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Studies on Phytochemical Screening, Antimicrobial and Anti Radical Scavenging Effect Coastal Salt Mash Plant of a *Suaeda monoica*

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ABSTRACT

Salt mash plants have used in many country for food and feed. The coastal people utilized the folk medicine based on the traditional knowledge to many diseased. Generally salt tolerance plants have more phyto chemical and more anti oxidant effect indicated that lot of literature, So in this way in our present study antioxidant, antimicrobial and phytochemcial screening of coastal salt mash plant of a *Suaeda monoica*. To evaluate the medicinal value of *Suaeda monoica* and we choose tree types of extracts, (Petroleum ether, ethyl acetate and methanol), three types of concentration (100, 50, 25 mg mL^{-1}) and three types of activity (photochemical screening, anti microbial and antioxidant). In our result point out maximum anti microbial effect observed in petroleum ether extract at 100 mg mL^{-1} concentration and minimum anti microbial effect observed in menthol at 25 mg mL^{-1} concentration. In the case of phytochemical screening was observed in phenolic group for all the extract and alkaloids, anthraquinones, catechins, flavonoids, gum, oils and resins and saponins are completely absent in all extracts.. Antioxidant effect maximum (71.4) in methonal extrat at 100 mg mL^{-1} concentration and minimum in 28.5 for at 25 mg mL^{-1} concentration of petroleum ether extract.

Key words: Salt mash, *Suaeda monoica*, phenolic compounds, DPPH free radical scavenging, Fe^{2+} chelating, antimicrobial

INTRODUCTION

Suaeda monoica Forsk. ex. Gmel (Chenopodiacea) is a salt marsh mangrove herb similar to *Suaeda maritima* L. Dumort in appearance, growing in hypersaline soils. It is distributed throughout the East West coast mangroves in India viz., Sunderbans in West Bengal State, Bitharkanika and Mahnadhi in Orissa State, Coringa, Godavari and Krishna in Andhra Pradesh State, Pichavaram, Karangadu and Muthupet in Tamil Nadu State. It is a shrub but much smaller in size (0.3-0.7 mm in length) when compared to *Suaeda maritima*. Leaves simple, succulent, linear, young twigs are slender ribbed. Locally it is called as Vellaikirai (or) Nilavumari (seaside Indian salt wort). The leaves have been used as edible green leaves. The ash obtained from burnt plant parts have been exported without knowing the purpose. It is a folklore medicinal plant used mainly against rheumatism, paralysis, asthma and snake-bites, skin disease, ulcer for local coastal people (Kathiresan and Ramanathan, 1997; Ramanathan, 2000). Traditionally, the leaf

from *Suaeda monoica* is known to use as a medicine for hepatitis (Bandaranayake, 1998) and scientifically it is reported to be used as ointment for wounds (Padmakumar and Ayyakkannu, 1992) and possess antiviral activity (Premnathan *et al.*, 1992) because of the presence of triterpenoids, sterols (Ghosh *et al.*, 1985; Subramanyam *et al.*, 1992). The hepatoprotective evaluation of crude ethanolic extract from leaves of *S. monoica* for possible development of hepatoprotective herbal medicine (Ravikumar *et al.*, 2010). Anti-oxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress (Ozsoy *et al.*, 2008). There is an increasing interest in natural anti-oxidants, e.g., polyphenols, present in medicinal and dietary plants which might help prevent oxidative damage (Silva *et al.*, 2005). Polyphenols possess ideal structural chemistry for free radical scavenging activity and they have been shown to be more effective anti-oxidants *in vitro* than tocopherols and ascorbate. Antimicrobial activity of different parts of this plant which has not been reported; hence, the present study was undertaken. The phytochemical literature reveals the presence of 2-benzoxazolinone, lignan glucosides, benzoxazinoide glucosides, flavone glycosides and phenylethanoid glycosides in this plant. Kanchapoom *et al.* (2001) and megastigmane glycosides Wu *et al.* (2003). According to various medical literatures, several adverse the drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immune compromised, AIDS and cancer patients (Diamond, 1991). It is likely that plant extract showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogen. However, very little information is available on such activity of medicinal plants (Lee *et al.*, 1998).

MATERIALS AND METHODS

Collection of plant material: The plant material (whole plant) of *Suaeda monoica* was collected from the Ariankuppam region near Pudhucherry and the collected plant material was botanically identified and confirmed by Herberia of C.A.S. in Marine Biology, Annamalai University, Tamil Nadu, India.

Preparation of the extracts: The collected materials (whole plant) were chopped into small pieces separately, shade-dried and coarsely powdered using a pulverizor. The coarse powders were subjected to successive extraction with organic solvents such as petroleum ether, ethyl acetate and methanol by Soxhlet method. The extracts were collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed *in vacuo*. The resulted extracts were used for preliminary phytochemical screening, antimicrobial activities and antioxidant activities.

Preliminary phytochemical screening: All the extracts were subjected to preliminary phytochemical tests followed by the methods of (Harborne, 1998; Sadasivam and Manickam 1996; Trease and Evans 1983).

Antimicrobial activities: Petroleum ether, ethyl acetate and methanol extracts of the selected plant were used to prepare various concentrations such as 25, 50 and 100 mg mL^{-1}, respectively. These were used for antimicrobial activity.

Test microorganisms: The following bacterial strains and fungal were used for the screening of antimicrobial activity. All the microbial strains of human pathogens used were procured from IMTECH, Chandigarh and the procured microbes are the gram-negative bacteria, viz., *Escherichia coli* (MTCC 724), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 741) and *Salmonella typhi* (MTCC 733), *Vibrio parahaemolyticus* (MTCC 451), *V. vulnificus* (MTCC 1145) and the gram-positive bacteria, *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 96), *Streptococcus pnemoniae* (MTCC 655) and fungi *viz.*, *Aspergillus flavus*, *A. fumigatus*, *A. niger* (MTCC 1344) and *Candida albicans* (MTCC 227), respectively.

Media used: Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were used respectively for testing the antibacterial and antifungal activity.

DPPH free radical scavenging activity: DPPH free radical scavenging activity was carried out by following the methods of Yaushisakono (1978). The 4.3 mg of DPPH (1, 1-Diphenyl-2-picrylhydrazyl) was dissolved in 3.3 mL methanol; it was protected from light by covering the test tubes with aluminum foil. The 150 µL DPPH solution was added to 3 mL methanol and absorbance was taken immediately at 516 nm for control reading. The test sample of 20 µL was taken and the volume was made uniformly to 150 µL using methanol. Sample was then further diluted with methanol up to 3 mL and to that 150 µL DPPH was added. Absorbance was taken after 15 min at 516 nm using methanol as blank on UV-visible spectrometer Systronics, India. Percentage of inhibition was calculated by using the equation given below:

$$\% \text{ of inhibition} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

Fe2$^+$ chelating assay: The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis *et al.* (1994). To 0.5 mL of extract, 1.6 mL of deionized water and 0.05 mL of $Fecl_2$ (2 mM) was added. After 30 sec, 0.1 mL ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe^{2+}-ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated as:

$$\text{Chelating rate } (\%) = \frac{(A_0 - A_1)}{A_0} \times 100$$

RESULTS

Preliminary phytochemical screening (qualitative analysis): The results of preliminary phytochemical screening of *Suaeda monoica* extracts are given in the Table 1. Phenolic groups are present in all the extracts viz., petroleum ether, ethyl acetate and methanol. Steroids and triterpenes are present both in ethyl acetate and methanol extracts but absent in petroleum ether extract. Tannins are present only in methanol extract but absent in petroleum ether and ethylacetate extracts. Carbohydrates and aminoacids are present only in petroleum ether extract but not in other extracts. Moreover, alkaloids, anthraquinones, catechins, flavonoids, gum, oils and resins and saponins are completely absent in all extracts.

Table 1: Preliminary phytochemical screening of various extracts of *Suaeda monoica* Forssk. ex Gmel

Phyto constituents	Petroleum ether	Ethyl acetate	Methanol
Alkaloids	-	-	-
Amino acids	+	-	-
Anthraquinones	-	-	-
Carbohydrates	+	-	-
Catechins	-	-	-
Flavonoids	-	-	-
Phenolic groups	+	+	+
Gum, oil and resins	-	-	-
Saponins	-	-	-
Steroids	-	+	+
Tannins	-	-	+
Triterpenes	-	+	+

+: Present, -: Absent

Table 2: Quantitative estimation of phytochemicals in *Suaeda monoica*

Phytochemicals	Estimated amount
Phenolic groups	49.1 mg GAE g^{-1}
Tannins	36.6 mg TAE g^{-1}

Table 3: Antimicrobial activity of various extracts of *Suaeda monoica* against various microorganisms

	Zone of inhibition (mm)									
	Petroleum ether extract (mg mL^{-1})			Ethyl acetate extract (mg mL^{-1})			Methanol extract (mg mL^{-1})			
Microorganisms tested	25	50	100	25	50	100	25	50	100	Standard
Gram-negative bacteria										
Escherichia coli	-	-	-	-	-	-	12±0.51	14±0.11	16±0.14	35(A)
Pseudomonas aeruginosa	-	-	25±0.27	12±0.16	13±0.16	19±0.21	-	-	-	32(A)
Proteus vulgaris	11±0.1	12±0.13	13±0.16	-	-	-	11±0.93	13±0.16	14±0.17	34(Cl)
Salmonella typhi	12±0.15	-	-	10±0.07	12±0.11	15±0.11	12±0.23	13±0.15	15±0.17	32(C)
Vibrio parahaemolyticus	-	15±0.14	20±0.17	13±0.12	15±0.19	17±0.14	11±0.64	13±0.13	17±0.15	33(K)
Vibrio vulnificus	-	-	-	10±0.12	13±0.11	17±0.13	11±0.11	13±0.17	16±0.15	36(K)
Gram-positive bacteria										
Bacillus subtilis	-	-	20±0.18	12±0.17	15±0.19	16±0.14	12±0.17	16±0.18	18±0.22	34(A)
Staphylococcus aureus	-	15±0.19	-	14±0.17	16±0.18	18±0.15	10±0.3	13±0.17	18±0.21	35(A)
Streptococcus pneumoniae	-	-	-	15±0.19	16±0.14	18±0.21	11±0.7	16±0.19	21±0.19	32(C)
Fungi										
Aspergillus flavus	-	-	-	16±0.14	18±0.15	20±0.17	12±0.14	13±0.15	14±0.16	33(P)
A. fumigatus	-	-	-	11±0.09	13±0.11	15±0.12	12±0.16	13±0.14	18±0.15	35(P)
A. niger	-	-	-	14±0.12	16±0.14	17±0.16	11±0.09	13±0.13	16±0.13	33(P)
Candida albicans	-	-	-	15±0.11	17±0.13	18±0.14	10±0.08	13±0.11	15±0.12	32(P)

Cl: Clotrimaxazole, A: Ampicillin, C: Ciprofloxacin, K: Kanamycin, P: Pencillin

Quantitative assay: The results of quantitative analysis of phytochemicals in *Suaeda monoica* are given in the Table 2. In quantitative analysis, total phenolic group estimated was recorded as 49.1 mg GAE g^{-1} and total tannins 36.6 mg TAE g^{-1} at 50 mg concentration.

Antimicrobial activity: In the present study, all the three extracts tested against various human pathogens are given in the Table 3 and Fig. 1-3. In the present study, the petroleum ether extract

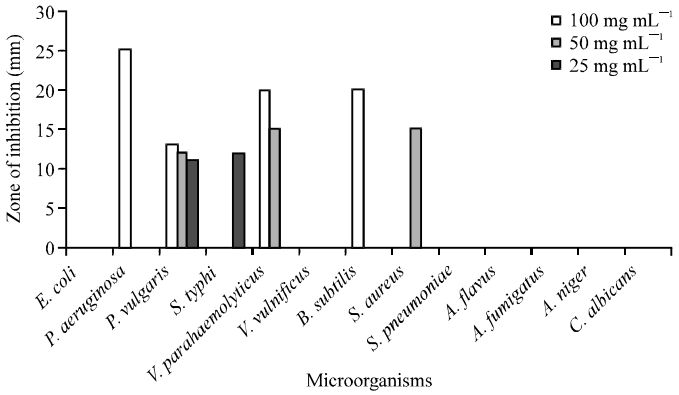

Fig. 1: Antimicrobial activity of petroleum ether extract of *Suaeda monoica* Forssk. ex Gmel against various microorganisms

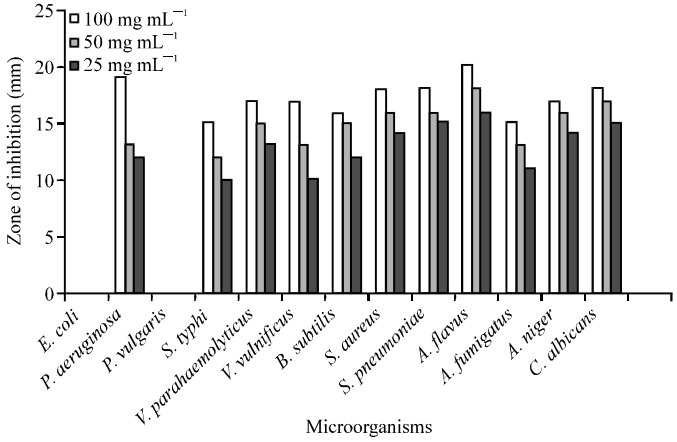

Fig. 2: Antimicrobial activity of ethylacetate extract of *Suaeda monoica* Forssk. ex Gmel against various microorganisms

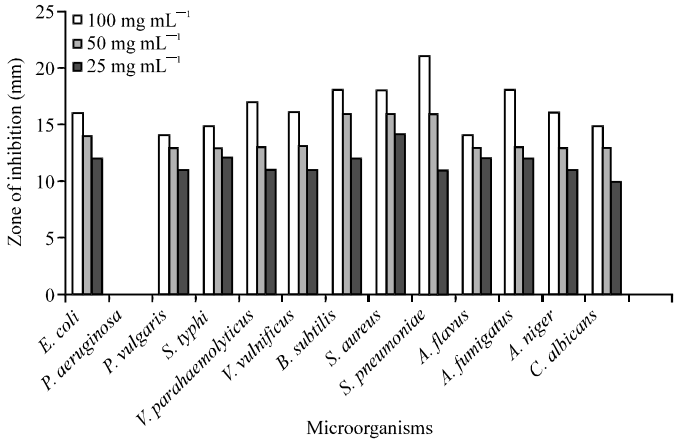

Fig. 3: Antimicrobial activity of methanol extract of *Suaeda monoica* Forssk. ex Gmel against various microorganisms

Table 4: Radical scavenging activity of various extracts of *Suaeda monoica*

Extracts	Concentrations (mg mL^{-1})	Inhibition (%)
DPPH method		
Petroleum ether	50	28.5
Ethyl acetate	50	42.8
Methanol	50	71.4
Fe^{2+} chelating method		
Petroleum ether	50	40.0
Ethyl acetate	50	46.7
Methanol	50	52.0

showed the maximum zone of inhibition as 25 and 20 mm against the gram negative bacteria *P. aeruginosa*, *V. parahaemolyticus* and 20 mm against gram positive bacteria *B. subtilis* at 100 mg mL^{-1} concentration and moderate zone of inhibition showed as 15 mm against gram negative bacteria *V. parahaemolyticus* and 15 mm against gram positive bacteria *S. aureus* at 50 mg mL^{-1} concentration.

At the same time, the minimum zone of inhibition as 13, 12 and 11 mm against gram negative bacteria *P. vulgaris* and *S. typhi* at 100, 50 and 25 mg mL^{-1} concentrations. This extract did not show any activity against the tested gram negative bacteria *E. coli* and *V. vulnificus* and gram positive bacteria *S. pneumoniae*. This extract did not show any activity against the tested such as *A. flavus*, *A. fumigatus*, *A. niger* and *C. albicans*.

Radical scavenging activity: The results of antioxidant activity are given in the Table 4. In DPPH activity, methanol extract showed high percentage of inhibition as 71.4 followed by 42.8 for ethyl acetate extract and 28.5 for petroleum ether extract, respectively. In Fe^{2+} chelating activity, methanol extract showed high percentage of inhibition as 52 followed by ethylacetate as 46.7 and petroleum ether extract as 40. For antioxidant activity, the methanol extract showed maximum percentage of inhibition at 50 mg mL^{-1} concentration and it revealed that the reducing properties are associated with the presence of chemicals in it.

DISCUSSION

Generally salt tolerance plant have more amount of phytochemical specially in phenolic compound. In our result point out phenolics compound pressed in all the extract (methal, petroleum ether ethyl acetate) (Table 1). Previous studies have reported that salt mash plant. Miftakhova *et al.* (1999) reported the phytochemical composition such as amino acid composition and quantitative character of the plant moisture content, carbohydrate and flavonoids of the plant, *Suaeda physophora* belongs to the family Chenopodiaceae..

An *et al.* (2008) isolated 4 compounds such as methyl 3, 5-di-o-caffeoyl quinate, 3, 5-di-o-caffeoyl quinic acid, isorhamnetin 3-o-**β**-D-galactoside and quercetin 3-o-**β**-D-galactoside from methanol extract of *Suaeda glauca* and also studied in vitro hepato protective activity for these isolated compounds. Banerjee *et al.* (2008) reported an antioxidant activity and total phenolics of 23 extracts of some selected mangroves associate plants including *Suaeda maritima*. So, in our result indicated moderated compre to previous studies.

Antimicrobial properties of substances are desirable tools in the control of undesirable microorganisms especially in the treatment of infections diseases. The active components usually interfere with growth and metabolism of microorganisms in a negative manner

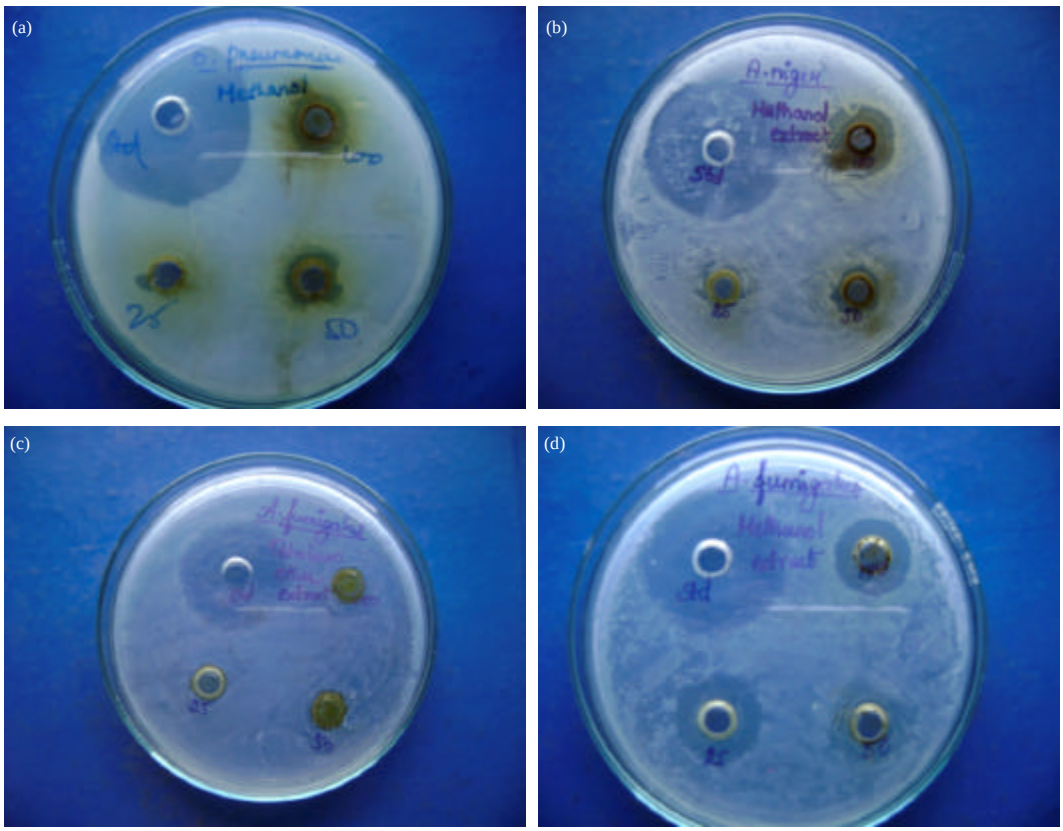

Fig. 4(a-d): Antifungal activity of *Suaeda monoica*, (a) *S. pneumoniae* (methonal) (b) *A. niger*, (methonal) (c) *A. fumigatus* (petroleum ether) and (d) *A. fumigatus* (methonal)

(Aboaba *et al.*, 2006). In the present study, petroleum ether extract showed the maximum zone of inhibition against *P. aeruginosa*, *V. parahaemolyticus* and *B. subtilis* (Fig. 1, 4 and 5), ethyl acetate extract showed the maximum zone of inhibition against *P. aeruginosa S. aureus* and *S. pneumoniae* and against the fungi *A. flavus*, *C. albicans*, respectively (Fig. 2, 4 and 5) and methanol extract showed the maximum zone of inhibition *B. subtilis, S. aureus, S. pneumoniae* and against fungi *A. fumigatus*, respectively. (Fig. 3-5). This may be due to the presence of phenolic groups. Steroids and triterpenes are present both in ethyl acetate and methanol extracts but absent in petroleum ether extract. In our study, the maximum zone of inhibition against *B. subtilis*, *S. aureus* may be due to the presence of secondary metabolites such as phenolic groups as suggested by previous reports by Pereira *et al.* (2007). This result mention that moderate compare to (Chandrasekaran *et al.*, 2009). The significant activity of the results against the fungi, *Candida albicans* provides additional confirmation to the phenolic compounds which are more effective in higher concentration inhibited the growth of all fungi (Winkelhausen *et al.*, 2005; Pereira *et al.*, 2007). Antimicrobial activity of salt marsh and coastal medicinal plants against human pathogenic microorganisms was reported by Ramanathan (2000) and Sithragaboopathy (2003). For marine drug discovery, antimicrobial and phytochemical investigation of medicinal mangroves and other coastal flora reported by Latha (2005).

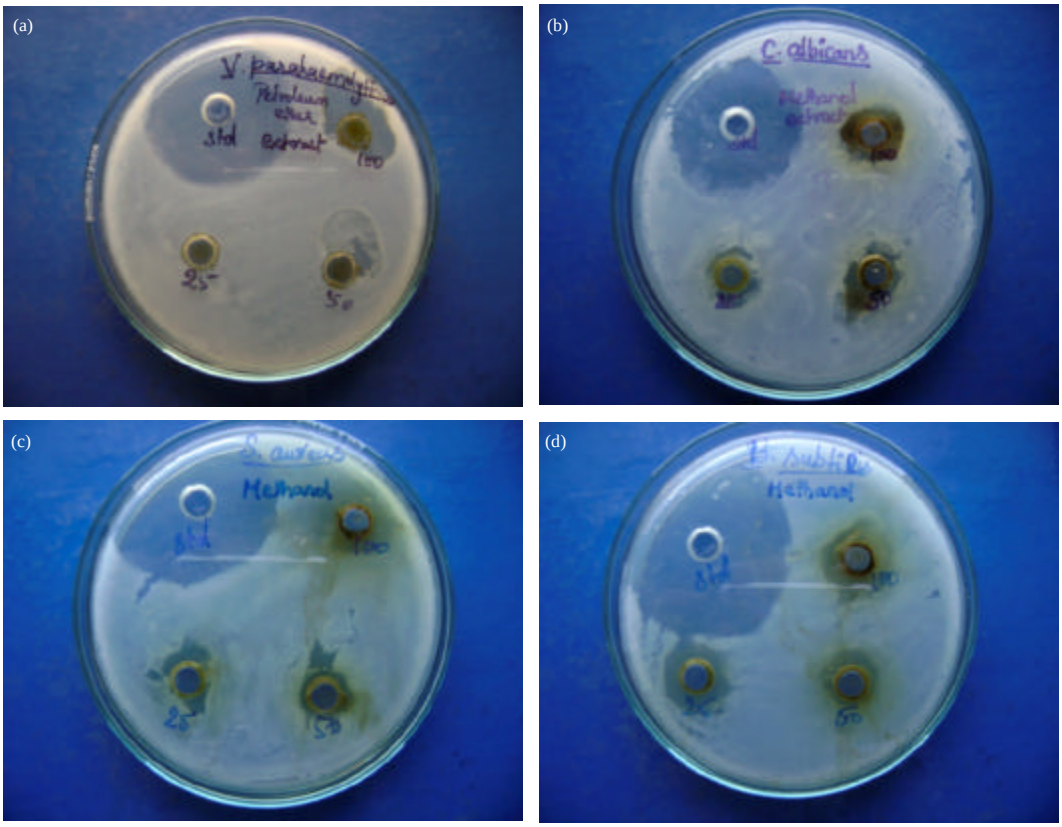

Fig. 5(a-d): Antibacterial activity of *Suaeda monoica*, (a) *V. parahaemolyticus* (petrolium ether), (b) *C. albicans* (methonal), (c) *S. aureus* (methonal) and (d) *B. subtilis* (methonal)

Even in hospitals, majority of disinfectants such as phenols, lysol, cresols used are belongs to phenolic groups. The present findings of antimicrobial activity against the fungi *A. flavus*, *A. fumigatus*, *A. niger* and *C. niger* revealed its medicinal potential as fungicides to develop new leads against fungal infections. Thus from our findings, it was concluded that the active principles responsible for an antimicrobial activity against these tested microorganisms should be isolated and identified to develop a new lead of therapeutic interest.

The antioxidant activity of different extracts may depend on the presence of polyphenols as reported by Jayaprakasha *et al.* (2003) and Ozkan *et al.* (2005), respectively. Thus the plants *Suaeda monoica* possess the property of antimicrobial and antioxidant property to develop a new of therapeutic interest. This result suggested that many previous study (Rhee *et al.*, 2009; Thirunavukkarasu *et al.*, 2011). In our previous study DPPH radical scavenging activity of *Suaeda monoica* extract was higher than *Sesuvium portulcastrum* and among the other coastal medicinal plants (Thirunavukkarasu *et al.*, 2010), so in our result remain indicate that suggestion. We recommended that it would be a good antioxidant drug. From our findings, it was concluded that the bioactive principles responsible for the antimicrobial activity and antioxidant activity should be identified, isolated and elucidate its structure for new drug development in pharmaceutical industry.

CONCLUSION

The present investigation is only a beginning in the direction of search for eco-system natural antimicrobial in our coastal environment future work needs to include standardization of active fractions, identification of the active compounds and extensive field testing especially challenge through different routes for finding out the effective administrative route and commercialization of the antimicrobials. Further work is needed to identify the compounds which are responsible for antimicrobials and radical scavenging activities. The information summarized about the *Sueada monoica* intended to serve as reference tool to researcher's in all field of ethno pharmacology and natural product chemistry.

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