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Phytochemical investigation and antibacterial activity of salt marsh plant extracts

Sathish P, Jaswanth G, Gurudhathan K.B, Gopinath J, Gayathri P.K, Yuvaraj D*

[1]Department of Biotechnology, Vel Tech High Tech Dr. Rangarajan, Dr. Sakunthala Engineering College, Avadi, Chennai-600062

***Corresponding author: E-Mail: yuvarajdinakarkumar@gmail.com**

ABSTRACT

The need for the search of new phytoconstituents from unexplored salt marsh plants. Phytochemical analysis of methanolic extracts of salt marsh plants viz., *Suaeda maritima, Salicornia brachiata and Sesuvium portulacastrum* were investigated and the presence of phenols, tannins, flavonoids, alkaloids, steroids and saponins were observed. Antibacterial activity of methanolic extracts of three salt marsh plants were tested against three human pathogenic bacterial strains (*Pseudomonas aeruginosa, Bacillus cereus* and *Escherichia coli*) by disc diffusion method. *Suaeda maritima* extract exhibited maximum zone of inhibition against *E.coli* followed by *Salicornia brachiata. Salicornia brachiata* extract showed maximum zone of inhibition against *Bacillus cereus* (7.2 mm) whereas methanolic *Suaeda maritima* extract showed maximum zone of inhibition against *Pseudomonas aeruginosa*. *Sesuvium portulacastrum* did not show any activity against the three human pathogens.

KEY WORDS: *Suaeda maritima, Salicornia brachiata, Sesuvium portulacastrum*, Antibacterial activity, tannins, saponins.

1. INTRODUCTION

For a long period of time in history, plants have been valuable and indispensable sources of natural products for the health of human beings and they have a great potential for producing new drugs (Nascimento, 2008; Littleton, 2005). Even today people who live near to the forests use plant products to cure chronic diseases. Tropical and sub-tropical areas of the world are bestowed with abundant flora and herbs which have untapped properties, such as antimicrobial, antiviral and antifungal. According to the World Health Organization, plants are a source of compounds that have the ability to combat disease, antimicrobial, antiviral and antifungal activities (Gazim, 2008). In addition, medicinal plants have been used for centuries as remedies for human ailments and diseases because they contain components of therapeutic value (Panda, 2009). Also they are less toxic to humans and environmentally friendly due to fewer pollutants produced in production and have minimal health hazards (Opra and Wokocha, 2008).

However literature related to the ethno-medicinal importance of salt marsh plants are scarce, knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances, etc. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Farnsworth, 1966). In the present work, qualitative phytochemical analysis and anti-bacterial studies was carried out in few salt marsh plants collected from Pulicat lake zone, Tamil Nadu, India.

2. MATERIALS AND METHODS

2.1. Collection and preparation of plant material: Healthy, disease free plants of *Suaeda maritima, Salicornia brachiata* and *Sesuvium portulacastrum* were collected from Pulicat, Ennore, Chennai, Tamilnadu. The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. Plants were cutted into small pieces using knife.

2.2. Plant extraction: Extract was prepared by weighing 20 g of *Suaeda maritima, Salicornia brachiata* and *Sesuvium portulacastrum* in soxhlet apparatus with methanol (200 ml) for 8 h at temperature closer to the boiling point. The extracts were concentrated to dryness in rotary pressure evaporator and stored at room temperature for further study.

2.3. Preliminary phytochemical screening: The preliminary phytochemical screening was performed by standard method (Gibbs, 1974; Harborne, 1973).

2.3.1. Test for phenols: a) Phenol test: To the extract, 0.5 mL of ferric chloride solution was added; formation of intense blue green color indicates the presence of phenolics.

2.3.2. Test for tannins: a) Ferric chloride test: 1 mL of 5% ferric chloride solution was added to the extracts. The formation of greenish black reveals the presence of tannins.

2.3.3. Test for flavonoids: a) Flavonoid test: Few magnesium turnings were added to the plant extract and conc. sulphuric acid was dropped through the sides of the test tubes. The formation of magenta color indicates flavonoids, scarlet color indicates flavones and deep cherry color indicates flavonoids.

b) Ferric chloride test: The extracts were treated with neutral ferric chloride solution; formation of blackish green color indicates the presence of flavonoid.

2.3.4. Tests for alkaloids: a) Dragendroff's reagent test: 2 mL of Dragendorff's reagent and 1 mL of dilute hydrochloric acid were added to the extract, orange precipitation indicates the presence of alkaloids.
2.3.5. Tests for steroids: a) Salkowski test: the formation of wine red color when concentrated sulphuric acid was added to the extracts indicates the presence of steroids.
b) Liebermann – Burchard's test: To the extract, few drops of acetic anhydride was added and mixed well. 1 mL of conc. sulphuric acid was from the sides of the test tube. The formation of red ring at the junction of the two layers indicates the presence of steroids.
2.3.6. Tests for saponins: a) Foam test: The extract was shaken vigorously with water. The formation of honeycomb like foam indicates the presence of saponins.
2.4. Disc diffusion assay: The disc diffusion method (Bauer, 1966) was employed for the determination of anti-microbial activity. The nutrient agar plates were prepared and 0.1 ml of inoculum (*Pseudomonas aeruginosa, Bacillus cereus* and *Escherichia coli*) was uniformly spread onto the plates by means of sterile swabs. Plates were allowed to stand for 15 minutes. 6 mm diameter sterile discs were soaked with the extracts and the discs were symmetrically placed onto the medium by means of sterile tweezers. Disc soaked with tetracycline was used as a positive control. The plates were incubated for 24±2h at 37ºC under anaerobic conditions. The results were evaluated by measuring the areas around the disc with no bacterial growth. The experiments were made in triplicates. The results were obtained using the following formula:
Inhibition value = Inhibition diameter (mm) – (Disk diameter (6mm) / 2)

3. RESULTS AND DISCUSSIONS

3.1. Phytochemical investigation: The present investigation on the methanolic extracts of salt marsh plants *Suaeda maritima, Salicornia brachiata* and *Sesuvium portulacastrum* revealed the presence of medicinally active constituents. The phytochemical constituents are phenols, tannins, flavonoids, alkaloids, steroids and saponins which are summarized in Table.1. *Suaeda maritima* has many phytochemical constituents when compared with other two plant extracts. Each phytochemical constituent has unique medicinal properties that can be tapped for future research. The presence of phenols, flavanoids, alkaloids indicate the anti-inflammatory, anti-oxidant, anti-proliferative (Rocha et al, 2014) of the extracts (Cushnie and Lamb, 2005). The presence of tannins, saponins and steriods indicates the wound healing, immunity booster and anti-inflammatory characterisitics respectively and the presence of all the three compounds may also contribute towards their anti-microbial properties.
3.2. Antibacterial activity: Antibacterial activity of methanol extracts of three salt marsh plants *Suaeda maritima, Salicornia brachiata, Sesuvium portulacastrum* were tested against three human pathogenic bacterial strains (*Pseudomonas aeruginosa, Bacillus cereus* and *Escherichia coli*) by muller hinton agar disc diffusion method. *Suaeda maritima* extract exhibited maximum zone of inhibition against *E.coli* (9±0.6 mm) followed by Pseudomonas *aeruginosa* (7.8±0.45 mm) and showed no activity against Bacillus *cereus*. *Salicornia brachiata* of extract showed maximum zone of inhibition against *Bacillus cereus* (6 ±0.75 mm) followed by *E.coli* (5.2±0.55 mm) and showed no activity against *Pseudomonas aeruginosa*. Whereas, the extract of *Sesuvium portulacastrum* was resisted completely by all the three bacterial pathogens. (Table. 2).

The presence of tannins and saponins in the phytochemical analysis of *Suaeda maritima* and *Salicornia brachiata* may contribute to the anti-bacterial properties. The antiseptic properties of tannins may be attributed to the phenolic groups present. (Felix, 2014) and the saponins were reported to possess anti-microbial and anti-biotic properties (Oakenfull and Fenwick, 1981)

Table.1. Preliminary phytochemical investigation (+ indicates presence, - indicates absence)

TEST	*Suaeda maritima*	*Salicornia brachiata*	*Sesuvium portulacastrum*
Phenolics: a. Phenol test	+	+	+
Tannins; a. Tannin test	+	-	-
b. Sodium chloride test	+	-	-
Flavonoids: a.Flavonoid test	+	+	+
b. Shinoda test	+	+	+
Alkaloids; a. Dragendroff's reagent test	+	+	+
b. Mayer's reagent test	+	+	+
Steroids			
a. Liebermann-Burchard's test	-	-	+
b. Salkowski test	-	-	+
Saponins; a. Foam test	+	+	-

Table.2. Anti-bacterial activity of *Suaeda maritima*

Microbial cultures	Solvent extracts (Zone of inhibition in mm)			
	Suaeda maritima	***Salichornia brachiata***	***Sesuvium portulacastrum***	**Tetracycline**
E.coli	9 ± 0.6	5.2 ± 0.55	R	20±2.2
Bacillus cereus	R	6 ± 0.75	R	21±1.7
Pseudomonas aeruginosa	7.8 ± 0.45	R	R	11.4±0.88

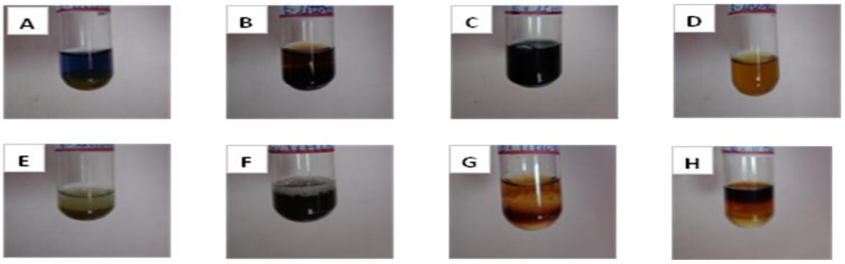

Figure.1.Phytochemical analysis of methanolic extract of three marsh plants. A – Blue/green indicates presence of phenol, B,C – Red and blackish green indicates presence of flavonoids, D – Orange color indicates presence of alkaloids, E – Greenish black color formation indicates presence of tannins, F – Formation of foam indicates presence of saponin, G, H – Wine red and red ring formation indicates presence of steroids

4. CONCLUSION

Phytochemical investigation were carried out in three salt marsh plants viz., *Suaeda maritima, Salicornia brachiata and Sesuvium portulacastrum* in which *Suaeda maritima* has many phytochemical constituents when compared with other two plant extracts. Methanolic extracts of three salt marsh plants were studied for the antibacterial activity against three human pathogenic bacterial strains in which *Suaeda maritima* showed maximum zone of inhibition when compared with other plants. These results indicate the potential of these plant extracts in the pharmaceutical industry. Further research can be helpful in identifying many bioactive compounds which would prove to be efficient and cost effective alternative source of potent drugs.

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