

## RESEARCH ARTICLE



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# Phytochemical Analysis, Antimicrobial and Antioxidant Activity of Mangrove Plants *Bruguiera gymnorhiza* (L.) Lam. and *Excoecaria agallocha* L.

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## Abstract

**Objectives:** To perform a detailed phytochemical analysis, antimicrobial and antioxidant activity of leaf extracts of mangroves *Bruguiera gymnorhiza* and *Excoecaria agallocha*. **Methods:** A comparative phytochemical analysis was done using qualitative and quantitative methods followed by antimicrobial activity and antioxidant analysis using DPPH. Aqueous and chloroform extracts of both mangroves were subjected to UV-Visible spectrum analysis. Further, the extracts were subjected to TLC, FTIR and GC-MS analysis to determine the different bioactive compounds present in the extract. **Findings:** The preliminary phytochemical analysis of different extracts revealed the presence of various phytoconstituents namely alkaloids, terpenoids, phenols and quinones. They had also shown significant antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. In DPPH analysis, the maximum antioxidant activity (% of inhibition) was observed in aqueous extract (67.2%) followed by methanol extract (67%) of *Bruguiera gymnorhiza*, whereas in the case of *Excoecaria agallocha*, it was 64.9% in ethanol and 46.2 % in chloroform extracts. Further, GC-MS along with FTIR analysis of the extracts revealed the presence of numerous compounds that may contribute to the antimicrobial, and antioxidant potential and have great promise in treating various diseases. **Novelty:** Comparative study of the mangrove species *Bruguiera gymnorhiza* and *Excoecaria agallocha* with five different solvents has been documented for the first time. In this study, both plants showed maximum activity against skin pathogens and further studies might help find new compounds against MDR pathogens. The findings of this study shed light on the folklore claim and immense potential of *Bruguiera gymnorhiza* and *Excoecaria agallocha* to treat various ailments.

**Keywords:** Phytochemicals; Antioxidant; *Bruguiera gymnorhiza*; *Excoecaria agallocha*; Mangroves

## 1 Introduction

The increased occurrence of lifestyle-related diseases, the emergence of drug-resistant microbes, and related diseases have put extensive pressure to identify of new drugs. Traditional and modern medicines have always turned towards plant-derived secondary metabolites to identify novel therapeutic compounds<sup>(1)</sup>. One such flora, the Mangroves are rich in pharmacologically active compounds. They are extensively found in tropical and subtropical tidal water, with about 77 species identified worldwide, and about 65 of them are located on the Indian coastline which accounts for 3% of the earth's total mangrove vegetation. The unique habitat with high salinity, moisture content, and changing tidal levels facilitates these plants to synthesize secondary metabolites, enabling them to survive the harsh environment<sup>(2)</sup>. Phytochemical analysis of various mangrove species has revealed the presence of all the major secondary metabolites<sup>(3)</sup>. These have also shown pharmacological activity in treating fungal infections, inflammation, diabetes, and control of cholesterol levels<sup>(4,5)</sup>. Due to their varied metabolite presence, it is imperative to screen mangrove plants to identify new antimicrobial agents.

*Bruguiera gymnorhiza*, commonly known as black mangrove, is a member of the Rhizophoraceae family of mangroves and is acknowledged for its application in treating viral fever, burns, diarrhoea and diabetes<sup>(6)</sup>. Methanolic extracts of leaves of *B. gymnorhiza* have shown antibacterial activity as well as anti-hemolytic activity<sup>(7)</sup>. *Excoecaria agallocha* belonging to the Euphorbiaceae family, commonly called the blinding tree, is found extensively in the Indian, Myanmar and Australian coastal regions. Its extracts are known to have high antioxidant activity as well as antidiabetic antineoplastic activity and antimicrobial<sup>(8)</sup>.

Though various bioactive compounds have been identified in mangrove extracts, they are not much exploited in South India. Further due to climatic changes and coastal development one in six mangrove species worldwide is in danger of extinction thereby necessitating restricted use of the whole plant. Utilizing only leaves of mangroves limits the over utilization of the mangrove, at the same time, helps to identify new bio active compounds. We performed a simultaneous comparative phytochemical analysis of leaf extract of *Bruguiera gymnorhiza* and *Excoecaria agallocha* using five different polar and non-polar solvents for this study. Antioxidant and antibacterial activity of various extracts were conducted to determine their pharmacological value. Ultraviolet (UV)-Visible spectrum, Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed to identify the multiple metabolites present in the extracts. With the higher incidences of multi-drug resistant bacteria, new antimicrobial compounds are the need of the hour. We have found out that both plants have antibacterial activity, especially against skin pathogens. Since these plants are used traditionally for many diseases, further research can lead to a promising discovery of new compounds which is useful to mankind.

## 2 Methodology

### 2.1 Collection and Identification of leaf material

Fresh Leaves of *Bruguiera gymnorhiza* and *Excoecaria agallocha* were collected from the Kumarakom Kerala Tourism Development Corporation (KTDC) bird sanctuary and Kollam Munro Island of Kerala State, respectively. The plant materials were identified from Tropical Botanical Garden and Research Institute (TBGRI), Kerala, India. The herbarium of *Bruguiera gymnorhiza* (collection No. 95948) and *Excoecaria agallocha* (collection No. 94680) was deposited at TBGRI. The identity of the specimens was found to be correct as per the International Code of Nomenclature for algae, fungi, and plants (ICN).

### 2.2 Preparation of leaf extracts

The young leaves were collected, and washed properly with tap water to remove debris. The leaves were dried in shades using blotting papers and then pulverised into a fine powder using a household blender and stored in an airtight bottle for further use. The powdered leaves of the two different plants were soaked (10g/100mL) in different solvents of increasing polarity (petroleum ether, chloroform, ethanol, methanol and water) overnight in a rotary shaker<sup>(9)</sup>. The extract obtained was stored in a refrigerator at 4°C until further use. All chemicals used in the study were obtained from Hi-Media, Sigma, and spectrum (AR, LR).

### 2.3 Preliminary Phytochemical analysis

#### 2.3.1 Qualitative analysis

Qualitative phytochemical analysis was carried out by the addition of the specific reagents and the results were tabulated based on the colour change or precipitate formation<sup>(9)</sup>.

### 2.3.2. Quantitative analysis

2.3.2.1 Total Phenol. Each 0.5 mL extracts were mixed with 2.5 mL Folin- Ciocalteu reagent and 2 mL of 7.5% (w/v) sodium carbonate. The reaction mixtures were kept in the dark for 30 minutes. Gallic acid was used as standard. Absorbance was measured at 765 nm, and the total phenol content was determined as gallic acid equivalents (GAE) in mg/g<sup>(10)</sup>.

2.3.2.2 Total flavonoid. Total flavonoids were determined by the Aluminium chloride method<sup>(9)</sup>. An aliquot (1 mL) of extract was added to 0.3 mL of 5% (w/v) NaNO<sub>2</sub> and incubated for 5 minutes. 0.3 mL AlCl<sub>3</sub> (10% w/v) and 2 mL of 1N NaOH was added, and the total volume was made up to 5 mL with distilled water, incubated for 10 minutes at ambient temperature. The absorbance was measured at 510 nm by using a UV-visible spectrophotometer. Three replicates were made for each test sample. The total flavonoid contents were expressed as Quercetin equivalence (QE) in mg/g.

## 2.4 *Invitro* free radical scavenging activity

Free radical scavenging potential was determined using 1,1-Diphenyl-2-picrylhydrazyl-DPPH. To each extract, 1mL of freshly prepared DPPH (200  $\mu$ M dissolved in ethanol) was added and vortexed thoroughly. Then the solution was incubated in a dark place for 30 minutes. The absorbance of stable DPPH was recorded at 517 nm by UV visible spectrometer Labtronics, Model Lt-291. Ascorbic acid was used as standard.

The percentage of inhibition was calculated using the formula.

$$\% \text{ Inhibition} = [1 - (A_{BS\text{SAMPLE}}/A_{BS\text{CONTROL}})] \times 100.$$

## 2.5 UV-Visible spectrum analysis

Both aqueous and chloroform extracts of *Bruguiera gymnorrhiza*, and *Excoecaria agallocha* were subjected to UV-Visible spectrum analysis. The spectra were recorded using Labtronics, Model Lt-291. The  $\lambda$  -peaks were measured in the 200-800 nm range at an absorbance interval of 5 nm. The spectra were used to characterize the extracts by determining the functional groups corresponding to each peak.

## 2.6 Thin layer Chromatography (TLC)

The aqueous and methanol extracts were added as spots using capillary tubes on the one end of the pre coated Silica gel using Merck KGaA TLC Silica gel60 F<sub>254</sub> of dimension 20×20 at above 1 cm. The TLC plate was allowed to dry, and then it was placed in a beaker containing solvent n-butanol, acetic acid and water in the ratio of 3:2:2. The samples were allowed to run 3/4<sup>th</sup> of the plate's length. Then the plate was removed from the chamber and allowed to dry. 2% of iodine was sprayed over the plate, and then dried for another 10 minutes. The plate was visualised under UV light to identify violet colour spots, and their RF values were measured.

## 2.7 Fourier -Transform Infrared Spectroscopy (FTIR spectrum analysis)

Based on the preliminary phytochemical screening, the methanol extract of both *Bruguiera gymnorrhiza*, and *Excoecaria agallocha* were subjected to FTIR analysis. FTIR spectra were recorded using Shimadzu FTIR Spectrometer 8000 series in the region 4000–400 cm<sup>-1</sup> by employing the standard KBr pellet technique. The extract was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet.

## 2.8 Gas Chromatography-Mass Spectrometry (GC-MS analysis)

GC-MS analysis was performed to identify individual constituents in the extract<sup>(11)</sup>. The analyses were performed using a GC-MS system (Agilent Technologies, USA) equipped with an HP-5 MS capillary column (30 m × 0.25 mm, 0.25 mm, Agilent Technologies USA). The injection volume of each sample was 1  $\mu$ L. Helium (99.999%) was used as the carrier gas at a flow-rate of 1 mL/min. The temperature of the injection port was 250°C, and the column temperature program was as follows: 50°C for 2 minutes, followed by an increase to 180°C at a rate of 5 C/min, an increase to 270°C at a rate of 20°C /min, and maintenance at 270°C for 5 minutes. The MS conditions included an EI ion source temperature of 230° C, ionization energy of 70 eV, and a mass scan range of 40–500 amu.

## 2.9 Determination of Antimicrobial activity

Clinical isolates of *Escherichia coli* (KY787193), *Bacillus cereus* (MT131177), *Klebsiella pneumoniae* (OL601967) and *Staphylococcus aureus* (MT126466) were procured from Centre for Bioscience and Nanoscience Research, Coimbatore, Tamil Nadu. Standard antimicrobial activity test with some modification as outlined was used<sup>(10)</sup>. 24hours old bacterial cultures (70  $\mu$ L) were swabbed using sterile cotton on the Mueller-Hinton agar plates. 5 mm diameter agar well was prepared with the help of a sterilized stainless cork borer. 20  $\mu$ L of extract or controls were loaded to each well. The plates were incubated at 37°C for 24 hours. Antibacterial activity was calculated by measuring the diameter of zones of inhibition against the tested bacteria. Commercial antibacterial disc Levofloxacin (5mcg) was used as positive control; water and methanol were used as negative controls.

## 2.10 Statistical Analysis

All the experiments were performed in triplicate values and expressed as  $\pm$  Standard deviation.

## 3 Results and Discussion

### 3.1 Phytochemical analysis

The preliminary phytochemical analysis of all the extracts revealed the presence of some essential phytochemical compounds (Table 1). Simultaneous determination of phytochemical activity using 5 different solvents provides us with a broader understanding of the different phytochemical groups present in the plant extracts. Being native to the coastal regions, *Bruguiera gymnorhiza* extracts might be rich in polar compounds<sup>(7)</sup>. However, no flavonoids are present in any *Bruguiera gymnorhiza* extracts, while it was detected in all extracts of *Excoecaria agallocha* except petroleum ether. The compounds, namely alkaloids, terpenoids and phenols, were present in most of the extracts of both *Bruguiera gymnorhiza* and *Excoecaria agallocha*, which are in accordance with the earlier finding. Quinones are present in all the extracts of *Bruguiera gymnorhiza*, whereas only the water extracts showed the presence of Quinones in *Excoecaria agallocha*. Further, the presence of saponins was seen only in the higher polarity solvents of both plant samples.

**Table 1.** Phytochemical constituents of *Bruguiera gymnorhiza*, *Excoecaria agallocha* extracts

Sl.No.	Phytochemical Constituents	<i>Bruguiera gymnorhiza</i>					<i>Excoecaria agallocha</i>				
		W	M	E	C	P	W	M	E	C	P
1	Alkaloids	+	+	+	+	+	+	+	+	+	+
2	Terpenoids	+	+	+	+	+	+	+	+	+	+
3	Phenol	+	+	+	+	+	+	+	+	+	-
4	Sugar	+	-	-	+	-	+	-	-	-	-
5	Saponins	+	+	-	+	-	+	-	-	-	-
6	Flavonoids	-	-	-	-	-	+	+	+	+	-
7	Quinines	+	+	+	+	+	+	-	-	-	-
8	Proteins	+	-	-	-	-	-	-	-	-	-
9	Steroids	+	-	-	+	-	-	+	+	+	+

W: Water, M: Methanol, E: Ethanol, C: Chloroform, P: Petroleum Ether, +: Present, -: Absent

### 3.2 Total Phenol

The maximum percentage yield of phenol was obtained in the aqueous extracts for *Bruguiera gymnorhiza* 14 mg/g GAE and *Excoecaria agallocha* 56 mg/g DAE followed by chloroform and ethanol extracts (Figure 1 A). Less phenol content is present in the petroleum ether extract of *Bruguiera gymnorhiza*. A recent study observed higher phenolic content in methanolic leaf extract of *Bruguiera gymnorhiza*<sup>(7)</sup>. The difference in phenolic content observed in our study could arise due to reduced polyphenol production in plants due to various environmental factors and the location of the source plant<sup>(12)</sup>.

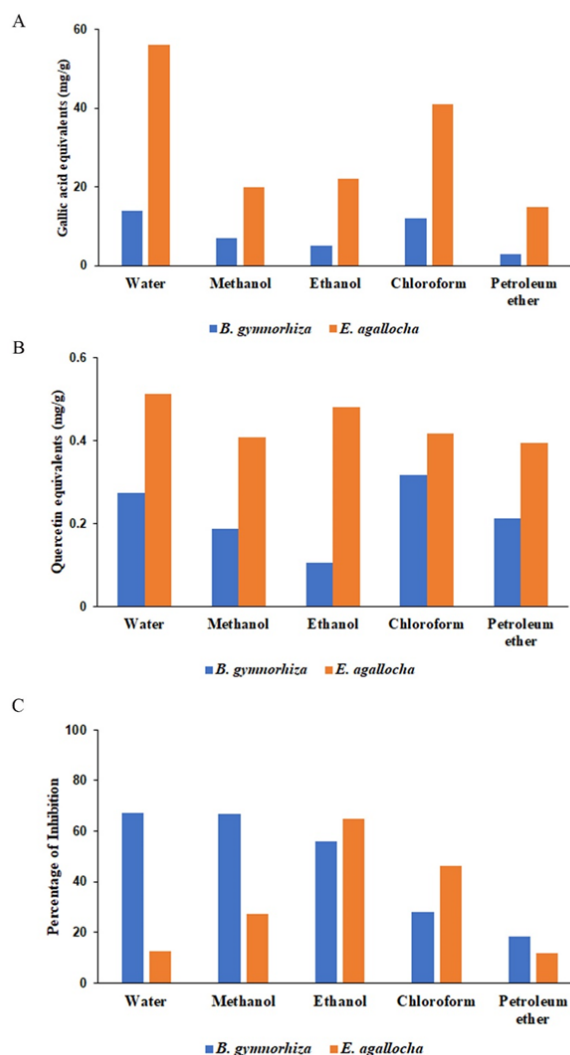


Fig 1. Comparative analysis of Total Phenol, Flavonoids, and antioxidant activity of *Bruguiera gymnorrhiza* and *Excoecaria agallocha* extracts

### 3.3 Total Flavonoids

The percentage yield of flavonoids was higher in the aqueous extract of *Bruguiera gymnorrhiza* (0.273 mg/g QE) and *Excoecaria agallocha* (0.513 mg/g QE). The earlier studies showed similar results where the highest total flavonoid content was observed in aqueous extract in terms of quercetin equivalents<sup>(13)</sup>. However, a relatively less amount of flavonoid content was present in the other extracts of *Excoecaria agallocha* (Figure 1 B).

### 3.4 Antioxidant activity

The DPPH radical scavenging activity of *Bruguiera gymnorrhiza* and *Excoecaria agallocha* was determined for all the extracts. The result of the DPPH scavenging assay is shown in (Figure 1 C). It is found that antioxidant compounds in all the extracts of both *Bruguiera gymnorrhiza* and *Excoecaria agallocha* have exhibited effective free radical scavenging activity. The highest free radical (DPPH) scavenging activity of *Bruguiera gymnorrhiza* was observed in the water extract (67.2%) and methanol extract (67%). Similar results have been observed in methanolic extracts of leaves though they were lesser in comparison to extracts obtained from other plant parts<sup>(9)</sup>. Further, the maximum percentage of inhibition in *Excoecaria agallocha* was observed in the ethanol extract (64.9%) followed by (46.2%) in the chloroform extract, which may be due to alkaloids, terpenoids, and other phenolic compounds that act as primary antioxidants or free radical scavengers.

### 3.5 UV - Visible Spectra

The UV-Visible (UV-VIS) spectra were used to identify the chemical compounds containing  $\sigma$ - bonds,  $\pi$ -bonds, lone pairs of electrons, chromophores and aromatic rings. The UV -VIS absorption spectra of the aqueous extract and chloroform extract of *Bruguiera gymnorrhiza* (Figure 2 A, B) and *Excoecaria agallocha* (Figure 2 C, D) were determined. The UV spectra profile of both aqueous and chloroform extracts of *Bruguiera gymnorrhiza* collectively showed peaks at 250 nm, 320 nm, 350 nm, 365 nm, 610 nm and 670 nm. The same two solvent extracts of *Excoecaria agallocha* showed peaks at 295 nm, 320 nm, 365 nm, 430 nm, and 670 nm, indicating that the absorption bands are due to flavonoids, phenol and its derivatives <sup>[21]</sup>. However, UV -VIS spectral data should always be complemented by FTIR and GC -MS analysis techniques.

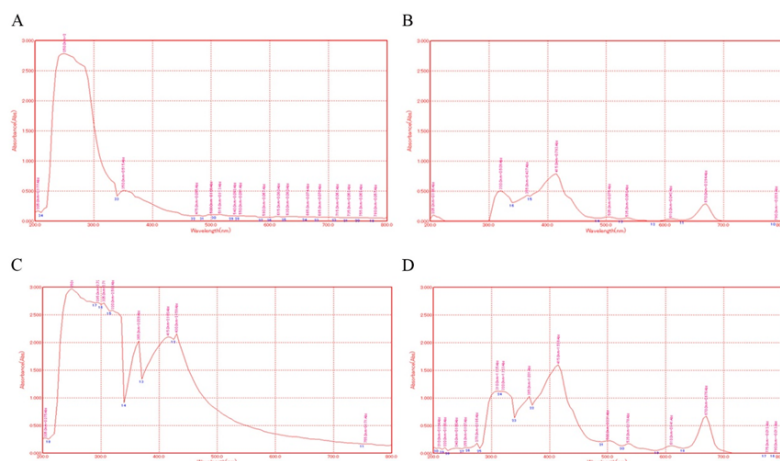


Fig 2. Chemical constituents in aqueous and chloroform extracts of mangroves leaf using UV-visible spectrum

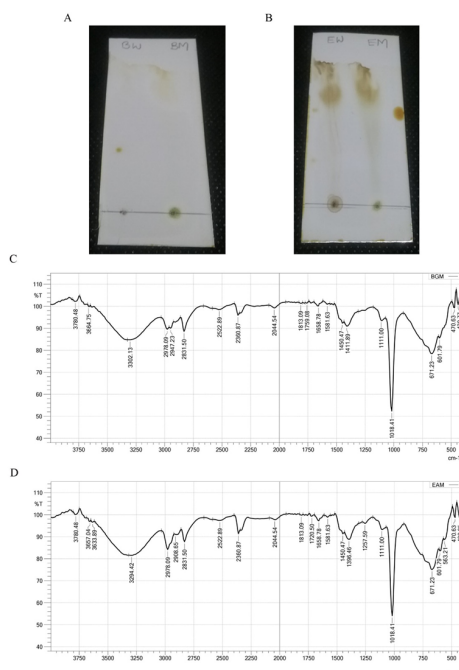
### 3.6 TLC analysis

TLC analysis of aqueous and methanol extracts of *Bruguiera gymnorrhiza* and *Excoecaria agallocha* using n-butanol, acetic acid and water (3:2:2) solvent system revealed the presence of promising spots, as shown in (Figure 3 A, B). TLC profiles of aqueous and methanol extract of *Bruguiera gymnorrhiza* revealed the presence of a single compound with the Rf value of 0.771 and 0.643, confirming the presence of phenolic and antioxidant compounds (Table 2). Likewise, the aqueous and methanol extract of *Excoecaria agallocha* showed the presence of a single compound with the Rf value of 0.585 and 0.615 indicating the presence of alkaloids and phenolic compounds. TLC profile of ethanolic leaf extract of *Excoecaria agallocha* using dichloromethane yielded four spots with different Rf value <sup>(8)</sup>. Various phytochemicals show different Rf values in different solvent systems which further provides insight into their polarity. This aids in selecting an appropriate solvent system for purifying pure compounds. Our solvent system was able to separate single compounds which would be purified and studied in the future.

Table 2. Rf values and compounds for water and methanolic extracts as determined by thin layer chromatography

Sl.No.	Plant name	Extract	Rf value	Compounds
1	<i>Bruguiera gymnorrhiza</i>	Water	0.771	Alkaloids
		Methanol	0.643	Phenols
2	<i>Excoecaria agallocha</i>	Water	0.585	Flavonoids
		Methanol	0.615	Phenols





**Fig 3.** Analysis of chemical constituents of *Bruguiera gymnorrhiza* and *Excoecaria agallocha* extracts using TLC and FTIR

### 3.7 FTIR analysis

The FTIR spectrum helps to characterize the nature of the phytochemical molecules. In this study, The FTIR analysis was performed to determine the functional groups present in the methanol extracts of *Bruguiera gymnorrhiza* and *Excoecaria agallocha*. The FTIR peak values are illustrated in the spectrum (Figure 3 C,D). The broad peak band at  $3302.13\text{ cm}^{-1}$  is an OH stretching in the alcohols and phenols group.  $2978.09\text{ cm}^{-1}$  to  $2044.54\text{ cm}^{-1}$  attributed to the C-H stretching vibration in the alkanes group. The peaks around  $1658.78\text{ cm}^{-1}$  are due to the amide I and II region that are characteristic of protein and enzymes. The presence of secondary amide groups with C=O stretching is confirmed by the peak at  $1581.63$ . CH<sub>2</sub> bending vibration at  $1450\text{ cm}^{-1}$  also affirms the existence of alkanes group. Peak at  $1396.46\text{ cm}^{-1}$  shows C-H stretching alkanes group indicated the presence of nitrates. Thus, the FTIR spectrum confirmed the presence of alcohols, phenols, amines, alkanes, aromatic and nitro compounds in the methanol extracts.

### 3.8 GC-MS analysis

Mass spectral analysis of compounds separated during gas chromatography helps to pinpoint the chemical components present in the sample as their mass spectra are fingerprints of the compounds, which can be determined from the established data library<sup>(14)</sup>. The GC-MS characterization of methanol extract of *Bruguiera gymnorrhiza* and *Excoecaria agallocha* identified several major phytochemical compounds (Figures 4 and 5). Few medicinally important phytoconstituents present in the extract are listed in the table (Tables 3 and 4), namely butanoic acid, succinic acid, pentanoic acid, thiazolidine, quinoline, leucine etc. It is reported that butanoic acid is known to be beneficial in treatment of Parkinson's and Alzheimer's and its levels indicative of better treatment process in Schizophrenia<sup>(15)</sup>. Thiazoline and its scaffold motif are known to have anticonvulsant, neuroprotective and anti-inflammatory activities<sup>(16)</sup>. Quinoline and its derivatives are known to have a wide variety of pharmacological applications, including anticancer, antiviral, antimicrobial, anti-malarial, and anti-inflammatory<sup>(11)</sup>. Ethyl acetate extract of *Bruguiera cylindrica* showed the presence of pharmacologically active compounds upon GC-MS analysis<sup>(17)</sup>. GC-MS analysis of ethanolic extract of *Excoecaria agallocha* indicated the presence of 10 compounds<sup>(8)</sup>. Due to the differential affinity of compounds to solvents, methanolic extracts contains the presence of varied compounds when compared to those extracted using ethanol, ethyl acetate. Our analysis used methanolic extract and was able to detect a larger number of compounds though we have represented only the major pharmacologically relevant compounds. The wide variety of biologically active compounds indicates that these extracts may have potent therapeutic applications. Though the GC-MS identified active

pharmacology compounds, further research is necessary to identify and purify the active compounds responsible for therapeutic activity.

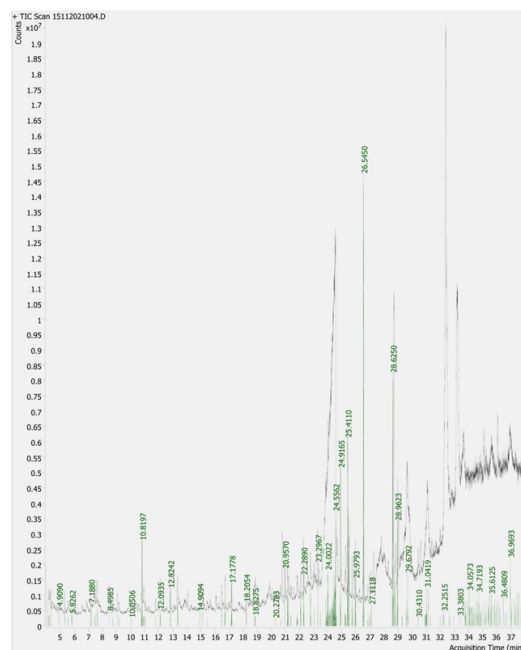


Fig 4. Identification of chemical constituents in methanol extracts of *Bruguiera gymnorrhiza* using GC-MS

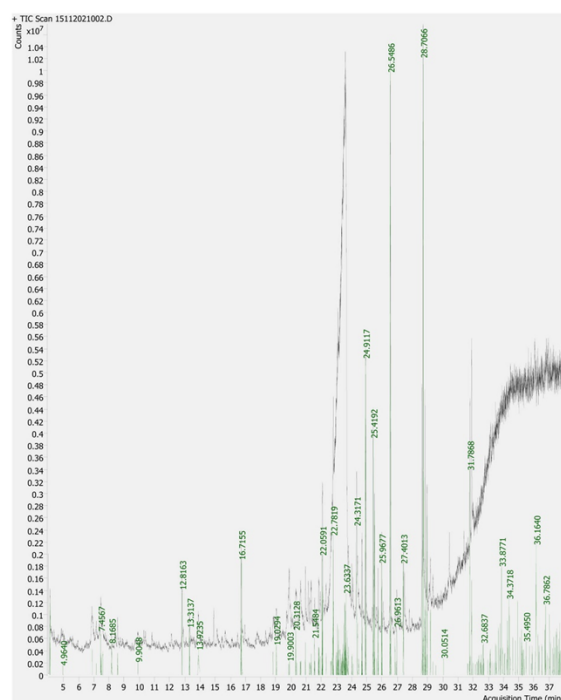


Fig 5. Identification of chemical constituents in methanol extracts of *Excoecaria agallocha* using GC-MS



**Table 3.** List of important phytochemicals present in GC-MS profile of methanol extract of *Bruguiera gymnorrhiza*

Retention time (RT)	Name of the compound	Mol. Formula	Component Area
23.4844	Butanoic acid	C <sub>10</sub> H <sub>14</sub> F <sub>5</sub> NO <sub>3</sub>	602754.8
23.9434	Succinic acid, pent-4-enyl propyl ester	C <sub>12</sub> H <sub>20</sub> O <sub>4</sub>	189025.1
24.0194	2-Pentenoic acid, 4-oxo-, methyl ester	C <sub>6</sub> H <sub>8</sub> O <sub>3</sub>	807404.6
24.3651	Thiazolidine, 3-methyl-	C <sub>4</sub> H <sub>9</sub> NS	207064.1
7.6253	6,7,8-Trimethoxy-3,4-dimethyl-1-methylsulfanyl-3,4-dihydroisoquinoline	C <sub>15</sub> H <sub>21</sub> NO <sub>3</sub> S	289170.0
23.8980	L-Leucine, methyl ester	C <sub>7</sub> H <sub>15</sub> NO <sub>2</sub>	189950.9
7.1880	2-Furancarboxitrile	C <sub>5</sub> H <sub>3</sub> NO	1265102.2
16.6974	Glutaric acid, di(4-cyanophenyl) ester	C <sub>19</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub>	323064.4
24.0022	1-Dodecanamine, N-dodecyl-	C <sub>24</sub> H <sub>51</sub> N	693094.9
28.6250	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	12106570.6
37.8775	Coumarine, 4-methyl-7-hydroxy-8-benzoyl-	C <sub>17</sub> H <sub>12</sub> O <sub>4</sub>	1151952.7

**Table 4.** List of important phytochemicals present in GC-MS profile of methanol extract of *Excoecaria agallocha*

Retention time (RT)	Name of the compound	Mol. Formula	Component Area
4.1048	Acetylacetone	C <sub>5</sub> H <sub>8</sub> O <sub>2</sub>	1792938.2
6.8916	Trifluoroguanidine	CH <sub>2</sub> F <sub>3</sub> N <sub>3</sub>	250364.5
8.1685	Picolinamide	C <sub>6</sub> H <sub>6</sub> N <sub>2</sub> O	781910.4
8.5621	p-Aminotoluene	C <sub>7</sub> H <sub>9</sub> N	214728.8
16.7231	Phthalic acid, hexyl 2-propylphenyl ester	C <sub>23</sub> H <sub>28</sub> O <sub>4</sub>	660795.2
20.3128	Enanthamide	C <sub>7</sub> H <sub>15</sub> NO	739821.0
20.3916	Cyclopentanecarboxaldehyde	C <sub>6</sub> H <sub>10</sub> O	123343.1
21.2097	Benzamide, 4-fluoro-N-allyl-	C <sub>10</sub> H <sub>10</sub> FNO	145294.8
22.0511	L-Leucine, ethyl ester	C <sub>8</sub> H <sub>17</sub> NO <sub>2</sub>	1088136.6
23.5800	1,3-Dioxolane	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	463354.2
26.5490	Octanamide, N-(2-butyl)-N-heptyl-	C <sub>19</sub> H <sub>39</sub> NO	18542826.6
31.7868	2H-1-Benzopyran-2-one, 3,4-dihydro-6-hydroxy-	C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>	3061558.0
37.7123	Glutaric acid, 2-methylpent-3-yl 2,2,3,4,4,4-hexafluorobutyl ester	C <sub>15</sub> H <sub>22</sub> F <sub>6</sub> O <sub>4</sub>	445565.3

### 3.9 Antimicrobial Activity of the Plant Extracts

Antibacterial activity of aqueous and methanol extracts of *Bruguiera gymnorrhiza* and *Excoecaria agallocha* were done on Gram-positive and Gram-negative test organisms. 20 µl Methanol extract of *Bruguiera gymnorrhiza* and *Excoecaria agallocha* showed maximum activity against *Staphylococcus aureus*, a skin pathogen and the aqueous extract of *Excoecaria agallocha* also showed maximum activity against *Klebsiella pneumoniae* (Figure 6).

Reviewing the previous studies, methanolic and combination extracts of *B. gymnorrhiza* leaves showed the ability to inhibit the growth of both Gram-positive bacteria such as *S. aureus* and Gram-negative bacteria such as *E. coli* and *P. aeruginosa*<sup>(18)</sup>.

Recently, ethanol and methanolic extract of *Excoecaria agallocha* showed considerable activity against both Gram-positive and gram-negative extracts. The highest activity was found against *Staphylococcus aureus*<sup>(8)</sup>. This is in accordance with our findings though the zone diameter is different. This could be due to the lower concentration of extract that was used for our study as well as variation in activity due to geographical and seasonal variation as recently reported in various mangrove plants<sup>(12)</sup>.

With the increasing occurrence of multi-drug resistant bacteria, new antimicrobial compounds are the need of the hour. Mangroves produce a wide array of natural products with immense medicinal and nutritional potential<sup>(19)</sup>. In the current study, *Bruguiera gymnorrhiza* and *Excoecaria agallocha* were shown significant inhibiting activity against *Staphylococcus aureus* which is responsible for skin infections.

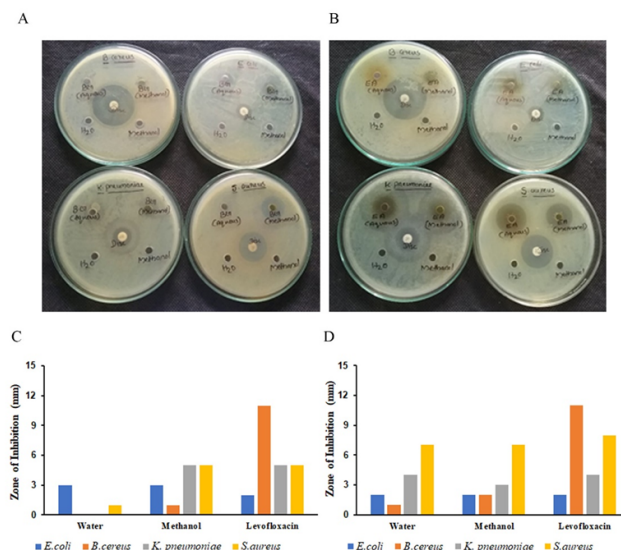


Fig 6. Antibacterial activity of *Bruguiera gymnorrhiza* and *Excoecaria agallocha* extracts

## 4 Conclusion

Among two plants studied *Excoecaria agallocha* showed a greater number of activities compared to *Bruguiera gymnorrhiza*. Phenols and flavonoids which are known to possess multiple biological activities are commonly found in plant extracts of *Bruguiera gymnorrhiza* and *Excoecaria agallocha*, due to its high level of in vitro free radical scavenging activity may be a valuable means to discover new healing drugs for various free radical-induced diseases and as a potential antimicrobial drug against MDRs. Further downstream work on purifying individual compounds identified using GC-MS would provide a better understanding of the main components having antimicrobial activity.

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