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Integrated Phytochemical Characterization and Biological Activity Assessment of Rhizophoraapiculata Leaf Extract: Anti-inflammatory, Antioxidant, and Antibacterial Potentials

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The study investigates the bioactive properties of the mangrove Rhizophoraapiculata, focusing on its antimicrobial, anti-inflammatory, and antioxidant activities. Mangroves, including Rhizophoraapiculata, are crucial for their ecological and medicinal significance. Qualitative phytochemical analysis revealed bioactive compounds such as flavonoids, tannins, glycosides, phenolic compounds, and saponins are present. The anti-inflammatory potential was evaluated through protein denaturation with a maximum inhibition of 96.05% at 100 µg/ml. Antioxidant activities were measured using DPPH, H202, and TAA. The extract demonstrated notable antioxidant activity, with a DPPH scavenging activity of 83.45% and H202 activity of 93.47% at 100 µg/ml. The TAA results confirmed significant antioxidant potential with increasing concentrations. Antimicrobial activity, assessed by the disc diffusion method against Escherichia coli and Streptococcus mutans, showed a concentration-dependent[1] inhibition, with the maximum inhibition of zones at 100 µg/ml. (FTIR) identified functional groups, including alcohols, esters,

alkanes, amines, and halo compounds, corroborating the presence of bioactive constituents. This research emphasizes Rhizophoraapiculataas a biosource of antimicrobial, anti-inflammatory, and antioxidant agents, validating its traditional medicinal use and its promise for developing new therapeutic agents.

Keywords: Mangrove, Rhizophoraapiculata, Leaf Extract, Phytochemical, Biological Activity.

1. Introduction

The intertidal zone in tropical and subtropical regions is occupied by a diverse community of halophytic plants and animals, collectively forming the intricate mangrove ecosystem. Mangroves, characterized by their salt-tolerant nature, are shrubs or trees growing in coastal saline or brackish water.Rhizophoraapiculata, widely known as the red mangrove, is a mangrove tree species native to tropical and subtropical areas (Kalasuba et al., 2023). It is an important component of coastal and estuarine ecosystems due to its ecological and economic significance. Mangrove plants have a diverse array of bioactive compounds such as steroids, tannins, alkaloids, and polyphenols, saponins, which collectively contribute to the medicinal properties inherent in mangrove vegetation (Habib et al., 2018). Rhizophoraapiculatais typically found in intertidal zones of estuaries, river mouths, and coastal areas. It is native to Southeast Asia, the western Pacific, and northern Australia (Yun et al., 2022). Rhizophoraapiculata acts as a carbon sink, sequestering substantial amounts of Co_2 and aiding in the mitigation of climate change. (Short et al., 2024).Rhizophoraapiculata extract, derived from Rhizophoraapiculata, has various applications due to its rich bioactive compounds. Tannins, resins, saponins, terpenoids are some common bioactive compounds present in the extract (Kumar et al., 2023). An extract is a concentrated substance obtained by removing or separating the active ingredients from a source material, such as a plant, using a specific solvent or method. The resulting product contains the essential compounds that provide the desired properties, such as flavor, fragrance, therapeutic benefits, or other characteristics (Aswathi et al., 2023;Selvaraj et al.,2024;Sivakumar et al). A crude extract is an unrefined mixture containing a wide range of compounds that are obtained directly from a biological material, such as plants, fungi, bacteria, or animals, without any further purification or fractionation (Abubakar et al., 2020). Rhizophoraapiculatacrude extract has proven antibacterial activity against several pathogens. This makes it a potential candidate for developing natural antimicrobial agents. It also has anti-inflammatory properties, which can be beneficial in treating conditions characterized by inflammation. The extract is traditionally used to promote wound healing. Its antimicrobial and anti-inflammatory properties aid in faster recovery and prevention of infections (Acharya et al., 2023). Plants are a vital source of potentially beneficial phytochemicals for the creation of new therapeutic agents. (Mahmud et al., 2019). The compounds hold potential in various fields like antibiotics, enzymes, vitamins, drugs, and biosurfactants (Sulaiman et al., 2022). These activities include antiferedant effects, antiviral, gastroprotective, antioxidant, antifungal, cytotoxic and inhibition, and anti-inflammatory to find its therapeutic uses (Dahibhate et al., 2019).

2. Materials and methods

2.1. Collection and extraction of mangrove Rhizophoraapiculata

The leaves of mangroveRhizophoraapiculata were gathered from the Kovalam coast coastline, Tamil Nadu, India. The samples were dried, and coarse powdered the extraction was made with ethanol. A 20 gram sample was mixed with 200 ml of 70 % ethanol in a conical flask. The sample containing the flask was kept in an orbital shaker for 3 days. Following three days the samples underwent filtration No.1 filter paper. The filtered extract was placed in a water bath at 60˚C and maintained until it reached a crude state (Fig.1).

Figure 1: Extraction of marine bioactive compounds from MangroveRhizophoraapiculata: a) Rhizophoraapiculata leaf b) Powdered sample, c) Extraction d) Filtration e)Crude Extract

2.2. Qualitative phytochemical analysis

In this study, qualitative phytochemical analysis was conducted on ethanol extracts from mangrove samples revealing bioactive compounds. The preliminary phytochemical screening of the extracts was performed following standard methods (Akbar et al., 2019).

2.3. Anti-inflammatory activity

The anti-inflammatory activity of the mangrove plant was performed in vitro with the modified protocol of (Yesmin et al. 2020) by inhibition of albumin denaturation assay. This mixture comprises the test samples (20 to 100 µg/ml) and an aqueous solution containing 1% bovine albumin. The mixture pH was adjusted using a minimal amount of 1 N HCl. Subsequently, samples were incubated at 37°C for 20 minutes, followed by heating at 51°C for an additional 20 minutes. Turbidity in the cooling sample was measured by a UV spectrometer at 660 nm. As a standard, diclofenac sodium was active at the con. range from (20 to 100 µg/ml). The triplicate manner experiment was performed. The inhibition of protein denaturation percentage was calculated as follows:

2.4 Antioxidant activities

2.4.1 DPPH Free Radical Scavenging Activity

The radical scavenger assay was conducted by testing various concentrations of extracts (20, 40, 60, 80, and 100 µg/mL). These samples were placed in a 96-well plate with a final concentration of 0.1 mM DPPH in mix with methanol, allowing the mixture to react for 30 minutes without light conditions. The methanol solution was used as the negative control. After the incubation period, absorbance was measured at 517 nm using a microplate reader.

The assay was performed in triplicate, and the results were expressed as a percentage of DPPH reduction compared to the methanol-negative control (Biswas et al. 2018).

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control} \times 100}$$

2.4.2 Hydrogen Peroxide Activity

To evaluate the extract hydrogen peroxide scavenging ability, a 40 mM H2O2 solution was prepared in a phosphate buffer. A 100 µg/mL concentration of the Mangrove extract in distilled water was added to a 0.6 mL aliquot of the 40 mM H2O2 solution. Following a 10-minute incubation, the absorbance was measured at 230 nm compared to a blank solution containing only a phosphate buffer. The hydrogen peroxide scavenging activity of the plant extracts and standard compounds was then calculated (Keser et al. 2012).

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control} \times 100}$$

2.4.3 TAA

The plant crude extract from the mangrove was analyzed using a method adapted from (Ramasamy et al. 2014) with minor modifications. A sample volume of 0.3 mL was prepared with concentrations ranging from 25 to 100 µl. The samples were mixed with a reagent solution consisting of 4 mM ammonium molybdate, 0.6 M sulfuric acid, and 28 mM sodium phosphate (Na3PO4) in a 3 mL container. The reaction mixtures were incubated in a water bath at 95°C for ninety minutes. The absorbance of each sample was measured at a wavelength of 695 nm. The TAA was determined by quantifying the ascorbic acid content.

2.5Antimicrobial activity

Antibacterial activity was conducted with the disc diffusion method. Nutrient media was poured into a Petri plate and inoculated selected pathogens were swapped in the Petri dish. The Samples containing discs were placed on plates inoculated with pathogens. The plates were then incubated at room temperature for 12-24 hours. Observations included evaluating the formation and quantification of clear zones during the specified incubation period, following a slight modification of the method described by (Pushparaj 2014).

2.5 Characterization

2.5.1 FTIR

The Fourier Transform Infrared Spectrophotometer is a very useful instrument for determining the sorts of functional groups found in compounds. Each chemical bond absorbs light of a certain wavelength, which is reflected in the interpreted spectrum. Chemical bonding inside a molecule can be analyzed by its infrared absorption spectra. For the FTIR study, dried powder samples from various solvent extracts of each plant material were employed.

3. Result

3.1 Qualitative phytochemical analysis

Mangrove plants contain biologically active composites that are of medical importance. However, the dispersal of phytochemicals varies, especially in plant parts such as leaves, stems, and roots. In this study, phytochemical screening was accomplished using an Ethanol extract of Rhizophoraapiculata. The results indicated the presence of various bioactive compounds, specifically phytochemicals, in mangrove leaf extracts. These included flavonoids, tannins, glycosides, Phenolic compounds, and Saponins (Table.1). Notably, alkaloids, Cardiac glycosides, and Terpenoids were found to be absent in the sample. In (Debnath et al., 2020) phytochemical screening it was noted that the ethanol extract from Avicenniacorniculatum leaves contains a diverse array of Bio-compounds such as saponins, tannins, alkaloids, flavonoids, glycosides, steroids, terpenoids, and others. (Raffat et al., 2017) demonstrated that the seeds of Avicennia marina contain coumarins, glycosides, flavonoids, saponins, tannins, and carbohydrates, with minimal levels of sugars. Previous studies have also indicated that DMF, acetone, and ethyl acetate are highly efficient solvents for extracting diverse phytochemicals and antioxidants (Shaheena et al., 2019). According to (Roy et al. 2021), both the leaf and RS samples of Suedamonoicaexhibited the presence of phenolics, flavonoids, tannins, steroids, alkaloids, glycosides, terpenoids, and reducing sugars. (Julyasih et al. 2022) found that the ethanol extract of G. salicornia contained phenolics, flavonoids, and terpenoids, but in absence contain steroids, alkaloids, and saponins.

3.2 DPPH

The ethanol extracts from mangrove Rhizophoraapiculata exhibited significantly distinct DPPH scavenging activities ($p<0.05$) at a 100 µg/mL concentration. As shown in Figure. The findings revealed a concentration-dependent increase in the scavenging capability of the Mangrove, with a maximum value of 83.45 ± 1.17% at 100 µg/ml, compared to the standard value of 89.71 ± 0.83%. At a minimum of 20 µg/ml, the mangrove extract displayed a value of 72.67 ± 0.31%, while the standard Ascorbic acid exhibited a value of 76.25± 1.22% (Fig.2). Antioxidants are rich in free radicals and reduce the risk of disease, in addition, natural antioxidants are non-toxic and powerful for biological activity (Mahendran et al. 2021). According to (Divya et al. 2020) The extract of A. marina has notable dose-dependent inhibition of DPPH activity with IC50 concentrations of 31.25-50 µl compared to the standard ascorbic acid (49.98±0.39). The methanol extracts from B. gymnorrhiza showed concentration-dependent DPPH scavenging activity with IC50 values of 113.79 ± 0.168 µg/mL (Karimet al.2020). Similar studies reported by (Eswaraiah et al. 2020) indicate that the leaf extract of Suaedanudiflora showed minimum scavenging activity of 37.78% at a con. of 50 µg/mL, whileLumnitzeraracemosa exhibited scavenging activity at a con. of 200 µg/mL, reaching 95.62%. In (Chelliah et al. 2023), the plant extract exhibited 86% antioxidant activity and 75% DPPH activity at concentrations of 350 µg/mL compared to ascorbic acid.

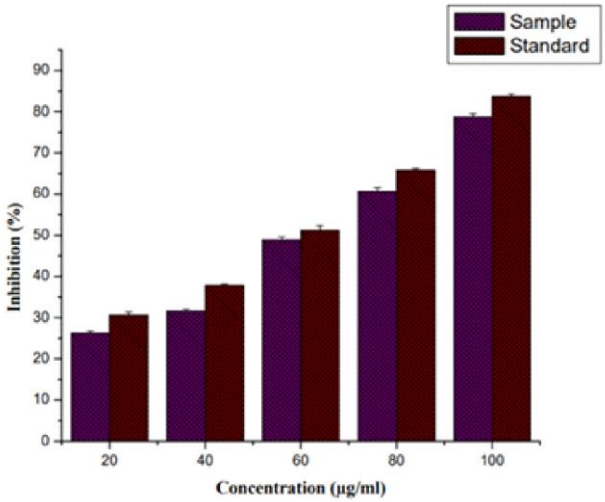

Figure 2: DPPH assay in different concentrations of MangroveRhizophoraapiculata

3.3 Hydrogen Peroxide Activity

The assessment of the scavenging potential of thecrude extract of Rhizophoraapiculata mangrove against the standard in the hydroxyl scavenging assay revealed an upward trend in scavenging percentage with an increase in the extract concentration shown in the (Fig.3). At the lowest concentration (20 µg/ml), the scavenging percentage was only 88.58±0.78%, whereas the standard, ascorbic acid, exhibited a scavenging percentage of 90.54 ± 0.91%. However, scavenging activity increased with concentration at 100µg/ml of con., the scavenging percentage was 93.47± 1%, whereas the standard was 74.5± 2.4%. As a result, the extract scavenging potential was significantly lower than the standard. The hydrogen peroxide radical scavenging activity in Rhizophoraapiculata, demonstrated a scavenging activity of 96.15 ± 1.11%. (Karim et al. 2020) found that B. gymnorrhizaleaf extract demonstrated a con. dependent ability to scavenge H_2O_2, with an IC50 value of 112.91 ± 0.164 µg/mL. In (Roy et al.2021) reported that at a con. of 100 µg/mL, the H_2O_2 radical scavenging abilities of Suedamonoicaextract and ascorbic acid were 88 ± 2.2% and 100 ± 2.1%, respectively. In the H_2O_2 scavenging assay, both the extract of Sonneratiacaseolaris and ascorbic acid scavenged the formed radicals, with calculated IC50 values of 66 µg/ml and 11 µg/ml (Kundu et.al.2022).

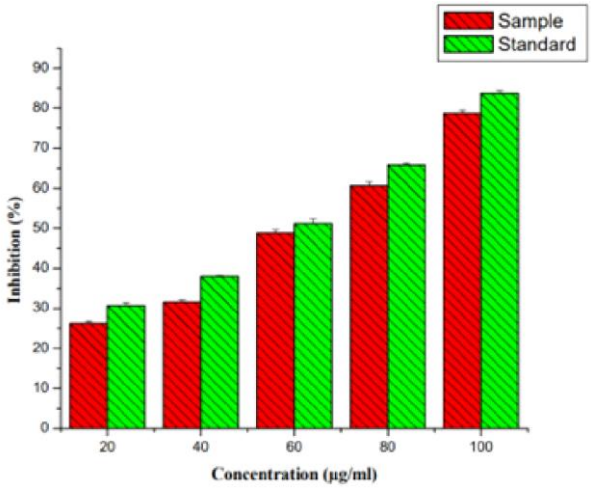

Figure 3: Hydrogen peroxide assay in different concentrations of Mangrove Rhizophoraapiculata

3.4 TAA

The antioxidant activity of Rhizophoraapiculata mangrove plant extract was assessed at different con. (20 to 100 µg/ml) and compared to a standard. (Fig.4) At 20 µg/ml, the extract exhibited an antioxidant activity of 26.19 ± 0.57 compared to the standard 26.6 ± 0.74. In the highest 100 µg/ml, the extract's activity reached 78.73 ± 0.79, compared to 79.29 ± 0.62 with a standard. These results indicate that the R. apiculataextract exhibits significant antioxidant activity with increasing activity observed as the concentration increases. (Mansoori 2020) found that flowers had a significantly higher relationship between, total flavonoid content, total polyphenols and antioxidant activity capacity compared to Leaves. (Abdelhamid et.al 2018) found that the total antioxidant activity of C. sedoides was the maximum for three species of brown macroalgae, with a value of 71.30 mg followed by C. spongeosis at 26.13 mg.

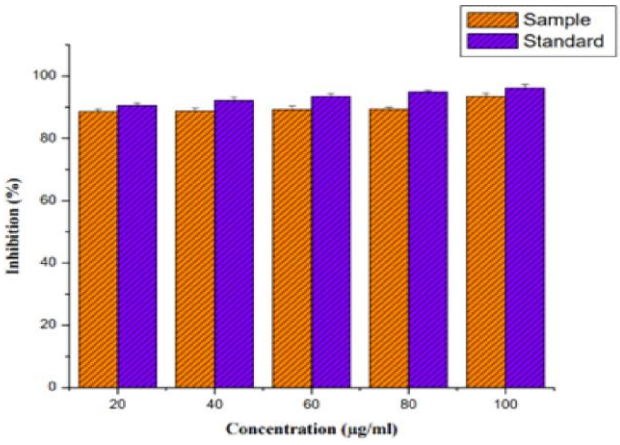

Figure 4: TAA assay in different concentrations of Mangrove Rhizophoraapiculata

3.5 Anti-inflammatory activity

The Mangrove Rhizophoraapiculataanti-inflammatory activity was evaluated. This result shows the ability to extract protein denaturation and the anti-inflammation activity was minimum inhibition (64.23 ± 1.23%) at the con. level of 20 µg/ml and the maximum (96.05 ± 1.29 %) con. a level of 100 µg/ml was observed from leaf crude extract (Fig.5). Increase the concentration of the leaf extract, and observe a stronger inhibition of protein denaturation, which is often associated with a higher anti-inflammatory effect. This finding indicates that the Mangrove Rhizophoraapiculata leaf extract has anti-inflammatory properties, and the degree of inhibition of protein denaturation is related to the various concentrations of the extract used. Higher concentrations of the extract appear to be more effective. In an investigation into the mechanism of anti-inflammatory activity, (Kaur et al. 2018) that the root extract exhibited the highest inhibition of protein denaturation (296.26%), bark (259.48%), and leaf (237.62%) extracts. Fucoidans sourced from seaweeds like Fucusvesiculosus have shown the ability to reduce pro-inflammatory cytokines and enzymes in various experimental models (Xie et al., 2023).

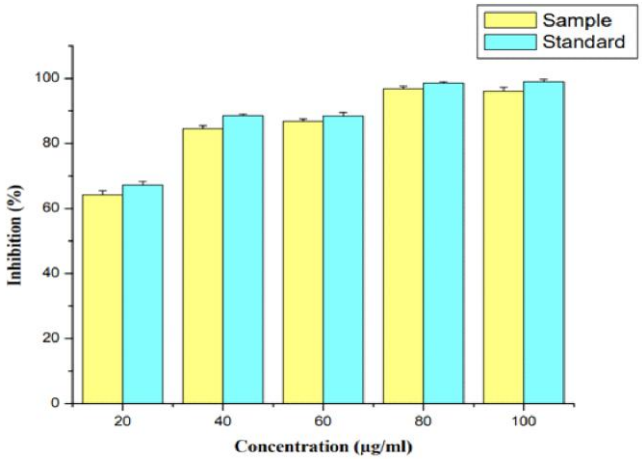

Figure 5: Anti-inflammatory assay in different concentrations of Mangrove Rhizophoraapiculata

3.6 Antibacterial test

The antimicrobial activity of Rhizophoraapiculata mangrove plant extract was assessed against Escherichia coli and Streptococcus mutans at concentration (25t o100 µg/ml). The study revealed a concentration-dependent inhibition effect. For E. coli the zones of inhibition were 12 mm for the control, 0 mm for 25 µg/ml, 14 mm for 50 µg/ml, 16 mm for 75 µg/ml, and 16.5 mm for 100 µg/ml. For S. mutans the zones of inhibition were 19 mm for the control, 0 mm for 25 µg/ml, 16 mm for 50 µg/ml, 18 mm for 75 µg/ml, and 19 mm for 100 µg/ml (Fig.6). These results indicate that R. apiculata extract is more effective against S. mutans at higher concentrations, with maximum inhibition observed at 100 µg/ml the zone of inhibition control, respectively, using the agar disc diffusion method. Ampicillin is used as a positive control and it confirmed that the bacteria were susceptible to Ampicillin. R. apiculata could be a potent natural antimicrobial agent, particularly useful in oral health for managing S. mutans

infections (Karim et.al.,2020) Bruguieragymnorrhiza exhibited the highest activity against MIC E. cloacae with a minimum inhibitory concentration of 20.1 mg/mL and E..coilof 1.8 mg/mL and In Heritieralittoralis showed strong lethality against S. enterica with an MIC of 17.6 mg/mL. (Zhou et al., 2022) reported R. mucronatahas higher levels of phenols, flavonoids, and bioactive compounds, all of which efficiently bind to the negatively charged surfaces of K. pneumoniae. (Alsaadiet. Al.2023)In mangrovesAvicennia marina, saponins showed modest antibacterial activity against both Gram-negative K. pneumoniae and Gram-positive S. aureusbacteria.According to (Saraswatiet.al., 2021) the extract of G. salicornia can inhibit the growth of both S. aureus and E. coli. (Audah et al. 2022) extract of A. marina leaves inhibited growth of S. aureus an inhibition zone of approximately 10.7 mm.

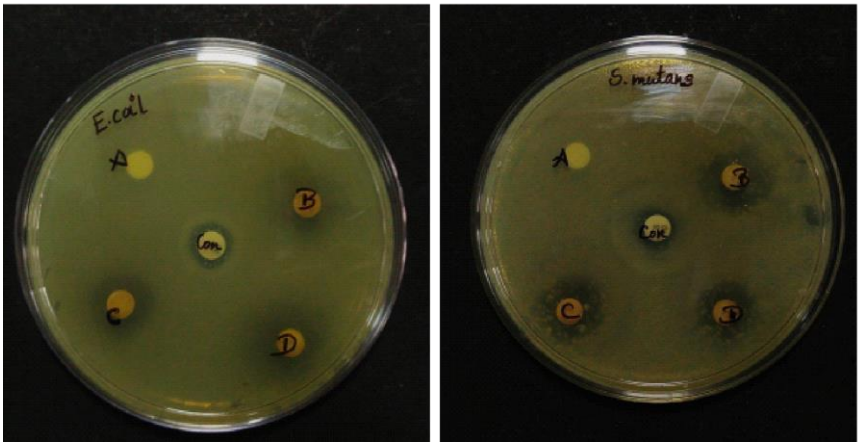

Figure 6:Antimicrobial potential using Mangrove crude extract against different pathogens a) Escherichia coli b) Streptococcus mutans

3.7 FTIR

The FTIR spectrum exhibits characteristic absorption bands corresponding to various functional groups. A medium, sharp peak at 3650 cm^{-1} indicates O-H stretching vibrations typical of alcohols. A strong, broad peak at 2950 cm^{-1} also corresponds to O-H stretching in alcohols. Strong absorption at 1750 cm^{-1} is attributed to C=O stretching in esters and δ-lactones. The medium peak at 1450 cm^{-1} is due to C-H bending vibrations in alkanes. C-N stretching in amines is identified by a medium peak at 1250 cm^{-1}. Primary alcohols show a strong C-O stretching peak at 1050 cm^{-1}. A medium peak at 840 cm^{-1} is indicative of C=C bending in alkenes, while a strong peak at 650 cm^{-1} corresponds to C-Br stretching in halo compounds. In (Roy et.al 2021) FTIR spectra in various solvent systems showed absorption bands in the 1,600–1,670 cm^{-1} range, indicating the presence of C=C stretching vibrations in alkene groups.The FTIR spectroscopy of the antibacterial compound in crude methanol extracts of Mangrove species indicated the presence of amide and alkane groups, while the crude methanol extract of mangrove revealed phenol, amide, nitro, and alkane groups (Duraipandianet.al 2022).

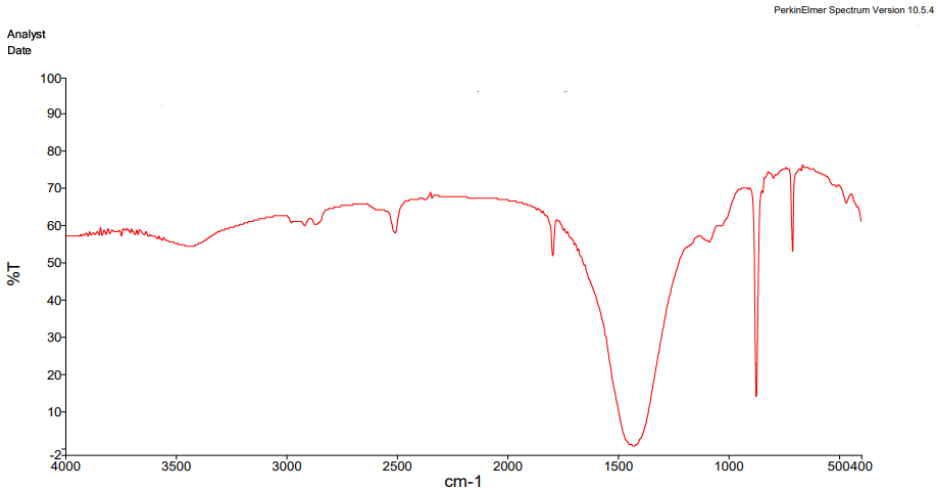

Figure 7: Characterization FTIR MangroveRhizophoraapiculata

4. Conclusion

In current investigation, Rhizophoraapiculata crude extract out as a promising natural resource rich in bioactive compounds, including flavonoids, tannins, glycosides, phenolic compounds, and saponins, which highlight its medicinal potential. The extract has notable anti-inflammatory properties by inhibiting protein denaturation, along with impressive antioxidant capabilities in scavenging free radicals. Additionally, its concentration-dependent antimicrobial activity against Escherichia coli and Streptococcus mutans potential as a therapeutic agent. FTIR analysis confirmed the presence of diverse functional groups, further validating its bioactive profile. These findings collectively position Rhizophoraapiculata as a valuable for developing new pharmaceuticals, affirming its traditional uses and ecological importance within mangrove ecosystems. Continued research into its mechanisms and clinical applications promises to yield further medical advancements.

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