry and medicine (Kusmana et al. (2016)) [1]. Because they are so frequently found in the intertidal zones of tropical and also subtropical nations, they have been given the scientific name halophytes, which refers to plants that are able to thrive in saline settings (Mitra et al. (2021)) [2]. A genus of trees and shrubs known as Rhizophora grows in saline marshes and tidal areas all along protected tropical and subtropical coastlines. Rhizophora can be found in both tropical and subtropical regions. Their taxonomic classifications encompass a diverse array of animal and plant life. Rhizophora are able to grow in salty environments having daily inundation across average sea level along with the greatest astronomical tides; so, they are vital in the functioning of ecosystems (Setyawan et al.(2019)) [3], and they supply key habitats and also food for all similarly suited resident and also migratory species. Rhizophora are able to thrive inside saline environments having daily inundation across average sea level and also the greatest astronomical tides. Rhizophora are able to flourish in environments that experience daily inundation that ranges from mean sea level to the greatest astronomical tides. Due to the characteristics of their natural environment, Rhizophora are able to resist conditions, like high salinity, along with strong winds, and also gradual tidal shifts, high temperatures, and also anaerobic tidal swamps (Srikanth et al. (2015)) [4]. Thus, rhizophora serve as a habitat for a diverse range of microorganisms and macroorganisms, which result in the production of an abundance of beneficial bioactive compounds and enzymes (Thatoi et al (2013)) [5]. All living things, including Rhizophora’s extensive supporting roots, Avicennia’s breathing roots, salt-excreting leaves, and also the water-dispersing viviparous seedlings, produce bioactive metabolites. These bioactive metabolites serve as 'chemical signals' that enable living things to reply to, and also avoid, or even defend versus environmental cues (Mitra et al. (2021)) [2]. Since ancient times, people have used rhizophora as a source of food, fuel, charcoal, building materials, furniture, boats, and fishing gear. Rhizophora can be found in many different environments. Tannins, which are utilized in the process of leather manufacturing and coloring, can also be extracted from rhizophora and used as a source of tannins (Habeebula et al. (2018)) [6]. The term "phytochemical" comes from the Greek word "phyto," which literally translates to "plant." Phytochemicals are the non-nutritive, biologically active components of plants. Traditional medical practitioners have for a very long time relied on their extensive knowledge of native plants and other natural products as a bio-resource of pharmaceuticals to treat various disorders, which ultimately resulted in the development of the notion of modern medicine. It was discovered that the utilization of rhizophora plants is associated to the production of bioactive substances as well as amino acids, along with carbohydrates, and also proteins, all of which are necessary for life preservation. Numerous diterpenoids, sesquiterpenes, triterpenoids, daucosterol, palmitone, atranorin, polymeric tannins, polyphenols, and hydrolyzable tannins are among the many phytochemical compounds present in Rhizophora species (e.g., Rhizophora apiculata, R. mangle, R. mucronata, and R. stylosa) that have significant medicinal potential. Extracts from various Rhizophora species have been credited with various pharmacological actions (Sormin et al (2020)) [7], including anti-inflammatory, anti-ulcer, anti-ulcerative, anti-inflammatory, antiseptic, and antibacterial characteristics. Rhizophora plants can yield various bioactive compounds, such as alkaloids, along with flavonoids, and also phenolics, along with tannins, and also terpenoid saponin metabolites (Lalitha et al. (2021) [8]. This review aims to deliver in-depth comprehension of the phytochemical profiles, as well as the antimicrobial activity, of several Rhizophora species.

2. Phytochemistry of Rhizophora species

The chemicals obtained from Rhizophora species' leaves, flowers, fruits, stems, and twigs in the study done by Kalasuba et al. (2023) [9]. To obtain the extract for the isolation process, many different solvents were used, such as n-hexane, chloroform, ethyl acetate, methanol, petroleum ether and n-butanol. A crude extract is made by grinding the dried sample in a 1:1 mixture of $CHCl_3$ and MeOH at room temperature, then diluting it with water and separating it into different solvents. The rough extract was cleaned up using a number of different techniques, such as Sephadex LH-20, column chromatography on RP-18 silica gel, and semipreparative HPLC on an RP-18 column. The chemicals were identified using a variety of techniques, including NMR, mass spectrophotometry, UV,

polarimetry, and FTIR. These chemical reactions yielded compounds with triterpenoid, lignan, steroid, megastigmadien, alanine-derived monoterpenoid, apoc-arotenoid, aromatics/phenolics, fatty acid, flavonoid, and secondary alcohol groups [9].
In the investigation done by Sachithanandam et al. (2022), [10] ten distinct Rhizophora leaf methanolic extracts were first analysed to determine the types of phytochemicals present, then the antibacterial, antioxidative, and anticancer activities of each extract were determined. The *Rhizospora mucornata* effective methanolic crude extract was further refined and characterized to look for the existence of the bioactive component. The bioactive component was determined to be 1,4-dihydroanthraquinone based on the results of UV-visible spectroscopy, NMR, FTIR, and HRMS analyses. Quinizarin molecule had greater binding affinity (6.2 kcal/mol) and substantial structural stability toward anti-apoptotic Bcl-2 protein, as described by the combined findings of molecular docking and also molecular dynamics simulation experiments. As a result, the study suggested that the naturally occurring chemical known as quinizarin, which was isolated from *R. mucornata*, could play an important part in the progress of therapeutic medications to treat bacterial infections and cancer. With the sequential extraction method, the leaves of Rhizophora apiculata, Rhizophora mucronata, and Rhizophora stylosa were taken out of the plant by Bulan et al. (2022) [11]. Based on the results of the phytochemical screening, the ethyl acetate extract has a wider range of molecules than the methanol extract. Anti-DPPH radical action was found in both methanol extracts and ethyl acetate. All of the samples underwent a qualitative analysis of the primary phytochemical properties, including alkaloids, along with anthocyanin, as well as cardiac glycoside, phenols, saponins, and also flavonoids, steroids, tannins, and also terpenoids. Also, in R. mucronata’s methanol extract, there were found to be alkaloids, cardiac glycosides, saponins, tannins, phenols, and steroids. Terpenoids were also present. On the other hand, there were no anthocyanins or flavonoids present.

The methanol extract of Rhizophora stylosa does not have any anthocyanins, cardiac glycosides, or saponins in it. In the meantime, there was evidence of the presence of saponins, alkaloids, and phenols within R. apiculata’s methanol extract. R. mucronata’s ethyl acetate extract did not contain any anthocyanins or cardiac glycosides. However, R. stylosa’s and R. apiculata’s ethyl acetate extract included virtually all phytochemicals, with the exception of anthocyanins. The outcomes of the preliminary phytochemical screening displayed that the ethyl acetate extract included a wider diversity of chemical components than the methanol extract did, in particular for R. stylosa and also R. apiculate species. The phytochemical qualities of each plant differed in response to the two different extractive solvents..

R. stylosa was found to contain the highest concentration of flavonoids compared to any other secondary metabolite and Yang et al. (2008) [12] were the first researchers to discover astilbin and also rutin in the leaves of R. stylosa [12]. In addition, Huong et al. (2014) [13] were able to separate kaempferol 3-rutinoside from the plant's leaves. Rhizophora mucronate and Rhizophora apiculata are both types of mangrove plants that are members of the family Rhizophoraceae. The investigation that was carried out by Vittaya et al. (2022) [23] was carried out with the intention of determining the bioactive phytochemical components of these two species of mangrove plants. Maceration in a solvent composed of methanol made it possible to prepare eight separate extracts from each of the four different components of each plant (the pod, the twig, the leaf, and also the bark). The phytochemical analysis occured with the assistance of a standard test, and the results were validated by quantitative analysis. Also, the quantitative analysis was based on the configuration of the total phenolic, saponin, and flavonoid contents of the sample.

3. Rhizophora plants' biological activity:

Antibacterial, antioxidant, anticancer, cytotoxic, antiproliferative, insecticidal, antimalarial, anti-fungal, antifeedant, antidiarrheal, central nervous system depressant, antimitotic, antileukemic, and anti-plasmodial activities are some of the biological properties possessed by rhizophora plants as suggested by Subekti, et al. (2019) [14].

3.1. Beneficial antibacterial activity of Rhizophora Species

Antibiotic resistance in many bacteria is becoming increasingly common, and the negative effects that are connected with the use of antibiotics are also a big barrier in the way of treating infectious diseases. As a result, the research and development of new antibacterial chemicals have become crucially important goals. There has been a significant amount of research conducted on the antibacterial properties of mangroves due to the fact that these trees display highly bactericidal effects against various infectious agents. Antibacterial action has been discovered in vitro as a result of the high concentration of secondary metabolites found in these plants, suggested by Mitra et al (2021) [15]. R. stylosa bark's chloroform extracts exhibited the toughest antibacterial capabilities related to the leaf extracts by Muafi et al. (2014) [16]. They had MIC values of 0.1 mg/mL and MBC values of 6.3 mg/mL versus Gram-positive and also Gram-negative bacteria, which indicate that they are more effective against certain bacteria. The leaf extracts only had MIC values of 0.1 mg/mL. This effect is probably caused by the fact that bark extracts contain a variety of active components and phytochemicals that are distinct from those found in leaf extracts. In addition, against Escherichia coli, R. stylosa leaf extracts that had been treated with ethyl acetate had the biggest inhibitory zone, which varied from 11 to 19 millimetres [16]. It was hypothesised that these extracts would work versus a broad range of Gram-negative bacteria; hence, this raises the possibility that they may be used as a BSA to treat many diseases caused by bacteria. Also, Gopal et al. (2019) [17] discovered that R. stylosa's chloroform extract demonstrated the strongest antibacterial action against S. epidermidis, Staphylococcus aureus, S. pyogenes, Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa.

The purpose of the research performed by Saywal et al. (2020) [21] was to identify the bacterial pathogens that were inhibited by the leaf extract of the mangrove species *R. apiculata* and to identify the chemical compounds that made up that extract. In this experiment, CRD was utilized by first creating a leaf extract with the concentration levels - 12.5% (T1), along with 25% (T2), as well as 50% (T3), and also 100% (T4), as well as a positive control (T5) and a negative control (T0), with three repeats. The development of the potentially harmful bacteria was stopped by the extract, with inhibition zones: S. aureus - 5.30 - 3.10 mm, *A. hydrophila* - 6.07 - 3.30 mm, & *P. aeruginosa* - 6.33 - 3.23 mm [21].

Extraction of the bioactive components from *Rhizophora apiculata* leaf extracts was carried out by Dahibhate et al. (2020) [22] via the Soxhlet technique along with methanol (MtOH) serving as the organic solvent. Also, the extracts were tested S. agalactiae and also E. coli growth (in isolation). For the antibacterial assay, the Kirby-Bauer test was utilized, and an in vitro experiment was carried out for the determination of the MIC and MBC. The toxicity of R. apiculata was determined using a lethality assay with brine shrimp. The antibiotic susceptibility test revealed that E. coli had a high level of resistance to neomycin, but that it was sensitive to tetracycline and amoxycilin. On the other hand, S. agalactiae was seen to be sensitive to all antibiotics. R. apiculata leaf extracts demonstrated inhibition zones against the microorganisms ranging 2 to 9 millimeters in size. Increasing the concentration of plant extracts is necessary for expanding the inhibitory zone. The minimum inhibitory concentration (MIC) testing range of R. apiculata against S. agalactiae and E. coli was 6.25 mg/ml to 12.5 mg/ml, respectively. Bactericidal action was observed in S. agalactiae's MBC/MIC ratio, whereas bacteriostatic action was observed in E. coli's MBC/MIC ratio. The LC50 value for R. apiculata was determined to be 81 mg/ml. According to this study's conclusions, Rhizophora apiculata could be an important contender in the search for antimicrobial compounds that are effective against fish infections.

In vitro research was conducted by Vittaya et al. (2022) [23] using DPPH and also ABTS techniques to investigate free radical scavenging activity. The hole-plate diffusion approach was used to test for the presence of antibacterial activity, and both the MIC and the min. bactericidal concentration were determined. R. mucronata's pod predominantly exhibited the highest overall saponin content [8.05 0.50 mg escin equivalent (EE)/g CE], whereas the bark displayed a great quantity of phenolic and also overall flavonoid contents (2.12 0.11 mg gallic acid equivalent/g CE and 6.73 0.25 mg RU/g CE). However, R. mucronata's pod predominantly showed the highest overall saponin content. At concentration - 100 g/ml, R. mucronata shown a higher capacity for scavenging free radicals than Rhizophora apiculata did. This was demonstrated by the DPPH and ABTS tests. Antibacterial testing revealed that R. mucronata extracts exhibited the greatest zone of inhibition against Aeromonas hydrophila, Streptococcus agalactiae, Vibrio harveyi, and also Vibrio parahemolyticus at a wavelength range of 8.50–13.56 nm. Only the A. hydrophila pod extract had a lower MIC than the R. mucronata pod extract did. In contrast, the minimum inhibitory concentration (MIC) of the R. mucronata bark extract was the lowest of all those tested for S. agalactiae and A. hydrophila. The stronger antibacterial action of R. mucronata extracts was stable with the increased saponin, along with phenolic, and also flavonoid contents, as well as the higher antioxidant action of the extracts of these species, which had a significant value of p less than 0.05. According to the findings of this study, R. mucronata has the potential to serve as a source of bioactive chemicals that combat the bacteria that cause disease in aquatic environments.

The purpose of the investigation carried out by Thorati et al. (2017) [24] was to investigate the antibacterial properties of an extract of an endophytic fungus called Aspergillus niger that had been isolated from the stilt roots of Rhizophora apiculata. The ethyl acetate extract of the fungus species was assayed for bioactivity against five human pathogenic bacterial strains Staphylococcus aureus (MTCC96), Micrococcus luteus (MTCC106), Pseudomonas aeruginosa (MTCC326) and Enterococcus faecalis (MTCC439), Proteus mirabilis (MTCC1429) by disc diffusion method. The sensitivity of the strains ranged from P. mirabilis, which was the most sensitive, through P. aeruginosa, M. leuteus, S. aureus, and E. faecalis in that order. P. mirabilis was shown to be the most sensitive. It was suggested that both gram positive and negative bacterial strains responded to the action of the crude extract by demonstrating the action zone with a range of 23mm to 15mm at concentrations of 50l Âµl and 100l Âµl, respectively. The presence of terpenoids, saponins, and proteins in the crude extract, leads one to believe that these biomolecules have the potential to function as antibacterial substances.

In the course of research by Gopal et al. (2017), [17] the leaves and barks of the Rhizophora stylosa plant were gathered and then extracted with a variety of organic solvents including methanol, chloroform, and petroleum ether. The antibacterial action of these crude extracts was tested against a variety of bacteria, like Staphylococcus aureus, along with Staphylococcus epidermidis, and also Streptococcus pyogenes, along with Escherichia coli, as well as Klebsiella pneumoniae, and also Pseudomonas aueroginosa, using a variety of different methodologies. R. stylosa's bark extracts were shown to possess a higher medicinal value than leaf extracts. The antibacterial action of the chloroform extracts was found to be the highest, with MIC - 0.1 mg/ml and a maximum inhibitory concentration (MIC) of 6.3 mg/ml. I Duraipandian et al (2020) [25] found that the presence of terpenoids, phenolics, and alkaloids contributed to the plant's A. officinalis, A. marina etc potential antibacterial action. Therefore, the pharmacological investigation of natural compounds has the potential to be a wellspring of an infinite number of therapeutic medicines.

4. Other Biological Activity

4.1. Antiviral rhizophora: Extracts from rhizophora plants have been shown to be effective against several different types of viruses. They were effective against a wide variety of human, animal, and plant pathogens, such as the HIV, and also Tobacco Mosaic virus, along with Hepatitis-B viruses, and also Encephalomyocarditis virus found that certain rhizophora species, especially those in the family Rhizophoraceae, exhibit potent anti-viral activity. Antiviral properties have been attributed to compounds and derivatives identified from rhizophora plants and their allies. Acid polysaccharides (galactose, galactosamine, glucose, and arabinose) and other purified active fractions exhibit significant anti-HIV activity, suggested by Mitra et al, (2021) [15].

4. Conclusion:

The Rhizophora woods that are found in the intertidal zone of tropical and also subtropical locations all over the world are extremely valuable coastal resources. The global community benefits from these ecosystems in a variety of ways, including receiving a diverse range of resources, services, and goods. On the other hand, the capabilities of these plants have not been harnessed to their utmost potential. The current article elucidates the early compounds that were extracted from a variety of rhizophora plants. These chemicals have the potential to be beneficial antimicrobial treatments in the future. Therefore, a more in-depth grasp of science is required in order to unveil the potential of phytochemicals that can be acquired from rhizophora. The overview of this article can help in discovery of novel biological substances that have applications in the various field such as medicinal chemistry, pharma industry, science filed for welfare of humans.

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