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Phytochemistry and Antibacterial Potentiality of Selected Mangrove Plants from Coringa Wild Life Sanctuary, Andhra Pradesh, India.

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ABSTRACT
Mangrove plants are well known for their resilience in saline and stressful environments, leading to the biosynthesis of a variety of secondary metabolites with significant pharmacological potential. The objective of this study is to explore phytochemical composition, total phenolic content (TPC), functional group characterization and antibacterial potentialities from four different mangrove species— Aegiceros corniculatum, Bruguiera gymnorrhiza, Ceriops decandra, and Sonneratia apetala leaf and stem in different solvents. Preliminary phytochemical screening revealed that B. gymnorrhiza plant exhibited diversity of secondary metabolites, including flavonoids, alkaloids, tannins, saponins, steroids and terpenoids whereas Aegiceros corniculatum and Sonneratia apetala also exhibited the presence of all metabolites except alkaloids and quinones, and C. decandra is devoid of tannins. Quantification of total phenolic content further confirmed that the richest phenolic contents in A. corniculatum and B. gymnorrhiza and S. apetala non polar solvents (120 mg, 73 mg and 76 mg GAE/g dry weight respectively) whereas C. decandra polar extract exhibited highest phenolic concentration (79 mg GAE/g). FTIR spectroscopy identified key functional groups such as hydroxyl (-OH), carbonyl (C=O), alkenes (C=C), alkanes (CH), amine salts (NH) and aromatic rings supporting the presence of polyphenolic compounds such as flavonoids, tannins, terpenoids, saponins more from non-polar extracts than polar solvents responsible for antimicrobial effects. All plants screened for antibacterial efficacy against multi drug resistant (MDR) Escherichia coli, Salmonella typhimurium, Shigella flexineri, Klebsiella pneumonia, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Vibrio parahaemolyticus. Of the four plant extracts B. gymnorrhiza ethyl acetate and chloroform had the highest inhibitory effects against Gram negative bacteria than the Gram-positive bacteria tested. These findings highlight the potential of non-polar extracts of mangrove species, particularly B. gymnorrhiza, as propitious source of natural antibacterial agents for pharmaceutical applications.
Key words: *AMR, Mangroves, B. gymnorrhiza, A. corniculatum, C. decandra, and S. apetala; Mangrove ecosystem; phytochemicals; FTIR, bioactivity.*

INTRODUCTION

The discovery of antibiotics has led to a tremendous improvement in medical history to treat pathogenic infections, and these became one of the commonly used therapeutics against various diseases. Unfortunately, each discovery followed rigorous and indiscriminate use of these magic bullets led the development of resistance in pathogens thereby posing high risks of side effects to the individuals (Baym et.al, 2016). Over the past decades, multidrug resistance (MDR) has become a major threat in the treatment of infectious diseases. The drug resistance in bacteria is an evolving phenomenon because of genetic mutations and/or acquired genomes. The long-term usage of the antibiotics imposes serious effects on the host physiology by disturbing natural microbiota that further results in gastrointestinal abscesses, inflammation, and allergies etc. (Cox et al., 2014). Today, research is active in finding alternatives for combating multidrug-resistance in pathogens. The World Health Organization (WHO) published a global priority pathogens list and categorized them as critical, high, and medium antibiotic-resistant bacteria that need to be addressed urgently through research and development of new alternative drug treatments. So, development of new therapeutic alternatives in place of antibiotics is the pressing priority.

The plants are richest source of therapeutic agents for ages back of human civilisation. Plant based bioactive compounds which are less toxic to humans proved to have strong inhibitory activity against the plant, animal, and human pathogens (Saranraj and Sujitha, 2015). Mangroves have a long tradition of medicinal use and extensively studied for their antimicrobial components and its pharmaceutical applications (Bandaranayake ,1998,2002). Traditionally, different parts of mangrove plants have been used for ages by the local people as folk medicine for curing many diseases including liver diseases. For instance, *R. racemosa* bark is used to cure boils

and fungal infections. An infusion of its leaf and bark is used to treat diarrhoea, dysentery, fever, malaria and leprosy (C.N. Duke, J.A. Allen et al., 2006). Traditionally, the sap of mangrove plants is used to treat cutaneous pruritus, herpes, scabies, and thrush (Chong et.al, 2009), the fruits of *B. gymnorrhiza* were extensively used as therapeutic agents to treat eye diseases and herpes (Haq et al, 2011).

Essential phytochemicals present in medicinal plants as secondary metabolites such as alkaloids, phenolics, steroids, terpenoids, tannins, saponins, flavonoids, glycosides and phenolic compounds have been characterized from mangroves and have toxicological, pharmacological and ecological importance. (Bandaranayake, 2002, Haq et al, 2011; Ravikumar et al, 2010; Das et al., 2018, Lalitha et.al, 2021). *Acanthus ilicifolius* have proved to possess analgesic and anti-inflammatory activities due to the presence of metabolites like long-chain alcohols, tri terpenes, etc for which plant is used in the treatment of paralysis, asthma, rheumatic pains. Acanthicifolin, an alkaloid has been isolated from this species (Kokpol et al. 1984).

Previous studies on mangroves families Avicenniaceae, Rhizophoraceae, Sonneratiaceae reported to be a rich source of tannins (Bandaranayake, 1995). Reportedly, an alkaloid "rhizophorin" is extracted from *Rhizophora mucronata,* "naphthoquinones" from *Avicennia alba,* "brugin" another alkaloid isolated from *Bruguiera sexangular* and *Acrosticum aureum* and *Rhizophora apiculata* are rich in terpenoids, steroids, and novel terpenoid esters. Findings on *Bruguiera sexangular* bark extracts are proved effective against two tumors, Sarcoma 180 and Lewis Lung Carcinoma (Loder and Russell, 1969). According to Bandaranayake, 2002 research study on fruits and barks of the *Sonneratia* have remedial activity against asthma, febrifuge, ulcers, swellings, sprains, bleeding, and haemorrhages. *Acanthus ilicifolius has* a steroid called stigmasterol which has hypercholesterolemic effects (Kokpol et al. 1990; Peng and Long 2006; Firdaus et al. 2013). This mangrove also has antifungal properties due to the presence of 2- Benzoxazolin which is extensively used as central nervous system depressants (Bandaranayake, 2002). As mangroves are rich in potent source of antioxidants and antimicrobial substances, they have been used in various herbal medicinal products to treat diseases like cancer, diabetes, HIV etc has been reported by various researchers. (Premanathan et al. 1999, Bandaranayake 2002, Banerjee et al, 2008, Patra et al 2009a; Patra JK, Das et al, 2009b). Several research studies and traditional knowledge among the coastal population have proved the medicinal properties such as an effective anti – inflammatory, antiviral, antimicrobial, anticancer, anti-diabetes, antifungal and insecticidal properties of mangrove plants (Habib et al., 2018; Sachithanandam et al., 2021).

Mangroves are the plants of extreme environmental conditions found in intertidal zones or estuaries of tropical ecosystem. Mangrove forests of India are situated at alluvial deltas of the major rivers such as the Ganga, Mahanadi, Godavari, Krishna, Cauvery and also on the bay of Andaman and Nicobar Islands (Mishra et al., 2005; Kathiresan and Rajendra, 2005; Thatoi and Biswal, 2008; Mandal and Naskar, 2008). The Godavari Mangroves at the Coringa Wildlife Sanctuary (CWLS) are said to be the second largest mangroves in India. Coringa wildlife sanctuary, a part of Godavari mangrove wetland forests, is outspread in 235.7 sq. km. This sanctuary has three Reserved Forests (RF) - Corangi, Corangi Extn. and Bhairavapalem. The Mangrove plant species that are commonly found in this forest are *Rhizophora apiculata, Rhizophora mucronata, Aegiceras corniculatum, Bruguiera gymnorrhiza, Ceriops decandra, Xylocarpus moluccensis, Excoecaria agallocha, Avicennia marina, Avicennia officinalis, Lumnitzera racemosa* and *Sonneratia apetala.* Mangroves play a significant role in the life style of the people who reside along the tropical shoreline. Coringa mangrove forest providing huge economic advantages for timber, fuel, fish poison, medicine, health benefits, food and fodder for raring animals for local residents. The bark of *C. decandra* is used for colouring (dye) the fishing nets by local fishermen community to prevent damage from saline water, thus increasing the permanence of the net (Raju et al., 2008) As they provide a unique range of resources and services to the residents, the huge area has been lost and significantly under threat (Bandaranayake, 1998). In the present study, *Aegiceras corniculatum, Bruguiera gymnorrhiza, Ceriops decandra* and *Sonneratia apetala* plants were selected for phytochemical investigation, determining polyphenol content, functional group analysis through FTIR and antibacterial activities of various solvent extracts of both leaf and stem in order to develop novel compounds with environmental and pharmaceutical significance. *A. corniculatum* belong to family Myrsinaceae is a small evergreen tree or shrub, occurs on the banks of tidal streams and inter-tidal zones (Ellison 2010). Bark and roots are used as fish poison and also used to cure asthma, diabetes (Gurudeeban et al., 2012), rheumatism (Bandaranayake 2002), arthritis, inflammation (Reddy ARK, Grace JR 2016). *B. gymnorrhiza* belong to Rhizophoraceae, grows on the seaward edge of estuaries in mud areas of well-watered and frost-free sites. It is salt-tolerant large evergreen tree, bark is used to treat hemorrhages, malignant ulcers and leaves are used to control blood pressure in India (Bandaranayake 2002). *C. decandra* is Rhizophoraceae family mangrove habituated at the edges of mangrove swamps in estuarine zone of intertidal

regions. It is a tall shrub used traditionally to cure hepatitis (Magwa, 2005), ulcers, astringent, anti hemorrhage (Lalitha et al. 2019) and to treat pain (Bandaranayake, 1998; Premnathan, 1992). *S. apetala* is a Lythraceae mangrove found in the upstream estuarine zone in the low to mid-intertidal regions of India, Bangladesh, Malaysia, Australia etc. It is used as anti-HIV, antibacterial, anti-estrogenic agent and the leaves are used to treat hepatitis. Numerous studies reported that these mangroves infused in different solvent extracts possesses several biological activities such as antioxidant [Nurjanah et al, 2016; Sur et al, 2016; Rozirwan et al, 2023], antifungal, cytotoxic [Kumar et al, 2011; Dahibhate et al., 2020, 2021], antimalarial [Haq et al, 2011], antidiabetic [Karimulla et al, 2011;], and antibacterial activities (Anil Kumar, 2011; Ravikumar et al., 2011; Selvam, 2014; Madhurima Bhakshi, 2014; Rajendra et al, 2016; Eswaraiah et al, 2020; Khadeeja & Ragunathan, 2022; Karim et al, 2020). H. Jahnmanchi & Raju et al, (2017) studied *Aegiceras corniculatum* and reported that purified fraction of ethyl acetate has very potent inhibition against *Mycobacterium tuberculosis* in microgram range with negligible side effects in mammalian cells. Though various bioactive compounds have been identified in mangrove extracts, they are not much exploited in South India. Further, there have been no detailed in vitro studies on mangrove medicinal plants from Corangi reserve forest, Kakinada, East Godavari district, Andhra Pradesh. Comparative study of the mangrove species *A. corniculatum, B. gymnorrhiza, C. decandra* and *S. apetala* with six different solvents of leaf and stem has been documented for the first time.

With this background, the present study was undertaken and performed a simultaneous comparative phytochemical analysis of leaf and stem extracts using six different polar and non-polar solvents, total phenol content, FTIR and antibacterial activity of various extracts were conducted to determine their pharmacological value. With the higher incidences of multi-drug-resistant bacteria, new therapeutic compounds are the need of the hour. In this study we have found out that stem parts of all plants have significant antibacterial activity, especially *Bruguiera gymnorrhiza* in non-polar extracts has high potential against all tested MDR pathogens. The findings of this study shed light on the folklore claim and immense potential of Bruguiera gymnorrhiza to treat various ailments

MATERIALS AND METHODS

Sample Collection Area

The plant samples were collected from different sites of the Estuarine Coringa Reserve Forest, Kakinada, Andhra Pradesh, India (between 16˚39'-17˚ N and 82˚ 14'- 82˚ 23˚ E) during rainy season in the month of August.

Plant material

The Leaves and Stems of *Aegiceras corniculatum, Bruguiera gymnorrhiza, Ceriops decandra, Sonneratia apetala* were collected randomly from different parts of the sample collection areas and were subsequently used for screening their antibacterial potentialities.

Collection of plant material and preparation of extracts:

Sample Collection and Processing

Collected leaves and stem parts were washed with tap water followed by distilled water to remove the dirt and any undesirable material. Washed leaves were shade dried and powdered using a mechanical grinder and stored in airtight containers until further use.

Preparation of Plant Extracts:

60 grams of powdered leaf and stem materials of *Aegiceras corniculatum, Bruguiera gymnorrhiza, Ceriops decandra, Sonneratia apetala* were extracted in 300 ml. of different solvents of increasing polarity (hexane, chloroform, ethyl acetate, ethanol, methanol and water). The extract obtained was stored in a refrigerator at 4ºC until further use. All chemicals used in the study were obtained from Hi-Media, Qualigens. Resulting extracts were concentrated to dryness under vacuum and reduced pressure using rotary evaporator to obtain concentrated extracts. Percentage yield was calculated from the dry extract powder and they were dissolved in 5% dimethyl sulfoxide (DMSO).

Bacterial Strains:

The antibacterial activity of the crude extracts was determined on two Gram positive and six Gram negative bacteria. Strains viz., *Escherichia coli* (MTCC 41), *Salmonella enterica typhimurium* (MTCC 98), *Shigella flexineri* (MTCC 1457), *Klebsiella pneumonia* (MTCC 4030), *Staphylococcus aureus* (MTCC 87), *Streptococcus pyogenes* (MTCC 442), *Pseudomonas aeruginosa* (MTCC 424), *Vibrio parahaemolyticus* (MTCC 451). They were cultured in nutrient broth for 24 h to yield a final concentration of 10^7 CFU/ml and the fresh inoculums were subjected to susceptibility test.

Preliminary Qualitative Phytochemical Analysis

The crude leaves and stem extracts of *Aegiceras corniculatum, Bruguiera gymnorrhiza, Ceriops decandra, Sonneratia apetala* in hexane, benzene, ethyl acetate,chloroform, acetone, methanol and distilled water were qualitatively analysed for the identification of secondary metabolites such as alkaloids, flavonoids, saponins, phenol, tannins and glycosides. by using standard protocols. (Mishra et al. 2018)

Quantitative phytochemical investigation

The leaf and stem plant extracts of *Aegiceras corniculatum, Bruguiera gymnorrhiza, Ceriops decandra, Sonneratia apetala* in hexane, benzene, ethyl acetate,chloroform, acetone, methanol and distilled water subjected for total poly-phenol content according to the *Senguttuvan et al. 2014*.

Total Phenol estimation:

Total phenol content was determined using Folin-Ciocalteu (FC) reagent method. The TPC was estimated by dissolving 10mg mL-1 of dried flower extract in methanol and filtered using Whatman No 1 filter paper. 2 mL of extract or standard solution was taken into test tubes and added 1 mL of FC reagent (1:10 v/v). 1 mL of 7.5% sodium carbonate was also added to this solution and kept for incubation up to 2 hours. The absorbance was read at 765 nm wavelength using UV-Vis Spectrophotometer (Shimadzu UV 2450) against blank without extract. The outcome data was expressed as mg/g of gallic acid equivalents per gram of dry weight (GAE/g) extract.

Fourier Transform Infrared Spectroscopy (FTIR spectrum analysis)

Based on the preliminary phytochemical screening, all extracts of both leaf and stem of four mangrove plants were subjected to FTIR analysis. FTIR spectra were recorded using Bruker Alpha II FTIR Spectrometer in the region 4000–400 cm^{-1}. the dried powder of different solvent extracts of both leaf and stem of each plant materials was evaluated using ATR-FTIR in OH transmittance mode to determine the functional groups such as hydroxyl, amide, epoxy/ether and carboxyl groups that are associated with core carbon from 400-4000 cm^{-1} wave number. The obtained raw data was smoothened and a graph is plotted using Origin Pro 2023b Software.

Antimicrobial activity assay

Disc diffusion method was followed to detect antibacterial activity of leaves and stem extracts prepared from *Aegiceras corniculatum, Bruguiera gymnorrhiza, Ceriops decandra, Sonneratia apetala.* The disc size of 6 mm was prepared from Whatmann No.1 filter paper and they were placed on the agar surface and 20 µL of crude solvent extracts (hexane, chloroform, ethyl acetate, ethanol, methanol and water) dissolved in 5% Di methyl Sulphoxide (DMSO) of concentration 200 mg/ml was spotted on each disc (4 mg/disc). [Arivuselvan et al 2011]. For the test, Mullen-Hinton agar plates were swabbed with culture of pathogenic bacteria, the disc were placed at equal distance and incubated at 37 ^{0}C for 18hrs. After incubation, the diameter zone of inhibition around the disc was measured in millimetre. The positive and negative controls in this study were streptomycin (200 µg/ml) and 5% Di-methyl sulfoxide (DMSO) in water, respectively. All the experiments were carried out in triplicates.

Statistical Analysis:

All experimental measurements were carried out in triplicate and are expressed as average of three analyses ± standard deviation were calculated by using Microsoft excel software.

RESULT & DISCUSSION:

Preliminary Qualitative Phytochemical Analysis

Mangroves and their parts were analysed for various phytochemical constituents like alkaloids, flavonoids, glycosides, saponins, steroids, terpenoids and tannins using qualitative analysis. Several researchers screened different mangrove species (2, 8, 10, 22, 30, 33 and 36) for their antioxidant and antimicrobial properties in order to identify potent new molecules with therapeutic value against many Gram positive and Gram negative multi drug resistance human pathogens. Studies confirmed that mangrove plants contain biologically active phytochemicals such as steroids, terpenoids, flavonoids, tannins, saponins, and phenols that are responsible for their bio activities including their blood glucose lowering effects, improving the blood circulation to the brain in Alzheimer's disease (Sharma et al2009), antioxidant, anticancer and antimicrobial properties [Bandaranayake 2002]. Sterols used in the treatment of cardiovascular disease, breast cancer and prostate cancer. [Grattan 2013].

Table-1: Preliminary phytochemical analysis of *A. corniculatum* and *B. gymnorrhiza* in different solvents

Phytochemical		*Aeg ceras corniculat um*						*Bruguiera gym ori hiza*					
	Plant part	**H**	**EA**	**C**	**E**	**M**	**Aq**	**H**	**EA**	**C**	**E**	**M**	**Aq**
Tannins	Leaves	-	+	+	+	+	+	-	-	-	+	+	+
	Stem	-	-	-	-	-	-	-	-	-	-	+	+

Alkaloids	Leaves	-	-	-	-	-	+	-	-	-	+	-	+
(Mayer's test)	Stem	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	Leaves	-	-	-	-	-	+	-	-	-	+	-	+
(Wagner's test)	Stem	-	-	-	-	-	+	-	-	+	-	+	-
saponins	Leaves	+	+	+	+	+	+	+	-	+	-	-	-
	Stem	-	+	-	+	+	+	+	-	-	+	+	+
Glycosides	Leaves	-	+	-	+	+	+	+	-	-	-	-	-
	Stem	-	+	+	+	+	+	-	+	+	+	-	+
Steroids	Leaves	+	-	-	+	+	+	-	+	-	+	+	+
	Stem	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	Leaves	+	+	+	+	+	+	-	-	-	+	+	+
	Stem	+	+	+	+	+	+	+	+	+	+	-	+
Flavonoids	Leaves	+	+	+	+	+	+	+	-	+	-	-	-
(Shinoda)	Stem	+	+	-	-	-	-	+	-	-	-	-	-
Flavonoids	Leaves	-	-	+	-	+	+	-	-	-	+	+	-
	Stem	-	-	+	-	+	+	-	+	+	+	-	-
Anthraquinones	Leaves	-	-	-	-	-	-	-	+	+	-	-	+
	Stem	-	-	-	+	+	+	-	-	-	+	+	-
Reducing sugars	Leaves	-	+	-	+	-	-	-	-	-	-	-	-
	Stem	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrates	Leaves	-	+	+	+	+	+	+	+	+	-	-	-
	Stem	-	+	-	-	+	+	+	+	+	-	-	-

H-Hexane; EA-Ethyl acetate; C-Chloroform; E-Ethanol; M-Methanol; Aq-Water: Present (+): Absent (-)

The phytochemical composition of *A. corniculatum* leaf hexane extract showed the presence of flavonoids. Ethyl acetate and chloroform extracts revealed the presence of flavonoids, glycosides, saponins, terpenoids and tannins. Remaining extracts showed the presence of glycosides, saponins, steroids, terpenoids, and tannins (Table 1). The stem chloroform extract of *A. corniculatum* revealed the presence of glycosides, steroids, terpenoids and flavonoids, and ethyl acetate extract showed glycosides, saponins, steroids, flavonoids and terpenoids. Ethanal and methanol extracts have flavonoids, glycosides, saponins, terpenoids, tannins and Anthraquinones. Flavonoids, glycosides, phenols, steroids, saponins, tannins and terpenoids are present in the remaining extracts as depicted in table1. A. corniculatum is being used folklore medicine practices in the treatment of various diseases and it is due to these phytochemical principles that control diseases. The results obtained are similar to Kulkarni et al., 2019. The phytochemical composition in leaf Ethyl acetate extract of *B. gymnorrhiza* showed the presence of steroids and Anthraquinones and chloroform has saponins and Anthraquinones. Leaf ethanol, methanol and aqueous extracts have tannins, steroids and terpenoids. No flavonoids found in leaf extracts except from chloroform and methanol (table1). The results obtained are in are in accordance with the earlier finding of Khadeeja et al.2022. The stem hexane extract of *B. gymnorrhiza* indicated the presence of saponins, and Flavonoids. EA, C and Ethanol stem extracts showed the presence of glycosides, steroids, terpenoids and flavonoids. Glycosides, saponins, steroids, terpenoids and tannins are present in water extract. The findings were similar to study of Mahmud et al. 2017.

Table 2: Preliminary phytochemical analysis of *C. decandra* and *S. apetala* in different solvents

Phytochemical		***Cer ops decandra***						***Son eratia apetala***					
	Plant part	**H**	**EA**	**C**	**E**	**M**	**Aq**	**H**	**EA**	**C**	**E**	**M**	**Aq**
Tannins	Leaves	-	-	-	-	+	-	-	-	-	+	+	+
	Stem	-	-	-	-	+	-	-	-	-	+	-	+
Alkaloids (Mayer's	Leaves	-	-	-	+	-	+	-	-	-	-	-	-
test)	Stem	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	Leaves	-	-	-	+	-	+	-	-	-	-	-	-
(Wagner's test)	Stem	-	+	-	-	+	-	-	-	-	-	-	-
saponins	Leaves	-	-	-	+	+	+	+	+	+	-	-	-
	Stem	-	-	+	-	-	-	-	+	+	-	-	-
Glycosides	Leaves	+	-	+	+	-	+	+	+	+	-	+	+
	Stem	+	+	+	+	-	-	-	-	-	-	-	-
Steroids	Leaves	+	+	+	+	+	+	+	-	+	+	+	+

Terpenoids	Stem	+	+	+	+	+	+	+	+	+	+	+	+
	Leaves	-	+	+	+	+	+	+	+	+	+	+	+
Flavonoids (Shinoda)	Stem	+	-	+	+	+	+	+	+	+	+	+	+
	Leaves	-	+	-	-	-	-	+	+	+	-	-	-
Flavonoids	Stem	-	+	-	-	+	+	-	-	-	-	-	-
	Leaves	-	-	-	+	+	-	-	-	-	-	-	-
Anthraquinones	Stem	-	+	-	+	-	-	-	-	-	+	-	-
	Leaves	-	-	-	+	+	+	-	+	-	+	-	+
Reducing sugars	Stem	-	+	-	+	+	+	-	-	-	+	-	+
	Leaves	-	-	-	-	-	-	-	+	-	-	-	+
Carbohydrates	Stem	-	-	-	-	-	-	-	+	-	-	+	-
	Leaves	+	+	+	+	+	+	+	+	-	+	+	+
	Stem	-	-	+	+	+	+	+	-	+	+	+	+

H-Hexane; EA-Ethyl acetate; C-Chloroform; E-Ethanol; M-Methanol; Aq-Water: Present (+): Absent (-)

The phytochemical composition of leaf ethanal extract of *C. decandra* showed the presence of alkaloids, flavonoids, glycosides, steroids, terpenoids and Anthraquinones. In addition to these methanol extract contained saponins, tannins. The aqueous extract showed the presence of all metabolites except flavonoids. The stem ethanolic extract of *C. decandra* contained flavonoids, glycosides, steroids, terpenoids and Anthraquinones. As like ethanol all the compounds present except glycosides, and in addition tannins are present in stem methanol. The aqueous extract showed thepresence of flavonoids, steroids, terpenoids and anthraquinones (Table: 2). Many studies proved that the whole plant C. decandra predominantly composed of diterpenoids, tri terpenoids, phenolic compounds, and steroids (Premanathan et al, 1996). Thongdeeying (2005) study reported three new lupane tri terpenoids and twenty-three known triterpenoid and Wu Jun et al, (2012) reported Forty-three diterpenes and twenty-nine triterpenes and exhibited antimicrobial, anticancer, antitumor and larvicidal activities. Preliminary phytochemical analysis of non-polar extracts hexane, EA and C of *S. apetala* revealed the presence of saponins, glycosides, steroids, terpenoids and flavonoids. Glycosides are absent from all the solvent extracts of stem and in addition anthraquinones are present in EA extract. This is in accordance with the previous findings of V. Prabhu Teja et. al, 2013 in which ethanol extracts exhibited all the metabolites except glycosides and proteins. In our study tannins are present in polar extracts of both leaf and stem; absent from tested nonpolar solvents. Alkaloids are completely absent from all the extracts of plant parts. saponins and flavonoids are absent from polar solvents of leaf. Flavonoids are present only in ethanol extract of stem. whereas stem ethanol, methanol and aqueous extracts of *S. apetala* showed the presence of tannins, steroids, terpenoids and anthraquinones. The obtained results are similar to study of Patra et al, 2014.

Quantitative phytochemical investigation

Total Phenol estimation:

The results (Fig.1) revealed that there was a wide variation in the amount of total phenolics in mangrove plant materials ranging from 1.12 to 120.68 mg GAE/g dry material (Table 3) *A. corniculatum* stem carried the highest phenolic contents compared to all other mangroves, while the lowest phenolic contents identified in *S. apetala* stem extract. Whereas in leaf extracts *S. apetala* carried highest amount and *B. gymnorrhiza* has least.

The amount of total phenolic content of leaves of the plants under study can be arranged in descending order viz. S. apetala (H) > B. gymnorrhiza (H) > C. decandra (EA) > A. corniculatum (EA). The descending order for total phenolic content of stem being A. corniculatum (H) > C. decandra (E) > S. apetala (H) > B. gymnorrhiza (C).

Table 3: Total Phenolics content (mg GAE/g of Extract) of different extracts of selected mangroves

Plant Sample / Solvent Extract	A. Corniculatum (Stem) mg GAE/g	A. Corniculatum (Leaf) mg GAE/g	B. Gymnorhiza (Stem) mg GAE/g	B. Gymnorhiza (Leaf) mg GAE/g	C. decandra (Stem) mg GAE/g	C. decandra (Leaf) mg GAE/g	S. apetala (Stem) mg GAE/g	S. apetala (Leaf) mg GAE/g
Hexane	120.68	6.14	12.8	73.85	11.4	22.75	72.62	76.23
Ethyl Acetate	10.52	39.89	40.07	12.95	48.43	55.09	62.09	64.53
chloroform	14.03	9.64	60.93	72.7	42.33	15.78	1.12	23.2
Ethanol	98.44	25.5	18.89	8.91	79.043	46.35	6.994	57.37
Methanol	10.32	5.14	6.29	8.89	38.9	12.8	34.28	55.74

Water	1.738	15.84	50.5	5.12	42.12	20.38	6.071	46.35

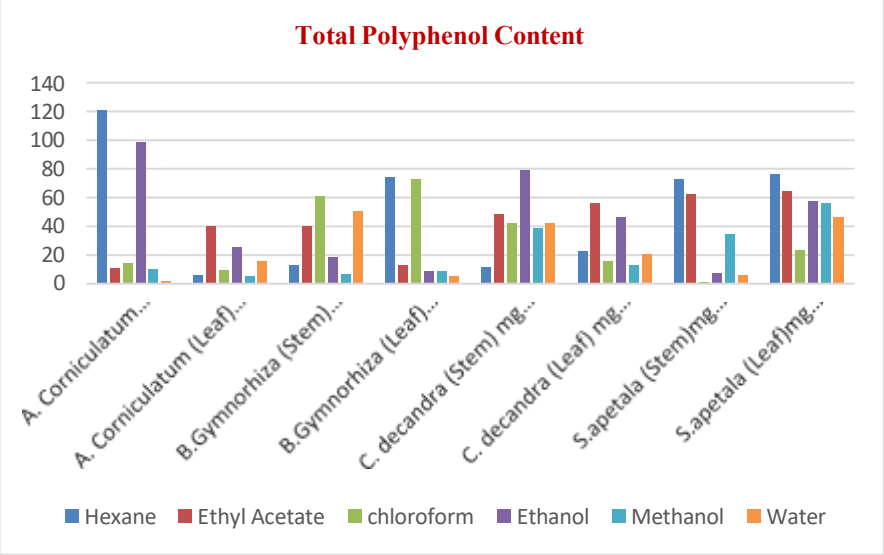

Fig 1. Comparative analysis of Total Phenol content of both leaf and stem extracts of tested mangrove plants

Among the fractions of *A. corniculatum*, stem hexane fraction significantly showed the highest (120.68 mg GAE/g) phenolic contents followed by the ethanol fraction (98.99 mg GAE/g), chloroform (14 mg GAE/g), methanol and ethyl acetate (10 .03mg GAE/g) (Table 3). This is in alignment with the results of Kulkarni et. Al, 2019. Our results obtained are higher than the results of H. Janmanchi et. al, (2017). In their findings methanol extracts (70.86mg GAE/g extracts) exhibited high TPC. Present study can be corelated with previous findings evidenced by Reddy et al, 2016. In which leaf methanol extracts showed 24.06 µg GAE/100 µg. The evaluation of total phenolic contents of A. corniculatum revealed that this plant possesses a huge amount of phenol in different extracts.

B. gymnorrhiza, leaf showed the highest phenol content in hexane (73.85 mg GAE/g) followed by chloroform (72.7 mg GAE/g) fraction, whereas the stem has lesser than leaf. Stem chloroform showed 60.93 mg GAE/g followed by water fraction 50.5 mg GAE/g. A study observed higher phenolic content in methanolic leaf extract (58.917) of *Bruguiera gymnorrhiza* (Karim et al.2020). Study of S. Acharya, et al. 2020 reported bark methanolic extracts of the plant showed significant amounts of phenolics among other extracts. Mahmud et al. 2017, studied bioactivities of *B. gymnorrhiza* and polyphenols profiling; they reported 40 mg and 33 mg GAE/g of stem and leaf ethanol extracts. The study of Haq et al. (2011) also found the higher phenolic compound in barks compared with leaves of *B. gymnorrhiza*. The difference in phenolic content observed in our study could arise due to various environmental factors, plant parts and the location of the plant.

In *C. decandra* stem ethanol fraction (79.04 mg GAE/g) has highest followed by ethyl acetate (48.43 mg GAE/g) and chloroform (42.33 mg GAE/g) while in leaf, ethyl acetate (55.09 mg GAE/g) has high phenol content followed by ethanol fractions (46.35 mg GAE/g). In this study, the stem polar fractions of the mangrove, *C. decandra* were found to contain appreciable amount of polyphenolics when compared to leaf. The total phenolic content obtained in our study is higher in stem and lesser in leaf when compared to earlier study by Simlai and Roy 2012 (wood 42.33 and leaf 67.66 mg/g).

Both leaf and stem of the mangrove *S. apetala* showed highest polyphenols from hexane fractions (76.23 and 72.62 mg GAE/g respectively) followed by ethyl acetate extracts (64.53 and 62.09mg GAE/g respectively). Comparatively all leaf solvents have significantly high amount of polyphenols than stem extracts. These findings are higher than previous findings by Patra et al, 2014 in which all the solvents tested carried lesser phenol contents compared to our results. The total phenolic content was found 50.75 mg/gm of GAE in methanol extract of bark of Sonneratia apetala in the study of Mukul et al. 2016.

Quantitative analysis using the Folin–Ciocalteu method confirmed significantly higher TPC in non-polar extracts especially *Bruguiera gymnorrhiza* and *A. corniculatum* had the highest phenolic levels, reinforcing the correlation between phenolic richness and antibacterial potency.

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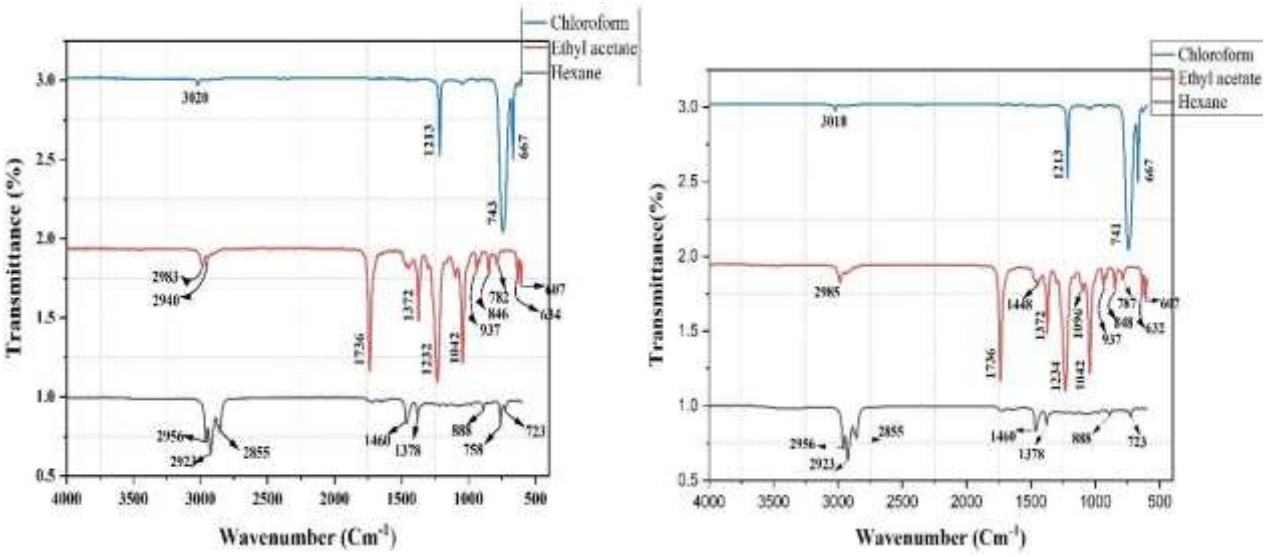
FTIR analysis:

The FTIR analysis is one of the most common analytical techniques used to characterize the nature of the phytochemical molecules. Depending on their corresponding peak values in the infra-red (IR) spectral region the functional groups of plant species are identified by following the references of Coates [16]. The FTIR analysis was performed to determine the functional groups present in all the six solvent (from non-polar to polar) extracts both leaf and stem of four mangrove plants *A. corniculatum, B. gymnorrhiza, C. decandra* and *S. apetala.* Based on the previous literature (Nurlaila and Tukiran, 2017, Gnanadesigan et al., 2011, Subayu et al,2021, Mohan Reddy et al., 2021, Oladunmoye et al., 2018, G.K. More et al, 2021 and Agi et al., 2018), the results of the FTIR spectra are analysed and used to identify the possible biomolecules responsible for their activity.



(a) **(b)**

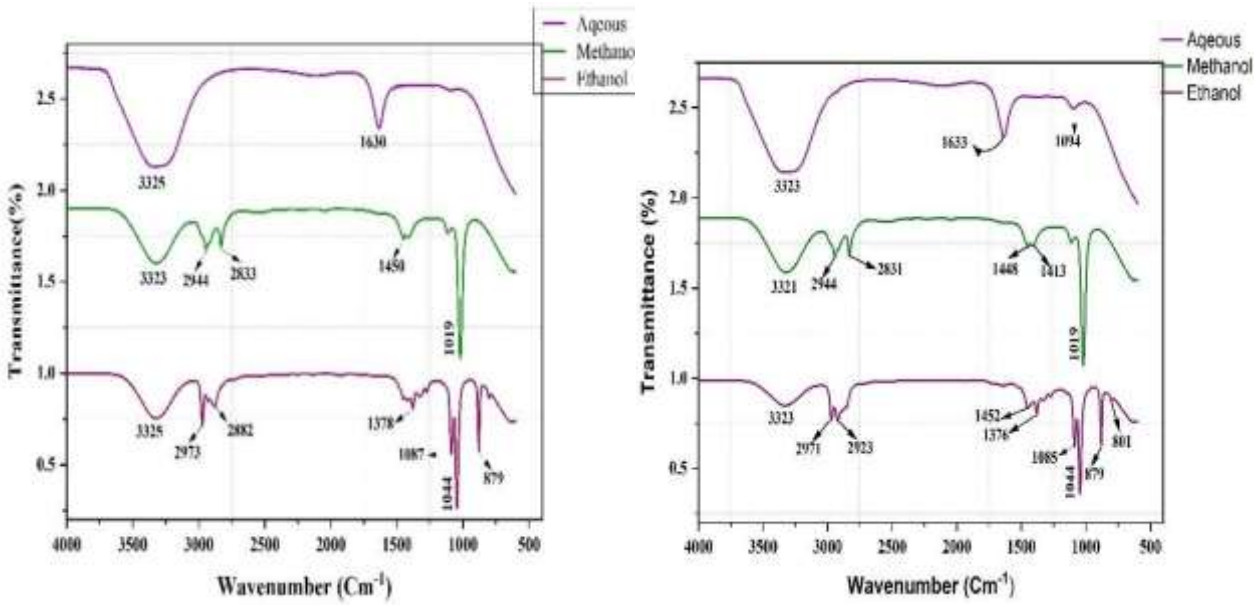
Fig 2: FT-IR spectra of *A. corniculatum* leaf (a) and stem (b) non-polar solvent extracts

Table 4: FTIR spectral peak values and functional groups obtained for non-polar solvents of leaf and stem extracts of *A. corniculatum*

Wavenumber (cm^{-1})			Assigned functional group	Intensity
Hexane	**Ethyl Acetate**	**Chloroform**		
		3018	C-H stretching vibration alkene	M
2855, 2956	2940, 2983, 2985		C-H stretching vibration alkane	M
2923			N-H stretching vibration amine salt	S &B
	1736		C=O stretching vibration ester	S
1460			C-H bending alkane	M
1378	1372		O-H bending alcohol	M
	1232, 1234	1213	C-O stretching vibration aromatic ester	S
	1042		C-O stretching vibration primary alcohol	S
758, 888	787	741	C-H bending 1,2,3-trisubstituted aromatic ring deformation	S
723			benzene derivative	

The FTIR spectrum of non-polar solvents of leaf and stem extracts of *A. corniculatum* were given in Fig. 2a & 2b. The analysis revealed that the spectral pattern of both leaf and stem is almost similar to each other (Table4). The signals in the IR spectra at 3018 in chloroform extract indicate C-H stretching vibration alkene. The appearance of bands at 2855, 2956, 2940, 2983 and 2985 cm^{-1} in hexane and ethyl acetate are attributed to alkane C-H stretching. The peak at 2923 in hexane correspond to N-H stretching and the absorption at 1736 from ethyl acetate is suggestive of the presence of C=O stretching. The peak at 1460 cm^{-1} of hexane corresponds to C-H bending alkane. The peaks recorded at 1232, 1234 and 1213 of ethyl acetate and hexane is belong to C-O

stretching vibration of aromatic ester. The peak at 1042 could be attributed to C-OH and the appearance of bands around 700 – 900 cm^{-1} are due to C-H out of plane bending (aromatic) vibrations. From the FTIR results, the observed peaks are considered as major functional groups such as flavonoids, triterpenoids, polyphenols, alkanes, amines and aromatic compounds



(a) **(b)**

Fig 3: FT-IR spectra of *A. corniculatum* leaf (a) and stem (b) polar solvent extracts

Table 5: FTIR spectral peak values and functional groups obtained for polar solvents of leaf and stem extracts of *A. corniculatum*

Wavenumber (cm^{-1})			Assigned functional group	Intensity
Ethanol	**Methanol**	**Aqueous**		
3323, 3325	3321, 3323		O-H & N-H stretching vibrations alcohol & aliphatic primary amine	S &B
		3323, 3325	O-H stretching vibration alcohol	S &B
2882, 2923, 2971, 2973	2944, 2831, 2833		C-H stretching vibration alkane	M
		1630, 1633	C=C stretching vibration alkene	M
1452	1448, 1450		C-H bending alkane	M
1376, 1378	1413		O-H bending alcohol	M
1085, 1087, 1044	1019		C-O stretching vibration primary alcohol	S
879			C-H bending vibration 1,3-disubstituted benzene deformation	S

The IR spectral bands of mangrove plant *A. corniculatum* polar solvents of both leaf and stem extracts were given in Fig. 3a & 3b.

The prominent peaks of the FTIR results are showing the correspond values to the amide (N-H stretching-3325 of stem ethanol) and to alcohol (O-H stretch at 3321,3323 of leaf) in all polar solvents, aliphatic group (CH2 – 2971 and 2973), alkane group (CH-2831, 2833 and 2882) alkene (CC-1630 and 1633) and primary alcohol group (CO-1019, 1044, 1085 and 1087). The peaks at 1448, 1450, 1452 and 879 belongs to C-H bending vibrations correspond to alkane. The observed peaks are showing the occurrence of major functional groups in different chemical classes such as flavonoids, triterpenoids and polyphenols

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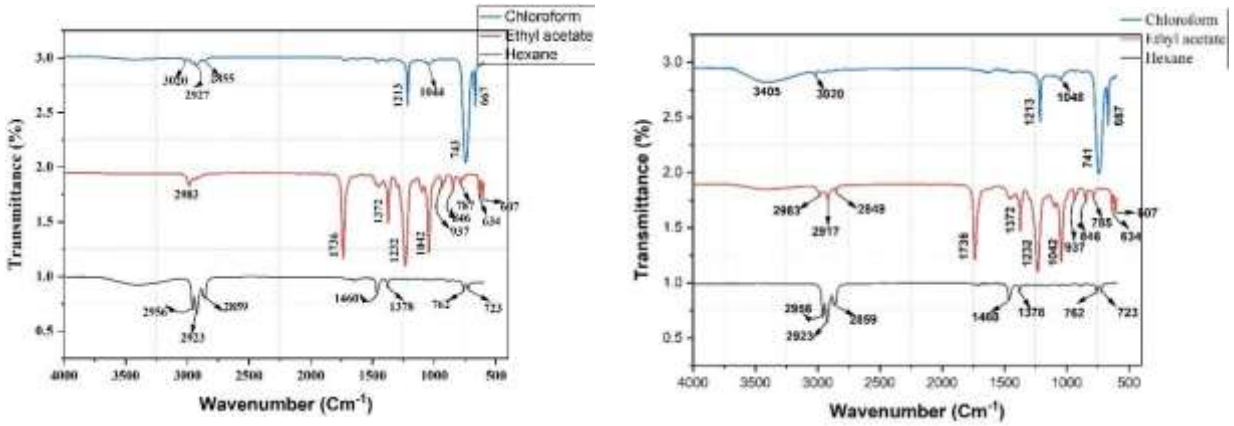


(a) **(b)**

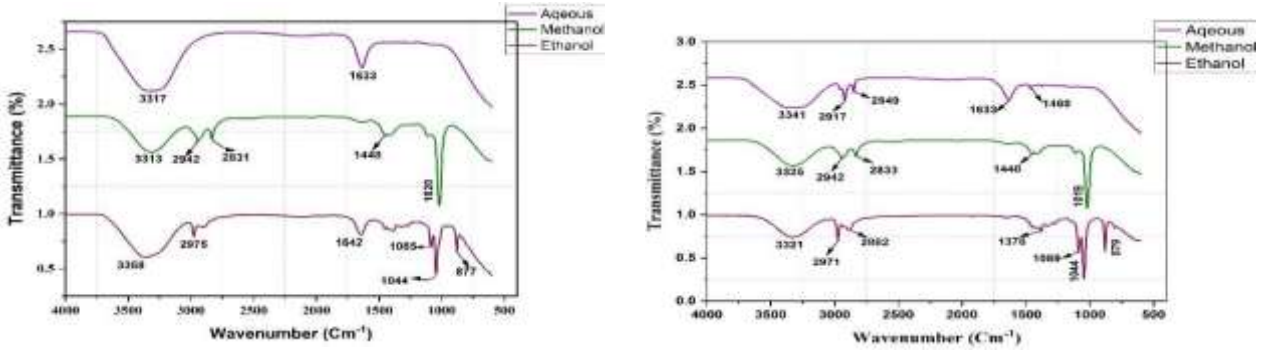
Fig 4: FT-IR spectra of *B. gymnorrhiza* leaf (a) and stem (b) non-polar solvent extracts

Table 6: FTIR spectral peak values and functional groups obtained for non-polar solvents of leaf and stem extracts of *B. gymnorrhiza*

Wavenumber (cm^{-1})			Assigned functional group	Intensity
Hexane	**Ethyl Acetate**	**Chloroform**		
		3405, 3020	O-H stretching alcohol	S&B
2956, 2923	2983, 2917, 2849	2927, 2855	C-H stretching vibrations alkane	M
2859			C–H stretch (symmetric, CH_2)	S&B
	1736		C=O stretch – ester carbonyl	S
1460			C-H bending vibration alkane	M
1378	1372		O-H bending vibration alcohol	M
	1232		C-O stretching aromatic ester	S
	1213		C-O stretching alkyl aryl ether	S
	1042	1044, 1048	C-O stretching primary alcohol	S
	937		C=C bending alkene	S
762			C–H out-of-plane bending (aromatic)	S
	787, 785, 846		C–H out-of-plane bending deformation	S
		741, 743	C-Cl stretching	S
		667	C=C bending alkene	S

The FTIR spectrum of non-polar solvents of leaf and stem extracts of *B. gymnorrhiza* were given in Fig. 4a & 4b. The analysis revealed that the spectral pattern is almost similar for both leaf and stem (Table 6). The hexane extract IR spectral bands from both leaf and stem are entirely same. According to the literature (Khadeeja et al. 2022, R. Ray et al. 2012, H. Zhao et al, 2016, Memon et al, 2018, Das et al. 2018) the signal bands in the IR spectra at 3405 cm^{-1} of stem, 3020 cm^{-1} chloroform extract of both leaf and stem indicate O-H stretching for alcohol. The wavenumbers around 2956, 2923 in hexane, 2983, 2917 and 2849 cm^{-1} in ethyl acetate and 2957, 2855 cm^{-1} are attributed to alkane C-H stretching. The peak at 2859 in hexane correspond to C–H stretch of saturated alkyl chains and the absorption at 1736 from ethyl acetate is suggestive of the presence of C=O stretch – ester carbonyl. The peak at 1460 of hexane corresponds to C-H bending alkane. The peaks recorded at 1378 and 1372 of hexane and ethyl acetate correspond to O-H bending vibration of alcohol. The absorptions at 1232, 1213 of ethyl acetate of both leaf and stem belongs to C-O stretching aromatic ester and C-O stretching alkyl aryl ether respectively. hexane belong to C-O stretching vibration of aromatic ester. The peaks around 1042 of ethyl acetate and 1044, 1048 in chloroform of both stem and leaf could be attributed to C-OH. The signal at 937 cm^{-1} belong to C=C bending alkene. The appearance of bands 762 in hexane, 787, 785, 846 cm^{-1} in ethyl acetate could be attributed to aliphatic and aromatic groups in the plane deformation vibrations of ethyl groups. The peak at 667 from chloroform extract of both leaf and stem indicated C=C bending alkene. FTIR spectrum

analysis supports the presence of alcohols, poly phenols such as flavonoids and tannins, saponins, terpenes, amines, alkanes and aromatic compounds in all the tested solvent extracts.



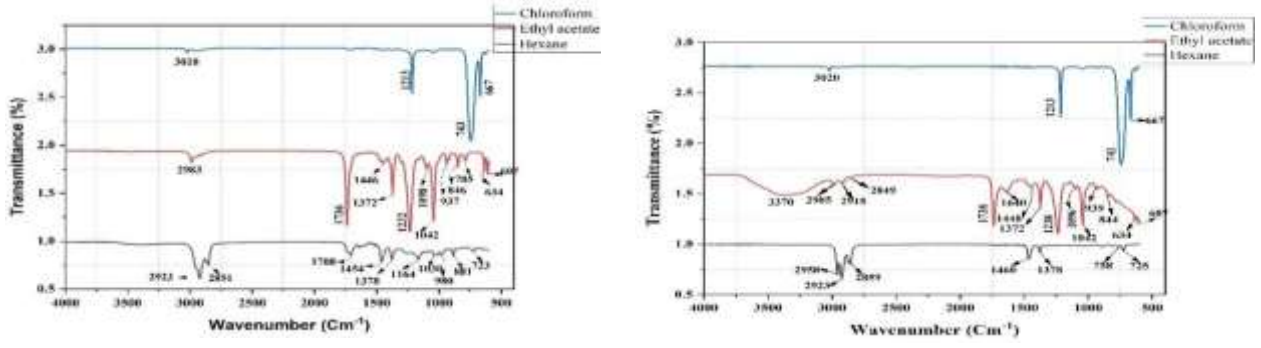
(a) **(b)**

Fig 5: FT-IR spectra of *B. gymnorrhiza* leaf (a) and stem (b) Polar solvent extracts

Table 7: FTIR spectral peak values and functional groups obtained for polar solvents of leaf and stem extracts of *B. gymnorrhiza*

Wavenumber (cm^{-1})			Assigned functional group	Intensity
Ethanol	**Methanol**	**Aqueous**		
3321, 3358	3313, 3325	3341, 3317	O-H stretching vibration alcohol	S&B
2971,2975, 2882	2942, 2831, 2833	2917, 2849	C-H stretching alkane	M
1642		1633	C=C stretching alkene	S
	1448	1460	C-H bending vibration alkane	M
1378			O-H bending vibration alcohol	M
1044, 1085, 1089	1019, 1020		C-O stretching primary alcohol	S
877, 879			C–C out-of-plane bending of aromatic ring deformation	S

The FTIR peak values are illustrated in the spectrum (Figure 5 a & b). The strong broad and intense peak bands of *B. gymnorrhiza* leaf at 3358 cm^{-1} in ethanol, 3313 in methanol and 3317 in aqueous extracts are an OH stretching in the alcohols and phenols group. Comparatively less intense but broad peak bands of stem at 3321, 3325 and 3341 cm^{-1} appeared for OH stretching. 2833 cm-1 to 2975 cm^{-1} attributed to the C-H stretching vibration in the alkanes group. The peaks around 1633 in stem and 1642 cm^{-1} leaf ethanol extract are suggestive to C=C stretching alkene. CH2 bending vibration at 1448 and 1460 cm^{-1} also affirms the existence of alkanes group. Peak at 1378 cm^{-1} from stem ethanol extract shows O-H bending vibration for alcohol. The spectral bands between 1019 – 1089 cm^{-1} from ethanol and methanol extracts of both leaf and stem belonged to C-O stretching primary alcohol. The bands around 877 and 879 cm^{-1} of ethanol leaf and stem could be attributed to C–C out-of-plane bending vibrations. Thus, the FTIR spectrum (Table 7) confirmed the presence of alcohols, poly phenols, terpenoids, amines, alkanes, alkaloid, anthraquinone, terpenoids, steroids and aromatic compounds in all the tested solvent extracts.



(a) **(b)**

Fig 6: FT-IR spectra of *C. decandra* leaf (a) and stem (b) non-polar solvent extracts

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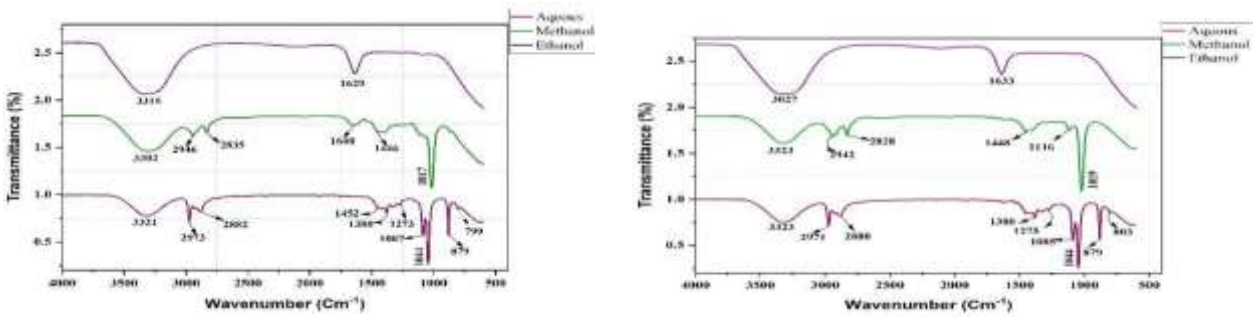
Table 8: FTIR spectral peak values and functional groups obtained for non-polar solvents of leaf and stem extracts of *C. decandra*

Wavenumber (cm^{-1})			Assigned functional group	Intensity
Hexane	**Ethyl Acetate**	**Chloroform**		
	3370		O-H stretching alcohol	S&B
		3018, 3020	C-H stretching alkene	M
2923, 2851	2983, 2985, 2915, 2849		C-H stretching alkane	M
2958, 2923, 2859			N-H stretching amine salt	S&B
	1736, 1738		C=O stretching ester	S
1708			C=O stretching aliphatic ketone	S&B
	1640		C=C stretching alkene	M
1454, 1460	1446, 1448		C-H bending alkane	M
1378			C-H bending vibration alkyl	M
	1372		O-H bending alcohol	M
		1213	C-O stretching ester	S
	1232, 1238		C-O stretching alkyl aryl ether	S
1164			C-O stretching tertiary alcohol	S
1030	1042		C-O stretching primary alcohol	S
980			C=C bending alkene	S
881	785	741, 743	C-H bending vibrations	S
		667	C–Cl stretch	S
723, 725			benzene deformation	

The FTIR spectroscopy was done to identify the functional groups of the antibacterial compounds present in the crude solvent extracts of *C. decandra* leaf and stem (Fig6 a& b). For this mangrove plant also the IR spectral pattern is almost similar in both leaf and stem extracts. The prominent peaks of the FTIR results are showing the correspond values to the alcohol (O-H stretching at 3370 in stem ethyl acetate extract), alkene (CH stretching at 3018 of leaf, 3020 of stem in chloroform extract), C-H stretching alkane (2923, 2851, 1454, 1460 in hexane and 2983, 2985, 2915, 2849, 1446, 1448 in ethyl acetate), amine salt (N-H stretching 2958, 2923, 2958, in stem hexane), ester (C=O stretching 1736, 1738, in ethyl acetate and 1213 in chloroform), C=O stretching aliphatic ketone (1708 in hexane), alkene (C=C stretching 1640 in stem ethyl acetate and C=C bending at 980), methyl group (C-H bending at 1378 in hexane) and alkyl aryl ether groups (CO stretch 1232, 1238 in ethyl acetate), alcohol group (C-O stretching 1164, 1030, 1042) . The observed peaks from our study are considered as major functional groups in different chemical classes such as flavonoids, triterpenoids, amides and polyphenols from the solvent extracts. The data presented here is in accordance with previous findings where *C. decandra* was characterized and functional compounds such as diterpenoids and alkenes were detected (Simlai et al., 2016).



(a) **(b)**

Fig 7: FT-IR spectra of *C. decandra* leaf (a) and stem (b) Polar solvent extracts

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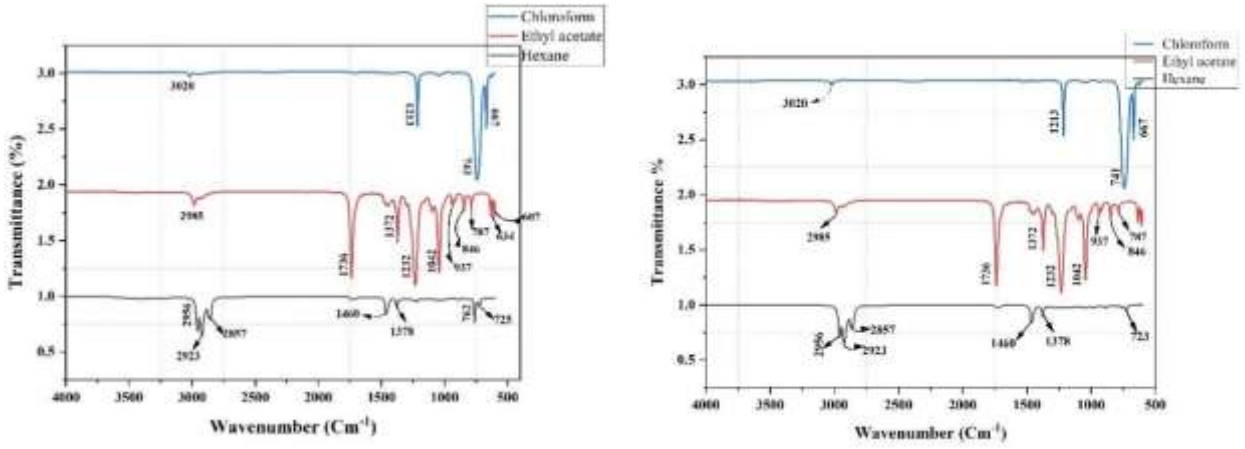
Table 9: FTIR spectral peak values and functional groups obtained for Polar solvents of leaf and stem extracts of *C. decandra*

Wavenumber (cm^{-1}) Ethanol	Methanol	Aqueous	Assigned functional group	Intensity
3321, 3323	3302, 3323	3315	O-H stretching alcohol	S&B
		3027	O-H stretching carboxylic acid	S&B
2971, 2973, 2880, 2882	2942, 2946, 2828, 2835		C-H stretching alkane	M
	1640	1633	C=C stretching cyclic alkene	M
		1629	C=C stretching conjugated alkene	M
1452			C-H bending alkane	M
1087, 1085	1017, 1019		C-O stretching primary alcohol	S
1044			CO-O-CO stretching anhydride	S&B
879			C-H Out-of-plane bending	S

FTIR analysis of *C. decandra* revealed (Fig 7) the similar spectral bands among all the polar solvents of both leaf and stem. The signals in the IR spectra at 3321, 3323, 3302, 3323 and 3315 cm^{-1} from all polar solvents tested indicate the presence of hydroxyl (–OH) group in both the leaf and stem. The peak at 3027 could be attributed to O-H stretching carboxylic acid (G.K. More et al. 2021). The absorptions at 2971, 2973, 2880, 2882 in ethanol, 2942, 2946, 2828, 2835 in methanol are correspond to C-H stretching alkane. The peaks at1640 in methanol, 1633 and 1629cm^{-1} in aqueous are attributed to unsaturation present such as cyclic and conjugated (C=C) alkene groups in both leaf and stem extracts. The appearance of the bands at 1085, 1087 in ethanol and 1017, 1019 cm^{-1} in methanol from both plant parts can be suggestive to the presence of C-O stretching alcohol. The strong and broad intense peak at 1044 in ethanol indicated the presence of CO-O-CO stretching in both leaf and stem. The FTIR study revealed the presence of alkaloids, terpenoids, flavonoids and phenols from the tested polar solvents of leaf and stem.



(a) **(b)**

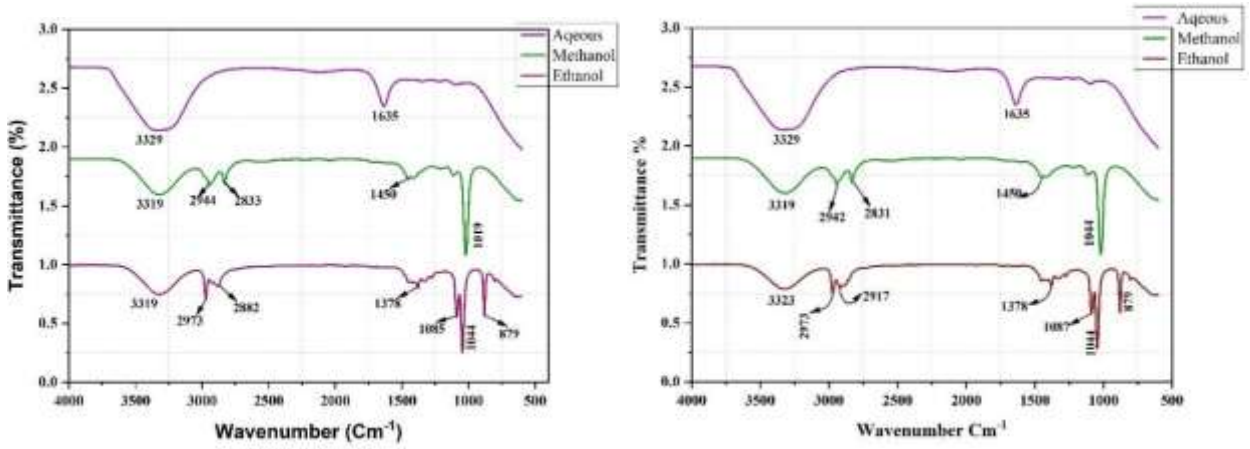
Fig 8: FT-IR spectra of *S. apetala* leaf (a) and stem (b) Non polar solvent extracts

Table 10: FTIR spectral peak values and functional groups obtained for non-polar solvents of leaf and stem extracts of *S. apetala*

Wavenumber (cm^{-1}) Hexane	Ethyl Acetate	Chloroform	Assigned functional group	Intensity
		3020	C-H stretching vibration alkene	M
2956, 2923, 2857	2985		C-H stretching vibration alkane	M
	1736		C=O stretching ester	S
1460, 1378			C-H bending vibration alkane	M
	1372		O-H bending alcohol	M

	1232	1213	C-O stretching aromatic ester/ether	S
	1042		C-O stretching primary alcohol	S
	937		C=C bending alkene	M
762	787, 846	743, 667	C-H out of plane bending (benzene)	S
723, 725			Rocking vibrations long chain alkanes (–CH_2–)n	S

The FTIR spectrum of non-polar solvents of leaf and stem extracts of *S. apetala* were given in Fig. 8a & 8b. The analysis revealed that the spectral pattern of all the non-polar solvents is same for both leaf and stem extracts (Table10). The signals in the IR spectra at 3020 in both leaf and stem chloroform extract indicate C-H stretching vibration alkene. The appearance of bands at 2956, 2923, 2985 and 2857 cm^{-1} in hexane and ethyl acetate are attributed to alkane C-H stretching (–CH2– and –CH3 groups) (Wu, Xu, Zhu et al., 2020). The absorption peak at 1736 from ethyl acetate is suggestive of the presence of C=O stretching. The peak at 1460 and 1378 cm^{-1} of hexane corresponds to C-H bending alkane. The peaks recorded at 1232, and 1213 of ethyl acetate and chloroform is belong to C-O stretching vibration of aromatic ester or vinyl ether. The peak at 1042 could be attributed to C-OH and the appearance of bands around 700 – 900 cm^{-1} are due to C-H out of plane bending vibrations for benzene and long chain alkanes. The observed FTIR peaks are considered as major functional groups in different chemical classes such as flavonoids, triterpenoids and polyphenols from the tested solvents. A detailed study (Patra et al, 2014) of partial characterization of the methanol extracts of leaf and bark revealed the presence of phenolics observed similar absorption bands.



(a) **(b)**

Fig 9: FT-IR spectra of *S. apetala* leaf (a) and stem (b) polar solvent extracts

Table 11: FTIR spectral peak values and functional groups obtained for polar solvents of leaf and stem extracts of *S. apetala*

Wavenumber (cm^{-1})			Assigned functional group	Intensity
Ethanol	**Methanol**	**Aqeous**		
3319, 3323	3319	3329	O-H stretching alcohol	S&B
2973	2942, 2944		C-H stretching alkane sp*	M
2882	2833, 2831		C-H stretching alkane sp^2	M
		1635	C=C stretching alkene	S
	1450		C-H bending alkane	M
1378			O-H bending alcohol	M
1085, 1087, 1044	1044, 1019		C-O stretching alcohol	S

879	C-H Out-of-plane bending aromatic or alkyl groups	S

The FTIR spectrum of polar solvents of leaf and stem extracts of *S. apetala* were given in Fig. 9a & 9b. The strong and broad signals in the IR spectra at 3319, 3323 and 3329 cm^{-1} of all polar solvents tested in leaf stem indicate the presence of alcohol group –OH stretching (J. Liu et al.2021). The absorptions at 2973 from ethanol and 2942, 2944 in methanol are correspond to (C-H stretching sp*) alkane. The peaks at 2882 in ethanol, 2833 and 2831 cm^{-1} in methanol are attributed to alkane (C-H stretching alkane sp^2). The strong and intense peak at 1635 affirms the unsaturation present such as cyclic and conjugated (C=C) alkene groups in both leaf and stem aqueous extracts. The appearance of the bands at 1085, 1087 in ethanol, 1044 in ethanol and methanol and 1019 cm^{-1} in methanol from both plant parts can be suggestive to the presence of C-O stretching alcohol. The C-H out-of-plane bending for aromatic or alkyl groups can be observed with strong and intense peak at 879 in both leaf and stem ethanol extract. Thus, the FTIR spectrum confirmed the presence of alcohols, poly phenols, amines, alkanes and aromatic compounds in all the tested polar solvent extracts of both stem and leaf.

Antimicrobial activity assay

Antibacterial efficacy was assessed via agar well diffusion method, against *Escherichia coli*, *Salmonella enterica typhimurium*, *Shigella flexineri*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus* showed that non polar solvents especially ethyl acetate and chloroform of both leaf and stem from *B. gymnorrhiza* had the highest inhibitory effects against all tested pathogens of both Gram-positive and Gram-negative, while polar extracts demonstrated limited to moderate activity.

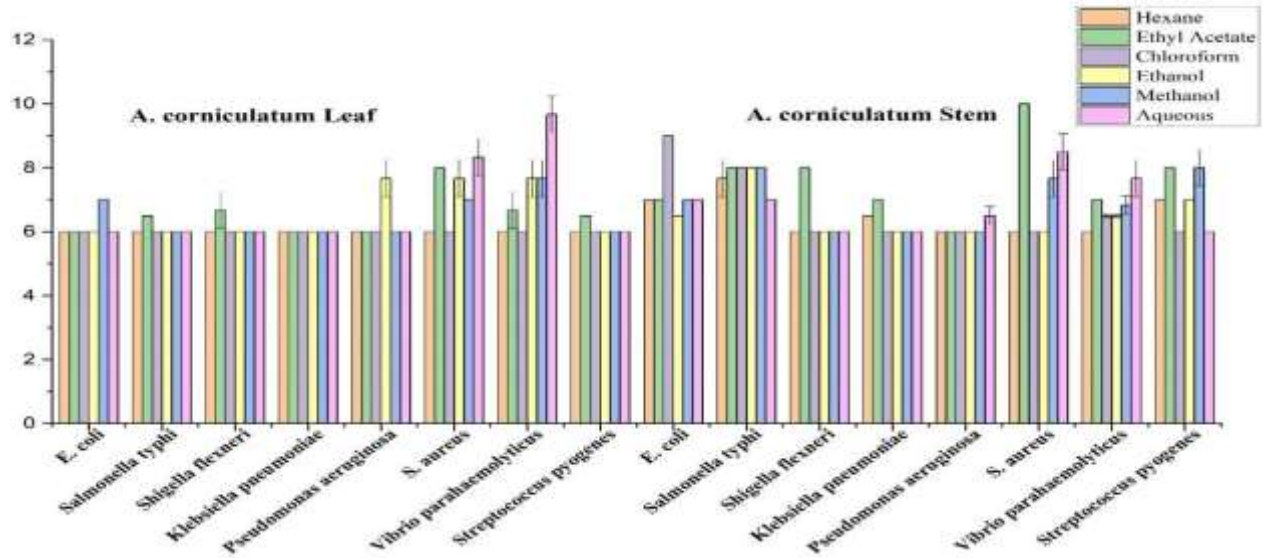


Fig 10. Antibacterial activity of *A. corniculatum* Leaf and Stem solvent extracts

The crude aqueous extract of *A. corniculatum* leaf showed high inhibitory zone of 9.67 mm against *V. parahaemolyticus* and 8.33 mm against *S. aureus*. The crude ethyl acetate exhibited 8mm against *S. aureus* (Figure 10), followed by ethanol extract 7.67mm against *S. aureus, V. parahaemolyticus and P. aeruginosa.*

The crude ethyl acetate extract of *A. corniculatum* Stem showed high inhibitory zone of 10 mm against *S. aureus* and 9 mm against *E. coli* by chloroform extract. The crude aqueous extract exhibited 8.5 mm against *S. aureus* (Fig 10).

A. corniculatum stem in non-polar solvents exhibited the most potent antibacterial effects, especially against *Staphylococcus aureus* and *E. coli*. The polar extracts, while showing some inhibitory activities were comparatively less effective, suggesting limited solubility in these fractions. Interestingly, *A. corniculatum* showed comparatively higher phenolic content in hexane extracts, although its antibacterial activity was slightly less than *B. gymnorrhiza*, indicating that bioactivity may also depend on specific compound structure and synergy, not merely concentration. The antimicrobial effectiveness of plant extracts also depends upon parts of the plant used and the solvent utilized for extraction (Okla et al. 2021).

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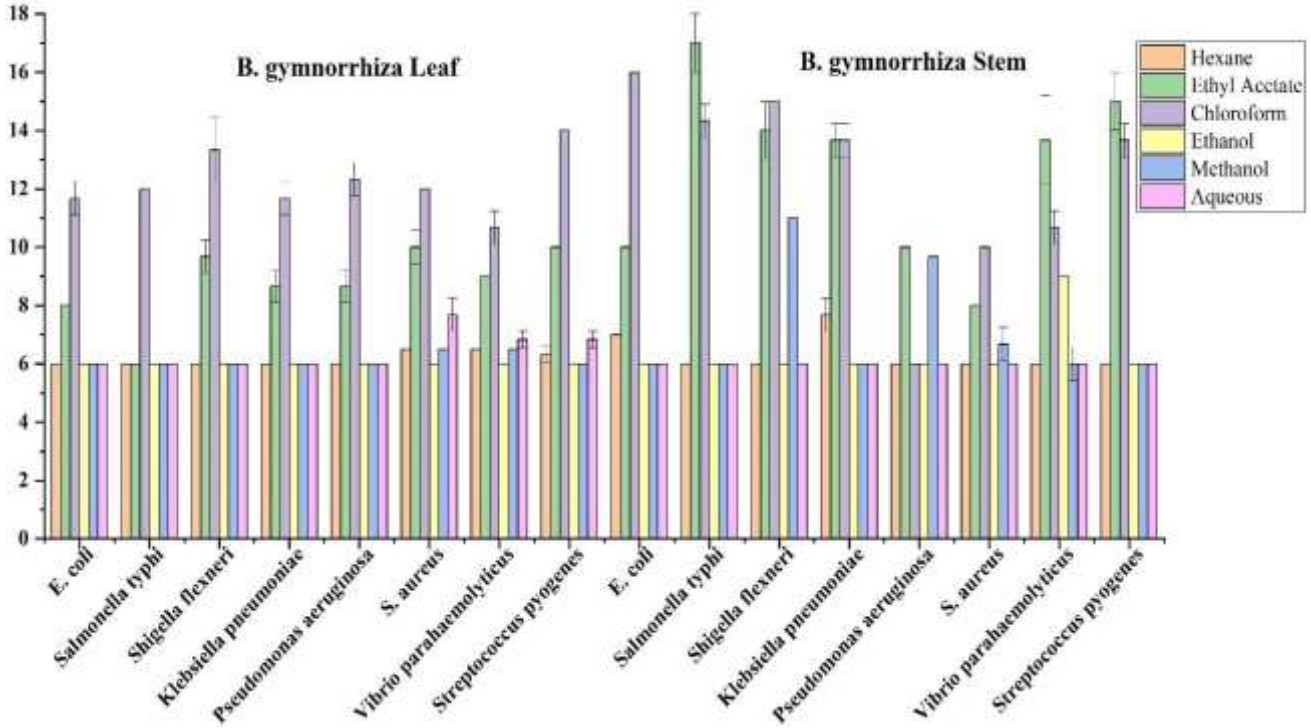

Fig 12. Antibacterial activity of *B. gymnorrhiza* Leaf and Stem solvent extracts

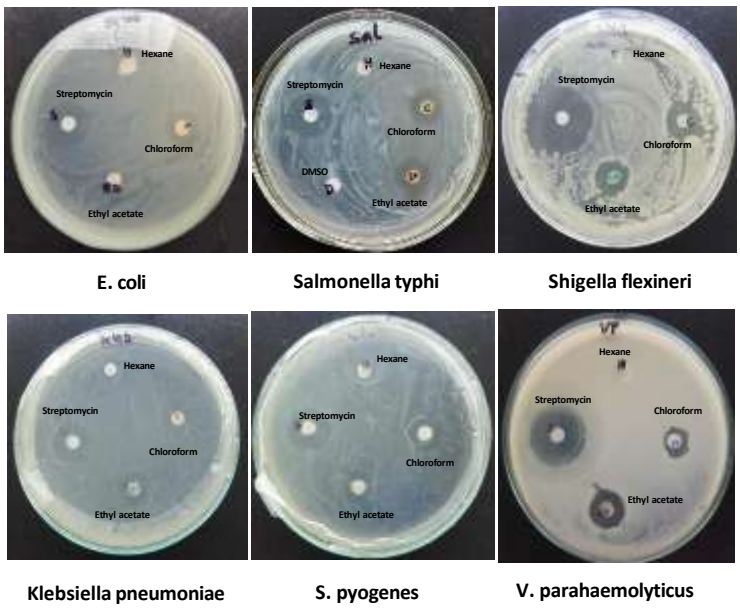

Fig 13. Antibacterial activity of *B. gymnorrhiza* Stem solvent extracts on MH Agar

The *B. gymnorrhiza* leaf chloroform showed zone of inhibition of 14 mm against *Streptococcus pyogenes,* followed by *Shigella* with 13mm, Pseudomonas with 12.33 mm and *Salmonella typhi* with 12 mm. The crude ethyl acetate exhibited similar inhibitions against Gram positive bacteria *S. aureus* and *Streptococcus* with 10mm (Fig12). *B. gymnorrhiza* stem possesses various degrees of antibacterial activity at different concentrations (2-8mg/ml) against tested bacteria (Fig 13). Ethyl acetate extract exhibited highest zone of inhibition against Salmonella 17mm, Streptococcus 15mm, Shigella 14mm, followed by chloroform extract exerted a zone of inhibition of 16mm against E. coli. *Shigella and Streptococcus* have showed similar zone of inhibitions 15mm towards chloroform and ethyl acetate respectively. Ethyl acetate and chloroform produced similar results 13.67mm against *Klebsiella, Vibrio* and *Streptococcus.* Hexane and aqueous extracts showed minimum activity and remaining extracts have shown moderate inhibition against tested bacteria.

Doifode et al, 2023 results suggest that *B. gymnorrhiza* has potential effects against bacterial infections of the respiratory and urinary systems and Acharya et al. 2020 studies on *B. gymnorrhiza* leaf methanolic and combination extracts 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.* Findings of Karim et al, 2020 demonstrated methanol extracts of *B. gymnorrhiza* active against opportunistic pathogens such as *E. coli, S. typhi and S. aureus*. This is in accordance with our findings though the solvent extracts different. In our study *B. gymnorrhiza* shows promising results against Enterobacteriaceae members as compared to the other works (Rajendra Seepana et al. 2016). Our study revealed that non-polar extracts of *B. gymnorrhiza* has broader spectrum of antibacterial activity against both Gram positive and Gram-negative pathogens.

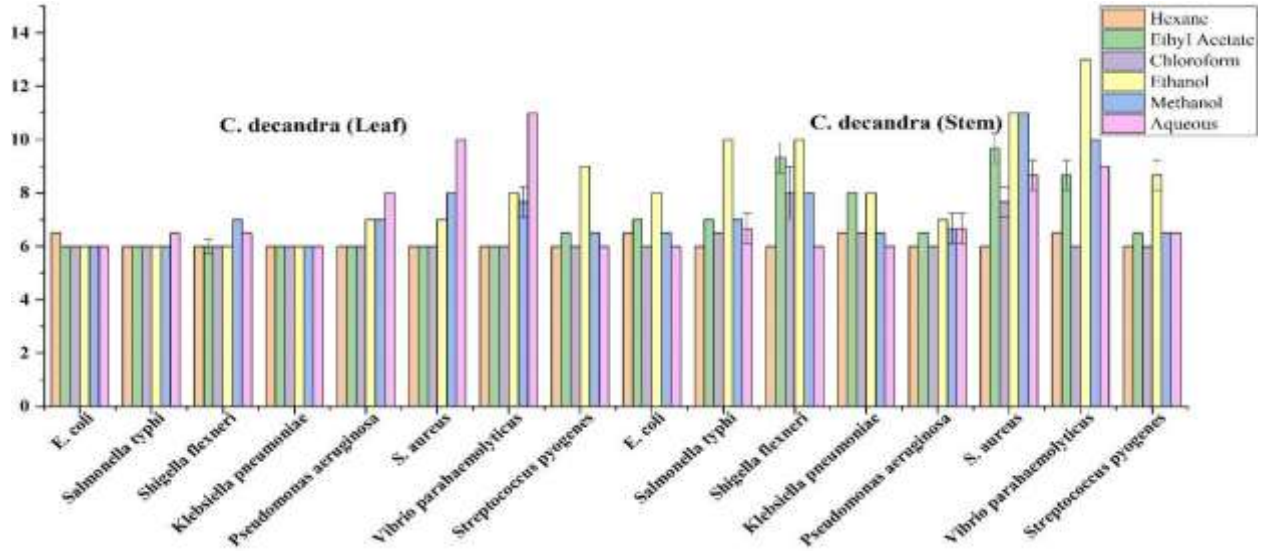

Fig 15. Antibacterial activity of *C. decandra* Leaf and Stem solvent extracts

Antibacterial activity of leaf *C. decandra* shown in fig 15. The high inhibitory activity is exhibited by crude aqueous leaf extracts of *C. decandra* against *V. parahaemolyticus* (11mm) and *S. aureus* with 10mm followed by ethanol extract against *Streptococcus pyogenes* with 9mm inhibition.

Among all the tested solvents of *C. decandra* stem extracts, ethanol showed highest zone with 13mm against *V. parahaemolyticus* and 11mm against *S. aureus*. Both *Salmonella* and *shigella* showed similar inhibition with 10mm for ethanol. The methanol extract exhibited high inhibition against gram positive *S. aureus* with zone of inhibition 11mm followed by 10mm against *V. parahaemolyticus*. Ethyl acetate also exhibited moderate activity against *S. aureus* and S*higella* (Fig 16). Antibacterial results obtained from leaf of this plant is in line with an earlier study by Simlai and Amit Roy,2012 and Simlai et al, 2016 in which methanol and aqueous extracts of leaf and bark has exhibited maximum broad-spectrum activity against both Gram-positive and Gram-negative bacteria.

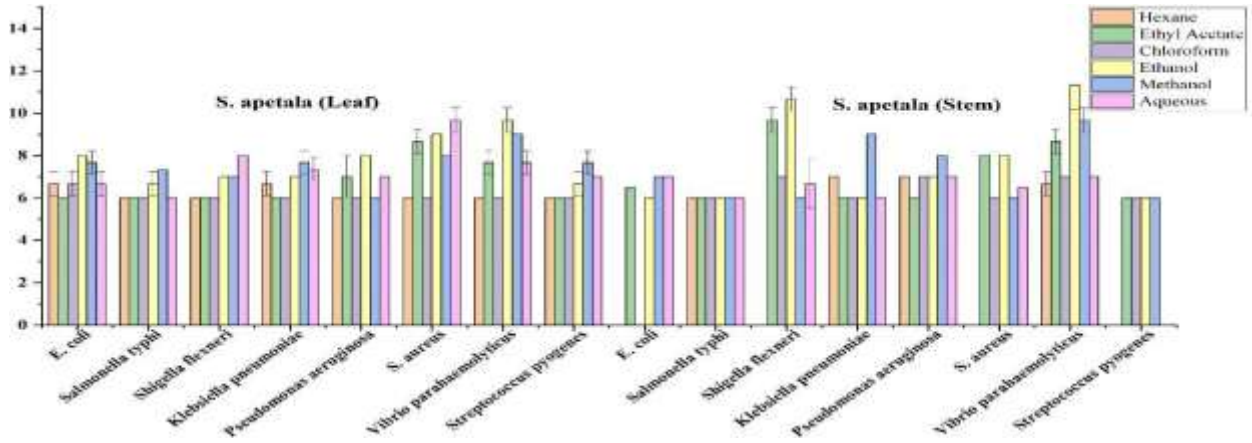

Fig 17. Antibacterial activity of *S. apetala* Leaf and Stem solvent extracts

The *S. apetala* Leaf crude aqueous and ethanol extracts only showed moderate inhibition against *S. aureus* and *V. parahaemolyticus* with 9.6mm and against *S. aureus* with 9mm zone of inhibition respectively (Fig 17). Remaining all exhibited minimum inhibition. Bakshi and Chaudhuri (2014) studies on *S. apetala* leaf has given antibacterial activity against *Agrobacterium tumefaciens, Escherichia coli, Streptococcus mutans, Staphylococcus aureus* in different solvents such as hexane, ethyl acetate, acetone and methanol.
The stem ethanol crude extract of *S. apetala* showed 11.33 mm and 10.67 mm against *V. parahaemolyticus* and *Shigella* respectively. The methanol and ethyl acetate showed similar inhibition 9.67 against *V. parahaemolyticus* and *Shigella* respectively (Fig 18). The acetone extract of leaf and bark of *S. apetala* showed significant antibacterial activity with the zone of inhibitions against both Gram positive and Gram-negative bacteria ranging from 12.0 to 22.0 (Patra et al, 2014). In their study Ethanol, methanol and aqueous showed inhibition against few bacteria like *V. cholerae*, but no activity against the bacterial strains such as *S. flexneri, S. epidermidis and E. coli*. Exceptionally our findings showed significant activity against *S. flexneri, V. parahaemolyticus and E. coli.* In another study (V. Prabhu Teja et. al, 2013) ethanolic extract of *S. apetala* plant exhibited antibacterial activity in a concentration dependant manner at higher concentrations (25 – 100 mg/ml). Comparatively in our study plant parts showed good inhibition at lower concentrations itself.

CONCLUSION:

In this study, non-polar extracts yielded higher levels of secondary metabolites and displayed stronger antibacterial activity, particularly in *Bruguiera gymnorrhiza* and *A. corniculatum*, as evidenced by qualitative screening and total phenolic content (TPC) analysis, and reinforcing the correlation between phenolic richness and antibacterial potency. The presence of compounds such as tannins, alkaloids, and flavonoids in these extracts likely contributed to the observed inhibition of both Gram-positive and Gram-negative bacteria.
FTIR spectral analysis supported the phytochemical screening by confirming the presence of functional groups corresponding to hydroxyl (-OH), carbonyl (C=O), and aromatic rings were observed, particularly indicating the presence of polyphenols, flavonoids, alkaloid, anthraquinone, terpenoids, quinones and steroids. These compounds are known to contribute to antibacterial mechanisms, including protein precipitation, membrane permeability alteration, and inhibition of nucleic acid synthesis.
Among four plants studied *Bruguiera gymnorrhiza* non polar solvents especially ethyl acetate and chloroform of both leaf and stem showed the highest inhibitory effects against all tested pathogens. Stem polar solvent extracts of *C. decandra* and *S. apetala* had the significant inhibitory activity especially in ethanol extracts, while leaf extracts demonstrated moderate inhibition in aqueous. In case of *A. corniculatum,* stem ethyl acetate extract had good activity. The results affirm that both species-specific phytochemical diversity and solvent polarity play pivotal roles in determining extract bioactivity.
The comparative study among mangrove species highlighted significant interspecific variation. *Bruguiera gymnorrhiza* consistently ranked highest in antibacterial efficacy and phytochemical richness, followed by *Aegiceras corniculatum*. *Sonneratia apetala* and *Ceriops decandra* also demonstrated moderate activity, suggesting that even lesser explored mangrove species detain therapeutic promise. These differences may be attributed to genetic, ecological, seasonal and physiological factors influencing secondary metabolite biosynthesis. Hence, the study proved that the mangrove extracts can be used for development of novel bioactive molecules for therapeutic applications against MDRs to treat both acute and chronic diseases. Further investigation on purifying individual compounds identification using GC-MS would provide a better understanding of the main components having antimicrobial activity.

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