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Phytochemical Screening And Anti-Bacterial Studies In Salt Marsh Plant Extracts (*Spinifex littoreus* (BURM.F) MERR. and *Heliotropium curassavicum* L.)

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Abstract: Phytochemical screening of salt marsh plants *Spinifex littoreus* (Burm.f) Merr. and *Heliotropium curassavicum* L. were investigated for the presence of bioactive compounds. The phytochemical screening was conducted using methanol, chloroform and aqueous extract of these plants. The study for antibacterial activity of plants *Spinifex littoreus* (Burm.f) Merr. and *Heliotropium curassavicum* L. was conducted against Gram Negative (*Escherichia coli, Pseudomonas aueroginosa and Acetobacter motfi*) and Gram positive bacteria (*Enterococcus hirae and Bacillus cereus)* by disc diffusion method. The presence of alkaloids and flavonoids were confirmed in *Heliotropium curassavicum* L. The extracts showed significant antibacterial activity.
Key words: Salt marsh, *Spinifex littoreus*, *Heliotropium curassavicum.*

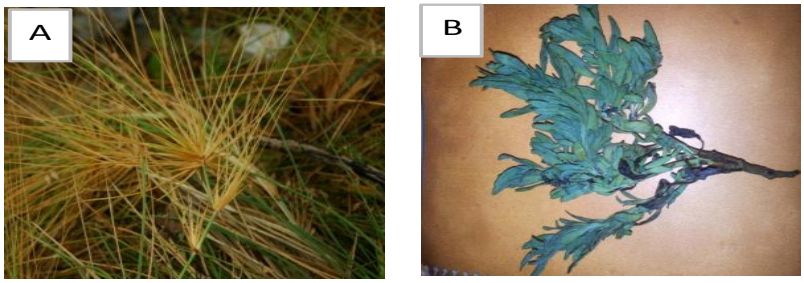
Introduction

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases [1]. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. These phytochemicals are divided into (1) primary metabolites such as sugars and fats, which are found in all plants; and (2) secondary metabolites –compounds which are found in a smaller range of plants, serving a more specific function. Pharmacognosy is the branch of modern medicine about medicines from plant sources. Plants included here are those that have been or are being used medicinally; in at least one such medicinal tradition. Modern medicine now tends to use the active ingredients of plants rather than the whole plants. The phytochemicals may be synthesized, compounded or otherwise transformed to make pharmaceuticals [2].

Marine plants and animals are reported to possess a wide spectrum of bioactive compounds which are attracting pharmaceutical industry to innovate new biologically active novel compounds [3]. Halophytes are adapted to tolerate harsh saline and arid conditions by synthesizing a number of active chemicals to maximize their fitness, many of which are a source of potent medicine against a number of chronic ailments. Coastal environments were reported to be more stressful than inland due to higher soil salinity, greater light intensity and more frequent diurnal and seasonal climatic conditions [4]. Numerous reports have documented the traditional uses of medicinal plants in rural and tribal areas all over the world as a successful home remedy

against different ailments[5]. For the present study, two plants have been selected are *Spinifex littoreus* (Burm.f) Merr.and *Heliotropium curassavicum*L., which are found in seashores and marshy areas (Figure- 1). *Spinifex littoreus* (Burm.f) Merr is one of the coastal plants that grow in a costal dunes, *Spinifex littoreus*, popularly called as Ravan's mustache or Beach Spinifex, is a perennial grass with incredible stolon forming stems. The culms (hollow jointed stem of a grass or sedge) are about 40-80 cms long, hard and stout with the presence of many nodes. Root is used in join muscle pain [6]. *Heliotropium curassavicum* L. is a sand binder salt marsh and a perennial herb which can take from a prostrate creeper along the ground to a somewhat erect shrub approaching 0.5m (1.6ft) in height and contains smooth nutlet fruits found in Southeast Asia, America and Europe. *Heliotropium curassavicum* had been traditionally used for ulcers, wounds; local inflammations cure gonorrhea, erysipelas, enema constipation, edema, bacterial infections cancer and diabetes [7]. Hence, aim of the work is phytochemical screening, antibacterial activity, Antioxidant activity and GC-MS analysis of crude extraction of selected salt marsh plants.

Figure 1: (A) *Spinifex littoreus* (B) *Heliotropium curassavicum*



Materials and Methods

Materials Required

Methanol, Chloroform and Distilled water, soxhlet apparatus (Franz von Soxhlet), Whattman filter paper, Dragondroff's reagent**,** iodine/potassium iodide, sodium hydroxide, hydrochloric acid, ferric chloride, sulfuric acid, *Enterococcus hirae* (MTCC No.10507), *Pseudomonas aurginosa* (MTCC No. 424), *Acetobacter mofti, Escherichia coli* (MTCC No.40) *and Bacillus cereus*, agar.

Collection and Extraction of Plants

The whole plants were collected from the seashores and Salt marsh areas of Covelong. The plants were washed in running tap water and cut into pieces; shade dried and powdered into fine particles. 20 g of the dried plant powder was extracted using 200 ml solvents i.e. Methanol, Chloroform and Distilled water respectively in soxhlet (Franz von Soxhlet) apparatus. The cycles were continued till the plant gets completely decolorized. The extracts were collected and evaporated for 20 mins. The concentrated extract was filtered using Whattman filter paper and stored in the refrigerator for further use.

Phytochemical Analysis

The presence of phytoconstituents in the extract was determined by the methods discussed earlier [2,8]. The brief procedure is given below:

Test for Alkaloids (Dragondroff's reagent)

A fraction of extract was treated with 3-5drops of Dragondroff's reagent and observed for the formation of reddish brown precipitate (or colouration).

Test for Carbohydrates (Iodine test)

2 ml of a sample solution is placed in a test tube. Two drops of iodine solution and one ml of water are added. The appearance of blue-black complex indicates the presence of carbohydrates.

Test for Flavonoids (Alkaline reagent test)

2 ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for Phenols (Ferric chloride test)

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

Test for Phlobatannins (Precipitate test)

Deposition of a red precipitate when 2 ml of extract was boiled with 1ml of 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for Amino acids and Proteins (1% ninhydrin solution in acetone)

2 ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

Test for Saponins (Foam test)

To 2 ml of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for Sterols (Liebermann-Burchard test)

1 ml of extract was treated with drops of chloroform, acetic anhydride and conc. H_2SO_4 and observed for the formation of dark pink or red colour.

Test for Tannins (Braymer's test)

2 ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Test for Terpenoids (Salkowki's test)

1 ml of chloroform was added to 2 ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

Test for Quinones

A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration).

Antibacterial Activity

The antibacterial assay was done by agar disc diffusion method [6]. The activities of the extracts were tested against five bacterial species such as *Enterococcus hirae, Pseudomonas aeroginosa, Acetobacter mofti, Escherichia coli* and *Bacillus cereus.*

Results and Discussions

Phytochemical Analysis

The plant extracts were analysed for the presence of phytochemicals as described earlier. The results of the analysis are shown in (Table- 1). The presence of tannins, protein, phenol, carbohydrates, saponins and terpenoids were confirmed in the aqueous extract of both *Spinifex littoreus* (Burm.f) Merr and *Heliotropium curassavicum* L. It has been proved that plant poly phenols such as phenols, terpenoids, flavonoids as a potential are antioxidants under invitro studies [9]. Tannins are also reported to have various physiological effects like anti-irritant, antiphlogistic, antimicrobial and ant parasitic effects [10]. Hence the presence of tannins, flavaoids and phenols in the methanolic extract of both *Spinifex littoreus* (Burm.f) Merr and *Heliotropium curassavicum* L shall drive the future research of this study into determining the antioxidant, anti-irritant and

antiphlogistic study. Proteins and carbohydrates are very good source of energy. So these species shall be used as feed supplement to under nutritioned patients when formulated appropriately.

Table 1: Phytochemical analysis of *Spinifex litoreus* (Brum F) Merr and *Heliotropium currasavicum L.*

Phytochemicals	Methanol		Chloroform		Aqueous	
	S. littoreus	H. curassavicum	S. littoreus	H. curassavicum	S. littoreus	H. curassa Vicum
Alkaloids	+	+	+	+	_	_
Carbohydrates	+	+	_	_	+	+
Flavonoids	++	++	+	+	+	+
Phenols	+	+	_	_	+	+
Phlobatannins	_	_	_	_	_	_
Proteins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Sterols	_	_	_	_	_	_
Tannins	_	_	_	_	++	++
Terpenoids	_	_	_	_	_	_
Quinones	_	_	_	_	_	_

Antibacterial Activity

Antibacterial activity of various extracts of *Spinifex littoreus* (Burm.f) Merr. and *Heliotropium curassavicum* L. was assessed using disc diffusion method which is shown in (Figure- 2 & 3) The disc was dipped in the various extract of the plants and placed on the petri dish. Ampicillin is used as positive control. The zone was measured after 24 h of incubation at 37ºC. In both the cases methanolic extracts showed antibacterial activity against all the five species. Further research on the cytotoxic activity and their respective active ingredient shall be done to isolate any unique bioactive compound from the methanolic extract.

The possible compound responsible for the antibacterial activity is the presence of tannins. Tannins have been reported to prevent the development of microorganisms by precipitating microbial proteins. The growth of many fungi, yeasts and virus have been inhibited by tannins. In ancient medicines, these compounds were used to treat diarrhea, inflammation and skin injury [11].

Figure 2: Antibacterial activity of *Heliotropium curassavicum* against gram positive and gram negative organisms

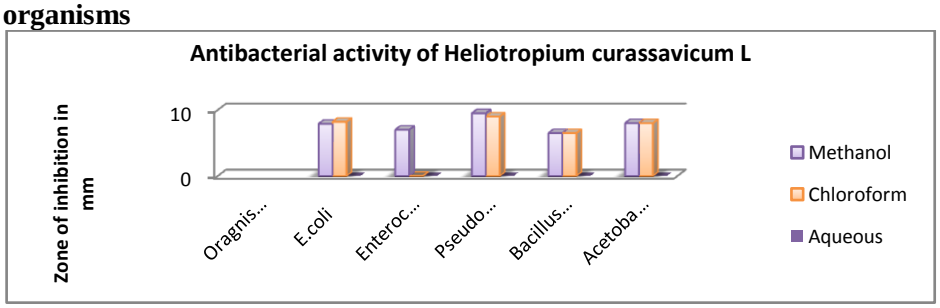
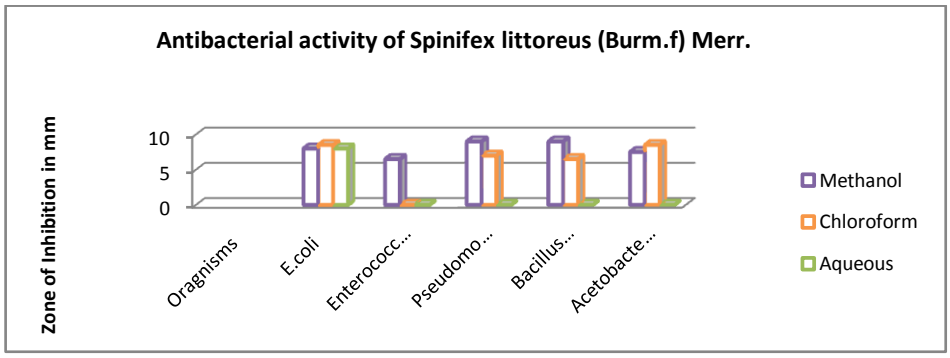


Figure 3: Antibacterial activity of *Spinifex littoreus* against gram positive and gram negative organisms



Conclusion

The selected two coastal plants were screened for novel bioactive phytochemicals. Antibacterial activity of the selected coastal plants was performed successfully. Phytochemical analysis of methanolic extracts of *Spinifex littoreus* (Burm.f) Merr indicates the presence of tannins, flavonoids, terpenoids, proteins, phenol, and Carbohydrates. The phytochemical analysis of both methanolic and aqueous extract *Heliotropium curassavicum* (Table- 1) indicates the presence of phytochemicals like alkaloids, carbohydrates, phenols, proteins, saponins. Methanolic extract of both the plants (*Spinifex littoreus* and *Heliotropium curassavicum)* showed antibacterial activity against *Pseudomonas aeruginosa.*

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