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Original Research Paper

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COMPARATIVE STUDY OF ANTIOXIDANT ACTIVITY , POLYPHENOLICS, CONTENT AND FTIR ANALYSIS OF VARIOUS EXTRACTS, FROM DIFFERENT PARTS OF EXCOECARIA AGALLOCHA .

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ABSTRACT Excoecaria agallocha a milky mangrove, blind your eye mangrove, or a river poison tree is widely distributed in Indian coastal regions. The present study focuses on evaluation of phenolics and flavanoids present in Excoecaria agallocha. The FTIR analysis of various mangrove extract is also studied .The FTIR analysis showed the presence of flavones, alcohol,arenes,aliphatic functional group. Total phenolic content (TPC) expressed as gallic acid equivalents (GAE) ranged from 6.3±0.4 (whole plant petroleum ether extract) to 10.83±0.7 (stem methanol extract) mg GAE/g dry weight. Total flavonoid content expressed as quercetin equivalents (QE) ranged from 9.2±0.9 (whole plant ethyl acetate extract) to 33.8±0.9 (leaf methanol extract) mg QE/g dry weight. Antioxidant activity determined by DPPH assay is highest(83.3 ±3.2%) in methanol extract. Keywords: Excoecaria agallocha ,halophyte, TPC ,TFC, antioxidant, DPPH assay, FTIR.

KEYWORDS :

Introduction:

Mangroves are facultative halophytic plants found in tropical and sub-tropical areas of inter tidal zones 37. Mangrove and mangrove associates contain biologically active antiviral, antibacterial and antifungal compounds. They provide a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins.35

The polyphenols found in different halophytes, contain high levels of bioactive compounds which help them in stress conditions 2. Plant phenolic compounds play an important role in protecting the plants from biotic and abiotic stress.

The reactive oxygen species (ROS) like singlet oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radicals are generated during the abiotic stress causes oxidative damage to the plants11. The polyphenol content in plants is correlated with its antioxidant capacity .The human body is also constantly exposed to free radicals or ROS which causes oxidative stress if produced in high concentration . Free radicals are associated with various physiological and pathological events such as inflammation, aging, mutagenicity and carcinogenicity 15. The principle of IR spectroscopy is based on the fact that various functional groups in a chemical structure gives rise to characteristic bands both in terms of intensity and position (frequency).7

Excoecaria agallocha, known as a back mangrove, is found at higher elevations back away from the ocean where salinity is lower1. It is widely distributed abundant in Pichavaram mangrove forest, Indian coastal regions, Australia from northern New South Wales, along the northern coastline around to Western Australia. According to Red list criteria it is a least concern position2. The plant exudes white latex from any broken part. It is also deciduous, and usually sheds its leaves just before the onset of flowering.

The potent anti-HIV21, anticancer21,22, and antimicrobial23,24 effects of the various extracts are also reported. Study showed EA was able to decrease the acidity and increase the mucosal defense in gastric areas, justifying its use as an antiulcerogenic agent25

The present study deals withphytochemical evaluation of TPC TFC , antioxidant activity and chemical identification of using IR spectroscopy.

2. Materials and Methods

2.1 Collection of Plant materials : Exocaeria agallocha was collected from Vashi creek Mumbai, Maharashtra and the plant samples was identified. with the help of manual published by Kathiresan K 11 .

2.2 Plant extract preparation: For the extraction of crude bioactives, plant powder was initially diluted with different solvents, with solvent to sample ratio of 10:1 (v/w). The diluted plant sample was kept at room temperature undisturbed for 24 h. Then, it was kept in the orbital shaker for next 48 h. Solvents ethanol, methanol, acetone, hexane, and diethyl ether were used for the extraction process.The diluted sample was then filtered through Whatman No. 1,dried and stored.

2.3. Total Phenolic content (TPC): 24 0.5 ml of plant extract (1000 µg/ml) or gallic acid was treated with 1 ml of Folin–Ciocalteu reagent (1:10 v/v) , and incubated for 15 minutes. 2.5 ml of sodium carbonate solution (7.5%, w/v) was added to the mixture. After 30 min of incubation at room temperature in the dark, the absorbance was measured at 760 nm. The content of total phenolic compounds was calculated as mean ± standard deviation (SD) (n=3) and expressed as mg gallic acid equivalent (GAE)/g dry extract.

2.4. Total Flavanoid content (TFC)6 : The reaction mixture consisting of, 1 ml of plant extract (1000 µg/ml) or quercetin , 0.5 ml of 10% aluminum chloride, 0.5 ml of 1M potassium acetate was incubated at room temperature for 30 min. The absorbance of the sample and the standard was measured at 415 nm .The content of total flavonoid compounds was calculated as mean ± SD (n=3) and expressed as mg quercetin equivalent (QE)/g dry extract.

2.5 DPPH radical scavenging Assay:

The assay was conducted on the basis of scavenging activity of the stable DPPH free radical5 Ascorbic acid (2.5–15 µg/ml) was used as the standard. 1 ml of plant extract (50–500 µg/ml) or standard was treated with 1 ml of 0.2 mM DPPH solution in methanol. The reaction mixture was incubated in the dark at room temperature for 90 min. The absorbance of the sample and standards was measured at 517 nm. The ability of the plant extract and standard to scavenge the DPPH radical was calculated as the percentage of inhibition using the following formula.

DPPH scavenging activity (%) = [(AControl-(ASample–ASample Blank))/AControl]×100 Where Acontrol indicates the absorbance of control containing 1 ml of DPPH and 1 ml of methanol. ASample is the absorbance of the sample. Due to the high concentration, the sample also absorbs at this wavelength, so it is required to perform blank measurement. ASample Blank is the absorbance of sample blank containing 1 ml of plant extract and 1 ml of methanol. Sample blank is prepared separately for each concentration.

2.6 IR Spectroscopy analysis: The IR Spectroscopy was performed on Perkin elmer Inc. Spectrum RX FT-IR spectrophotometer. It has an autosampler with fibre optic interfaces and range of microscope. Spectral resolution is better than 0.8 cm-1 It is equipped with dynascan interferometer.

2.7. Statistical analysis: All the experiments were done in triplicates, and the results are expressed as mean ± SE.

3. RESULTS AND DISCUSSION

3.3 Total Phenolic content:

Phenolic compounds are secondary metabolites and play an imperfect in profiting the plants from biotic and abiotic stresses 23. For assessing the total phenolic compound present various parts in S. portulacastrum, four different solvent were used for the extraction. The TPC were different in the above four solvents viz.,methanol, ethanol,ethyl acetate and petroleum ether .The content of total phenolic compounds was calculated and expressed as mg GAE/g dry extract. (Table 3).The overall TPC based on the plant part , and solvent used for extraction can be arranged as LET>LME>LEA>LPE>WPME>WPET>WPPE>WPEA.

The least TPC is seen in Whole plant ethyl acetate (WPEA) which is 5.2±0.79 mg GA/g.

3.4 Total Flavanoid content:

Plant-derived polyphenolic compounds include phenolic acids, flavonoids, tannins, and the less common stilbenes and lignin. These compounds are considered to be a rich source of antioxidants. Flavonoids are the most common polyphenols ,are considered as more powerful antioxidants than Vitamin C and E and carotenoids in vitro. The compounds have the ability to reduce free radicals by rapidly donating hydrogen atoms and thereby break the chain of reactions that lead to free radical formation 4. The content of total flavanoids was calculated and expressed as mg QE/g dry extract. (Table 3).

The overall TFC based on the plant part , and solvent used for extraction can be arranged as,

LME>LET>LEA>LPE>WPME>WPET>WPEA>WPPE.
The least TFC is seen in Whole plant petroleum ether (WPPE) which is 7.7±0.8 mg GA/g.

Table 3: Total phenolic content(TPC) andTotal flavonoid contents(TFC) of various plant parts in different solvents

Plant parts in various solvents	TFC expressed as mg QE/g extract	TPC expressed as mg GAE/g extract
WP PE	7.7±0.8	6.3±0.4
WPEA	8.2±0.9	5.2±0.79
WPET	9.4±0.7	5.3±0.57
WPME	10.1±0.7	6.5±0.85
LPE	15.7±0.7	9.1±0.84
LEA	22.6±1	9.3±1.1
LET	29±.0.9	9.76±0.6
LME	33.8±0.9	9.6±0.9

The data is presented as mean ±SE in triplicates, where WP PE is whole plant petroleum ether, WP EA is whole plant ethyl acetate, WPET is whole plant ethanol, WPME is whole plant methanol, LPE is leaf petroleum ether, LEA is leaf ethyl acetate, LET is leaf ethanol, LME is leaf methanol.

3.5 DPPH radical scavenging Assay:

DPPH assay is simple, rapid, economic, and a widely used method to evaluate antioxidant activity. DPPH shows maximum absorption at 517 nm because of the presence of an unpaired electron.Antioxidant reduces the unpaired electron on DPPH (nitrogen atom of hydrazine contains the odd electron) by donating a hydrogen atom 19. Due to activity of DPPH as free radical, the compound is being used to assess the reducing ability of an unknown plant extract. Therefore it is considered as a valuable agent for determining the free radical scavenging activity of unknown solution 13.The highest activity was found in, methanol extract followed by , ethanol, ethylacetate and petroleum ether. Therefore, the high content of phenolic compounds in diethyl ether extract explains its high antioxidant potential .

Table 4: DPPH assay of various extracts of Excoecaria agallocha along with standard.

Concentration	Vitamin C	Methanol	Ethanol	Ethyl acetate	Pet. Ether
50	67.5±1.04	49.7±0.6	45.9±0.81	45±0.89	40.5±1
100	71.11±1.5	55.4±1.1	47.7±1.6	45±0.6	44±1.0
150	85.3±1.22	61.5±1.4	48.1 ±1.7	47.7 ±1.6	43.2± 1.02
200	88.89 1.0	64.4 1.84	51.5 1.1	52.6 1.2	50.1 1.1
250	90 ±1.2	70.3± 1.3	60.1± 0.7	53.2± 0.7	51.0 ±1.2
300	93.8± 1.2	75.5± 0.8	70.8 ±0.9	57.5± 0.6	51.3± 0.9
350	94.6 ±1.28	80.6± 0.4	72.7 ±1.14	62.2± 3.6	53.6± 0.9
400	95.1± 1.3	83.4 ±2.0	73.0 ±2.1	64.9 ±2.4	54.9 ±3.0
450	95.3± 1.6	83.3 ±3.2	76.9± 3.0	66.3 ±1.77	59.5± 2.2

FTIR analysis of Exocaeria leaf extract:The IR spectrum is shown in figure .The SNF extract yielded maximum peak level 3600 cm-1 and minimum peak 783cm-1 .FT-IR studies confirm the presence of functional groups in extract listed in table no the compound listed in the table 2,

FTIR analysis of Exocearia whole plant extract:The IR spectrum is shown in figure .The SNF extract yielded maximum peak level 3448 cm-1 and minimum peak 723 cm-1 Many of the functional present in leaf and WP extract are similar like aliphatic group ,alcohol group but the leaf extract contains Arenes and whole plant contains flavones. So that the compound may be phenolic. This identification is possible using IR spectroscopy as it give rise to characteristic bands both in terms of intensity and position (frequency)10

Conclusion:

Screening of Phytoconstituents of all the extracts from the halophyte indicated the presence of phytochemicals of biological importance.The FTIR analysis indicated the presence of probable functional groups present in the mangrove. The halophyte also contains high amount of phenolics and flavanoids which help in increasing its medicinal value. Further studies are underway in isolating and characterizing compounds with antioxidant properties.

Table no 1: FTIR Result for Excearia agallocha leaf extract

Wavelength in cm-1	Functional groups	Name of the Functional groups
3600-3000	O-H	Alcohol
2926 , 2854	C-H	Aliphatic
1745	C=O	Carboxylic acid
1643	C=C	Arenes
1159 ,1097	C-O	Alcohols/ Phenols
715	=C-H bending	(out-of-plane bending) cis – RCH=CHR

Table no 2: FTIR Result for Exocearia agallocha whole plant extract

Wavelength in cm-1	Functional groups	Name of the Functional groups
3600-3000	O-H	Alcohol
2931	C-H	Aliphatic
1745	C=O	Carboxylic acid

1651	C=C	Conjugated carbonyl (may be flavone)
1159 ,1097	C-O	Alcohols/ Phenols
783	=C-H bending	(out-of-plane bending) cis – RCH=CHR

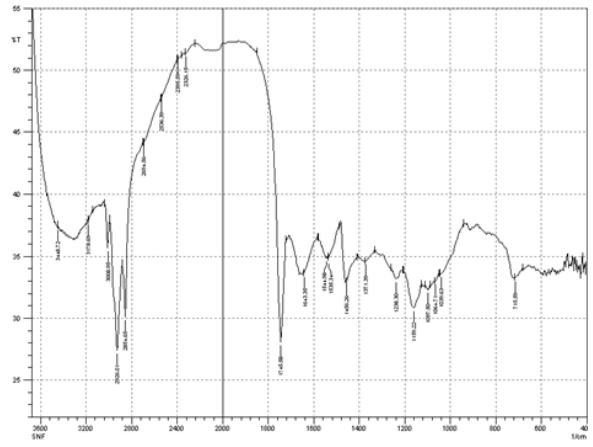

Figure no1: FTIR spectrum of Exocearia agallocha leaf extract

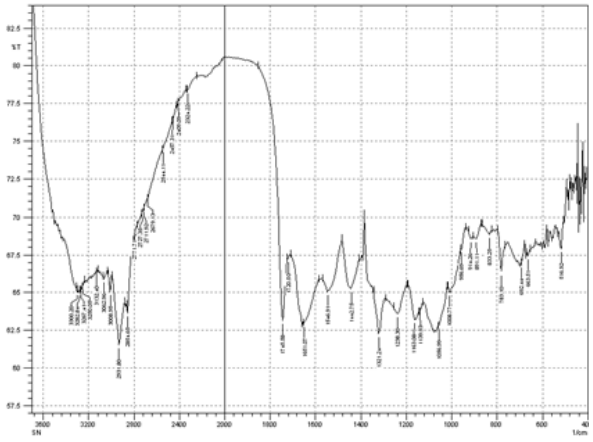

Figure no 2: FTIR spectrum of Exocearia agallocha whole plant extract

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