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INVESTIGATION OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF *EXCOECARIA AGALLOCHA*, L.

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ABSTRACT: Mangrove survives in the most hostile environment with fluctuating tidal and saline regime. The present work focused on a detailed study on quantitative estimation of phytoconstituents and DPPH radical scavenging activity of *Excoecaria agallocha* L., a mangrove from Kattiparambu and Chirackal of Ernakulam District, Kerala. Preliminary phytochemical investigation indicated the presence of biologically active metabolites of leaf, stem and root of the plant in different solvents. Methanol and hexane gave maximum results. Since the plant grows in stress conditions showed the presence of amino acids. Recorded values for high protein in leaf (4.8%), stem (1.93%), Carbohydrate in the root (11.17%) at Chirackal. Phenols (6.02%), flavanoids (3.6%) and tannins (5.68%) was observed in leaf samples of the species at Chirackal. Methanolic extract showed maximum free radical scavenging activity (IC_{50}-141.56µg/ml) in leaf samples and stem showed minimum (IC50-931.3µg/ml) in chloroform extract. The range of DPPH radical scavenging activity in the solvent is Methanol > Ethyl acetate > Ethanol > Chloroform.

INTRODUCTION: The mangroves are marvel of nature, ecological wonder and scenic splendor. These plants grow in mud flats and shallow water coastal areas where the water is generally brackish. Mangrove ecosystems provide a wide range of ecological and economic products and services, and also support a variety of other coastal and marine ecosystems [1]. They contain many bioactive compounds of toxicological, pharmacological and ecological importance. These compounds are synthesized by primary or secondary metabolism of living organism. The knowledge of chemical constituents of these plants is desirable to understand herbal drugs and their preparation [2].

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Due to their medicinal values, mangrove plant extracts have been used for centuries by the local people as folklore medicine for curing many health disorders.

Antioxidants save as potential agents for preventing and treating oxidative stress-related diseases. The antioxidant effects of mangrove plants, which may be up to 20 times that of tocopherol, a powerful antioxidant, are stemmed from phenolic hydroxyl groups. Polyphenols are capable of suppressing cholesterol levels, the incidence of pathogens, blood pressure levels, halitosis and allergic rhinitis. Its strong free radical scavenging activities and subsequent antimutagenic activity prove effective for preventing various life style diseases in adults, including antiatherogenic effects, gastric ulcer, colorectal cancer, cataract and diabetic complications [3]. Kattiparambu and Chirackal mangrove areas are increasingly threatened by the heavy metal contamination and their impact on the

flora. The aim of the study was to evaluate the qualitative and quantitative analysis of phytochemicals and the antioxidant activity of *Excoecaria agallocha*, L..

Plant Description: *Excoecaria agallocha*, L. belongs to the family Euphorbiaceae, is a medium dioceous tree, deliquescent, woody, perennial, partially deciduous, much branched, very dense with dark leaves and spreading branches. This plant is known as ‘‘milk mangrove’’, is an important medicinal plant and among the few non-viviparous mangrove species. Continuous habitat loss or deterioration accompanied with little compensation has caused great challenges for survival and regeneration of non-viviparous mangrove species like *E. agallocha*, L. The plant has been used in traditional medicine against rheumatism, leprosy, epilepsy, paralysis, conjunctivitis and dermatitis [4, 5]. The plant is reported to have anti HIV [6], anti microbial [7], anti oxidant [8] anti-ulcer [9] and anti filarial properties [10].

MATERIALS AND METHODS: The leaves of *Excoecaria agallocha*, L. were collected from Chirackal and Kattiparambu of Ernakulam District, Kerala. The collected leaves were washed with tap water and shade dried at room temperature. The dried leaves were powdered using electrical blender. Ten grams of material was stirred overnight for 72 hours in various solvents (100 mL) and then centrifuged at 10,000 rpm for 10 min at 4^0C. The resultant supernatant was collected and the solvents were removed by evaporation. This extract was used for further phytochemical analysis.

Qualitative profiling: Extracts of *E. agallocha*, L. leaf, stem and root were used for qualitative assessment for the major classes of phytochemicals namely phenols, flavonoids, steroids, amino acids, tannins, terpenoids, glycosides, saponins, alkaloids in addition to the major biomolecules namely lipids, carbohydrates and proteins. The tests were performed according to various standard methods [11, 12, 13].

Quantitative profiling: Quantitative estimation of various phytochemicals viz. Total Proteins [14], Total Carbohydrates [15], Total Lipids [16], Total Phenols [17], Total Tannins and Lignin [18], Total Flavonoids [19], Total Amino Acids [20], DPPH antioxidant activity [21] present in the leaf, stem and root of *E. agallocha*, L. was analyzed according to the standard protocols.

RESULTS:

Qualitative phytochemical Analysis: Preliminary screening tests revealed the presence and absence of the contents in extracts. Important phytochemicals like phenols, alkaloids, steroids, glycosides, saponins, tannins, flavonoids, amino acids, proteins, carbohydrates and lipids (**Table 1**) were screened for their presence in various extracts.

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *EXCOECARIA AGALLOCHA*, L. IN DIFFERENT EXTRACTS

	Methanol			Ethanol			Chloroform			Benzene			Acetone			Petroleum Ether			Hexane			Water		
	L	**S**	**R**	**L**	**S**	**R**	**L**	**S**	**R**	**L**	**S**	**R**	**L**	**S**	**R**	**L**	**S**	**R**	**L**	**S**	**R**	**L**	**S**	**R**
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavanoids	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+	+	-
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aminoacids	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-	-	-
Saponin	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	+	-	-	+	+	-	+	-	-
Starch	+	+	+	+	+	-	+	-	-	+	-	+	+	-	-	+	+	-	+	+	+	+	+	-
Steroids	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Triterpenoids	+	-	+	+	-	-	+	-	+	-	-	-	+	+	-	+	-	-	+	-	+	-	-	-
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	-	-	-	+	-	-	-	+	-	+	-	-	-	+	+	-	+	+	-	-	-	-
Proteins	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-

‘+’= present, ‘-’ absent, L-Leaf, S-Stem, R-Root

Methanol and hexane extracts gave maximum results of primary and secondary metabolites. Minimum results were found in ethanol, benzene and water extracts. The leaf of *E. agallocha*, L. showed maximum results in most of the solvents, while stem and root showed minimum.

Quantitative phytochemical analysis: Studies on the bioactive compounds of mangrove plants often lead to the discovery of new therapeutic agents. It has been observed that *Excoecaria agallocha*, L. can yield high amount of phenolic compounds**.** Presence of bioactive compounds are varied in these areas. The above study showed that protein content was recorded maximum in leaf (4.85±0.109) and minimum in root (1.635±0.04). Phenol, flavanoids and tannin contents ranged from 2.722±0.0002 to 6.53±0.0023, 1.1±0.001 to 2.46±0.003 and 1.849±0.0015 to 4.449±0.0015 in Chirackal and 4.26±0.023 to 5.29±0.0278, 1.24±0.0006 to 2.74±0.0004 and 2.63±0.004 to 3.65±0.0326 of Kattiparambu respectively (**Fig. 1**). High carbohydrate and lipid contents were recorded in the root (11.06±0.057 and 7.066±0.058) of *E. agallocha*, L. at Chirackal (**Fig.2**).

Antioxidant activity of different tissues by DPPH method: DPPH activity was determined in leaf, stem and root samples of *E. agallocha*, L. Leaf showed maximum (IC_{50}-141.56µg/ml) antioxidant property in methanolic extract and stem showed minimum (IC_{50}-931.3µg/ml). DPPH activity is increased when IC_{50} value decreased, (**Fig. 3, 4, 5, 6**). The range of DPPH radical scavenging activity in the solvent is Methanol > Ethyl acetate > Ethanol > Chloroform.

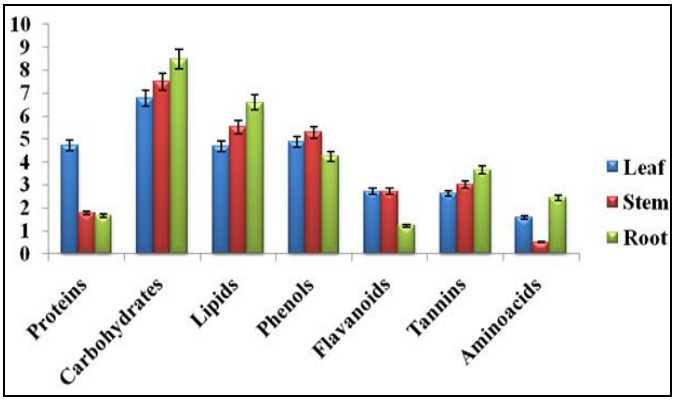

FIG. 1: (KATTIPARAMBU) SHOWING THE PHYTOCHEMICAL CONSTITUENTS OF *E. AGALLOCHA*, L.

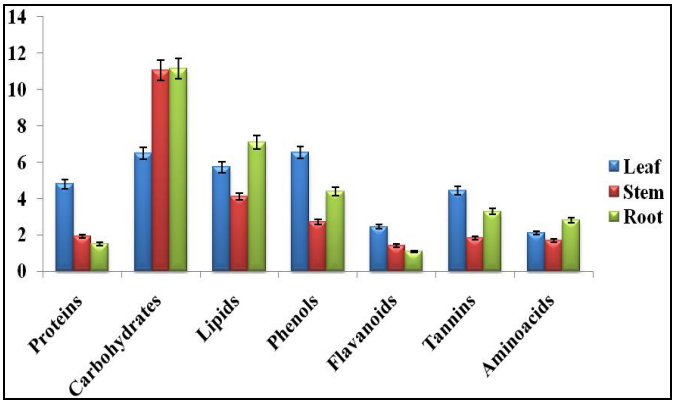

FIG.2: (CHIRACKAL) SHOWING THE PHYTOCHEMICAL CONSTITUENTS OF *E. AGALLOCHA*, L.

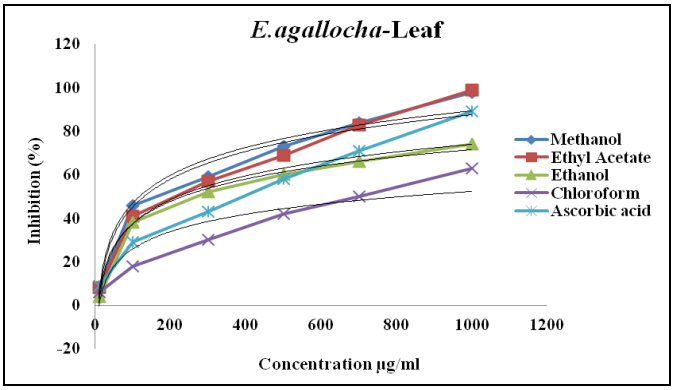

FIG.3: DPPH FREE RADICAL SCAVENGING ACTIVITY OF *E. AGALLOCHA*, L. LEAF

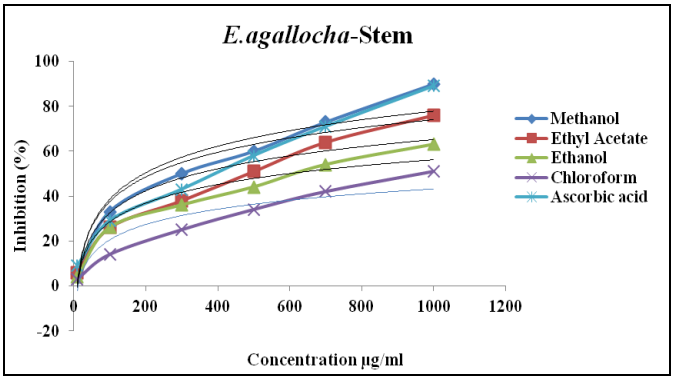

FIG. 4: DPPH FREE RADICAL SCAVENGING ACTIVITY OF *E. AGALLOCHA*, L. STEM

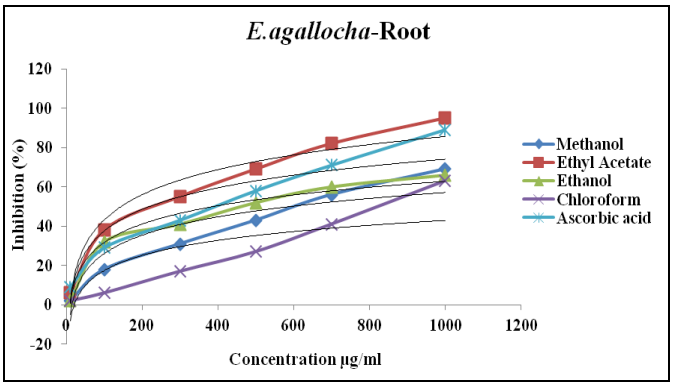

FIG.5: DPPH FREE RADICAL SCAVENGING ACTIVITY OF *E. AGALLOCHA*, L. ROOT

DISCUSSION: The marine environment is incomparable reservoirs of bioactive natural products, many of which exhibit structural features that have not been found in terrestrial natural products [22]. Recently, it has been strongly recommended that mangroves should be considered as a valuable source for chemical constituents with potential medicinal and agricultural values [23]. Qualitative and quantitative screening revealed the strong phytochemical relation within the plant under investigation. Mangrove plants can produce metabolites and toxins that are unique to these plants, which suggest that they may be a source of novel compounds [24]. They are biochemically unique, producing a wide array of natural products. They possess new agrochemical products, compounds of medicinal value and biologically active compounds.

On the other hand, quantitative estimation of the primary and secondary metabolites in different extracts of the plant gave a clear evidence for the high quantities of these by- products. Mangroves are a promising source of natural products. They have been a source of several bioactive compounds [25]. Phenolic compounds from plants are known to be good natural anti-oxidants. It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health beneficial effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) [26] order to survive and prevent molecular damage and damage by micro organisms, insects and herbivores. Environmental stress factors affect plant growth [27, 28]. Among the effects of environmental stress is the creation of reactive oxygen species (ROS), which cause damage to cell membranes, nucleic acid and chloroplast pigments [29]. The levels of reactive oxygen species are controlled by enzymatic antioxidants under normal conditions [30], however, ROS rise under stress and stimulate the activity of antioxidant enzymes. Two review articles have been published earlier on *in vitro* evaluation of antioxidant activity [31, 32].

CONCLUSION: From the above results we assume that different active secondary metabolites are present in the plant extracts and perhaps some of these compounds function in a synergistic manner. The antioxidant activity of phenolic compounds is therefore confirmed in mangrove plants. The scale of human impact on mangroves has increased dramatically in these areas over the past three decades. Environmental education can contribute to active involvement and greater public participation in issues related to mangrove conservation and management is inevitable. However, further studies like isolation and characterization are necessary to elucidate the mechanism lying with this effect. This report will serve as a stepping stone for future research on the biological and phytochemical activities of *E. agallocha*, L. plant extract.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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