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PHYTOCHEMICAL AND MICROBIOLOGICAL INVESTIGATION ON THE ROOT BARK OF *SUAEDA MARITIMA*

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ABSTRACT

Suaeda maritima is a mangrove plant belonging to family Amaranthaceae. The present investigation is based on chemical and biological aspects of root bark of the plant. Antimicrobial activity was investigated against *Bacillus subtilis, Staphylococcus aureus* and Gram –ve bacteria *Escherichia coli, Proteus vulgaris* and Antifungal activity was investigated against *Rhizopus oryzae, Aspergillus niger*. The extract has shown less antimicrobial activity and significant antifungal activity. Three chemical compounds were isolated and analyzed using H NMR, Mass and IR spectral studies.

KEYWORDS

Mangrove plants, Antimicrobial, Antifungal activity and *Suaeda maritima*.

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INTRODUCTON

The natural products remain one of the most important sources of lead compounds for the pharmaceutical industry, among them the ocean offers enormous biomedical potential in the last four decades as it provides a moist, chemically polar aqueous medium containing dissolved electrolytes and possessing characteristic conditions of temperature[1]. Research in the area of marine natural products grew geometrically and spread geographically beyond expectations and activities of bioactive secondary metabolites of various classes are alkaloids, steroids, terpenoids, etc[2]. Mangroves are a group of highly evolved halophytes and are open ecosystems which

exchanges matter and energy with the adjacent marine and terrestrial ecosystems which protects coastal area from sea erosion and from violent effects of cyclones and tropical storms[3,4].

Suaeda maritima belongs to family Amaranthaceae sub family suaedoideae and it has worldwide distribution but in North America it is confined to the north east coast. It is a habitat of aquatic, terrestrial and wet lands. Leaf and stem extracts has strong antioxidant and hepatoprotective properties and root extract has antibacterial and antifungal properties[5]. Because of its wide usage and availability, this study was set out to investigate microbiological activity of *Suaeda maritima*.

MATERIAL AND METHODS

Suaeda maritima was collected at Manali Island near Rameswaram in Tamilnandu and were identified and authenticated at Department of Botany, Andhra University, Visakhapatnam.

Preparation of extract

The powered root bark of the plant *S. Maritima* was soaked in methanol at room temperature for 24 hours and filtered and was repeated three times. The combined methanolic extract was concentrated to a small volume under reduced pressure and precipitate was collected. The precipitate is suspended in water and fractionated with ethyl acetate and separated into ethyl acetate and water soluble portions. The filtrate was fractionated with hexane and ethyl acetate, thus separating into hexane and ethyl acetate fractions and mother liquor.

Column chromatographic Methods

The ethyl acetate portions of filtrate and precipitate are mixed and chromatographed over a column of silica gel using solvents of increasing order of polarity starting from hexane, hexane-ethyl acetate, ethyl acetate-methanol fractions (100ml) were collected and monitored through silica gel TLC, The visualization of spots was carried out under UV light and iodine vapor or by spraying 20% sulfuric acid in methanol and heating at 110 Degrees and progress of chromatography was recorded and analyzed.

MICROBIOLOGICAL INVESTIGATIONS:

Anti-bacterial activity

The ethyl acetate soluble portion of methanolic extract was screened for antibacterial activity using nutrient agar medium against gram +ve bacteria i.e., *bacillus subtilis, staphylococcus aureus* and gram – ve bacteria *Escherichia coli, Proteus vulgaris*. Cups of 8mm diameter were made on the solidified media. Solution of ethyl acetate soluble portion at concentrations of 100mg/ml and 200 mg/ml were prepared in dimethyl sulfoxide and 20ml of each solution was placed in cups and two additional cups were also used for Benzyl pencillin as a standard. Inhibition Zones were measured after 24 hours incubation at 37^{o}C.

Anti-fungal activity

The ethyl acetate soluble portion of the methanolic extract was screened for anti-fungal activity using YEME agar medium (yeast extract and Meat extract) and organisms are *Rhizopus Oryzae* and *Aspergillums niger*. Cups of 8mm diameter were made on solidified media. Solution of ethyl acetate soluble portion at concentrations of 100mg/ml and 200mg/ml were prepared in dimethyl sulfoxide and 50ml of each solution was placed in cups by sterile micropipette under laminar flow. In each, two additional cups were also used for ketoconazole as standard. Inhibition zones were measured after 4 days of incubation period at room temperature.

RESULTS AND DISCUSSION

After performing column chromatography and TLC, three compounds were isolated.

The first compound obtained from hexane, ethyl acetate (99:1) from methanolic extract and crystallized from hexane as needles gave positive Liebermann-Burchard test for triterpenes whose formula was found to be $C_{30}H_{50}O$ from IR bands. ^{1}HNMR spectrum showed that it is a lupane derivative and ^{13}C NMR spectral data confirmed that it is Lupeol.

Second compound obtained from hexane ethyl acetate (99:1) fraction and crystallized from hexane gave positive Liebermann-Burchard test for sterols. ^{1}H NMR spectral data confirms it as a mixture of sterols and ^{13}C NMR data confirms it as a mixture

of sterols i.e., Campesterol, Stigmasterol and β-Sitosterol.

Third compound obtained from 2% ethyl acetate in hexane fraction gave positive test for triterpenoids, ^{1}H NMR and mass spectrum confirmed that it is Lupeal.

The root bark of *Suaeda maritima* was prepared in different solvents like n-hexane, benzene Ethyl acetate, petroleum ether, acetone, ethanol. Among the different extracts tested, extracts prepared in methanol and then fractionated with Ethyl acetate showed antibacterial activity against Gram +ve bacteria i.e., Bacillus subtilis, Staphylococcus aureus and Gram –ve bacteria Escherichia coli, Proteus vulgaris.

The Ethyl acetate extract possess not only antibacterial activity but also antifungal activity against *Rhizopus oryzae, Aspergillus niger*. The antimicrobial activity was assessed by zone of Inhibition values which are shown in Table No.1 and 2. The lowest antibacterial activity was recorded against Proteus vulgaris (Gram –ve) and highest activity was recorded against *Staphylococcus aureus* (Gram +ve). The lowest antifungal activity was recorded against *Aspergillus niger* and highest activity was recorded against *Rhizopus oryzae*. Three chemical compounds were isolated and analysed using ^{1}H NMR, Mass and IR spectral studies those are lupeol, mixture of sterols (Campesterol, β- Sitosterol, Stigmasterol) and lupeal (Figure No.1-4).

Table No.1: Inhibition Zone Diameters showing Antibacterial Activity

S.No	Compound Name	Inhibition Zone Diameter (mm)			
		Microorganism Used			
		Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Proteus vulgaris***
1	Ethyl acetate soluble portion	14	16	10	9
2	Benzyl penicillin (Standard-20µg/ml)	28	30	20	18

Table No.2: Inhibition Zone Diameters showing Antifungal Activity

S.No	Compound Name	Inhibition Zone Diameter (mm)	
		Microorganism Used	
		Rhizopus oryzae	***Aspergillus niger***
1	Ethyl acetate soluble portion	16	14
2	Ketoconazole (Standard-20µg/ml)	20	29

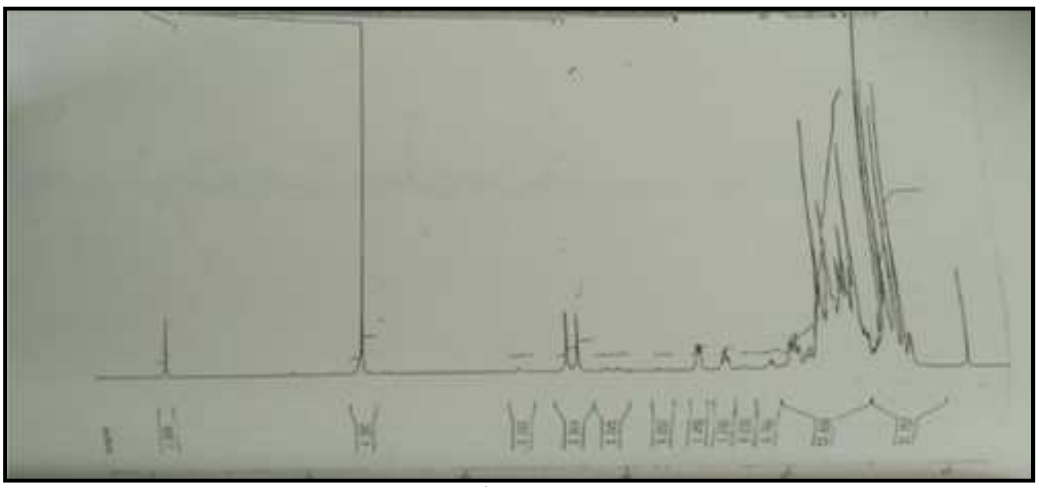

Figure No.1: ^{1}H NMR of Lupeal

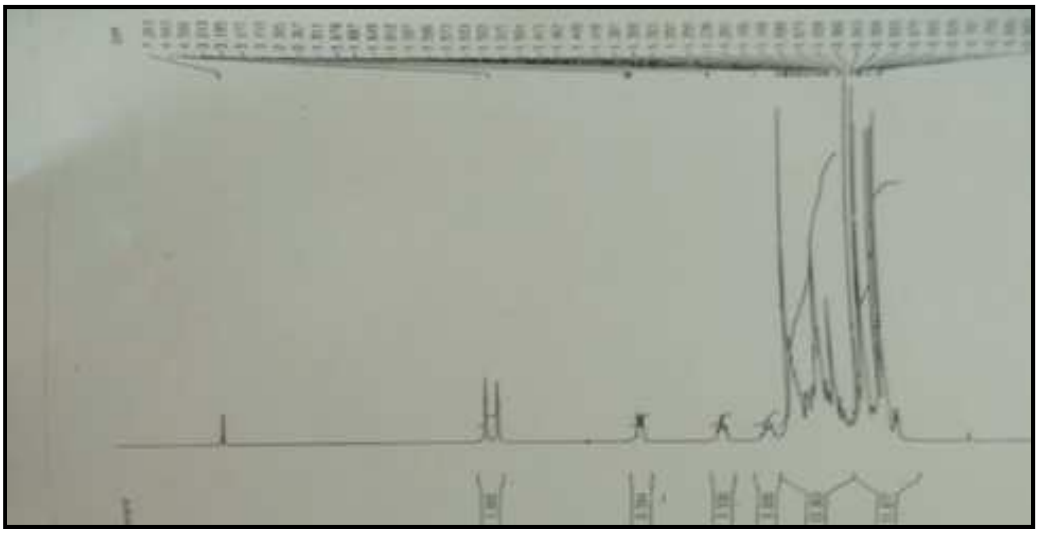

Figure No.2: ^{1}H NMR of Lupeol

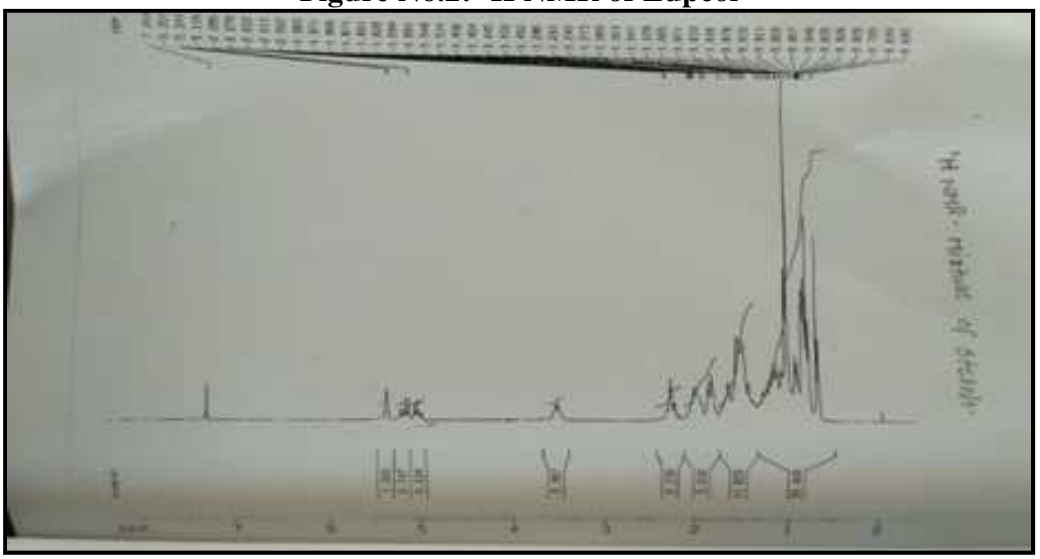

Figure No.3: ^{1}H NMR of Mixture of Sterols (Campesterol, β- Sitosterol, Stigmasterol)

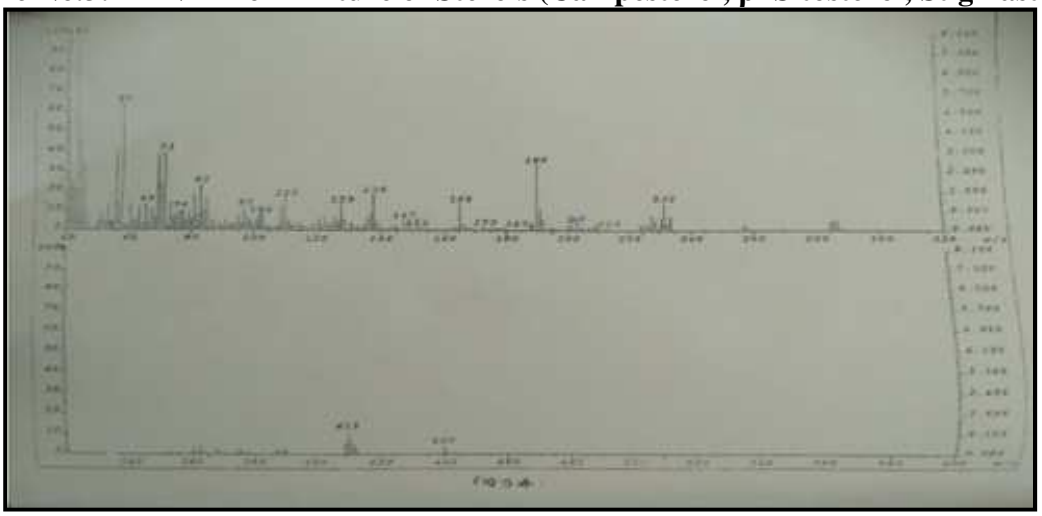

Figure No.4: Mass Spectrum of Lupeal

CONCLUSION

The extract has shown significant zone inbition after comparing the various strains of gram positive and gram negative bacteria against the standard Benzyl Penicillin (20µg/ml) and has shown significant antifungal activity after comparing the fungal strains with standard Ketoconazole. The extract has shown less antibacterial activity and significant antifungal activity.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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