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Antibacterial Activities of *Eriocaulon quinquangulare* L.

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Abstract

The use of plant and plant parts for the treatment of diseases and disorders is oldest practices by human beings. Still in the modern lifestyle, the rural and the tribal community depends on local plants for their primary health care. Among the plant diversity, less documentation has observed on medicinal plants of wetland. Keeping this in view, *Eriocaulon quinquangulare* was selected for antibacterial activities against selected pathogenic bacteria. The plant parts were collected from Khurda to Puri districts of Odisha state. The collected plant parts were subjected for plant extraction followed by phytochemical screening and antimicrobial activities. The results revealed that the experimental plant rich with diverse phytochemicals observed sound antimicrobial activities. The present work highlights the importance of wetland and unexplored floral wealth.

Keywords: Unexplored, Wetland, Anti-Microbial Resistance

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Introduction

Wetland plants are defined as those species normally found growing in wetlands of all kinds, either in or on the water, or where soils are flooded or saturated long enough for anaerobic conditions to develop in the root zone.[1-5] Wetland plants are often the most conspicuous component of wetland ecosystems. They are also referred to as hydrophytes, macrophytes, and aquatic plants.[5-12] Wetland plants are, with a few exceptions, angiosperms, or flowering plants. Of the known 250,000 angiosperm species, only about 3–5% are adapted to the wetland environment. Some ferns and fern allies (*Pteridophyta*), such as floating water fern (*Ceratopteris pteridoides*), and some gymnosperms, such as bald cypress (*Taxodium distichum*), tamarack (*Larix laricina*), and the south Florida slash pine (*Pinus elliotti*), do grow in wetlands.[13] Peat lands are inhabited by a number of moss species (*Bryophyta*), notably of the genus, Sphagnum. Most of our discussion here refers to wetland angiosperms. Although wetland plants are defined by their ability to inhabit wet places, they represent a diverse assemblage of species with different adaptations, ecological tolerances, and life history strategies.[14-20] Wetland plants play a number of vital roles in wetland ecosystems, which include[1] wetland plants are at the base of the food chain and, as such, are a major conduit for energy flow in the system. The primary productivity of wetland plant communities varies, but some herbaceous wetlands have extremely high levels of productivity, rivaling those of tropical rain forests.[2] Wetland plants provide critical habitat for other taxonomic groups, such as bacteria, epiphyton (algae that grow on the surface of plants), macroinvertebrates, fish, and birds. The composition of the plant community influences the overall diversity of the wetland community,[3] Wetland plants strongly influence water chemistry, acting as both nutrient sinks through uptake, and as nutrient pumps, moving compounds from the sediment to the water column. They improve water quality by taking up nutrients, metals, and other contaminants. Wetland plants influence the hydrology and sediments of wetlands by stabilizing shorelines, modifying currents, and abating the effects of flooding. Vegetation controls hydrologic conditions in many ways including peat accumulation, water shading (which affects water temperatures), and transpiration. Wetland plants are also among the tools used by wetland managers and researchers in the conservation and management of wetland areas, for example, (a) wetland plants are routinely used to help identify or delineate jurisdictional boundaries of wetlands in the United States and elsewhere. (b) The composition of the plant community can act as a biological indicator of the "health" of a wetland.

Wetland plants have a number of different growth types or morphologies. They grow as emergent, submergent, floating-leaved, or floating species.[2] Emergent plants are rooted in the soil with basal portions typically beneath the water surface, and with aerial leaves, stems, and flowers emerging above the water surface. Most of the plants in this group are herbaceous, but woody species can be included here (Bedford *et al*., 2001). Among all the types of wetland plants, emergent is perhaps the most similar to terrestrial species, relying on aerial reproduction and on the soil as their exclusive source of nutrients. Emergent herbaceous plants often inhabit shallow waters in marshes, along lakeshores or tidal creeks, and because of their ability to intercept sunlight before it reaches the water's surface.

With the exception of flowering, submergent plants typically spend their entire life cycle beneath the surface of the water and are distributed in coastal, estuarine, and freshwater habitats. Nearly, all are rooted in the substrate, although there are several rootless species. Stems and leaves of submergent species tend to be soft, with leaves that are either elongated and ribbon-like, or highly divided, making them flexible enough to withstand water movement without damage. Rooted submerged species acquire the majority of their nutrients from the sediments, although

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some nutrients may be absorbed from the water column.[21,22] Floating-leaved plants: The leaves of floating-leaved species float on the water's surface while their roots are anchored in the substrate. In most cases, flexible stems connect the leaves to the soil. The stomata, openings through which gas exchange occurs, are located on the aerial side of the leaf. Floating-leaved species shade the water column below and are often able to out-compete submergent species.[23-26] The flowers of these species either float, as in the water lilies (*Nymphaeaceae*), or are borne above the water surface on emergent peduncles (flower stalks), as seen in the water lotus (*Nelumbonaceae*).[27-30] Floating plants: The leaves and stems of floating plants (also known as floating unattached) float on the water's surface. If roots are present, they hang free in the water and are not anchored in sediments. Floating plants move on the water surface with winds and water currents. A widespread family of free-floating plants is the duckweeds (*Lemnaceae*). Some floating plants are larger species, such as water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*). Both have extensive branching roots that hang down into the water column.[31] The roots absorb nutrients and provide a weight that helps stabilize the plant on the water. These growth types are found in marshes and shallow water wetlands, such as ponds and prairie potholes, peat lands, and floodplains in a typical zonation pattern (Weis and Weis, 2004). Woody emergents are generally found out of standing water, except during periodic flooding. Herbaceous emergents are found both out of standing water and in shallow water areas.

An estimated 60% of wetland species have extensive geographical distributions that range over more than 1 continent, leading them to be classified as cosmopolitan. The widespread distribution of these plants indicates a well-developed facility for long distance dispersal of seeds and vegetative parts over inhospitable territory such as land and sea. Mechanisms of dispersal include wind and water transport, movement by migratory birds, and, increasingly, transport by humans. Wetland plants tend to cover a wide latitudinal gradient relative to land plants, even those that are not considered to be cosmopolitan. The distribution of many species tends to follow predictable patterns, with geographic ranges focused across large regions such as Eurasia, North Africa, continental Africa, or the tropical and subtropical latitudes of the Americas.[32] Some species inhabit the temperate latitudes of both North and South America, but not necessarily in between.

Keeping the importance, the present study has designed to study the unexplored medicinal plants from wetland through phytochemical screening and antibacterial activities. The present work highlights the importance of wetland flora.

Review of Literature

All human societies use plants as medicines. Many important medicines are derived from plants with a long history of traditional use, and new medicinal applications for plants as medicines continue to be discovered.[33] Plants with known medicinal uses and properties occur in most habitats to which people have access. Most medicinal plants are wild harvested rather than cultivated.[34-36] In animals, fungi, bacteria, and algae associated with wetlands are proving to be successful targets for discovery of new natural products with medicinal properties, some flowering plant taxa typical of wetlands have been valued as sources of medicine since ancient times.[37] These include the sundews (*Drosera*/*Droseraceae*), lotus (*Nelumbo*/*Nelumbonaceae*), reeds and grasses (*Phragmites*/*Poaceae*), and bulrushes (*Typha*/*Typhaceae*). Contribution of Wetlands to Plant-Sourced Medicines: A comprehensive global survey of the distribution of medicinally used plants in various habitats does not yet exist, without which it is not possible to evaluate the relative importance of wetlands as a source of medicinal plants. Therefore, most descriptions of medicinal plants in wetlands rely on well-known taxa.[37] However, local and regional surveys of biodiversity that includes socioeconomically important plants can provide insights into the importance of wetlands as sources of medicinal plants, their contribution to health and livelihood benefits, and their conservation challenges. For example, a survey of freshwater biodiversity in North Africa included 518 plant species, of which 43% have known social and economic value.[16] Among the documented uses (structural material, paper, other chemicals, household, fuel, aromatic, horticulture, aquarium, handicrafts, animal feed, ornamental, food, and medicinal), the largest number of species have medicinal use. The sedge (*Cyperaceae*), mint (*Lamiaceae*), knotweed (*Polygonaceae*), and daisy (*Asteraceae*) families provided the largest numbers of medicinal plants from the wetland habitats included in the survey. These are large families with cosmopolitan distributions, although the strong representation of species in the mint family may reflect its regional center of diversity in the Mediterranean-Central Asia.[38] The representation of plant taxa in the medicinal flora of freshwater habitats likely varies with the taxonomic composition of the regional flora. For example, in the Apo Kayan communities at the headwaters of the Kayan River, East Kalimantan (Indonesian Borneo), the ginger family (*Zingiberaceae*) provides the largest number of medicinal plant species associated with freshwater habitats, followed by the grass (*Poaceae*), aroid (*Araceae*), and mint (*Lamiaceae*) families,[19] reflecting the relatively large representation of these families in the Southeast Asian/Malesian flora.[38] High numbers of plant species with medicinal use are common in developing countries, where traditional systems of medicine are more accessible than modern alternative treatments. However, the use of herbal remedies is increasing worldwide, as governments and health practitioners accept and integrate traditional medicine and other complementary and alternative practices,[39] based on a growing scientific capacity to evaluate the medicinal properties, safety, and efficacy of traditional and new applications.[4] The economic value of trade in wetland plant species is not currently possible to assess as a distinct component of the global sales of herbal products, which is itself difficult to evaluate even on a national scale, since the substantial value of local use and trade is not captured in most market data. In China alone, the market value of Chinese Materia Medica was estimated to be US$83.1 billion in 2013.[39] The great majority of freshwater medicinal plants in the North Africa region is collected from the wild,[16] reflecting the global situation for wild sourcing of medicinal plants.[34] In North Africa, 20% of the wetland plant species identified as economically important are threatened with extinction. The main threat is habitat loss and degradation (affecting 95% of species), although non-sustainable level of harvest is identified as a major threat to some species.[16] Future challenges so little are known about medicinal plants in wetlands that there is clearly a need for better documentation of their presence and value in this habitat.

Methodology

Selection, Identification, and Enumeration of Selected Experimental Plants from Study Areas

The experimental plant (*Eriocaulon quinquangulare*) was collected as per availability in the coastal areas of Odisha. The selection

was also based on research objectives. The experimental plant species of this region were identified following Flora's book and published articles by Dr. Sanjeet Kumar. The selected plant species was characterized using morphological characteristics followed by Flora's book and published articles.

Collection of Experimental Plant Species

The plant sample was collected [Figure 1] and kept in ploy bags tagged with botanical name and sorted out as per standard sampling process. Samplings were preserved as voucher specimens (APRFH-19 Dt. December 27, 2019) and were deposited in the herbarium Deposition unit, Biodiversity and Conservation Lab., Ambika Prasad Research Foundation, Bhubaneswar. These experimental plant species were properly washed and dried for experimental work.

Percolation

The powder of whole plant part of *Eriocaulon quinquangulare* was measured. 2.5 of plant powder was taken in four beakers and four solvents were added in the quantity of 50 ml in each beaker in the order of their polarity, namely, n-hexane, acetone, methanol and distilled water. Hence, four extracts were prepared [Figure 2]. The mouths of the beaker were covered with aluminum foil and were preserved in refrigerator for 48 h. After 48 h, the extracts were filtered into fresh beakers with filter paper, then the phytochemical analysis was conducted. Phytochemical assays were carried out on different extracts of whole plant parts using standard procedure to identify the secondary metabolites. Nine phytochemical tests were conducted to identify the secondary metabolites of plant extract for each solvent. The tests were conducted as follows [Figure 3]:

Test of Tannin

Five milliliters of plant extract were added with five drops of 10% lead acetate. The acetone, methanol, and aqueous extracts formed a light yellow precipitate, which indicates the presence of tannin. N-hexane extract showed no results.

Test of Saponin

One milliliter of the extract was boiled in 10 ml of distilled water and filtered with filter paper. Five milliliters of the filtrate were mixed with 2 ml of normal distilled water and shaken vigorously. The stable persistent froth in aqueous extract indicated the presence of saponin. No results were observed in other solvents.

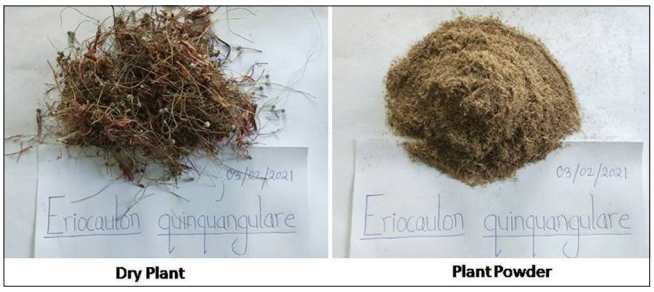

Figure 1: Plant parts of *Eriocaulon quinquangulare*

Test of Flavonoid

To 1 ml of the extract, few drops of dilute sodium hydroxide were added. An intense yellow color was produced in the aqueous

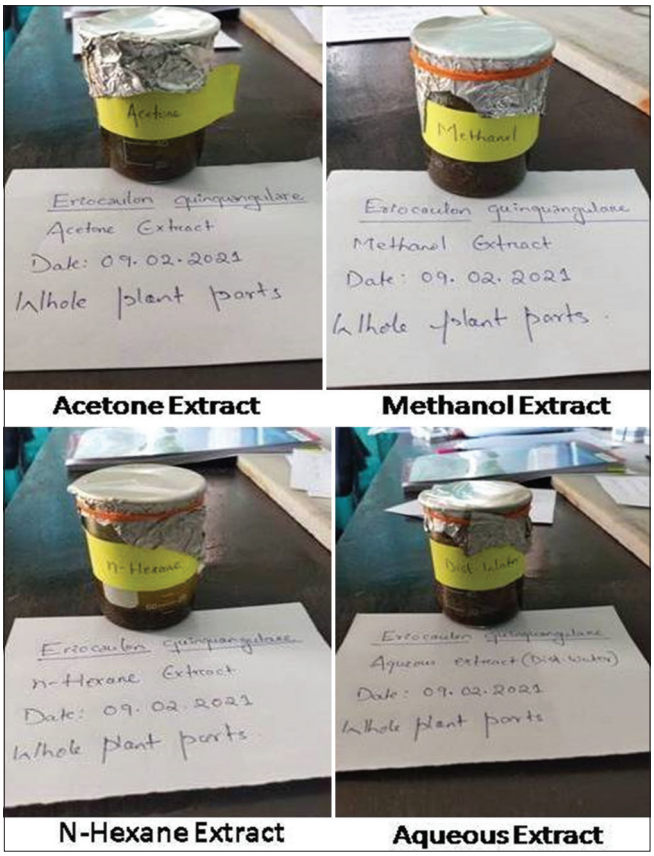

Figure 2: Extraction for qualitative analysis of bioactive compounds

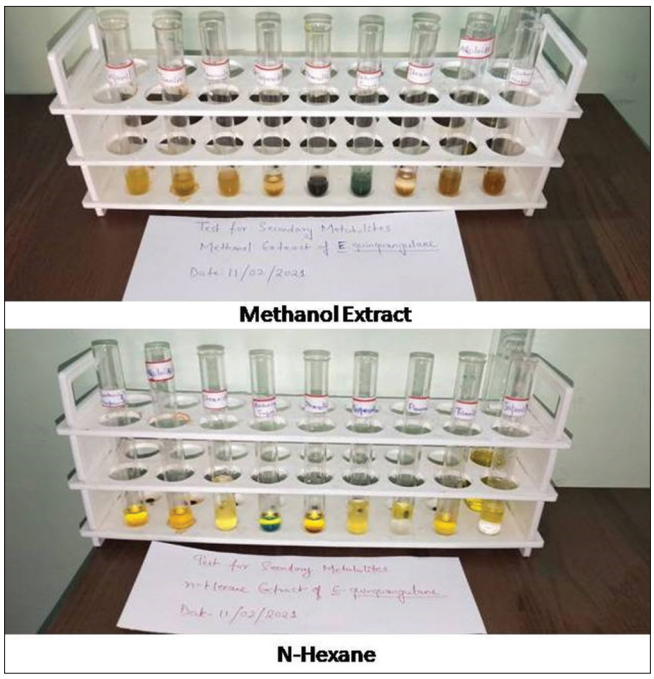

Figure 3: Phytochemical screenin

extract which became colorless on addition of 2–3 drops of 50% dilute sulfuric acid, indicated the presence of flavonoids. No results were observed in other solvents.

Test of Terpenoid

0.5 g of plant extract was mixed with 2 ml of chloroform and equal volume of conc. Sulfuric acid was added. No colorations were observed in any of the solvents.

Test of Phenolic Compounds

Two milliliters of plant extract were added with five drops of 1% ferric chloride and 1 ml of potassium ferrocyanide, a bluish-green solution in aqueous and methanol extracts showed the presence of phenolic compounds. No results were observed in other solvents.

Test of Reducing Sugar

0.5 g of plant extract was dissolved with distilled water and filtered. The filtrate was boiled with two drops of Fehling's solution A and B for 5 min. None of the solvents formed an orange precipitate.

Test of Steroid

Two milliliters of plant extract were dissolved in 5 ml chloroform and then 5 ml of conc. sulfuric acid wad added. No phases were formed in any of the solvents.

Test of Alkaloid

Five milliliters of plant extract were mixed with 3 ml of aqueous HCl on water bath and then filtered. One milliliter of Dragendorff's reagent was added in the filtrate. No precipitate was formed in any of the solvents.

Test of Carbonyl Compounds

Two milliliters of plant extracts were added with two drops of 2,4-dinitrophenyl hydrazine solution and thoroughly shaken. No results were observed in any of the solvents. After completion of phytochemical test, it was observed that the plant extract in distilled water as solvent and methanol as solvent showed 4/9 and 2/9 +ve results, respectively. Since both these solvents had most +ve results, these two solvents (aqueous and methanol) were taken for Soxhlet extraction.

Soxhlet Extraction

Soxhlet extraction was conducted for the whole plant parts using methanol and distilled water as solvents. Twenty-five grams of the whole plant part were taken in the Soxhlet tube and 250 g of solvent was taken in the round bottom flask of the Soxhlet apparatus. Then, the whole apparatus was setup and extraction was carries out at 50–60°C. For distilled water as solvent, Soxhlet extraction was carried out for 6 h for 3 days. After 18 h of extraction for aqueous solvent, the residue was collected from the round bottom flask and was kept in a water bath to get further concentrated. The same plant powder used in Soxhlet for d/w as solvent was dried and further used for Soxhlet for methanol solvent. The Soxhlet extraction for methanol was carried out until our rounds of condensation. Since methanol has a lower boiling point, the extraction was completed within 4–5 h in its concentrated form, which was collected from Soxhlet flask [Figure 4].

Antimicrobial Activity

Antibacterial activity

The aqueous and methanol extracts of *E. quinquangulare* were screened for antibacterial activity against two Gram-negative bacteria, *Vibrio cholerae* and *Salmonella typhi*. All used microbial type culture collection bacterial strains were collected from conservation laboratories of APRF, Bhubaneswar. Antibacterial activity was done using slight modification of standard methods of agar well diffusion assay and disc diffusion method.

Agar well diffusion assay

Agar well diffusion method was followed to test the antibacterial activity of extracts of *E. quinquangulare* against the two bacterial strains [Figure 5]. Nutrient agar plates were prepared as per manufacturer's instructions. Twenty-five milliliters of nutrient broth cultures of the test microbes prepared a day before poured over the plates. One milliliter of the microbes from the broth was added to agar medium and mixed under laminar hood and poured over plates uniformly. Two sets of each organism plate were prepared for two extracts and two separate plates for +ve and –ve control. Aqueous extract of sample was prepared in 5% DMSO and 2-fold serial dilutions

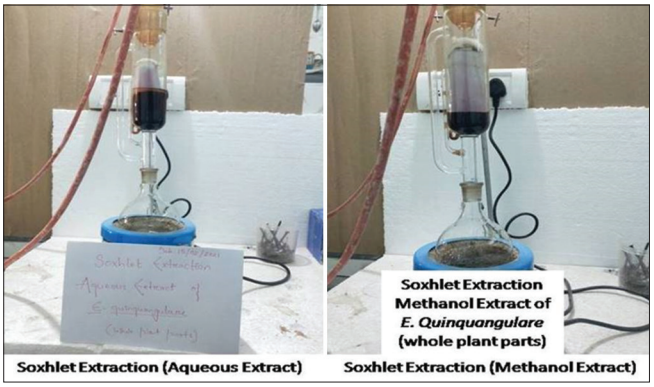

Figure 4: Extraction through Soxhlet apparatus

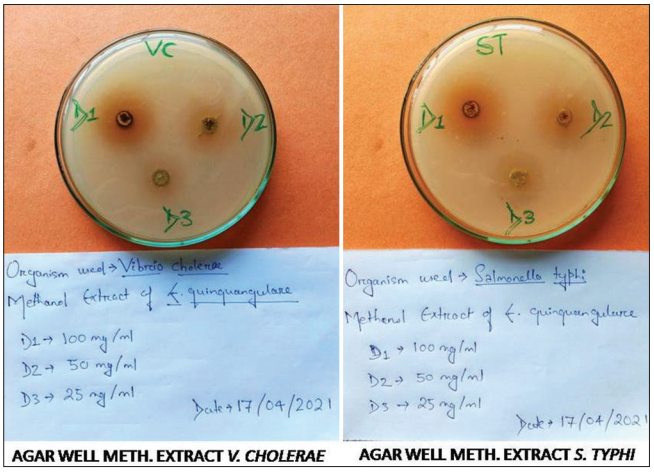

Figure 5: Antibacterial activity using agar well diffusion assay

were made in concentrations of 100 mg/ml, 50 mg/ml and 25 mg/ml. Methanol extract of sample was also prepared with three dilution concentrations 100 mg/ml, 50 mg/ml, and 25 mg/ml. One hundred microliters of samples were added by micropipettes into the walls in three above-mentioned concentrations and allowed to diffuse at room temperature for 30 min. Plates were incubated at 35 ± 2°C for 18–24 h. Ampicillin served as standard antibiotics control (+ve control) and distilled water as –ve control. Duplicates were maintained and the experiment was repeated twice. For each replicate, the readings (diameter of zone of inhibition in cm) were taken and the mean ± SD values (diameter of zone of inhibition) were recorded.

Disc diffusion assay

Antibacterial activity using disc diffusion assay was done using the 6 mm of disc prepared from Whatman filter paper. Each extract was made three sets of dilution by serial dilution with concentration of 100 mg/ml, 50 mg/ml, and 25 mg/ml. Six millimeters of discs were kept in the dilutions for 12 h before placing to the agar plates. The zones of growth inhibition around the discs were measured after 18–24 h of incubation at 37°C for bacteria. The sensitivities of the microbial species to the plant extracts were determined by the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the discs, and values < 8 mm were considered as not active against microorganisms. Penicillin discs were used as standard antibiotic control for both organisms.

Media used

Nutrient broth was used to maintain broth cultures. The constituents of the nutrient broth included 0.5 g NaCl (Deaker *et al*., 2011), 0.5 g peptone, and 0.3 g beef/100 ml. An additional 1.5 g of agar made up the nutrient agar medium.

Preparation of working slant

Stock cultures of *V. cholerae* and *S. typhi* were maintained at 4°C on slants of semi-solid media containing 1.5% of agar-agar, 0.5% beef extract, and 0.5% peptone. Active working cultures for experiments were prepared by transferring a loopful of culture mass from the stock. Slants were incubated for 24 h at 36 ± 1.0°C.

Broth preparation

Colonies of prepared slants of *V. cholerae* and *S. typhi* were picked off using sterile loop and inoculated in sterile conditions in autoclaved cool liquid broth medium containing 0.3% of beef extract and 0.5% peptone. The broth was incubated for 24 h at 36 ± 1.0°C until there was visible growth indicated by turbidity standard.

Swabbing and inoculation of drugs

Swabbing with autoclaved cotton swab was done using broth strain on Petri plates. Wells (6 mm) were made using sterile borer for agar cup well method. Stock solutions of samples were prepared in 5% DMSO and 2-fold serial dilutions were made in amount of 100 µl/well ranged from 100 mg/ml, 50 mg/ml, and 25 mg/ml. One hundred microliters of samples were added by sterile syringes into the wells in three above-mentioned concentration and allowed to diffuse at room temperature for 30 min. Only the solvent (distilled water) was poured in to the wells in another set of plates as part of negative control. The positive control set consisted of standard antibiotics ampicillin. For the disc diffusion assay, only swabbing was done using sterile swab. The discs of respective aforesaid concentration were placed on media. Both Petri plates (for agar well diffusion and disc diffusion method) were incubated at 36 ± 1.0°C for 18 h. Zones of inhibition free microbial growth appeared around each well and disc in the form of clear rings which confirmed the antibacterial activity of the respective samples. Those samples which did not have any inhibitory effect on the microbe did not form any clear ring. In this way, the antibacterial activity of the samples was confirmed. Duplicates were maintained and the experiment was repeated twice. For each replication, the readings (zone of inhibition) were taken and the mean values were recorded.

Data analysis

Mean and SD (standard deviation) was calculated taking duplicate values of zone of inhibition (mm for agar well diffusion assay; mm for disc diffusion of samples using Excel, Microsoft Corporation-2010, US).

Minimum inhibitory concentration (MIC) using broth dilution assay

The aqueous and methanol extracts of *E. quinquangulare* plant were screened for their antibacterial activity. Antibacterial activity was assessed by MIC by serial dilution method. Selected colonies of aforesaid bacteria were picked off to a fresh isolation plate and inoculated into the corresponding tubes containing 1 ml of Nutrient broth. The broth was incubated for 8 ± 1 h at 35 ± 2°C until there was visible growth.

Data interpretation

After incubation, the tubes showing no visible growth after 8 h till 12 h were considered to be inhibition of bacteria which represent MIC values of a respective concentration. Duplicates were maintained and the experiment was repeated twice, for each replicate. The readings were taken as foresaid.

Results and Discussion

Phytochemical Screening

The phytochemical screening revealed that saponin is present in aqueous extract. It was observed that tannin is detected in acetone, aqueous, and methanol. Flavonoids are detected in aqueous extract and phenolic compounds in aqueous and methanol extracts [Table 1].

Antibacterial Activity of Disc Diffusion assay [Figure 6 and Tables 2-7]

Results of aqueous extract

The zone of inhibition for *V. cholerae* having dilution concentration of 100 mg/ml was n1 = 2.3 cm and n2 = 2.4 cm. The mean for this concentration was 2.34 cm with SD of 0.05. Dilution concentration of 50 mg/ml had n1 = 2.0 cm and n2 = 1.9 cm zone of inhibition, having mean of 1.94 cm with SD of 0.05. 25 mg/ml dilution concentration creates a zone of inhibition of n1 = 1.8 cm and n2 = 1.6 cm, whose mean is 1.69 cm with SD 0.1. The zone of inhibition for *S. typhi* having dilution concentration of 100 mg/ml was n1 = 2.0 cm and n2 = 1.9 cm. The mean for this concentration was 1.94 cm with SD of 0.05.

Dilution concentration of 50 mg/ml had n1 = 1.8 cm and n2 = 1.6 cm zone of inhibition, having mean of 1.69 cm with SD of 0.1. Twenty-five mg/ml dilution concentration creates a one of inhibition of n1 = 1.5 cm and n2 = 1.0 cm, whose mean is 1.22 cm with SD 0.25.

Results of methanol extract

The zone of inhibition for *V. cholerae* having dilution concentration of 100 mg/ml was n1 = 2.9 cm and n2 = 2.5 cm. The mean for this concentration was 2.69 cm with SD of 0.2. Dilution concentration of 50 mg/ml had n1 = 2.6 cm and n2 = 2.1 cm zone of inhibition, having mean of 2.33 cm with SD of 0.25. Twenty-five mg/ml dilution concentration creates a zone of inhibition of n1 = 2.3 cm and n2 = 1.8 cm, whose mean is 2.03 cm with SD 0.25. The zone of inhibition for *S. typhi* having dilution concentration of 100 mg/ml was n1 = 3.1 cm and n2 = 2.7 cm. The mean for this concentration was 2.89 cm with SD of 0.2. Dilution concentration of 50 mg/ml had n1 = 2.6 cm and n2 = 2.4 cm zone of inhibition, having mean of 2.49 cm with SD of 0.1. Twenty-five mg/ml dilution concentration creates a one of inhibition of n1 = 2.3 cm and n2 = 1.9 cm, whose mean is 2.09 cm with SD 0.2. The standard antibiotic control (ampicillin) for *V. cholerae* had a zone of inhibition of n1 = 4.6 cm and n2 = 4.1 cm having mean 4.9 cm with SD of 0.2. The standard antibiotic control for *S. typhi* had a zone of inhibition of n1 = 5.0 cm and n2 = 4.8 cm having mean 4.89 cm with SD of 0.1.

Antibacterial Activity of Agar Well diffusion assay [Figure 5]

Results of aqueous extract

The zone of inhibition for *V. cholerae* having dilution concentration of 100 mg/ml was n1 = 0.9 cm and n2 = 0.8 cm. The mean for this concentration was 0.84 cm with SD of 0.05. Dilution concentration of 50 mg/ml had n1 = 0.7 cm and n2 = 0.8 cm zone of inhibition, having mean of 0.74 cm with SD of 0.05. Twenty-five mg/ml dilution concentration creates a zone of inhibition of n2 = 0.7 cm, whose mean is 0.7 cm with SD 0. The zone of inhibition for *S. typhi* having dilution concentration of 100 mg/ml was n1 = 1.0 cm and n2 = 0.9 cm. The mean for this concentration was 0.89 cm with SD of 0.06. Dilution concentration of 50 mg/ml had n1 = 0.8 cm and n2 = 0.8 cm zone of inhibition, having mean of 0.8 cm with SD of 0. Twenty-five mg/ml dilution concentration creates a one of inhibition of n2 = 0.7 cm, whose mean is 0.7 cm with SD 0.

Results of aqueous methanol extract

The zone of inhibition for *V. cholerae* having dilution concentration of 100 mg/ml was n1 = 1.5 cm and n2 = 1.3 cm. The mean for this concentration was 1.39 cm with SD of 0.1. Dilution concentration of 50 mg/ml had n1 = 0.9 cm and n2 = 0.8 cm zone of inhibition, having mean of 0.84 cm with SD of 0.05. Twenty-five mg/ml dilution concentration creates a zone of inhibition of n1 = 0.8 cm and n2 = 0.8 cm, whose mean is 0.8 cm with SD 0. The zone of inhibition for *S. typhi* having dilution concentration of 100 mg/ml was n1 = 1.4 cm and n2 = 1.2 cm. The mean for this concentration was 1.29 cm with SD of 0.1. Dilution concentration of 50 mg/ml had n2 = 0.7 cm zone of inhibition, having mean of 0.7 cm with SD of 0. Twenty-five mg/ml dilution concentration creates a one of inhibition of n2 = 0.7 cm, whose mean is 0.7cm with SD 0. The standard antibiotic control (penicillin) for *V. cholerae* had a zone of inhibition of n1 = 2.9 cm and n2 = 2.6 cm having mean 2.74 cm with SD of 0.15. The standard antibiotic control for *S. typhi* had a

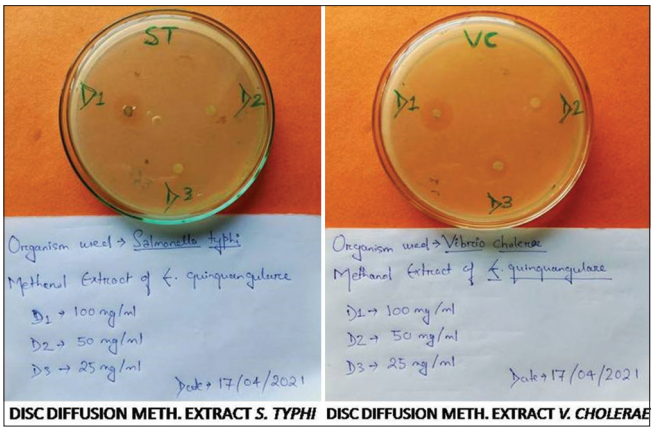

Figure 6: Antibacterial activity using disc diffusion assay

Table 1: Phytochemical assay of experimental plant

Extract	*Saponin*	*Tannin*	*Flavonoids*	*Phenolic compounds*
Acetone	−ve	+ve	−ve	−ve
Aqueous	+ve	+ve	+ve	+ve
n-Hexane	−ve	−ve	−ve	−ve
Methanol	−ve	+ve	−ve	+ve

Table 2: Disc diffusion assay for aqueous extract of *Eriocaulon quinquangulare*

Bacterial strain	*Concentration*	*Zone of inhibition*		*Mean*	*Standard deviation*
		n=1	*n=2*		
V. cholerae	100 mg/ml	0.9 cm	0.8 cm	0.84 cm	0.05
	50 mg/ml	0.7 cm	0.8 cm	0.74 cm	0.05
	25 mg/ml	Nil	0.7 cm	0.7 cm	0
S. typhi	100 mg/ml	1.0 cm	0.9 cm	0.89 cm	0.06
	50 mg/ml	0.8 cm	0.8 cm	0.8 cm	0
	25 mg/ml	Nil	0.7 cm	0.7 cm	0
+ve control penicillin *V. cholerae*		2.9 cm	2.6 cm	2.74 cm	0.15
+ve control penicillin *S. typhi*		2.8 cm	2.4 cm	2.59 cm	0.25
−ve control *V. cholerae*		Nil	Nil	Nil	Nil
−ve control *S. typhi*		Nil	Nil	Nil	Nil

V. cholerae: *Vibrio cholerae*, *S. typhi*: *Salmonella typhi*

zone of inhibition of n1 = 2.8 cm and n2 = 2.3 cm having mean 2.59 cm with SD of 0.25.

Estimation of MIC [Figure 7]

Results of aqueous extract

The initial concentrations (100 mg/ml and 50 mg/ml) show no growth of *V. cholerae* and only the first concentration (100 mg/ml) shows no growth of *S. typhi.*

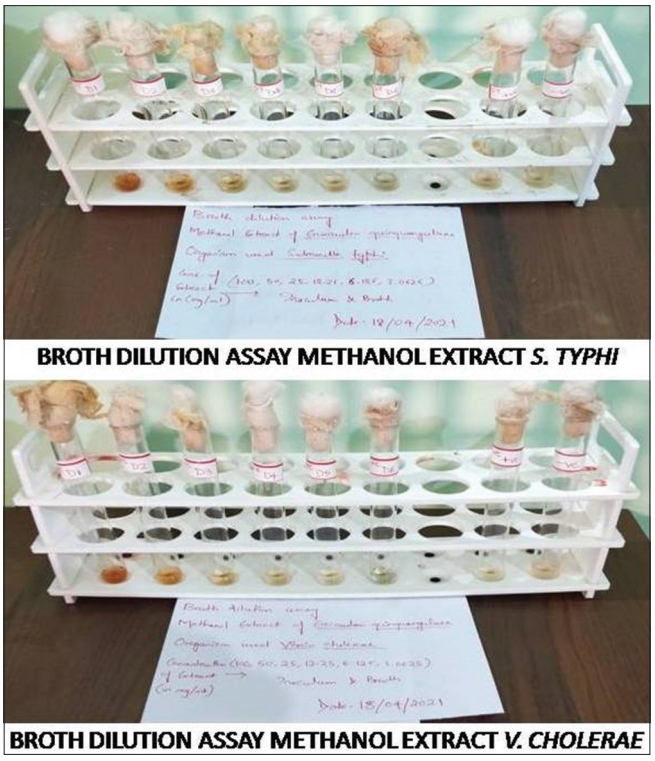

Figure 7: Estimation of minimum inhibitory concentration

Results of methanol extract

The initial concentrations (100 mg/ml and 50 mg/ml) show no growth of *V. cholerae* and only the first concentration (100 mg/ml) shows no growth of *S. typhi.* The observations of MIC were recorded on the basis of turbidity. MIC for *V. cholerae* was 50 mg/ml and MIC for *S. typhi* was 100 mg/ml for both aqueous and methanol extracts.

Conclusion

The present study concludes that the secondary metabolites detected in the experimental plant *E. quinquangulare* were saponin, tannin, flavonoids, and phenolic compounds. The secondary metabolites were predominantly found in aqueous and methanol extracts of the plant. The extraction of plant through Soxhlet extraction was found in a concentrated form which shows the

Table 4: Disc diffusion assay for methanol extract of *Eriocaulon quinquangulare*

Organism used	Concentration	Zone of inhibition		Mean	Standard deviation
		n=1	*n*=2		
V. cholerae	100 mg/ml	1.5 cm	1.3 cm	1.39 cm	0.1
	50 mg/ml	0.9 cm	0.8 cm	0.84 cm	0.05
	25 mg/ml	0.8 cm	0.8 cm	0.8 cm	0
S. typhi	100 mg/ml	1.4 cm	1.2 cm	1.29 cm	0.1
	50 mg/ml	Nil	0.7 cm	0.7 cm	0
	25 mg/ml	Nil	0.7 cm	0.7 cm	0
+ve control penicillin *V. cholerae*		2.9 cm	2.6 cm	2.74 cm	0.15
+ve control penicillin *S. typhi*		2.8 cm	2.3 cm	2.59 cm	0.25
−ve control *V. cholerae*		Nil	Nil	Nil	Nil
−ve control *S. typhi*		Nil	Nil	Nil	Nil

V. cholerae: *Vibrio cholerae*, *S. typhi*: *Salmonella typhi*

Table 3: Agar well diffusion assay for aqueous extract of *Eriocaulon quinquangulare*

Bacterial strain	Concentration	Zone of inhibition		Mean	Standard deviation
		n=1	*n*=2		
V. cholerae	100 mg/ml	2.3 cm	2.4 cm	2.34 cm	0.05
	50 mg/ml	2.0 cm	1.9 cm	1.94 cm	0.05
	25 mg/ml	1.8 cm	1.6 cm	1.69 cm	0.1
S. typhi	100 mg/ml	2.0 cm	1.9 cm	1.94 cm	0.05
	50 mg/ml	1.8 cm	1.6 cm	1.69 cm	0.1
	25 mg/ml	1.5 cm	1.0 cm	1.22 cm	0.25
+ve control ampicillin *V. cholerae*		4.6 cm	4.2 cm	4.39 cm	0.2
+ve control ampicillin *S. typhi*		5 cm	4.8 cm	4.89 cm	0.1
−ve control *V. cholerae*		Nil	Nil	Nil	Nil
−ve control *S. typhi*		Nil	Nil	Nil	Nil

V. cholerae: *Vibrio cholerae*, *S. typhi*: *Salmonella typhi*

Table 5: Agar well diffusion assay for methanol extract of *Eriocaulon quinquangulare*

Organism used	Concentration	Zone of inhibition		Mean	Standard deviation
		n=1	*n*=2		
V. cholerae	100 mg/ml	2.9 cm	2.5 cm	2.69 cm	0.2
	50 mg/ml	2.6 cm	2.1 cm	2.33 cm	0.25
	25 mg/ml	2.3 cm	1.8 cm	2.03 cm	0.25
S. typhi	100 mg/ml	3.1 cm	2.7 cm	2.89 cm	0.2
	50 mg/ml	2.6 cm	2.4 cm	2.49 cm	0.1
	25 mg/ml	2.3 cm	1.9 cm	2.09 cm	0.2
+ve control ampicillin *V. cholerae*		4.6 cm	4.1 cm	4.39 cm	0.2
+ve control ampicillin *S. typhi*		5 cm	4.8 cm	4.89 cm	0.1
−ve control *V. cholerae*		Nil	Nil	Nil	Nil
−ve control *S. typhi*		Nil	Nil	Nil	Nil

V. cholerae: *Vibrio cholerae*, *S. typhi*: *Salmonella typhi*

Table 6: MIC using broth dilution method (aqueous extract of *Eriocaulon quinquangulare*)

Bacterial strain	Concentration						Inoculums	Broth
	100 mg/ml	50 mg/ml	25 mg/ml	12.25 mg/ml	6.125 mg/ml	3.0625 mg/ml		
Vibrio cholerae	No growth	No growth	Growth	Growth	Growth	Growth	Growth	No growth
Salmonella typhi	No growth	Growth	Growth	Growth	Growth	Growth	Growth	No growth

MIC: Minimum inhibitory concentration

Table 7: MIC using broth dilution method (methanol extract of *Eriocaulon quinquangulare*)

Organism used	*Concentration*						*Inoculums*	*Broth*
	100 mg/ml	*50 mg/ml*	*25 mg/ml*	*12.25 mg/ml*	*6.125 mg/ml*	*3.0625 mg/ml*		
Vibrio cholerae	No growth	No growth	Growth	Growth	Growth	Growth	Growth	No growth
Salmonella typhi	No growth	Growth	Growth	Growth	Growth	Growth	Growth	No growth

MIC: Minimum inhibitory concentration

presence of medicinal values of the plant. The antibacterial activity of *E. quinquangulare* against Gram –ve *V. cholerae* and *S. typhi* was found to be effective at 50 mg/ml and 100 mg/ml, respectively. The conclusions were derived after observing the zone of inhibition of plant extracts with distilled water and methanol as solvents.

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