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ANTIMICROBIAL ACTION OF MANGROVE PLANT EXTRACTS AGAINST *SALMONELLA TYPHI* AND *CANDIDA PARAPSILOSIS* CHARACTERISED BY THEIR ANTIOXIDANT POTENTIALS AND BIOACTIVE COMPOUNDS

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Keywords:

Mangrove plants, *Excoecaria agallocha*, Antimicrobial, Antioxidant, Natural bioactive agents, Liquid chromatography-mass spectroscopy

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ABSTRACT: Plants present in the mangrove ecosystem are underexplored for their natural bioactive agents, including a neglected scope of inventing newer antimicrobials, to combat global crisis mediated by MDR microorganisms. In this study, we investigated four plants-*Excoecaria agallocha, Bruguiera gymnorhiza, Avicennia alba* and *Aegialitis rotundifolia* of the Sundarbans, world's largest mangrove ecosystem in West Bengal, India, for their antimicrobial activities against *Salmonella typhi* and *Candida parapsilosis*, in addition to their important bioactive resources including antioxidants. Ethanolic, methanolic, and DMSO extracts of leaves of these plants were studied by antimicrobial screening, determination of total phenolic and flavonoid contents, DPPH free radical scavenging activity, ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] assay, ferric reducing antioxidant power (FRAP), lipid peroxidation inhibition, thin layer chromatography, and liquid chromatography-mass spectroscopy (LC-MS). *E. agallocha* extracts showed excellent antimicrobial activities against *S. typhi*, while antifungal activity against *C. parapsilosis* was almost lacking. MIC values of all the extracts against *S. typhi* was as low as 3.96 µg/mL, however, growth inhibition was most with *E. agallocha* extract. Again total phenolic content (>300 mg/g), DPPH scavenging activity (75.55%), ABTS scavenging (78.53%), lipid peroxidation inhibition (64.35%) activities were found highest with *E. agallocha* extract. All chemicals retrieved by LC-MS of *E. agallocha* were found bioactive, among them hexanoylglycine, chorismic acid, tyramine, methyl jasmonate, khayanthone, chlorogenic acid were found particularly important. This study undoubtedly pointed out a good quality natural reservoir of important antimicrobials, antioxidants, and bioactive chemicals in the mangrove plants studied by us, predominantly in *E. agallocha*, which emerged a candidate mangrove plant for industrial development for such chemicals.

INTRODUCTION: Antibiotics are regularly used for the therapy of bacterial infections. However, overuse of antibiotics has become the major risk factor for the emergence of multi-drug resistant (MDR) strains of microorganisms [1].

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The worldwide emergence of *Escherichia coli*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, and many other β-lactamase producers has become a major therapeutic problem.

Water and food-borne infections caused by *Salmonella* and *Vibrio* is a global threat that needs to be controlled. Multi-drug resistant strains of *E. coli* and *K. pneumoniae* are widely distributed in hospitals and are increasingly being isolated from community-acquired infections [2, 3]. *S. typhi* is a clinically important bacterium which causes typhoid fever, while many other *Salmonella* spp.

like *S. eneritidis, S. typhimurium etc*. causes food poisoning and gastroenteritis in millions of people worldwide each year [4]. Fluoroquinolones and tetracyclines are the antibiotics most commonly used to treat *Salmonella* and until recently most strains were susceptible to these drugs [5]. However, a high incidence of *Salmonella* strains resistant to commonly prescribed antibiotics has been reported recently [6]. *Candida albicans, Canida tropicalis* and *Candida parapsilosis*, a few nosocomial fungal pathogens, have been reported to account for 50-70% cases of invasive candidiasis [7]. Alarmingly, the cases of nosocomial candidemia have sharply increased in the last decade [8]. As a result, this has led to the emergence of severe consequences including increased cost of medicines and mortality of patients. Therefore, the need to find alternate antimicrobial agents is of paramount importance. At the same time, literature studies and past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy [9]. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against MDR microbe strains [10].

Several research works to date have suggested the potential of mangrove floral community in traditional medicines [11-13]. For centuries, the tribal population employed mangrove plant extracts as their traditional folk medicine for healing several health disorders [14, 15]. However, unlike various herbs, the Indian Sundarbans, one of the most taxonomically diverse and physicochemically dynamic ecosystems of the Indian subcontinent, sustains some 34 species of true mangroves, among which members of the families Euphorbiaceae, Avicenniaceae, Plumbaginaceae, and Rhizophoraceae in which our present study plants present, however, Avicenniaceae family rank second in terms of prevalence [15, 16].

In comparison to the normal terrestrial flora, this halophytic mangrove community gets exposed to high and low tides twice in every 24 h [17, 18] and therefore, has developed a unique mode of adaptation, which could have enriched their phytochemical repertoire of medicinal importance. Mangroves are widespread in tropical and subtropical regions, growing in the saline intertidal zones of sheltered coastlines, and contain biologically active antimicrobial compounds. Previous studies on mangrove plant parts and its chief chemical classes exhibited various levels of biological activities such as antibacterial, antifungal, cytotoxic, hepatoprotective and free radical scavenging activities [19-25]. Mangrove plant parts have been used for centuries as popular medication for various natural products screening their antimicrobial property as well as to determine their mechanism of action.

According to the WHO, medicinal plants would be the best source for obtaining variety of drugs in the coming years [26]. This evidence contributes to substantiate and quantify the importance of screening natural products. The aim of our present study was to investigate the antibacterial and antifungal activity of mainly ethanolic, methanolic, and DMSO extracts of mangrove plants *Bruguiera gymnorhiza, Excoecaria agallocha, Avicennia alba* and *Aegialitis rotundifolia* against multi-drug resistant strains of bacteria and fungi isolated from nosocomial or hospital-acquired infections.

MATERIALS AND METHODS:

Collection and Preservation of Plant Samples: Fresh leaf samples of *Bruguiera gymnorhiza, Excoecaria agallocha, Avicennia alba* and *Aegialitis rotundifolia* were collected from Bali Island of the Indian Sundarbans (between 21°013'N and 22°040' N latitude and 88°003'E and 89°007'E longitude) during the month of June, 2018. The plant samples were washed with distilled water stored at 4 °C after collection and utilized within 7 days for extract preparation.

Collection and Maintenance of Microorganisms: The nosocomial MDR strain of *Salmonella typhi* and *Candida parapsilosis* were isolated from blood of patients at Peerless Hospital and B. K. Roy Research Centre, Kolkata, India and they were identified in the VITEK-2 automated system in the hospital. Fresh subcultures were made in the preceding day of the experiment from the stock cultures maintained in the laboratory.

Extract Preparation: The leaf samples were oven-dried at 60 °C till crisp and ground to fine powder using mortar and pestle. About 1 g of each of the finely powdered plant leaf material was soaked in

10 mL of solvents (ethanol, methanol, and dimethyl sulfoxide [DMSO]) for a period of 1 week at room temperature. Then, the extracts were filtered and concentrated by a rotary vacuum evaporator (RotaVap). The final concentration was adjusted to 1 mg/mL for screening the antimicrobial activity.

Antimicrobial Screening Assay: The minimum inhibitory concentration (MIC) assay was done by serial dilution method, using 96 well plates and plate reader (Erba Lisa Scan II Transasia Mannheim, Germany). 100 µL of Mueller-Hinton broth (HiMedia, India) was dispensed in all the wells of the plate. 100 µL of stock concentration of the extract was added to the first well of each column. Serial double dilution was done till the eighth well. Finally, 10 µL of 0.5 McFarland opacity culture suspension was added to each well of the plate. The plate was then gently shaken to mix the contents properly and immediately a baseline absorbance reading at 620 nm was taken. Then, the plates were kept for incubation for 16–18 h at 37 °C, and another absorbance reading at 620 nm was recorded [27].

Determination of Total Phenolic Content of the Plant Extracts: The amount of phenol in the four different extracts were determined by Folin-Ciocalteu reagent, according to the method using gallic acid as a standard phenolic compound [28] 1.0 mL of extract solution containing 1.0 g extract in a conical flask was diluted with 46 mL of distilled water in methanol. 1.0 mL of Folin-Ciocalteau reagent was added and mixed thoroughly. After three minutes 3.0 mL of 2% sodium carbonate was added and the mixture was allowed to stand for 3 h with intermittent shaking. The absorbance of the blue colour that developed was read at 760 nm. The concentration of total phenols was expressed as mg/g of dry extract [29]. All determinations were performed in triplicate.

Determination of Total Flavonoid Content of the Plant Extracts: Aluminium chloride colorimetric method was used with some modifications to determine the flavonoid content. 1 mL of plant extracts were mixed with 3 mL of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1M potassium acetate and 5.6mL of distilled water and remains at room temperature for 30 min. The absorbance was measured at 420 nm. Quercetin was used as standard (1mg/mL). All the tests were performed in triplicates. Flavonoid content was determined from the standard curve and expressed as quercetin equivalent (mg/g of the extracted compound) [30].

DPPH Free Radical Scavenging Activity: The stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) was used to assess the free radical scavenging activity of the different solvent extracts as a direct readout of their anti-oxidant activity. To 900 µl of each test sample (100 mg/mL), 100 µl of 95% methanol and 1 mL of freshly prepared DPPH solution in 95% methanol (1 mM) were added, mixed well and incubated at dark for 30 min. After 30 min, the absorbance was measured at 517 nm using methanol (95%) and de-ionized water with DPPH solution as reference and control, respectively [31]. The ability to scavenge the DPPH radical was measured using the following equation:

$$\% \text{ DPPH scavenged} = \{(Ac - At) / Ac\} \times 100$$

Where Ac is the absorbance of the control and at is the absorbance of the sample (solvent extracts). The antioxidant activity was expressed as IC_{50}.

ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] Assay: $ABTS^{\cdot}$ solution was prepared by mixing 7mM of ABTS and 2.45 Mm of Potassium persulphate in water, which was incubated for 12 hours in the dark at room temperature. Before use, the ABTS solution was diluted with ethanol to get an absorbance of 0.7± 0.002 at 734 nm. Briefly, to 5µl of the plant extract, 4 mL of ABTS solution was added. The samples were mixed thoroughly, incubated for 30 minutes at room temperature and absorbance was recorded at 734 nm [31].

$$\% \text{ of ABTS scavenging activity} = [\text{control- (test/control)}] \times 100$$

Ferric Reducing Antioxidant Power (FRAP) Assay: The method is based on the reduction of Fe^{3+} TPTZ complex (colourless complex) to Fe^{2+}-tripyridyltriazine (blue coloured complex) formed by the action of electron-donating antioxidants at low pH. The FRAP reagent is prepared by mixing 300mM acetate buffer, 10mL TPTZ in 40Mm HCl and 20Mm $FeCl_3.6H_20$ in the proportion of 10:1:1 at 37 °C. Freshly prepared working FRAP reagent is pipetted (3mL) and mixed with 5 µl of the plant

sample and mixed thoroughly. An intense blue colour complex is formed when ferric tripyridyl triazine (Fe^{3+} TPTZ) complex is reduced to ferrous (Fe^{2+}) form and the absorbance is recorded at 593 nm[31]. A blank is also prepared by adding FRAP to water.

Inhibition of Lipid Peroxidation: Egg homogenate (0.5mL, 10% in distilled water) and 0.1mL of each fraction were mixed separately in a test tube and the volume was made up to 1mL, by adding distilled water. Finally, 0.05mL FeSO4 (0.07M) was added to the above mixer and incubated for 30min to induce lipid peroxidation. Thereafter, 1.5mL of 20% acetic acid and 1.5mL of 0.8% TBA (w/v) in 1.1SDS and 0.05mL 20% TCA was added, vortexed and then heated in a boiling water bath for 1 hr. After cooling, 5mL of butanol was added to each tube and centrifuged at 3000rpm for 10mins. The absorbance of the organic upper layer was measured at 532nm with ascorbic acid (0.1mg/mL) as control [32].

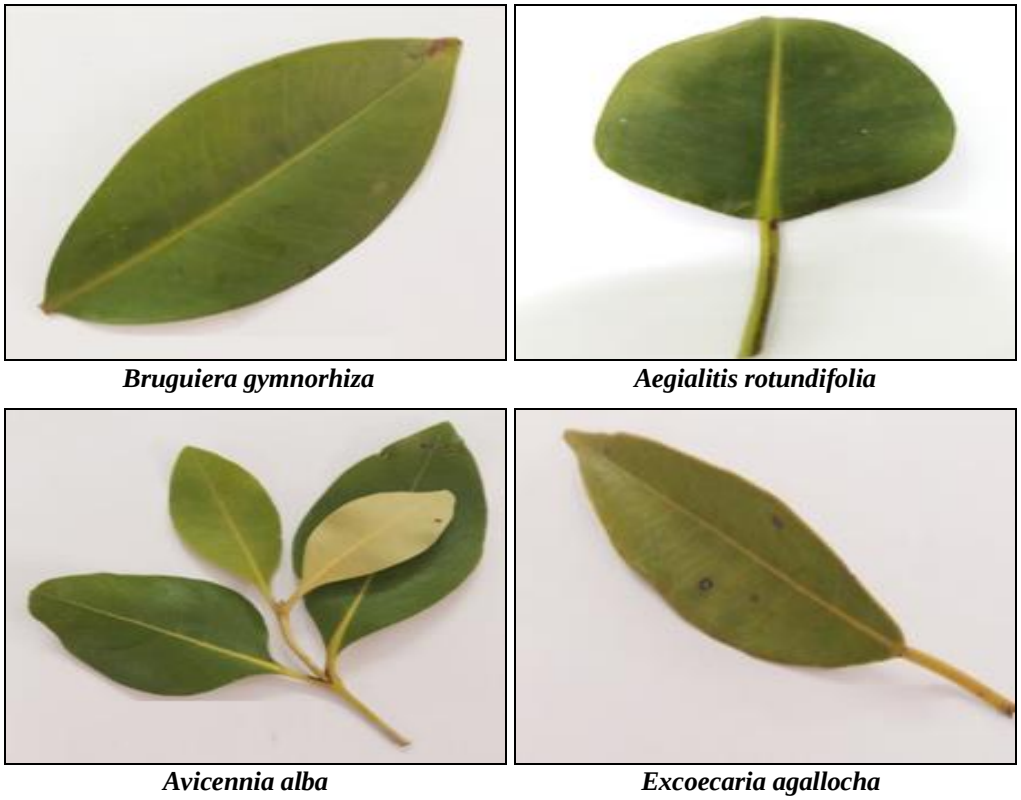
Thin Layer Chromatography: The crude plant extract was freshly prepared and filtered for TLC profiling. The solvent system used was Toluene: Ethyl acetate in 9:1 ratio (standardized by trials). Silica gel 60 F254 plate (Merck) of uniform thickness of 0.2 mm was used a stationary phase. 10µl of the extract was applied on the TLC plate and developed in the solvent system in a closed glass chamber to a height of about 8cm. The plate was sprayed with Vanillin spray reagent (0.5gm Vanillin in 100mL ethanol and 1.5 mL of conc. Sulphuric acid), and the Rf values of each band were recorded according to the formula [33]:

Retention factor (R_f) = Distance travelled by the plant extract/Distance travelled by the solvent

Liquid Chromatography-Mass Spectroscopy (LC-MS) of the Extracts: The LC-MS process was carried out at SAIF (Sophisticated Analytical Instrumentation Facility)-IIT Bombay. Q-TOF Mass Spectrophotometer was used (Model no.: G6550A). Column details - Syncronis C18 100 × 2.1, particle size 1.7µ.

RESULTS:

Collection of Plant Samples: The collected plant samples were verified from a taxonomist and botanist at Department of Botany, West Bengal State University. Four mangrove plants were selected based on ethnobotanical and literature study for conducting the experiments in the present investigation.



Bruguiera gymnorhiza ***Aegialitis rotundifolia***

Avicennia alba ***Excoecaria agallocha***

FIG. 1: THE COLLECTED MANGROVE PLANTS FROM SUNDARBANS USED IN THIS STUDY

TABLE 1: TAXONOMIC CLASSIFICATION AND IDENTIFICATION OF THE COLLECTED MANGROVE PLANTS

S. no.	Mangrove plant	Family	Common name
1	*Bruguiera gymnorhiza*	Rhizophoraceae	Kankra
2	*Aegialitis rotundifolia*	Plumbaginaceae	Tora
3	*Avicennia alba*	Avicenniaceae	Kalobaen
4	*Excoecaria agallocha*	Euphorbiaceae	Genwa

Collection and Maintenance of Microorganisms: After blood culture in an automated Bactec system, the microorganisms were isolated by standard laboratory methods. Antimicrobial sensitivity tests showed they were MDR strains. Their identifications were confirmed in VITEK automated system. They were maintained as stock cultures in the laboratory.

Extract Preparation: The extracts were prepared in the different solvents according to the mentioned protocol. The concentrated extracts were prepared with four different solvents with a final concentration of 1mg/mL.

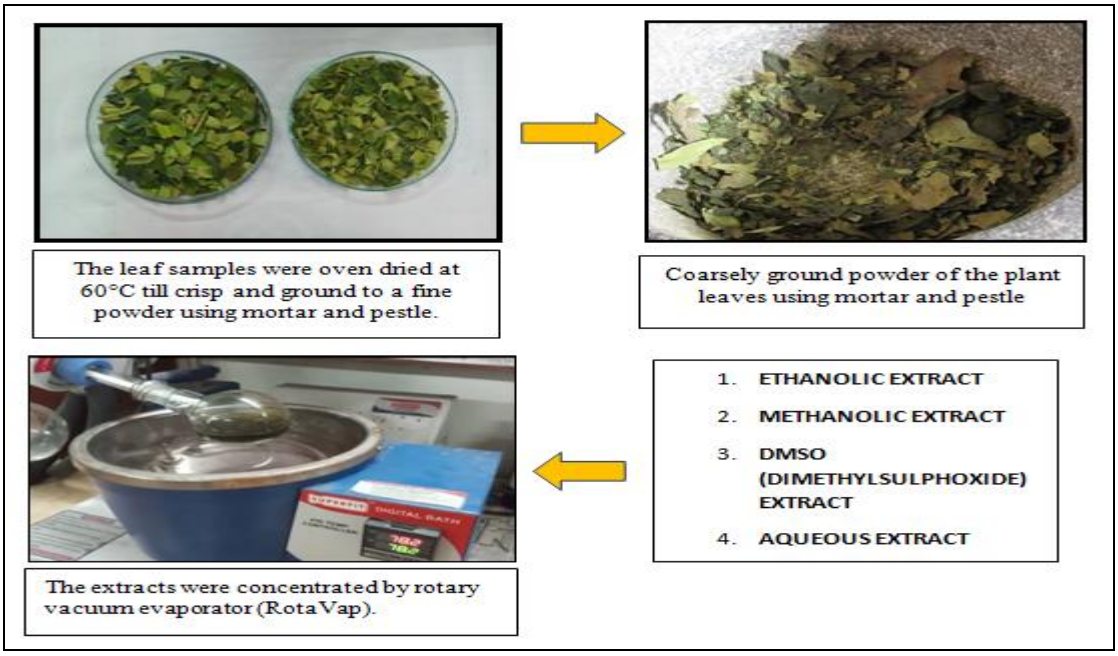
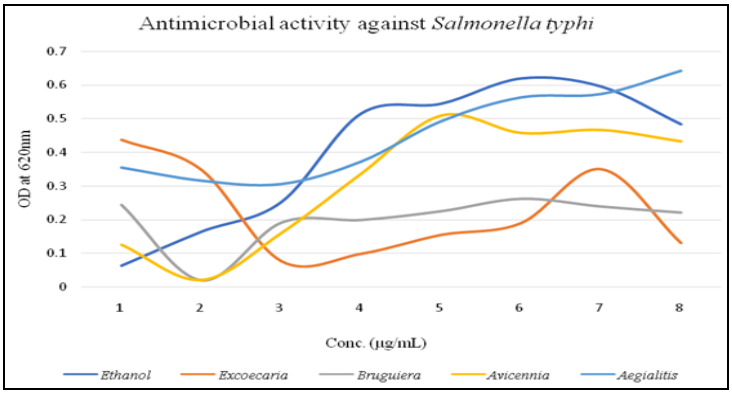


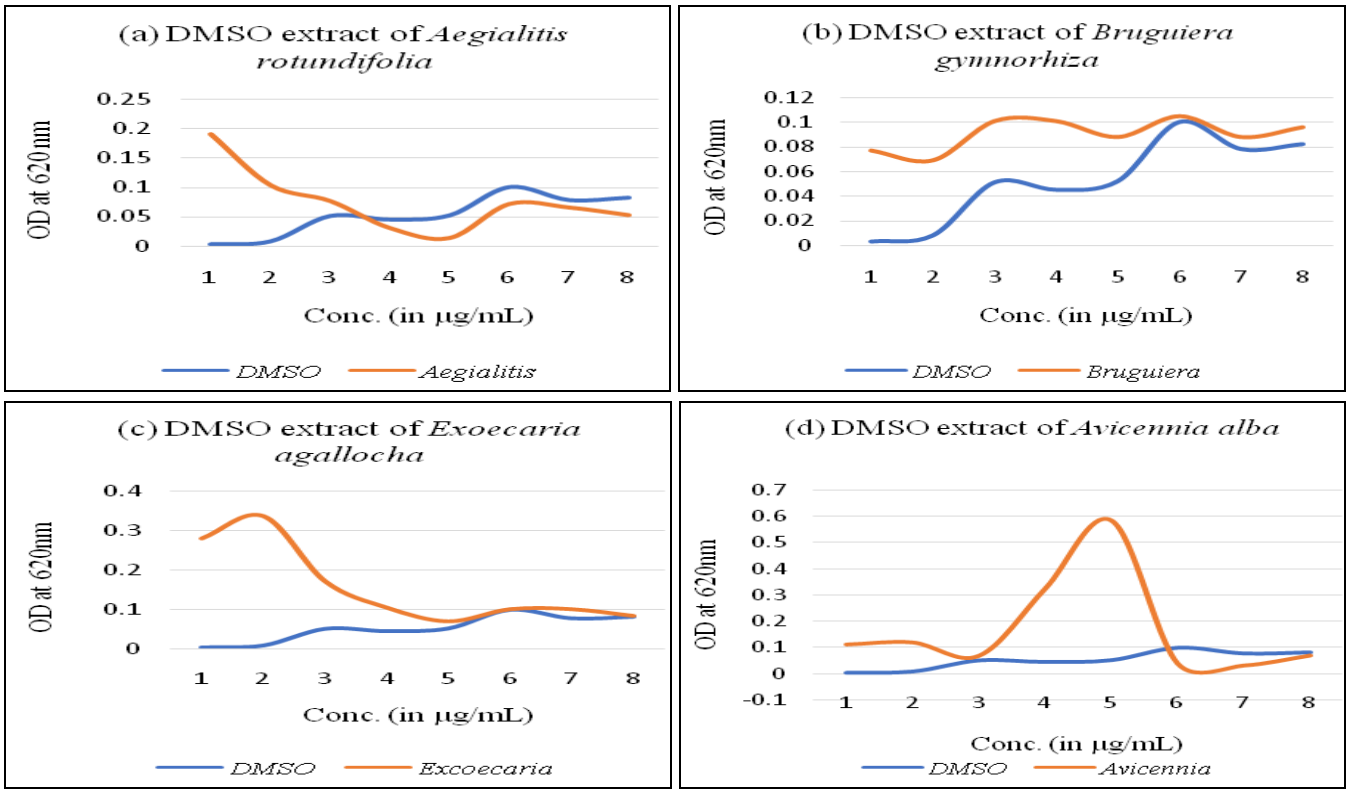
FIG. 2: EXTRACT PREPARATION

Antimicrobial Screening Assay: The ethanolic and methanolic plant extracts were screened against *Salmonella typhi*.



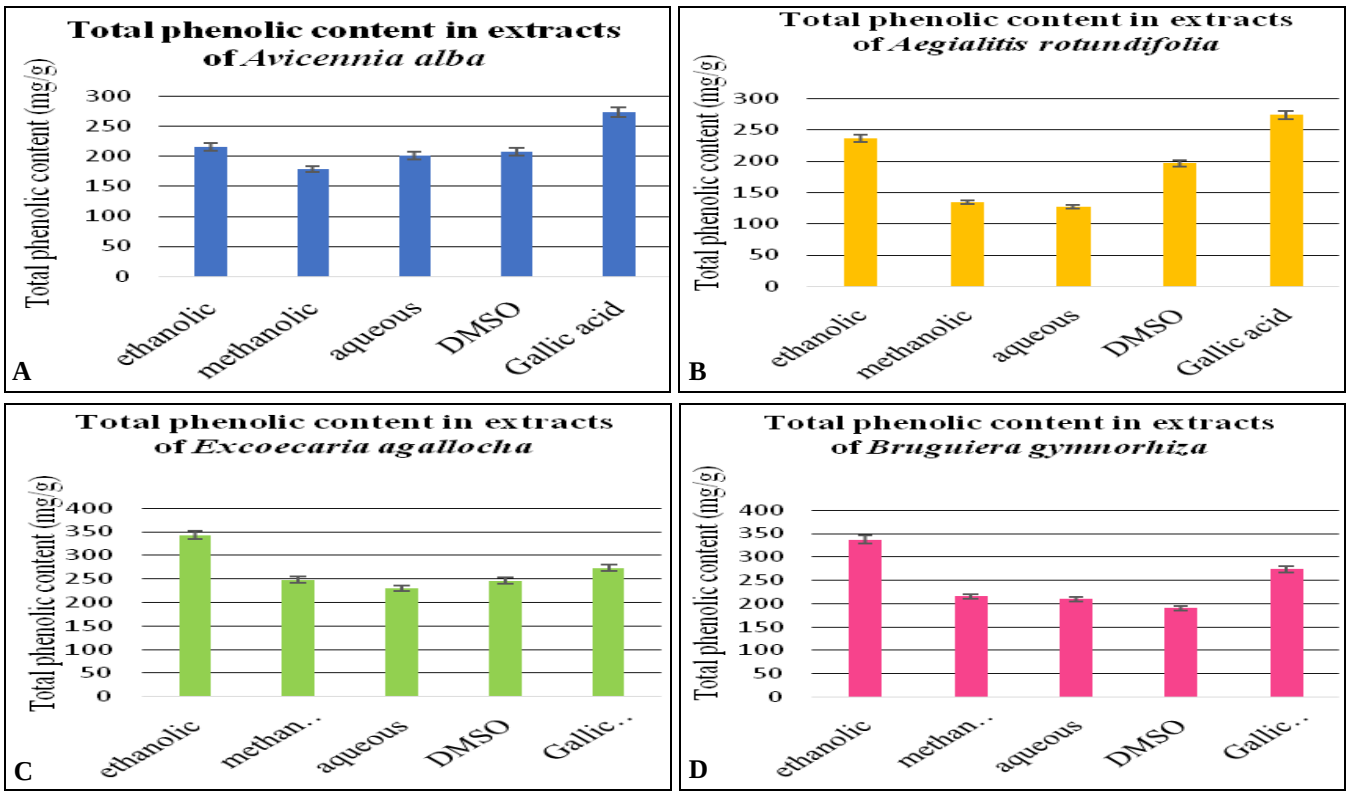
GRAPH 1: ANTIBIOTIC SCREENING ASSAY AND DETERMINATION OF MIC OF ETHANOLIC EXTRACT OF *EXCOECARIA AGALLOCHA* AGAINST *SALMONELLA TYPHI*. CONCENTRATIONS (1-8) USED: 500, 250, 125, 62.5, 31.75, 15.87, 7.93 AND 3.96 (ALL CONCENTRATIONS IN µg/mL)

The inhibitory action of the plant extracts was also evaluated against *Candida parapsilosis*. However, DMSO (dimethylsulfoxide) extracts were observed to be more potent against *C. parapsilosis*.



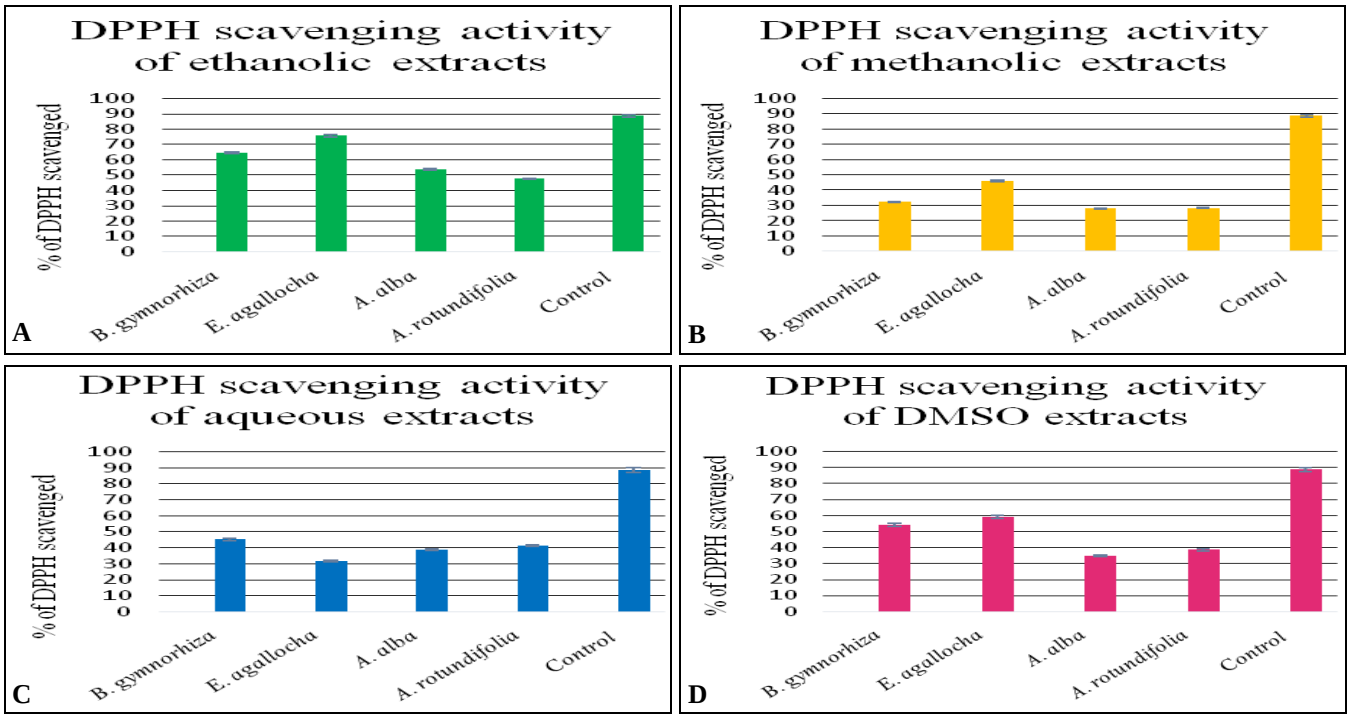
GRAPH 2 (A-D): ANTIBIOTIC SCREENING ASSAY AND DETERMINATION OF MIC OF DMSO EXTRACTS OF *AEGIALITIS ROTUNDIFOLIA*, *BRUGUIERA GYMNORHIZA*, *EXCOECARIA AGALLOCHA* AND *AVICENNIA ALBA* AGAINST *CANDIDA PARAPSILOSIS*. CONCENTRATIONS (1-8) USED: 500, 250, 125, 62.5, 31.75, 15.87, 7.93 AND 3.96 (ALL CONCENTRATIONS IN µg/mL)

Determination of Total Phenolic Content of the Plant extracts: Total phenolic content was observed to be highest in ethanolic extract of *E. agallocha* (342.56 mg/g of dry weight).



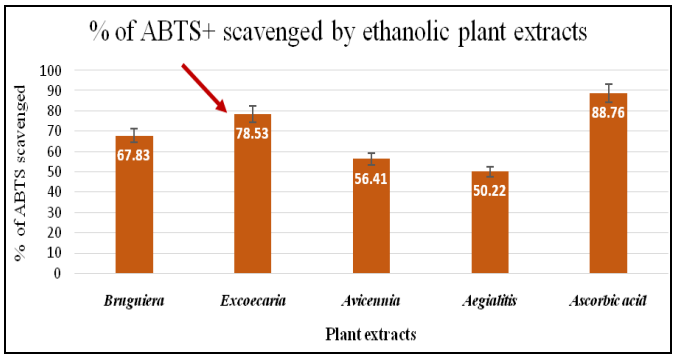
GRAPH 3: TOTAL PHENOLIC CONTENT OF THE PLANT EXTRACTS (A) *A. ALBA* (B) *A. ROTUNDIFOLIA* (C) *E. AGALLOCHA* AND (D) *B. GYMNORHIZA*

DPPH Free Radical Scavenging Activity: It can be inferred that ethanolic extract of *E. agallocha* has highest DPPH scavenging activity (75.55%) and thus, can be used as a potent antioxidant agent.



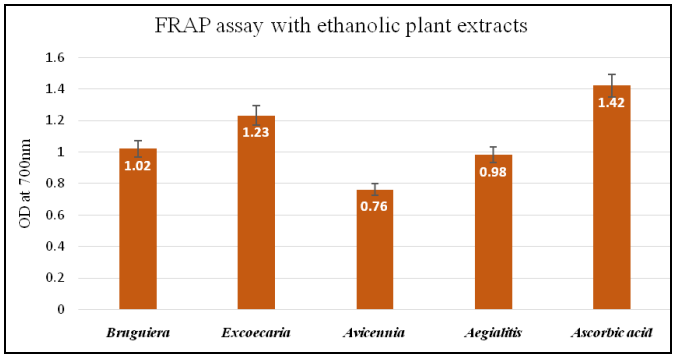
GRAPH 4: DPPH SCAVENGING ACTIVITY OF THE PLANT EXTRACTS (A) ETHANOLIC (B) METHANOLIC (C) AQUEOUS AND (D) DMSO EXTRACTS

ABTS [2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] Assay: ABTS scavenging activity was observed to be the highest in ethanolic extract of *E. agallocha* (78.53%), and this activity further substantiates its antioxidant activity.



GRAPH 5: ABTS SCAVENGING ACTIVITY OF THE ETHANOLIC MANGROVE PLANT EXTRACTS

Ferric Reducing Antioxidant Power (FRAP) Assay:



GRAPH 6: FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY WITH ETHANOLIC PLANT EXTRACTS

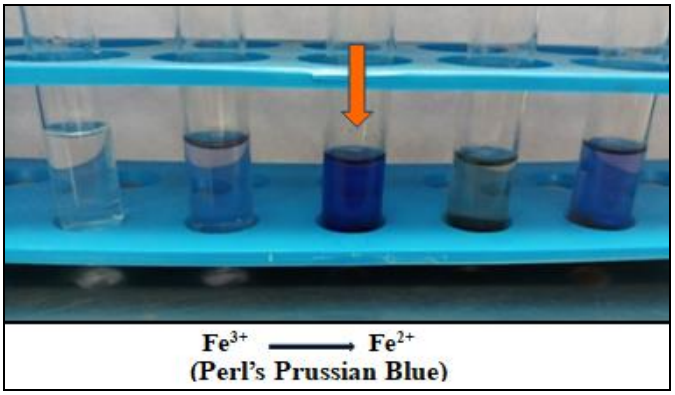
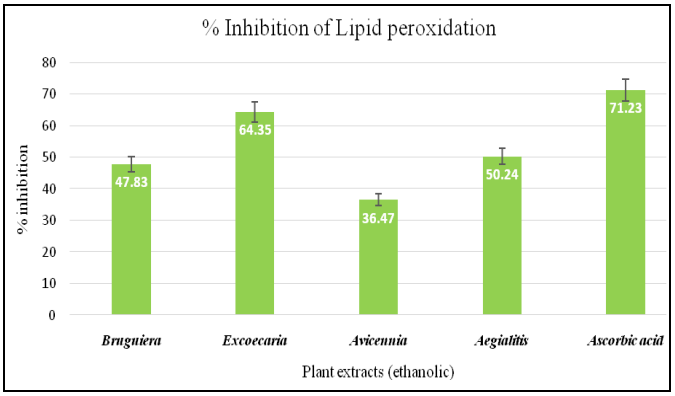


FIG. 3: FRAP ASSAY WITH ETHANOLIC EXTRACTS. THE REDUCTION of Fe^{3+} TPTZ COMPLEX (COLOURLESS COMPLEX) TO Fe^{2+}- TRIPYRIDYLTRIAZINE (BLUE COLOURED COMPLEX) FORMED BY THE ACTION OF ELECTRON DONATING ANTIOXIDANTS AT LOW PH IS INDICATED IN THE PICTURE

The reducing power was found to be highest in ethanolic extract of *E. agallocha* and it might be used as an effective antioxidant agent.

Inhibition of Lipid Peroxidation: The ethanolic extracts produced more potent lipid peroxidation inhibition.



GRAPH 7: GRAPHICAL REPRESENTATION OF THE PERCENTAGE OF LIPID PEROXIDATION INHIBITION FACILITATED BY THE ETHANOLIC PLANT EXTRACTS

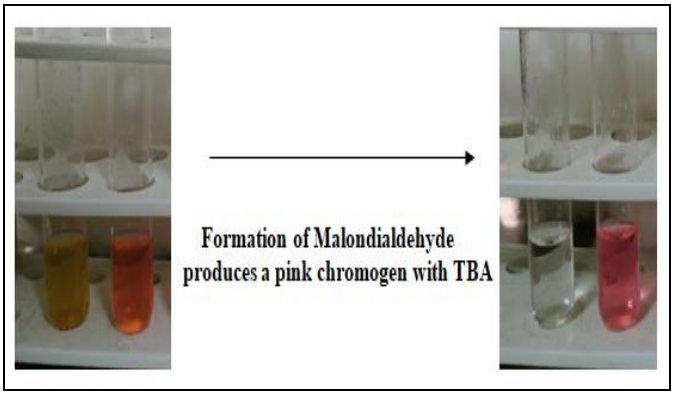


FIG. 5: LIPID PEROXIDATION INHIBITORY EFFECT EXHIBITED BY *EXCOECARIA* ETHANOLIC EXTRACT

Thin Layer Chromatography: Separate bands were observed in the TLC plate after derivatization with Vanillin spray reagent.

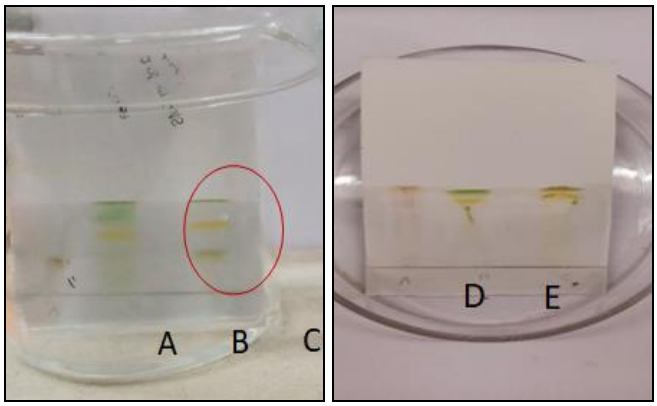


FIG. 6: TLC PLATES AFTER DERIVATIZATION WITH VANILLIN SPRAY REAGENT (MOBILE PHASE-TOLUENE: ETHYL ACETATE=9:1). A: *A. ROTUNDIFOLIA*, B: *E. AGALLOCHA*, C: *B. GYMNORHIZA*, D: *A. ALBA*, E: *A. OFFICINALIS*

Liquid Chromatography-Mass Spectroscopy (LC-MS): LC-MS results indicated the presence of several novel compounds as well as some known bioactive compounds

SAMPLE 1: *EXCOECARIA AGALLOCHA* (ETHANOLIC)

S. no.	Compound Name	Formula	Mass (G/Mol)	Rel. Abund.
1	Hexanoylglycine	$C_8H_{15}NO_3$	173.1051	3113960
2	Nicotinamide mononucleotide	$C_{11}H_{15}N_2O_8P$	334.0551	714833
3	1-L-Leucyl-L-Proline	$C_{11}H_{20}N_2O_3$	228.1472	725402
4	3-(4-Hydroxyphenyl) pyruvic acid	$C_9H_8O_4$	180.0421	642416
5	Chorismic acid	$C_{10}H_{10}O_6$	226.0473	1434743
6	6-Phosphogluconic acid	$C_6H_{13}O_{10}P$	276.0268	413388
7	N-Deacetylketokonazole	$C_{24}H_{26}C_{l2}N_4O_3$	488.1357	51085
8	Tyramine	$C_8H_{11}NO$	137.084	1121909
9	Methyl jasmonate	$C_{13}H_{20}O_3$	224.1409	1253355
10	Dihydromyricetin	$C_{15}H_{12}O_8$	320.0526	963860
11	Khivorin	$C_{32}H_{42}O_{10}$	586.2782	265755
12	Khayanthone	$C_{32}H_{42}O_9$	570.2835	2236552
13	Chlorogenic acid	$C_{16}H_{18}O_9$	354.0963	2228670
14	S, S, S, -Tributylphosphotrithioate	$C_{12}H_{27}OPS_3$	314.1017	304638
15	Alpha,4-Dihydroxytriazolam	$C_{17}H_{12}Cl_2N_4O_2$	374.0292	278962
16	Ellagic acid	$C_{14}H_6O_8$	302.0079	629690

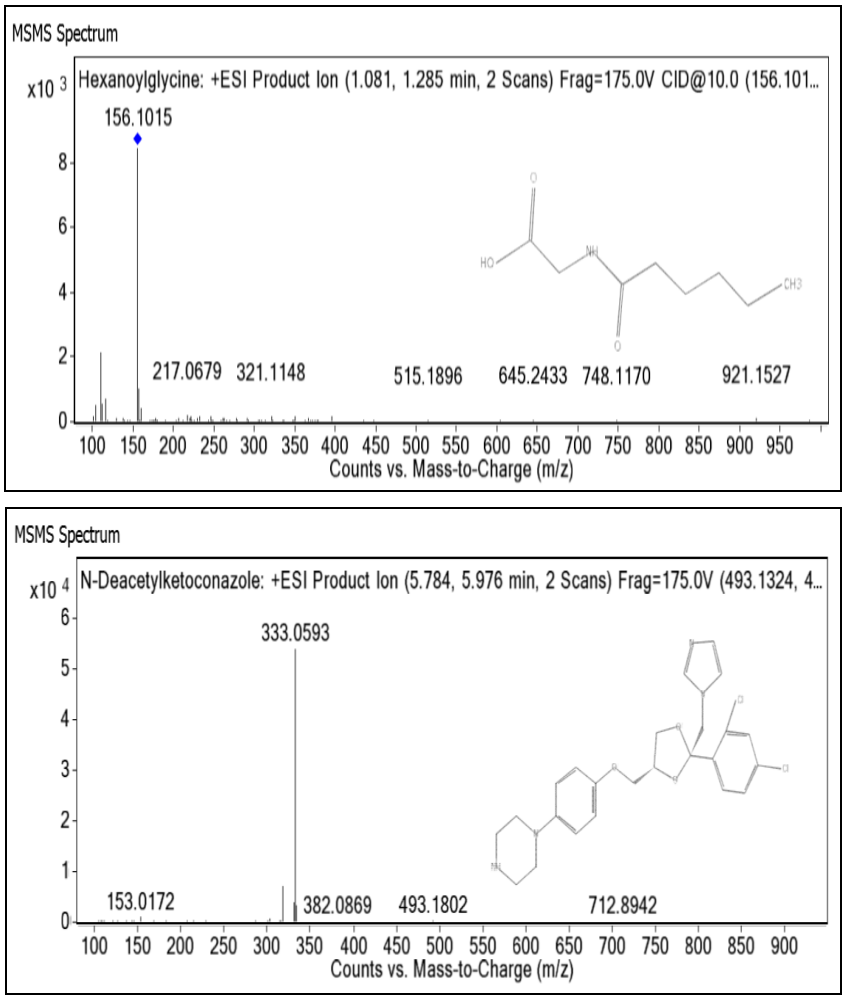


FIG. 7: LC-MS SPECTRA OF ETHANOLIC EXTRACT OF *EXCOECARIA AGALLOCHA* INDICATING THE PRESENCE OF HEXANOYLGLYCINE AND N-DEACETYLKETOKONAZOLE IN HIGH ABUNDANCE

SAMPLE 2: *BRUGUEIRA GYMNORHIZA* (METHANOLIC)

S. no.	Compound Name	Formula	Mass (g/mol)	Rel. Abund.
1	4-(2-hydroxy-3-isopropyl-aminopropyl) benzoic acid	$C_{13}H_{19}NO_4$	253.1308	523753
2	Isoamyl nitrite	$C_5H_{11}NO_2$	117.0785	2524152
3	Triparanol	$C_7H_{13}NO_2$	143.0943	860991
4	Methylsalicyluric acid	$C_{10}H_{11}NO_4$	209.0681	190860
5	Cephalotaxine	$C_{18}H_{21}NO_4$	315.1463	414152
6	Methyl jasmonate	$C_{13}H_{20}O_3$	224.1405	987172
7	2-Aminopropiophenone	C9H11NO	149.0838	1658526
8	4-Hydroxypropranolol	$C_{16}H_{21}NO_3$	275.1539	1316810
9	Acetylaminodantrolene	$C_{16}H_{14}N_4O_4$	326.1019	1055786
10	Diacetyldideisovalerylrhodomyrtoxin	$C_{18}H_{16}O_7$	344.0891	3736048
11	Koparin 2'-Methyl ether	$C_{17}H_{14}O_6$	314.0783	485286
12	Isotectorigenin, 7-Methyl ether	$C_{18}H_{16}O_6$	328.0938	513190
13	6-alpha-hydroxycastasterone	$C_{28}H_{50}O_5$	466.3669	410095
14	9S, 10-epoxy-10, 12Z-octadecadienoic acid	$C_{18}H_{30}O_3$	294.2188	669699
15	6-Deoxotyphastetrol	$C2_8H_{50}O_3$	434.3768	1171355
16	Campestanol	$C_{28}H_{50}O$	402.3871	364153
17	Harderoporphyrin	$C_{35}H_{36}N_4O_6$	608.2622	3510452
18	Rescinnamine	$C_{35}H_{42}N_2O_9$	634.2999	2651388
19	Epigallocatechin	$C_{15}H_{14}O_7$	306.0766	207831
20	Monodesmethylchlorpheniramine	$C_{15}H_{17}ClN_2$	260.1076	680001
21	Diltiazem	$C_{22}H_{26}N_2O_4S$	414.1556	123904
22	Tubaic acid	$C_{12}H_{12}O_4$	220.0757	19347
23	S-Methylcaptopril	$C_{11}H_{18}O_3S$	230.0975	516665
24	2,3-Dihydroxy-4-methoxy-4'-ethoxybenzophenone	$C_{16}H_{16}O_5$	288.0975	100373
25	Mackain	$C_{16}H_{12}O_5$	284.0686	454665
26	3-Deoxy-3-azido-25-hydroxyvitamin D3	$C_{27}H_{44}N_{30}$	426.3491	547774

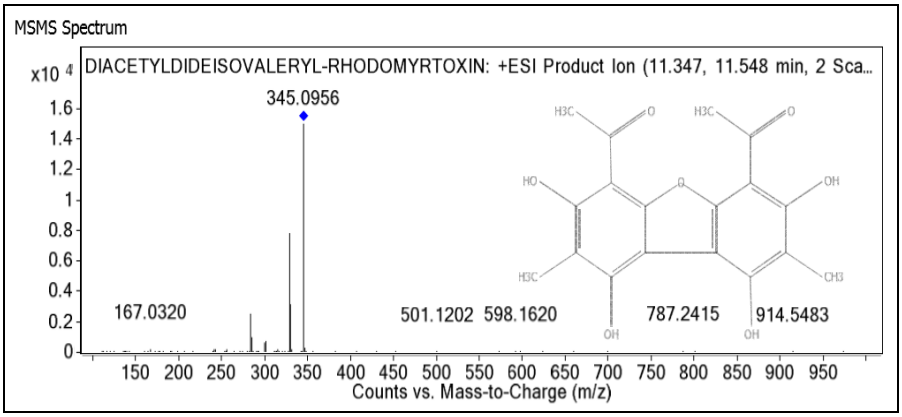


FIG. 8: LC-MS SPECTRA OF METHANOLIC EXTRACT OF *BRUGUIERA GYMNORHIZA* INDICATING THE PRESENCE OF DIACETYLDIDEISOVALERYL-RHODOMYROTOXIN IN THE HIGHEST ABUNDANCE

SAMPLE 3: *BRUGUIERA GYMNORHIZA* (ETHANOLIC)

S. no.	Compound Name	Formula	Mass (g/mol)	Rel. Abund.
1	4-Hydroxyclobazam	$C_{16}H_{13}ClN_2O_3$	316.0611	8226407
2	Bilirubin	$C_{33}H_{36}N_40_6$	584.2631	2414879
3	Harderoporphyrin	$C_{35}H_{36}N_4O_6$	608.2614	4386067
4	Khayanthone	$C_{32}H_{42}O_9$	570.2835	11926320
5	(3a, 5b, 7b, 12a) -(1,3-dihydro-5-nitro-1,3-dioxo-2H-isoindol-2-yl) methyl ester-3,7,12-trihydroxy-Cholan	$C_{33}H_{44}N_2O_9$	612.2945	1545728
6	Harderoporphyrinogen	$C_{35}H_{42}N_4O_6$	614.3114	4769120
7	Hexacosanedioic acid	$C_{26}H_{50}O_4$	426.3718	3636857
8	Teasterone	$C_{28}H_{48}O_4$	448.354	1650200
9	Quercitrin	$C_{21}H_{20}O_{11}$	448.1013	145282
10	Demeclocycline	$C_{21}H_{21}ClN_2O_8$	464.0968	426521
11	Hederagenin	$C_{30}H_{48}O_4$	472.3562	572804
12	3-alpha, 6-alpha, 7-alpha, 12 -alpha-Tetrahydroxy-5beta-cholest-24-en-26-oic acid	$C_{27}H_{44}O_6$	464.3163	152528

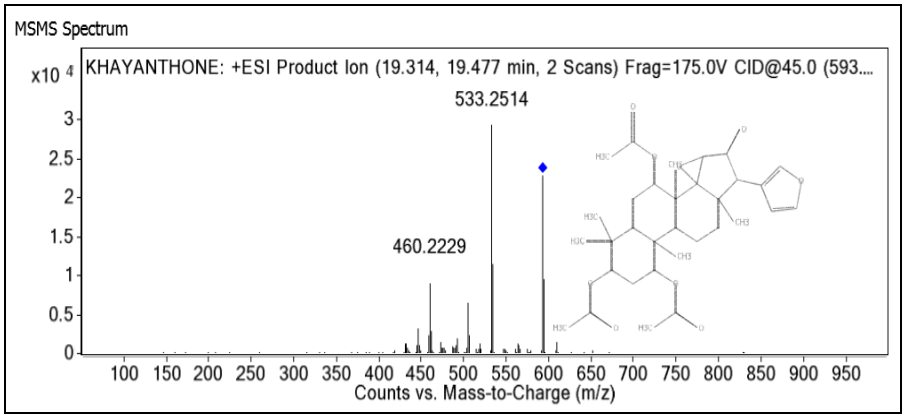


FIG. 9: LC-MS SPECTRA OF ETHANOLIC EXTRACT OF *BRUGUIERA GYMNORHIZA* INDICATING THE PRESENCE OF KHAYANTHONEIN HIGHEST ABUNDANCE

SAMPLE 4: *AVICENNIA ALBA* (ETHANOLIC)

S. no.	Compound Name	Formula	Mass (g/mol)	Rel. Abund.
1	Isoamyl nitrite	$C_5H_{11}NO_2$	117.0785	4432584
2	Cephalotaxine	$C_{18}H_{21}NO_4$	315.1487	1362816
3	2-Aminopropiophenone	$C_9H_{11}NO$	149.0836	1480729
4	Lecanoric acid	$C_{16}H_{14}O_7$	318.0736	1943103
5	Bilirubin	$C_{33}H_{36}N_4O_6$	584.2619	424348
6	Khivorin	$C_{32}H_{42}O_{10}$	586.2782	1224105
7	Khayanthone	$C_{32}H_{42}O_9$	570.2835	5025383
8	Rescinnamine	$C_{35}H_{42}N_2O_9$	634.2759	1496168
9	Dihydrogambogic acid	$C_{38}H_{46}O_8$	630.3048	585071
10	Trandolapril glucoronide	$C_{30}H_{42}N_2O_{11}$	606.2818	4488828
11	Harderoporphyrinogen	$C_{35}H_{42}N_4O_6$	614.3085	1571476
12	2,4,6-trimethyl-2, 15-tetracosadienoic acid	$C_{27}H_{50}O_2$	406.3816	809418

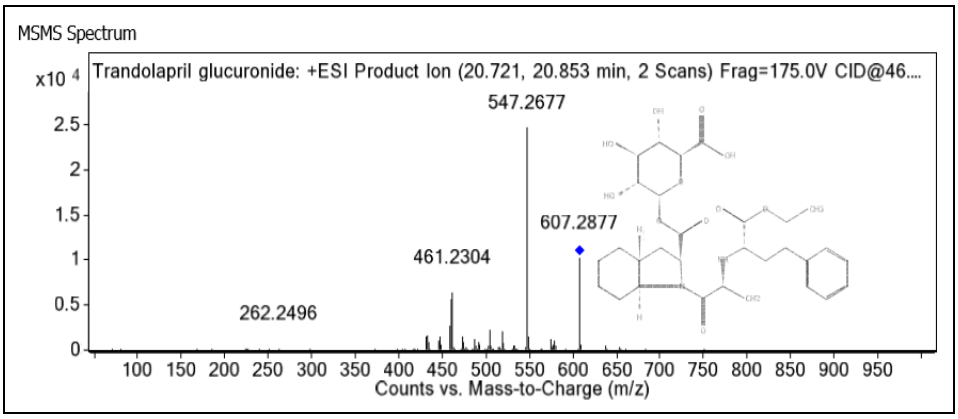


FIG. 10: LC-MS SPECTRA OF ETHANOLIC EXTRACT OF *AVICENNIA ALBA* INDICATING THE PRESENCE OF TRANDOLAPRIL GLUCURONIDE IN HIGHEST ABUNDANCE

SAMPLE 5: *AEGIALITIS ROTUNDIFOLIA* (METHANOLIC)

S. no.	Compound Name	Formula	Mass (g/mol)	Rel. Abund.
1	Isoamyl nitrite	$C_5H_{11}NO_2$	117.0785	3927555
2	4-hydroxyphenylethanol	$C_8H_{10}O_2$	138.0676	3275680
3	Alpha-[1-(ethylamino) ethyl]-p-hydroxy-benzyl alcohol	$C_{11}H_{17}NO_2$	195.1253	5093222
4	Lecanoric acid	$C_{16}H_{14}O_7$	318.0736	729272
5	Bilirubin	$C_{33}H_{36}N_4O_6$	584.2619	835516
6	Khivorin	$C_{32}H_{42}O_{10}$	586.2782	5881648
7	Khayanthone	$C_{32}H_{42}O_9$	570.2835	13443402
8	24, 24-Difluro-25-hydroxy-26,27-dimethylvitamin D3	$C_{29}H_{46}F_2O_2$	464.3481	1652171
9	Gallic acid	$C_7H_6O_5$	170.0204	157930
10	Epicatechin pentaacetate	$C_{25}H_{24}O_{11}$	500.1331	238764
11	Methyl 7-desoxypurpurogallin-7-carboxylate trimethyl ether	$C_{16}H_{16}O_6$	304.0968	104873
12	1,2-di-(9Z, 12Z, 15Z-octadecatrienoyl)-3-o-beta-D-galactosyl-sn-glycerol	$C_{45}H_{74}O_{10}$	774.5271	412871
	5-[2-(Hydroxymethyl)-5-methylphenoxy]-2,2-dimethyl-pentanoic acid (Gemfirozil M4)	$C_{15}H_{22}O_4$	266.1529	156364

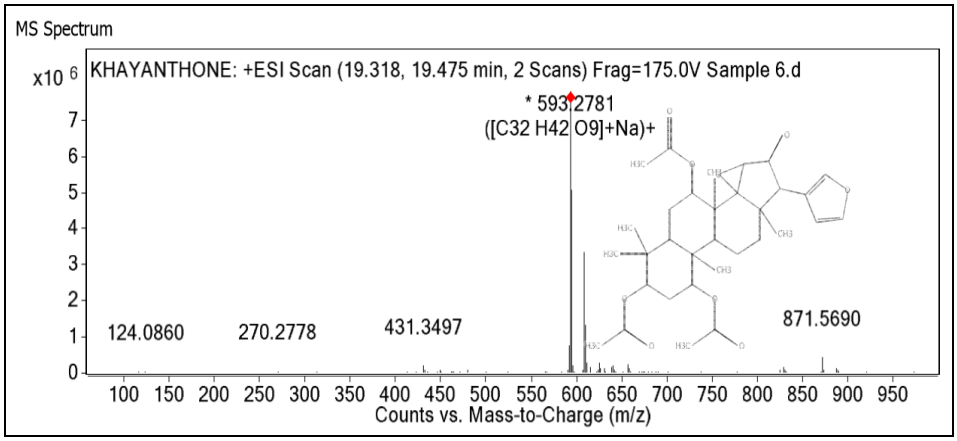


FIG. 11: LC-MS SPECTRA OF ETHANOLIC EXTRACT OF *AEGIALITIS ROTUNDIFOLIA* INDICATING THE PRESENCE OF KHAYANTHONE IN THE HIGHEST ABUNDANCE

Thus, A number of novel compounds have been detected by LC-MS and according to their relative abundances. The compounds will further be isolated and purified from the crude extracts by sophisticated chromatographic techniques to check their antibacterial, antifungal and anti-cancerous activity.

DISCUSSION: We mainly focused our study on two important human pathogens - *S. typhi* and *C. parapsilosis*. This is because these two microbes recently acquired antimicrobial resistance to a great extent, and they have become emerging pathogens in many countries throughout the globe. Only a few studies are present on antimicrobial activity of

mangrove plants against *S. typhi*. In one study, dried leaf samples of *E. agallocha* collected from Muthupettai mangrove forest of Thiruvarur district of Tamil Nadu, India, showed good antimicrobial activity against *S. typhi* [34]. In another study ethanolic extract of leaves of *Sonneratia alba* collected from Chorao Island, Goa, India, showed good antimicrobial activity against *S. typhi* [35]. Although anti-*Candida* activities of some common terrestrial plants are well known [36-38], but there are only a few studies on this activity in relation to mangrove plants [39].

Again it is important to note that some yeasts are also present in mangrove ecosystems [40, 41], where they play important role in the detritus food web [42], possibly involving marine invertebrates and zooplanktons. Some *Candida* spp. is also frequently found in mangrove ecosystem [43]. However, the role of yeasts in mangrove ecosystem is largely unknown. Among different *Candida* spp., *C. tropicalis* is most commonly found in this ecosystem as observed in an important study in China [44].

In one study leaves of *Avicennia officinalis*, collected from the mangrove forest of Mahanadi delta region of Odisha coast, India showed good antifungal activities against *Candida albicans* and *C. krusei* with MIC values of 200 and 100 µg/mL respectively [45]. In this study, although *E. agallocha* extract showed good antimicrobial activity against *S. typhi*, however, antifungal activity against *C. parapsilosis* was not established. This is possibly due to close natural habitat of *Candida* spp. with mangrove plants in this ecosystem.

Phytochemicals of mangrove plants have been explored in several studies, and many biologically active phytochemicals such as flavonoids, tannins, steroids, terpenoids, saponins and phenols are found to be present in significant amounts [46-48]. In this study total phenolic content was highest in ethanolic extract of *E. agallocha* (342.56 mg/g of dry weight). This may contribute to its anti-microbial action. Oxidative injury is an important pathogenetic marker in many diseases of human being such as inflammation, immunological disorders, neoplasia, viral infections *etc*. [49] Thus, natural antioxidants present in plants may have a pivotal role in the treatment of these diseases. In this study, we observed the highest DPPH scavenging activity (75.55%), ABTS scavenging activity (78.53%) and ferric reducing power in ethanolic extract of *E. agallocha*. Thus this plant may be exploited as a good natural source of antioxidants. The ethanolic extracts of the leaves of all the plants produced potent lipid peroxidation inhibition activity. Many interesting chemicals were identified in liquid chromatography-mass spectroscopic (LC-MS). Almost all chemicals present in *E. agallocha* are biologically important.

E. agallocha contains some amount of N-deacetyl ketoconazole (DAK), which is the major metabolite of ketoconazole, which undergoes further metabolism by the flavin-containing monooxygenases (FMO) to form a potentially toxic dialdehyde which damages the liver [50]. Heat inactivation of FMOs abolished the formation of this toxic chemical. Ketoconazole is a synthetic antifungal drug used to treat and prevent fungal Infections. It works by inhibiting an enzyme required for the synthesis of ergosterol and ultimately altering the fungal cell membrane and is primarily fungistatic. It is very lipophilic, which leads to accumulation in fatty tissues, and is best adsorbed at the highly acidic level. In conventional treatment, ketoconazole is usually prescribed for infections such as ringworm, candidiasis, *etc*. The decrease in testosterone caused by the drug makes it useful for treating prostate cancer and for preventing post-operative erections following penile surgery.

Hexanoylglycine is present in exceptionally large amount in *E. agallocha*. Hexanoylglycine (other names are Caproylglycine, N-(1-Oxohexyl)glycine, N-Caproylglycine, N-Hexanoyl-glycine, N-Hexanoylglycine) is an acyl glycine (Chemical formula: $C_8H_{15}NO_3$; Molecular weight 173.2096; CAS number 24003-67-6) present in the urine as minor metabolites of fatty acids. Disorders of mitochondrial fatty acid beta-oxidation are associated with increased excretion of hexanoylglycine in urine. It is particularly found in patients with hereditary medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, which is a genetic disorder [51] Normal level of hexanoylglycine in urine is 1-2 µg/mg of creatinine. In MCAD deficiency it becomes 3-170 µg/mg of creatinine (acute stage 20-600 µg/mg of creatinine). In other related congenital metabolic disorders, it is usually 1-3 µg/mg of creatinine, except glutaric aciduria type

II, where it becomes 2-15 µg/mg of creatinine (acute stage 20-100 µg/mg of creatinine), and in ethylmalonic - adipose aciduria it becomes 6-75 µg/mg of creatinine (acute 61-152 µg/mg of creatinine). This chemical is used as analytical control in urine tests.

Chorismic acid is present at a key branching point in aromatic acid biosynthesis. It is the precursor of tryptophan, tyrosine, and phenylalanine. It helps the biosynthesis of vitamin K and folate in plants and microorganisms. It can modulate t-RNA. It is also a precursor of salicylic acid. It is converted to para-aminobenzoic acid, and it is associated with ubiquinone biosynthesis in Gram-negative bacteria. In bacteria it is converted into 4-hydroxybenzoate and pyruvate [52, 53].

Tyramine is a naturally occurring catecholamine releasing trace amine formed from tyrosine. Tyramine is physiologically metabolized by monoamine oxidases into 4-hydroxyphenylacet-aldehyde and if there is intake of monoamine oxidase inhibitors (MAOIs) with foods high in tyramine *e.g.* cheese [54], a hypertensive crisis can result. Tyramine can induce migraine.

Methyl jasmonate (MeJA) is a volatile organic compound used in defense of plants, as well as in germination of seeds, root growth, flowering, fruit ripening, and senescence [55]. Methyljasmonate is derived from jasmonic acid. An herbivorous attack on a plant liberates MeJA both for internal defense and for defense signalling to other plants. It is also a plant hormone involved in tendril coiling, flowering, seed, and fruit maturation. It induces cytochrome C release in the mitochondria of cancer cells, leading to cell death, but does not harm normal cells.

Khayanthone is a limonoid (bitterness of lemon) formed from apotirucallane after loss of four terminal carbons [56]. Limonoids are also known as tetranortriterpenoids. They occur mainly in the Meliaceae, Rutaceae, and Cneoraceae families. The neem tree (*Azadirachta indica*), a limonoid producing plant produces a limonoid known as Azadirachtin.

Chlorogenic acid is an ester of caffeic acid and quinic acid found in coffee and coffee beans. It is also found in *Hibiscus sabdariffa*, peaches, prune, eggplants, potatoes. It produes a green colour when oxidized. It releases glucose slowly after meals and it has got antihypertensive anti-inflammatory effects. It can be used as a dietary supplement [57]. Ellagic acid is found in fruits and vegetables, in oak species and some mushrooms. It is a natural phenol antioxidant. It is also found in grapes, chestnuts, walnuts, cranberries, strawberries, *etc*.

Nicotinamide mononucleotide is a nucleotide derived from nicotinamide and ribose. It is a derivative of niacin, and in our body, it is converted to nicotinamide adenine dinucleotide (NAD) [58]. Dihydromyricetin is used as an anti-alcohol intoxication medication. 6-Phosphogluconic acid is an intermediate in the pentose phosphate pathway and the Entner-Doudoroff Pathway. 4-Hydroxy-phenylpyruvic acid is an intermediate in the metabolism of the amino acid phenylalanine. 1-L-Leucyl-L-Proline may inhibit ACE receptors. Khivorin has antibacterial and antifungal activities. S, S, S, -tributylphosphotrithioate is related to insecticidal activities. Alpha,4-Dihydroxytriazolam is a hypnotic.

Among other chemicals, diacetyl dideisovaleryl rhodomyrtoxin is an antibacterial agent acting on MDR hospital-acquired infections caused by Gram-positive bacteria. Rhodomyrtone is highly active against MRSA (methicillin-resistant *Staphylococcus aureus*), vancomycin-intermediate *S. aureus*, and vancomycin-resistant *Enterococcus* strains [59].

Trandolapril glucuronide is a non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor with antihypertensive activity [60]. It is converted into its active form, trandolaprilat, in the liver, which competitively inhibits ACE, blocking the conversion of angiotensin I to angiotensin II. It also decreases the secretion of aldosterone by the adrenal cortex. Trandolapril may improve survival in clinically stable myocardial infarction patients with left ventricular dysfunction, as an adjunct treatment, it is used in congestive cardiac failure, and it slows the progression of kidney damage in hypertension associated with diabetes mellitus and micro-albuminuria. Isoamyl nitrite is a well-known volatile chemical agent which is used in angina pectoris for more than 100 years. It directly causes vasorelaxation by nitric oxide and via cyclic GMP [61].

Thus, this study not only showed excellent anti-bacterial activity against *S. typhi* by the extracts of leaves of four mangrove forest plants, it also shows their high potentiality of bioactive agents and antioxidants.

CONCLUSION: The present study revealed excellent antimicrobial activities of extracts of leaves of mangrove plants, particularly of *E. agallocha* against *S. typhi*. There was no antifungal activity against *C. parapsilosis*, which appears due to their close habitat with these plants in mangrove ecosystem. Phenolic content and antioxidant activities are also prominent in these plants, particularly in *E. agallocha* extract. These mangrove plants are good natural reservoirs of many important bioactive chemicals, among them commercial venture for hexanoylglycine, and methyl jasmonate will be an important landmark of economic challenge in future utilizing the mangrove plant *E. agallocha.*

Contribution of Authors: Tamanna Sultana - Literature search, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation; Arup Kumar Mitra and Satadal Das – Concept, design, the definition of intellectual content, manuscript editing, and manuscript review.

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CONFLICTS OF INTEREST: Nil

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