

PUBLICATIONS

RESEARCH ARTICLES

- Chakraborty, N., Mandal, B. and Das, A.K., 2015. *Vallisneria spiralis* L. - Rapid aquatic colonizers to anti dandruff dermatitis, *Journal of Ecosystem & Ecography*, **5** (3), 169-175.
- Chakraborty, N., Das, A.K., and Mandal, B., 2016. Rapid Structural Characterization of Plant Extracts – a FT-IR study, *Chemical Sciences Review and Letters*, **5**(19), 250-255.
- Chakraborty, N., Mandal, B., Das, A.K., and Manna, R.K., 2018. Phytochemical, Antibacterial and Brine Shrimp Toxicity Studies of Green Banana Leaves, *Environment and Ecology*, **36** (3): 767 – 776.

ORAL PRESENTATIONS

- Nabanita Chakraborty***, B.M. Mandal and A.K. Das, *Vallisneria spiralis* - Rapid aquatic colonizers to invitro anti dandruff dermatitis and broad spectrum bacteriocide, UGC & DST National Seminar on “Natural Resources Development strategies through Biotechnological Approaches”, 23-24 March, 2015, Vidyasagar University.
- Nabanita Chakraborty***, A.K Das and A.P. Sharma, *Wetland colonizers to anti dandruff dermatitis: study with swamp cabbage*, 3rd International Conference and Exhibition on Pharmacognosy, Phytochemistry & Natural Products, 26-28th October 2015, Hyderabad International Convention Centre. *Journal of Natural product Chemistry & Research*, 3(6): 107.
- N. Chakraborty***, B. Mandal, A.K. Das, R.K. Manna and L. Kumar, *BIOACTIVE PHYTO-COMPOUNDS: FACET OF GREEN CHEMISTRY - A REVIEW*,

National Seminar on Medicinal Plants (NSMP - 2016), UGC-DRS-SAP & DST, Department of Botany & Forestry, Vidyasagar University, OP-12, Pg- 53, 16th - 17th February, 2016.

Nabanita Chakraborty*, Archan Kanti Das and Basudev Mandal¹, *Rapid Structural Characterization of Plant Extracts – a FT-IR study*, 2nd National Conference on Fundamental and Applied Chemistry, Salem, Tamil Naidu, 4th June 2016.

ABSTRACTS

Nabanita Chakraborty, B.M. Mandal and A.K. Das, *Vallisneria spiralis* - Rapid aquatic colonizers to invitro anti dandruff dermatitis and broad spectrum bacteriocide, UGC & DST National Seminar on “*Natural Resources Development strategies through Biotechnological Approaches*”, 23-24 March, 2015, Vidyasagar University.

N. Chakraborty, B. Mandal, A.K. Das, R.K. Manna and L. Kumar, *BIOACTIVE PHYTO-COMPOUNDS: FACET OF GREEN CHEMISTRY - A REVIEW*, *National Seminar on Medicinal Plants* (NSMP - 2016), UGC-DRS-SAP & DST, Department of Botany & Forestry, Vidyasagar University, OP-12, Pg- 53, 16th -17th February, 2016.

Nabanita Chakraborty, Basudev Mandal and Archan Kanti Das, *Studying FT-IR analysis for curative traits of Aquatic Macrophytes – A review*, National Symposium on Bio resource Management (NSBM-2016), Pg: 32, 26th – 27th April, 2016.

N. Chakraborty, B. Mandal, A.K. Das, R.K. Manna and B.K. Das, *Toxicity Assessment of Polar and Non-polar Phyto-chemicals: Brine Shrimp Lethality Assay*, World

Brackish Aquaculture Conference 2019, ICAR – CIBA, 23-25 June, Chennai.
ENV-23, Pg: 239.

N. Chakraborty, B. Mandal, A.K. Das, R.K. Manna and B.K. Das, *Inhibition of Fish Pathogenic Bacteria By Plant Hydrocarbons*, National Seminar and frontiers of Fisheries Research; Problems and Prospects, Vidyasagar University, 31st January – 1st February 2019, Midnapore, Pg: 48 .

WORKSHOPS

5 Days Training Programme on Algal culture at CCUBGA, Indian Agricultural Research Institute (IARI), Delhi.

Training Programme on proteomics Approaches for Women Scientists in Life Sciences sponsored By DST and organized by and at Banaras Hindu University from March 2-6, 2017

AWARDS

Young Scientist Award and *Best Presenter Award* at 2nd National Conference on Fundamental and Applied Chemistry by Aufau International at Salem, Tamil Nadu on 4th June, 2016.

REPRINTS

Leaves of *Vallisneria* Finds Source to Anti Dermatitis: Enriching Wetland Ecosystem

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Abstract

The renaissances of phyto-medicines lead to a resolute search of potential medicinal plants worldwide. In this perspective, predominantly, terrestrial allelochemistry has been an imperative tool to evaluate the drug potentiality of a plant. However, in aquatic ecosphere, the pharmaceutical evaluation of the aquatic macrophytes is an emerging aspect. Our study seeks to explore the curative traits of the exotic macrophyte, *Vallisneria spiralis* L. (Hydrocharitaceae), a perennial stoloniferous species and a key ecological community in freshwater wetland ecosystem. It is commonly used as aquarium plant and inhabits both in lentic and lotic environment of tropical and sub-tropical regions worldwide. Being rapid colonizers of aquatic ecosystem, literature study states the leaves of the macrophyte can excrete special function groups that can absorb, filter and precipitate chemical compound of water and through the auxiliary function of the microorganism. Considering these significances, we have extracted the leaf leachates of *Vallisneria* in 80% ethanol which had been purified by solid-liquid extraction process and further crystallized and subjected to biochemical analysis viz; phenols, flavonoids and tannins followed by antioxidant scavenging and microbial screening. Further the isolated compound was subjected to Mass and Fourier Infra-red Spectrometry. Comprehensively, all the experimental assays infer that *Vallisneria* leaves contain bioactive compound with mol wt. m/z 359 structured with microbial growth inhibiting functional groups which were found fungicidal against *Malassezia globosa* - the dandruff causing dermatitis fungus.

Keywords: *Vallisneria spiralis*, Wetlands; *Malassezia globosa*; Fungicidal

Abbreviations:

MIC: Minimum Inhibitory Concentration; MFC: Minimum Fungicidal Concentration; GAE: Gallic Acid Equivalents; QE: Quercetin Equivalent; TAE: Tannic Acid Equivalent; DPPH: 2, 2-diphenyl-1-Picrylhydrazyl; DMSO: Dimethyl Sulphoxide; FT-IR: Fourier Infra-red

Introduction

An integrative ecosystem demands biological interactions which accords for survival values amongst life. The competition between different photoautotroph [1-3] for resources in water body changes the succession of species which is otherwise an outcome of interactive secondary metabolites in progressive plant populations. Virtually, various theoretical and experimental citations have been stated decades back on the role of these metabolites not only for synthetic innovations as drug adjuvant [4,5] but also on natural ecospheres including aquatic macrophyte and their indulged impact on algal bloom and phytoplankton [6,7]. Field evidences and literature studies confer that all primary producing organisms (cyanobacteria, micro-and macroalgae as well as angiosperms) are capable of producing and releasing these active compounds [8]. However, utilizing there *in situ* exudations in outward pharmaceutical implications is a fascinating aspect and a fetch for ayurvedic sciences [9] Some Indigenous

technical knowledge (ITK) and a few chemical perceptions through experimentation lead to various knowledge outsourcing to insight into the medicinal traits of many plants.

Vallisneria spiralis L. is a common submerged rooted macrophyte found in many wetlands, shallow ponds, lakes, marshes and streams of West Bengal. It is an extensively wide stretched colonizer [10-12] and the dispersal of the species can take place both through human and natural means via wind or water. Literature cites its role as appetizer, refrigerant, demulcent and women complaint (leucorrhoea) and used for stomach ache [13,14]. The plant is also found to bio remediate the tannery effluents [15]. The fact that the species is also capable of changing the pore water chemistry towards a more oxidized state [16] reveals its microbial static traits. This study aims to use the leaf leachates of *Vallisneria* against the most troubleshoot dermatitis fungi *Malassezia globosa*. The experiment was initiated with four different fractions with 0%, 40%, 80% and 100% ethanol in water but only the 80% ethanol fraction was found to show activity and in accordance to that this paper demonstrates only the data relevant to this fraction.

Methods and Materials

Collection and preparation of plant sample

The plant samples were collected from wetland water bodies of Nadia district of West Bengal, India, where the growth was intense. The plants after uprooting were washed in tap water and carried to the laboratory in sterile polypropylene and rewashed thoroughly with

double distilled water, air dried and weighed in sterile bags and processed within 24 hrs of collection [17].

Extraction of bioactive compounds

Following the preparative steps, the plant leaves (7 kg fresh weight/set; excised of roots) were completely immersed in glass jars with perforated lids in increasing ratios from 0%, 20%, 40% and 80% ethanol using milipore water. The amount of solvent added was in the ratio of 10:1 with respect to the fresh weight of the plants in each jar. The jars were kept in room temperature with sufficient sunlight initially for a span of not more than three days to prevent auto toxicity resulting into decolouration and foul odour. Around 3 sets were required to obtain desirable amount. The crude extracts of the four fractions were collected and concentrated to dryness by rota vap and subjected to the following biochemical analysis.

Biochemical assay

Phenols

The total phenolic content was determined following Folin-Ciocalteu method [16] using 0.1 ml of the extract with a concentration range of 0.05-0.3 mg/ml of the leaf leachate. The extracts were mixed with Folin-Ciocalteu reagent and Sodium carbonate (Na_2CO_3) following incubation for 30 mins at room temperature. The change of colour was measured in spectrophotometer with absorbance reading at 765 nm. Gallic acid in the same concentration as the sample was used as positive control. The total phenolic content was expressed as GAE in milligram per gram of dry material using the calibration curve, where X was the absorbance and Y was GAE (mg/g).

Flavonoids

Flavonoid estimation was carried out following the method of Jia et al. [18]. The preferred concentration range for the leaf leachates were 0.2-1.2 mg/ml with 0.1 ml of the extract. Later the extract was added with 1.2 ml distilled water, 0.12 ml of 5% Sodium nitrite (NaNO_2) with uniform intermixing. Following incubation for 5 mins at 25°C temperatures, 0.12 ml of 10% AlCl_3 solution was added and mixed thoroughly. Then the tubes were further incubated at room temperature for 5 minutes and added with 0.8 ml of 1 mM Sodium hydroxide (NaOH) solution and 1.16 ml of distilled water. The absorbance was measured at 510 nm. Methodically, quercetin in the same concentration as the sample was used as positive control. Total flavonoids content as calculated as Quercetin (mg/g) using the calibration curve, where X was the absorbance and Y was QE (mg/g).

Tannins

European Commission [19] reference method was used to determine the total tannins content using tannic acid as standard curve. Briefly, 200 μL of extracts of 0.05 – 0.3 mg/ml was mixed with 200 μL of ferric ammonium citrate (0.35%) prepared freshly and 200 μL of ammoniac (0.8%). The absorbance of the mixture was measured at 525 nm. The results were expressed as TAE mg of per gram of extracts or fractions.

Antioxidant assay

DPPH radical scavenging activity: The free radical scavenging activity of extracts and fractions for the radical DPPH was measured as described [20,21]. Freshly prepared DPPH solution (25 mg/L) in

methanol was prepared and 3.9 ml of this solution was mixed with 0.1 ml of extract in methanol containing different concentration range (0.05-0.3 mg/ml conc.) of the extract. 30 minutes later, the absorbance was measured at 517 nm using Spectrophotometer. Butylated Hydroxy Toluene (BHT) in the same concentration as the sample was used as positive control. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\{Ac-At\}}{Ac} \times 100$$

Where Ac is the absorbance of the blank reaction and At is the absorbance in presence of the sample of the extracts.

IC50 which defines the concentration of the plant extract that's needed to scavenge 50% of the radical present was calculated by the following equation:

$$\text{IC50} = \frac{\{\text{Percentage Inhibition}\}}{\{\text{Concentration of the sample}\}} \times 50$$

Microbiological screening

Antimicrobial activities of different extracts were evaluated by the agar well diffusion method Mayser et al. [22] modified by Murray [23] and Minimum inhibitory concentration [24-26].

Sample and Media preparation

The crude plant extracts were concentrated to dryness and resuspended in 20% DMSO [27]. A dilution range of 1000 $\mu\text{g}/\text{ml}$ to 1 $\mu\text{g}/\text{ml}$ was prepared with DMSO as a control. For agar well diffusion method, the fungal sample was collected from human and cultured in sabouraud agar. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values were determined by serial broth dilution assay using sabouraud dextrose broth. All the media prepared was sterilized by autoclaving the media at (121°C) for 20 min.

Agar well diffusion method

The well diffusion method was used to determine the antifungal activity. The plates were swabbed (sterile cotton swabs) with the overnight fungal culture from sabouraud dextrose broth. Single well was punched in a petri plate using sterile cork borer. 100 μl of each concentrations of plant extracts were added into the wells and allowed to diffuse at room temperature for 3 hrs along with the control plates. The plates were incubated at 28°C for 36-48 hrs.

Minimal Inhibitory Concentration (MIC) and Minimal Fungal Concentration (MFC)

Broth dilution method was preferred to perform the MIC assay. The dilutions were chosen depending on the results of the well diffusion assay. The inoculum was loop transferred to 0.85% saline solution and the turbidity adjusted to 0.5 McFarland to ensure 5×10^5 CFU/ml after addition to the dilution tubes. 20 μl of the inoculums was transferred from saline solution to Sabouraud dextrose broth. 5 μl of the inoculums was transferred to all the experimental tubes within fifteen minutes after transferring in the broth. The tubes were incubated in 28°C for 18 hrs and the lowest concentrations without visible growth were the MIC. The higher concentrations above to MIC were plated in sabouraud agar and incubated in 28°C for 48 hrs; the lowest concentration without any visible growth was conferred as the MFC indicating 99.5% killing of the original inoculums [28].

Purification and Instrumental Analysis

The compound was isolated by chromatographic technique and deposited for Mass and FT-IR spectrometry to confer the molecular weight and functional groups.

Results

Biochemical analysis

The results of the biochemical assay for the four different fractions are tabulated in Table 1. Since fraction 4 was found to contain considerable amount of antioxidants, phenols and flavonoids [29], further work was carried out only with fraction 4 as well the data description for fraction 4 is stated below.

Fractio ns	Bio- Chemical Assay	Concentration (mg/ml)					
		0.05/0. 2	0.10/0. 4	0.15/0. 6	0.20/0.8	0.25/1.0	0.30/1. 2
0% ethanol	A	0.16	3.24	7.59	6.01	11.88	15.43
	B	0.43	0.68	1.92	1.28	2.6	3.77
	C	0.11	0.19	0.36	0.85	1.03	1.21
	D	2.53	6.47	12.58	10.01	16.01	20.22
20% ethanol	A	0.12	2.18	5.37	2.61	6.77	8.4
	B	0.18	0.32	0.69	1.34	2.18	4.82
	C	0.1	0.39	0.33	0.46	0.89	1.06
	D	2.68	5.37	7.98	9.56	21.32	26.49
40% ethanol	A	0.18	3.73	4.51	4.98	9.85	15.61
	B	0.1	0.32	0.38	0.08	1.06	3.49
	C	0.28	0.25	0.32	0.37	1.06	1.59
	D	1.65	5.29	9	9.82	22.91	25.66
80% ethanol	A	0.38	5.29	8.64	6.78	13.56	19.23
	B	0.68	0.76	2.64	3.53	3.58	6.91
	C	0.56	0.58	0.64	0.49	1.27	1.88
	D	5.2	9.56	15.46	11.28	21.01	28.97

Biochemical analysis of the four different fractions extracted from the leaves of *Vallisneria spiralis* L. A- Phenols; B-Flavonoids; C-Tannins and D-Antioxidant activity. The concentrations 0.5 mg/ml-3.0 mg/ml are for phenols, Tannins and Antioxidant activity and 0.2 mg/ml-1.2 mg/ml is specific for Flavonoid assay.

Table 1: Biochemical Analysis.

Phenols

The Total Phenol Content was expressed as Gallic acid equivalents (GAE) in mg/g fresh weight. The experimental data exhibits (Figure 1A) a proportionate increase along the concentration gradient although a sudden fall at 0.2 mg/ml with 6.78 GAE mg/g and highest noted at 0.3 mg/ml with 19.23 GAE mg/g of (0.05 – 0.3 mg/ml) extracts concentration. The result is validated with the coefficient of

determination ($R^2=0.963$) which reads the data as best fit along the regression line.

Flavonoids

The Total Flavonoid Content was expressed as Quercetin equivalents (QE) in mg/g fresh weight. The flavonoid content showed a consistent data (Figure 1B) evenly throughout the concentration range. It increased thoroughly with raise in concentration and 1.2 mg/ml extract had the highest flavonoid content with 6.91 mg/gm QE. Presence of flavonoid may result as alkaloids and glycosides in the plant extract. The R^2 value 0.935 was found to be significant and defines the goodness of fit of the data along the regression line.

Tannins

The Tannin Content was expressed as Tannic acid equivalents (TAE) in mg/g fresh weight. The progress of the data for tannin and flavonoid was found to be quite similar by way of steady increase with concentration of the leaf extract. The highest tannin content was recorded at 0.3 mg/ml of the leaf extract with 1.88 mg/gm TAE (Figure 1C). The strength of the response variables and the model was found to be significant with $R^2 = 0.959$.

Antioxidant assay

To determine the free radical scavenging as an index of antioxidant ability of the investigated plant extracts, DPPH assay was performed. The data followed that of the Total phenol content with a fall at 0.2 mg/ml of the extract with percent inhibition of 11.28. The highest percent inhibition of 28.97 was found for 0.3 mg/ml concentration. The co-linearity of the data trend (Figure 1D) for phenols and antioxidants reveals the fact that the active phenolics present in the plant extract are the antioxidants. The positive control BHT recorded the highest percent inhibition as 35.6 at 0.3 mg/ml concentration.

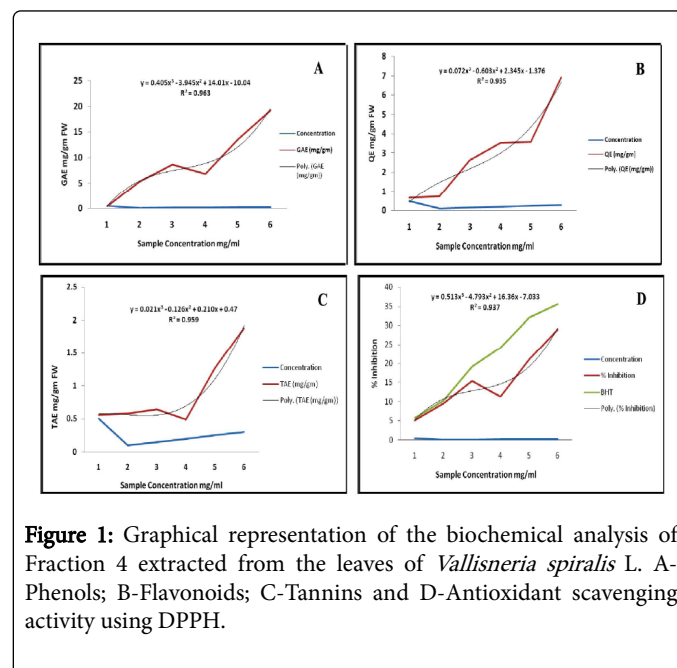


Figure 1: Graphical representation of the biochemical analysis of Fraction 4 extracted from the leaves of *Vallisneria spiralis* L. A- Phenols; B-Flavonoids; C-Tannins and D-Antioxidant scavenging activity using DPPH.

Microbial screening

The zone of inhibition was found to initiate at 100 µg/ml but the clear zone could be seen at 500 µg/ml and 1000 µg/ml (Figure 2). No anti-fungal activity was noticed in the other four concentrations. Hence forth, eleven tubes ranging from 10000 µg/ml to 39.0625 µg/ml of the extract and two controls one without inoculum and the other without extract was maintained for experimental assurance along with 1 ml of broth in each of the tube for MIC and MFC. The optical density at 600 nm was taken for all the samples. The data tabulated below shows MIC 156.25 µg/ml (Figure 3). All the six concentrations beyond that were streaked and kept in incubator at 37°C for 24 hrs/48 hrs and the MFC was found at 5000 µg/ml.

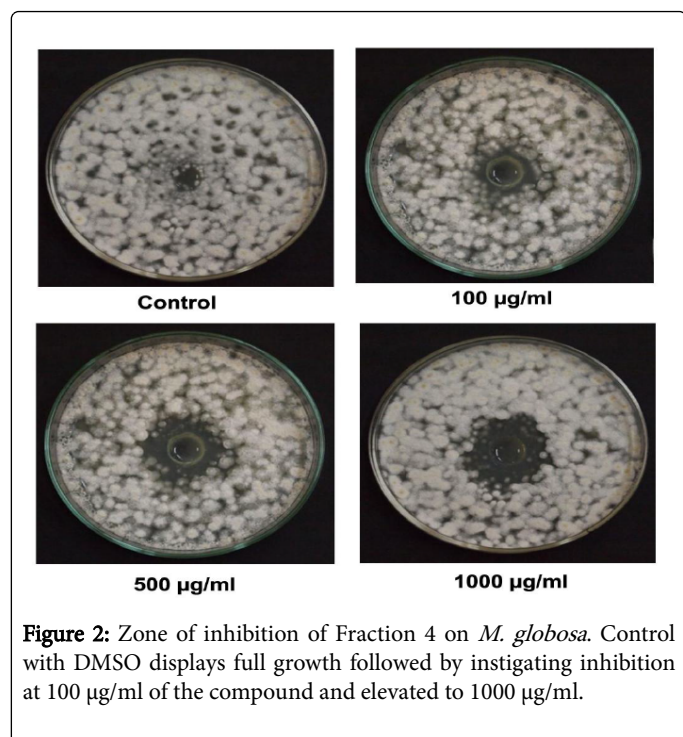


Figure 2: Zone of inhibition of Fraction 4 on *M. globosa*. Control with DMSO displays full growth followed by instigating inhibition at 100 µg/ml of the compound and elevated to 1000 µg/ml.

Purification and Structure activity relationship by Mass and FT-IR spectroscopy

The compound was ideally isolated using solvent system of 1% methanol in ethyl acetate by column chromatography using silica gel 100-230 mesh size and obtained as crystals with chloroform: methanol washes and finally flushed with toluene. Mass spectrum [30] with Time of Flight analyzer (TOF) shows the (Figure 4) molecular weight of the isolated compound to be m/z (%) = 359 [M]⁺ with the base peak at 360 [M+H]⁺. Infra-red spectrum (Figure 5) analysis helped to sort the functional group of the active compound which is tabulated (Table 2). The absorption spectrum of the 80% ethanol fraction shows ten major bands; the band at 3642.10 cm⁻¹ corresponds to hydroxyl group usually free hydroxyls. The other dominating bands at 1665.22 cm⁻¹ and 1554.80 cm⁻¹ are those of carbonyls and aromatic compounds. The presence of aromatic compound is further confirmed at 779.64 cm⁻¹ with C-H stretching which if in case of flavonoids, corresponds to the first aromatic ring. The S=O bond usually includes compounds [31,32] which shows radical trapping antioxidant property and act as antimicrobial, antiparasitic and antitumor agents, a common example of this compound is contained in *Allium sps.* [33,34].

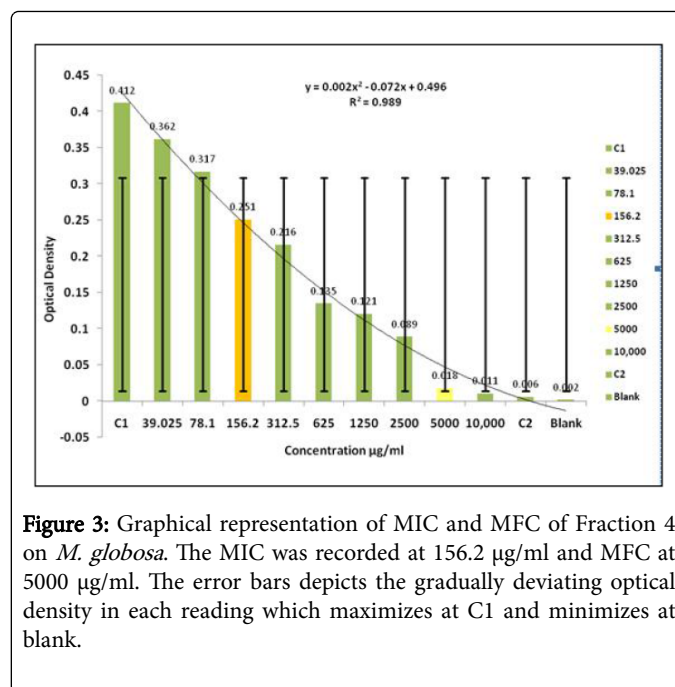


Figure 3: Graphical representation of MIC and MFC of Fraction 4 on *M. globosa*. The MIC was recorded at 156.2 µg/ml and MFC at 5000 µg/ml. The error bars depicts the gradually deviating optical density in each reading which maximizes at C1 and minimizes at blank.

SL No.	Peak value (cm ⁻¹)	Stretching	Inference
1	3642.1	O-H stretching	Alcohols/Phenols
2	2965.4	C-H stretching	Alkanes
3	2928.5	C-H stretching	Alkanes
4	1665.2	C=O stretching	C-O NH
5	1600.8	C=C stretching	Alkenes
6	1554.8	C=C stretching of aromatic ring	Aromatic Compounds
7	1360	S=O, sym	Sulphoxide
8	1080	C-O	Ethers
9	1076.9	C-O	Ethers
10	779.64	C-H stretching	Aromatics

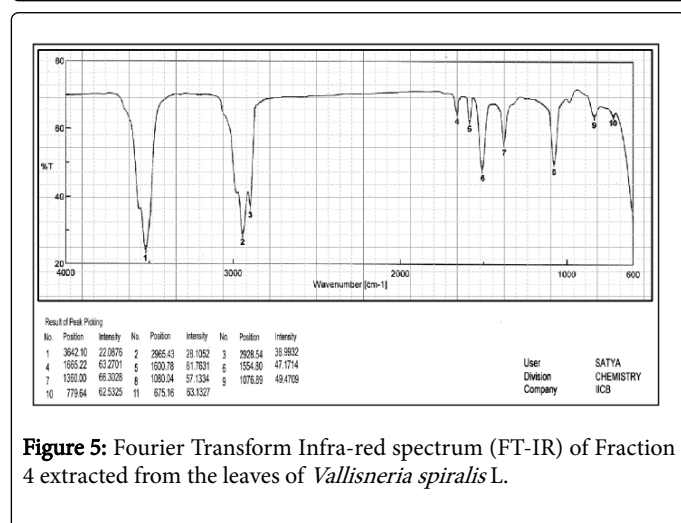
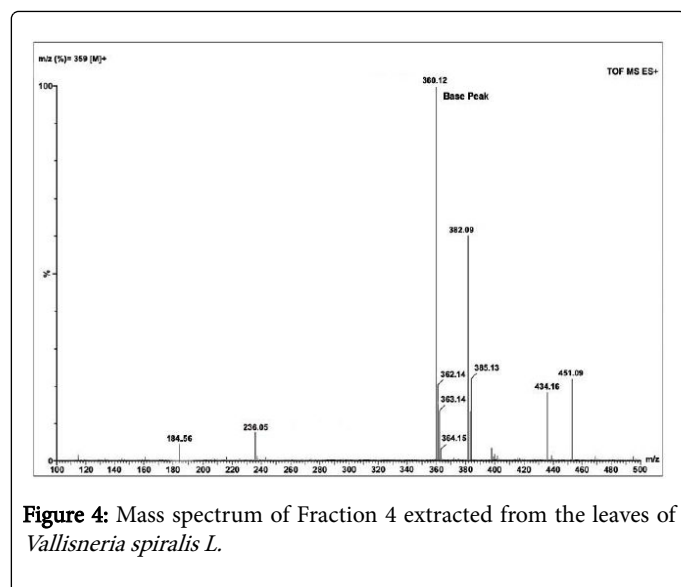
Structure activity relationship of the different functional groups present in the bioactive compound extracted by 80% ethanol from leaves of *Vallisneria spiralis* L. by FT-IR Spectroscopy.

Table 2: FT-IR Peaks Interpretation.

Discussion

Plants have been serving us as versatile sources of our basic needs [35,36]. The art of utilising the plant associated aspects including various metabolites is intriguing and holds a great prospect in pharmaceuticals and drug crafty [37] mostly due to its nearly nullified negative impacts [38-41] However, the ecosystem that harbours the plant is a vital factor for its outsourcing to nature. Science have much studied the terrestrial ecosystem which orients soil as a resource variable and quiet easy to monitor and modify, but water being a diluting variable needs advanced techniques and resources, hence comparatively much less explored. In this paper, we have screened a

rapid wetland colonizer, *Vallisneria spiralis* L. for antifungal traits against dandruff dermatitis following its uses as refrigent and skin lesions. The fourth fraction (80% ethanol) was assayed for possible biochemical evaluation and interestingly displayed a co-linearity in peaks for phenol and antioxidants which confirms that the antioxidant compound is phenolic in nature [42]. Further it was found to be antifungal against *M. globosa* initiating at 100 µg/ml and showed highly remarkable zone of inhibition at 1000 µg/ml and the minimum fungicidal concentration was observed at 5000 µg/ml. The compound was crystalline in nature with molecular weight m/z (%) = 359 [M]⁺. The FT-IR Spectrum depicts phenols, carbonyls, aromatics, sulphoxides and alcohols as major functional groups [43,44] of the structure which are antifungal [45,46] or mostly antimicrobial [47] and shows prospective research in herbal drug analysis. The structure contains a polyphenolic group. Most antioxidants isolated from medicinal plants are polyphenols [48-50] which show biological activities include anti-bacterial, anti-inflammatory, anti-obesity, antiviral, anti-carcinogenic and immune stimulating effect. Additively, the S=O group of the compound is one of the major antioxidant imparting factor [51,52] and lysis of microbial cell wall integrity.



The concise attempt to utilize the aquatic macrophyte as an antidandruff source provides a platform for research with wetland colonizers in an innovative aspect. Further research with NMR spectroscopy is required for demonstration the chemical structure of the compound.

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Research Article

Rapid Structural Characterization of Plant Extracts – a FT-IR study

Nabanita Chakraborty^{1,*}, Archan Kanti Das¹ and Basudev Mandal²¹ICAR- Central Inland Fisheries Research Institute (CIFRI) Monirampur, Barrackpore, Kolkata, West Bengal-700120²Aquaculture Management and Technology (AMT), Vidyasagar University, Midnapur, West Bengal- 721102**Abstract**

Plant natural product chemistry have ensued numerous pharmaceutical intermediates or drug precursors utilized for synthetic drugs. However lack of convenient qualitative tool for incessantly monitoring the potency of plant drugs, hinders application of herbal products. Molecular structure is the major theme in chemistry. A molecule absorbs a unique set of Infra Red light frequencies and as such beholds its own characteristic spectrum indicating characteristic functional group bands that assert a character to any phyto-compound. The application of FTIR spectroscopy in crude medicine authentication and quality evaluation is the most convenient tool to sort medicinal plants holistically at molecular level. I have tried to exemplify the fact with wetland macrophytes from my work viz; *Vallisneria spiralis* and *Ipomoea aquatic* as anti-dermatitis and anti-bacterial respectively.

The two prime regions are to studied *Functional group region* (4000 cm⁻¹-1450 cm⁻¹) and *Finger print region* (1450 cm⁻¹ – 500 cm⁻¹). The bands of spectra could be interpretative by scanning the sample table.

Keywords: FT-IR, Functional group, Finger print region, macrophytes, medicinal property.

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Introduction

The invention of Fourier Transform Infra-Red Spectroscopy (FT-IR) has revolutionized the natural product chemistry research. It not only detects very minute quantity of sample but also allows recovery of the sample. The fraction upon extraction followed by chromatographic purification or in a semi purified state can be subjected to IR spectrum analysis for construal of function groups or quality assurance fingerprints. Most of these instrumental analysis are high cost oriented and beyond the extend of most Indian students for multiple shots of a single samples in case of research need demands, despite that FT-IR could facilitate out compounds asserting the biotic activity of the fraction.

In this review paper an attempt has been made to illustrate the assisting IR spectra of purified compounds and their predictive chemical constituents of two aquatic macrophytes viz; *Vallisneria spiralis* (Hydrocharitaceae) and *Ipomoea aquatic* (convolvulaceae). Briefing about the plants - *Vallisneria spiralis* L. is a common submerged rooted macrophytes found in many wetlands, shallow ponds, lakes, marshes and streams of West Bengal. It is an invasive colonizer and of immense significance to maintaining an aquarium. *Ipomoea aquatica* Forsk (Water Spinach) is a commonly consumed leafy vegetable. It usually grows in wild areas in wetlands and confined water bodies. Well known for dermal medical relevance and a source of antioxidants.

Experimental***Seclusion of target compounds***

Vallisneria leaves were endured to influx extraction process plunged for chemical leaching in glass jars with perforated lids in increasing ratios from 0%, 20%, 40% and 80% ethanol using milipore water. The amount of solvent added was in the ratio of 10:1 with respect to the fresh weight of the plants. The jars were kept in room temperature with sufficient sunlight initially for a span of not more than three days to prevent auto toxicity resulting into decolouration and foul odour. The crude extracts of the four fractions were collected and concentrated to dryness by rota vap and subjected to the following biochemical analysis. The fourth fraction was found to contain highest antioxidants with a resonance with phenol curves. Henceforth the compound was ideally isolated using solvent system of 1% methanol in ethyl acetate by column chromatography using silica gel (100-230).The samples upon room

temperature retention for a week shaped as crystals which could be isolated with chloroform: methanol washes and finally flushed with toluene. Mass spectrum depicts molecular weight of the isolated compound to be m/z (%) = 359 $[M]^+$ [1, 2, 3].

For *Ipomoea aquatica*, following the preparative steps of sterilized wash, around 5kg leaves were processed by shade dry and powered to obtain a dry weight of approximately 1.7kg. It was soaked in methanol which was recovered after 24hours, concentrated in rota vap at 40°C. The fraction was mixed with equal proportion of methanol: water for liquid extraction and mixed with 250 ml hexane to obtain the hexane fraction. The presence of both methanol and water in the same chemical ambience is necessary for extraction of hexane or other solvents with low polarity index. The chloroform fraction was obtained from the aqueous fraction after methanol was evaporated. It was dried using anhydrous sodium sulphate. The ethyl acetate fraction was obtained in the similar manner. The methanol and the aqueous fraction were made free from non polar compounds and the aqueous fraction upon repetitive acetone precipitation [4] was almost pure and retained in freezer which was later purified by Pet. Ether: chloroform wash [5, 6]. The chloroform fraction was column purified with increasing percentage of methanol and along with the aqueous fraction subjected to IR spectrometric reading prepared in KBr plates under high pressure (solids) without absorptions from mulling agent. The FT-IR analysis was done partially from IICB, Kolkata and Kalyani University, Nadia.

Infra-red spectrum Interpretations

Infrared spectroscopy deals with the interaction of infrared light with theme. Matter of fact, the energy of infrared photon can be calculated using the Planck energy relation and Wave equation which states energy is directly proportional to frequency and wave number. A molecule absorbs infrared radiation when the vibration of atoms in the molecule produces waver electric field with the same frequency as the frequency of incident IR radiations. All of these motions can be described in terms of two basic types of molecular vibrations - stretching and bending. Stretch has higher energy requirement than bending and hence corresponds to higher wave number in a spectrum. However, the energy of the stretching mode is inversely proportional to the mass of the atoms which shifts the bands towards the lower wavelengths and as well dependent on its hybridization in the order of $sp > sp^2 > sp^3$. The Hooke's Law model for bond stretching frequencies helps us to understand various modes; it is useful to compare a vibrating bond to the physical model of a vibrating spring system. "The spring system as described by Hooke's Law is a good working approximation". The stronger the bonds the higher are the corresponding frequency. A stretch mode can be symmetric (ν_s) or asymmetric (ν_{as}) of which usually asymmetric stretch corresponds to higher energy than symmetric. Bending can occur in the plane of the molecule (scissoring, rocking) or out of plane (wig-wag and twisting). The number of basic stretching and bending modes also increases with the number of atoms in the molecule. For non-linear molecules $3N-6$ ($2N-5$ bending, $N-1$ stretching) vibrations are observed; hence for linear its $3N-5$ number of modes [7]. In Fourier transform of the Infra red Spectroscopy (FT-IR) converts the raw data in readable sample spectrum [8]. A primary requisite for this analysis must contain vivid sequence of the peaks corresponding to exact wavenumber from the spectrum (integer), the intensities (w/m/s/br) and scanning two prime regions: Functional group region, Fingerprint regions and Bohlmann bands for amines. The functional group region runs from 4000 cm^{-1} to 1450 cm^{-1} , and the fingerprint region from 1450 cm^{-1} to 500 cm^{-1} [9]. The functional group contains fewer peaks and is coupled with stretching mode of the atoms and the finger print region is much complicated and outlines the bending vibrations which are unique to every compound. Elucidation of the complete chemical structure is a long winded method and requires sophisticated instrumentations or financial aspects. In contrary, the aim of detailed IR spectral study is for forecasting of natural compounds which could further be compared with standard spectral database or even serve as attribute factor for unknown compounds [10, 11].

Results and Interpretations

Ipomoea aquatica Methanol fraction

Description: Compound status: Aggregate of white prismatic crystals.

The Infra Red spectra seem to be of a much pure compound (purity < 90%) (Figure1). There is a sp^3 C-H stretching at 2929 cm^{-1} . This is an alkane ($\nu_{as}\text{CH}_2$) stretching mode with medium strength. 3000 cm^{-1} can be assigned as a bar liner. Similar stretching mode would be absorbed left to 3000 cm^{-1} at around $3200\text{ cm}^{-1} - 3000\text{ cm}^{-1}$ if it would be of alkenes/aromatics. The narrow sharp peak at 3395 cm^{-1} corresponds to hydrogen bonded hydroxyl moiety. In this case

there is not much trough in the hydroxyl band. The prominent and sharp peak of non-conjugated C=O band appears at 1750 cm^{-1} . The corresponding C-O-H peak appears 1450 cm^{-1} . The compound is an ester group derivative (Table 1) and probable to be in monomeric state [12, 13].

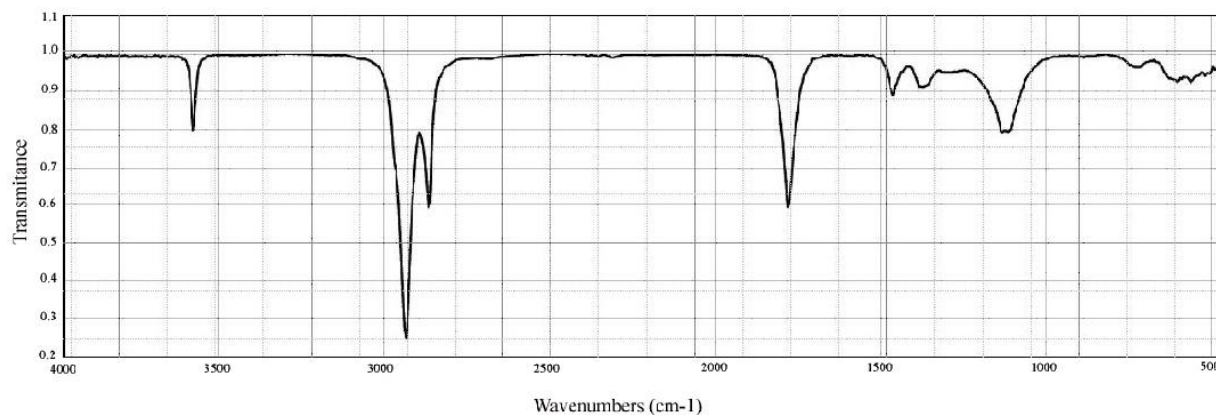


Figure 1 IR spectra of Methanol Fraction of *Ipomoea* leaves

Table 1 Peak Value with Corresponding Functional groups of Methanol Fraction of *Ipomoea* leaves

SL. No.	Peak value (cm^{-1})	Chemical groups
1	3385	OH
2	2929	C-H
3	2343	C-H
4	1750	C=O
5	1450	C-O-H
6	1382	C-O

Ipomoea aquatica Water fraction

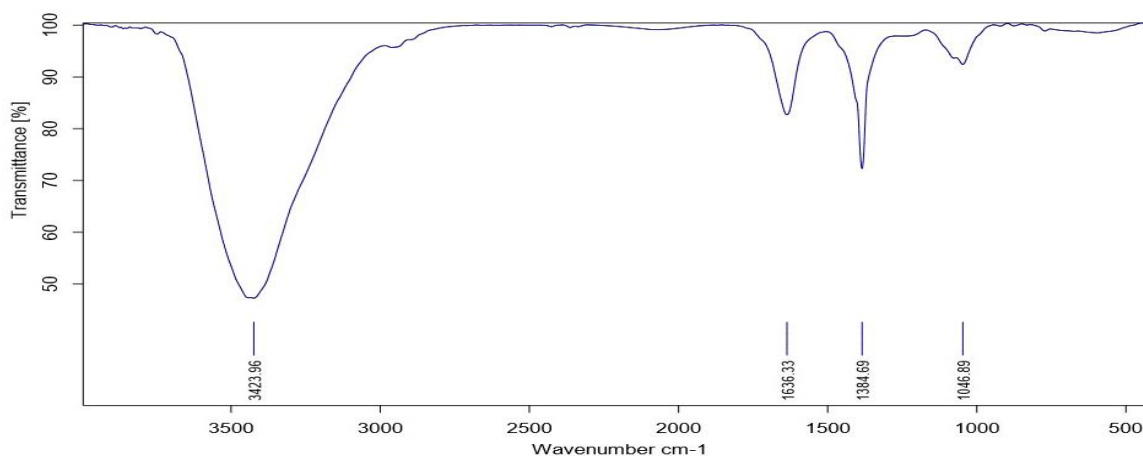


Figure 2 IR spectra of Water Fraction of *Ipomoea* leaves

Table 2 Peak Value with Corresponding Functional groups of Water fraction of *Ipomoea* leaves

SL. No.	Peak value (cm^{-1})	Chemical groups
1	3423.96	OH
2	1636.33	Presence of water molecules.
3	1384.69	C-H (bending/scissoring)
4	1046.89	C-O stretch

Description: Compound status: Aggregate of white cubic crystals.

The broad and strong peak at 3423.96 corresponds to free hydroxyl group (Figure 2). The compound is of low molecular weight and probable to be inorganic salt lacking functional groups. Due its hydrophilic nature it absorbed water in room temperature which gave a peak at 1636 cm^{-1} . The spectrum is see-through in the $1480\text{-}1850\text{ cm}^{-1}$ region and $3500\text{-}3000\text{ cm}^{-1}$ region indicating absence of C=C and C-heteroatom (Table 2). The IR spectrum therefore indicates the compound to be of aliphatic [14, 15]

Vallisneria spiralis Ethanol - Water fraction

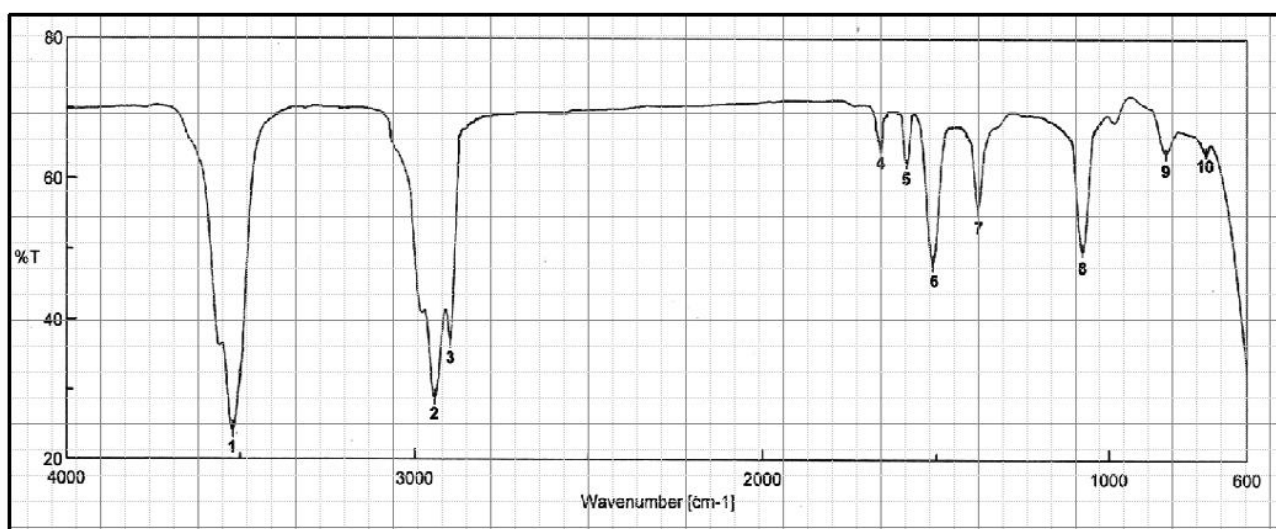


Figure 3 IR spectra of Ethanol Fraction of *Vallisneria* leaves

Table 3 Peak Value with Corresponding Functional groups of Ethanol fraction of *Vallisneria* leaves

SL No.	Peak value (cm^{-1})	Chemical group
1	3642.10	O-H stretching (free hydroxyl)
2	2965.43	C-H stretching
3	2928.54	C-H stretching
4	1665.22	C=O bending
5	1600.78	C=C stretching
6	1554.80	C=C bending of aromatic ring
7	1360.00	S=O, sym
8	1080.04	C-O
9	1076.89	C-O
10	779.64	C-H bending
11	675.16	C-Cl stretching

Description: Compound status: Aggregate of white cubic crystals.

The absorption spectrum of the 80% ethanol fraction shows ten major bands; the band at 3642.10 cm^{-1} corresponds to broad hydroxyl group band usually free hydroxyls. 2965.43 cm^{-1} and 2928.54 cm^{-1} represents the sp^3 C-H stretching with corresponding bending at 1432 cm^{-1} . The other dominating bands at 1665.22 cm^{-1} and 1554.80 cm^{-1} are those of carbonyls and aromatic compounds. The presence of aromatic compound is further confirmed at 779.64 cm^{-1} with C-H stretching. The S=O bond signifies the presence of the sulfoxides (Figure 3 & Table 3)[16, 17].

A review of Bioassay

These are the bioassay [18-20] done against most troubleshoot dermatitis fungi *Malassezia globosa* (Figure 4)

- The left figure is the inhibition zone assay by 80% ethanol extract of *Vallisneria* leaves. It displays an early zone of inhibition at 100 μ g/ml with a noteworthy zone at 1000 μ g/ml. The MIC was found to be at 156.25 μ g/ml and MFC at 5000 μ g/ml.
- The right figure is a non-monotonic dose response curve plotted for concentration of the water fraction of the *Ipomoea* leaves against inhibition zone diameter with MIC at 625 μ g/ml and MFC at 5000 μ g/ml.

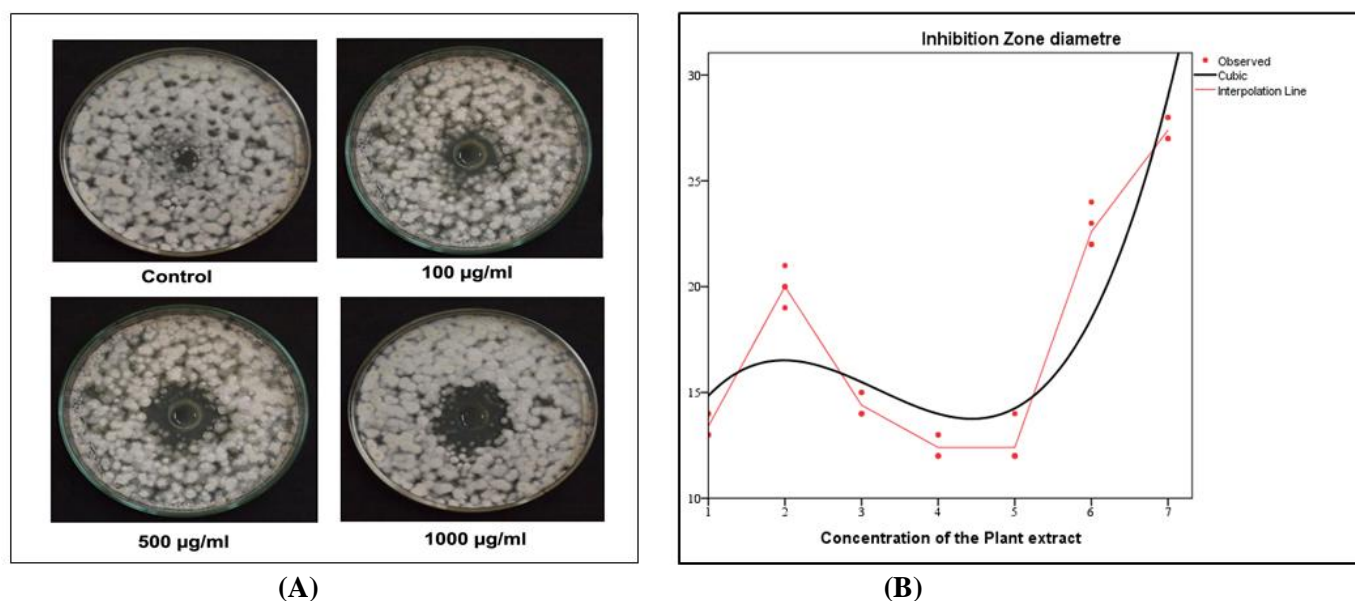


Figure 4: (A): Inhibition Zone activity of *Vallisneria* leaf extract at four different concentrations on *Malassezia globosa*.

(B): Non-Monotonic Dose Response Curve.

Conclusion

A mere diverse and versatile approach for interpretation of structure peak relationship in Infra-red spectroscopy could be accomplished by recognizing characteristic shapes and patterns or different modes of vibration within the spectrum, and by applying the information obtained from authentic database along with other attributes of the sample. Identification of the chemical groups of phytochemical compounds present in plants provides some information on the different functional groups accountable for their pharmacological traits. The presence of aromatic compound at 779.64 cm^{-1} in *Vallisneria* leaves with C-H stretching which if in case of flavonoids, corresponds to the first aromatic ring. The S=O bond includes compounds which shows radical trapping antioxidant property and act as antimicrobial, antiparasitic and antitumor agents. The FTIR analysis of methanolic and aqueous leaf extracts of *Ipomoea* revealed the presence of phenolic compounds, flavonoids and esters as major functional groups which are indeed present in broad spectrum antimicrobial compounds [21].

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Phyto-Chemical, Antibacterial and Brine Shrimp Toxicity Studies of Green Banana Leaves

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Abstract Green banana or cooking banana plants have been of great importance to man, serving diverse needs from food, culture and religion. The biochemical, antimicrobial and cytotoxicity activity of the ethyl acetate fraction of the dried leaf powder of cooking banana is evaluated following fractionation, soxhlet extraction and chromatography. The TPC, TQC and TTC content of the fraction is recorded as 12.85 GAE mg/g, 6.91 QE mg/g and 2.74 mg/g of the dry weight respectively which is comparatively higher than the preceding chloroform fraction. The MIC of the fraction against gram negative anaerobic bacteria *E. tarda* is recorded at 625 µg/ml and radical scavenging strength with IC₅₀ at 0.055 mg/ml with AAI of 0.0173. The cytotoxicity assay of the ethyl acetate fraction was examined on brine shrimp nauplii hatched from 250 µm cysts under suitable laboratory conditions with PHR at 92%. The threshold percent mortality was calculated from probit logistic regression analysis with LC₅₀ at 0.845 µg/ml at 6 h exposure. The

normal distribution of the data is assured by Shapiro Wilk significance.

Keywords *Musa paradisiaca*, *Edwardsiella tarda*, DPPH, IC₅₀, LC₅₀

Introduction

There is a growing concept that population and ecology complement each other but this complementation is greatly hampered by use of precarious chemicals and microcidal, insecticidal and numerous synthetic chemicals (Wang et al. 2009). Demands for high yield and numerous pathogenic outbreaks have instigated the fields of aquaculture and agriculture to depend extensively on synthetic chemicals, a large portion of which reaches the non-target system. In view of this concept, allelopathy can be used as an efficient bio-intensive tool to address ecosystem level aspects. Allelopathy is the study of allelochemicals which are low molecular weight compounds mostly produced as secondary metabolites of plants which they exude to the environment. The phyto-antimicrobial resources exemplify a wide range of plant secondary metabolites (Tamokou et al. 2011, Gatsing et al. 2009, Al-Mariri 2008) asserting diverse effects from extreme deadly to immense beneficial. The plant products may be phyto-anticipins or phyto-alexins (Reuben et al. 2008, Al-Bayati 2009). Though finding therapeutics in plants is an ancient idea but in most cases lack defined protocol for formulations and quality control constrains scientific validation. With this

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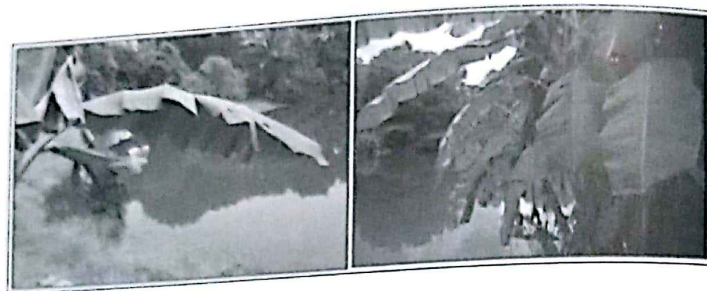


Fig. 1. *Musa paradisiaca* in natural habitat.

state of fact an attempt has been initiated to study the antimicrobial activity of banana leaves against fish pathogens followed by cytotoxicity effect (Fabrega et al. 2011). A very frequent sight which we come across most of the waterbodies is rows of banana plants (Fig. 1) growing contiguously, with leaves drooping down to water. *Musa* or banana is an exotic plant with its origin in the tropics of South Eastern Asia. They are large woodless flowering plants belonging to the Musaceae family (Bilba et al. 2007). Amongst 100 other species, *Musa paradisiaca* is the most widely cultivated alkaloid rich vegetable banana or green banana owing to its high iron content of 0.33 mg/100 mg of banana (Garcia et al. 2015). There are numerous articles on ethno pharmaceuticals (González-Lamothe et al. 2009) of banana fruits, flowers and stem on human health management (Aysal et al. 2007; Kanrar et al. 2010). In this study we worked on the biochemical and antimicrobial activity of the leaf extract of the cooking banana against fish pathogen and their cytotoxicity on brine shrimp nauplii.

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Materials and Methods

Collection and processing of the plant sample

The banana leaves approximately collected 3.5 kg from both terrestrial and flood plains. Samples carried to laboratory in sterile polypropylene upon washing. The leaves were cleansed thoroughly with double distilled water in the laboratory and lyophilised. Following the preparative steps, the leaves were powdered using electrical mixer to obtain a dry weight of approximately 800 g. The dried leaves were processed preferably within 24 h.

Extraction and isolation of active fraction

500 ml–800 ml of fresh solvents are preferred for the process. The leaf powder is loaded in the soxhlet at least an inch below the siphon tube to prevent its

Table 1. Solvents / Solvent system used in column chromatography.

Fraction No.	Solvents/ Solvent System	Ratio	Quantity (ml)
F ₁	Petroleum ether	—	250
F ₂	Pet ether : Chlo	7 : 3	500
F ₃	Chloroform	—	300
F ₄	Ethyl acetate	—	250
F ₅	Acetone : Water	7 : 3	300
F ₆	Ethanol : Water	7 : 3	250
F ₇	Ethanol : Water	3 : 7	—

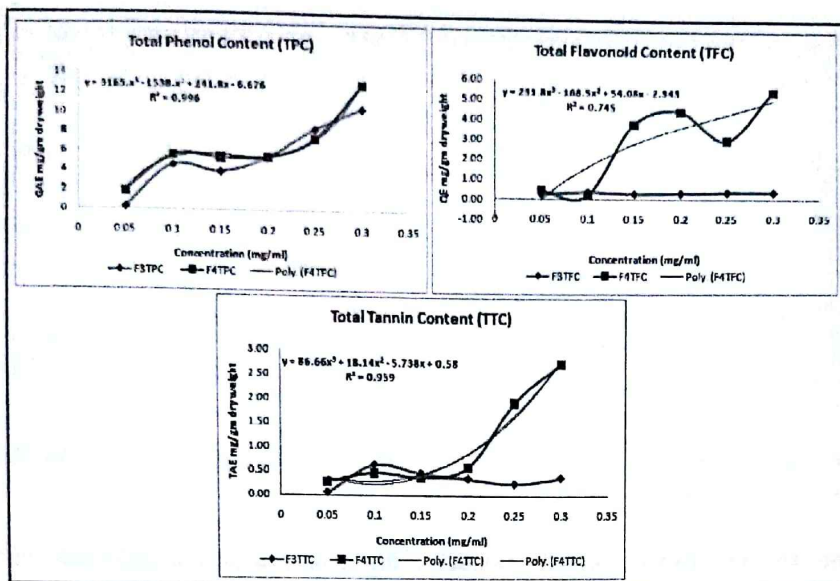
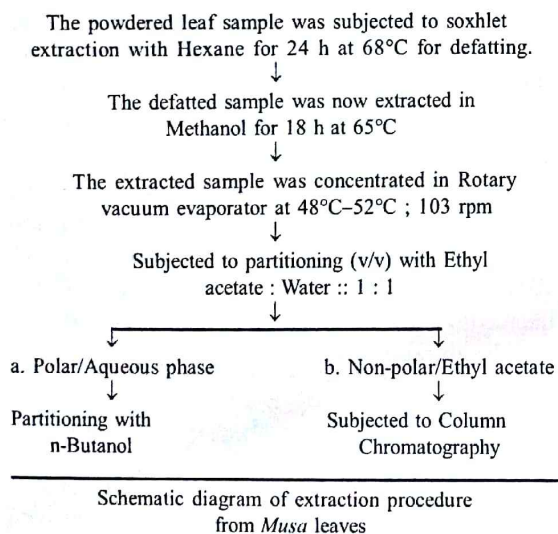


Fig. 2. Graphical representation of TPC, TFC and TTC of the F_3 and F_4 fraction of *Musa* leaves.

entry and blockage of solvent refluxing. The refluxing is done until there remains no deposit in the solvent that is being refluxed. Moisture removal is done by sodium sulfate. The process in brief is schemed below.



Chromatography

Column Chromatography

Glass columns (50 × 15 cm²) are tightly packed with

Silica gel with three different mesh sizes (69–120 initially and latter with 230–400) upto 30 cm. The sample coated in activated Silica gel is dried and poured into the column. Solvents / Solvent system with various combinatorial proportions following the elutropic series was added which is as charted below (Table 1). The selection of suitable solvent system depends much on the target compound to be eluted.

Thin Layer Chromatography (TLC)

Each column fraction is subjected to thin layer chromatography. The standardization of the Retention factor (Rf) value and the ideal solvent system is done using pre-coated TLC plated on aluminium sheets Silica gel F_{254} with binder and latter the isolation of the sample is carried on preparative TLC with glass plates (20 × 20 cm²) layered with slurry of uniformly homogenized Silica gel G and acetone with 1mm thickness with an applicator. The coated plates are allowed to dry at room temperature for 24 h. Thereafter, on the day of work the plates are activated in hot air oven at 110°C. Usually 10–15 µl of the fraction is spotted on the plates using a glass capillary. The loaded plated are placed in a glass chambers of (25 × 100 cm²) pre-saturated solvents. The spots are allowed to develop at a maximum height of 19 cm and minimum 15 cm

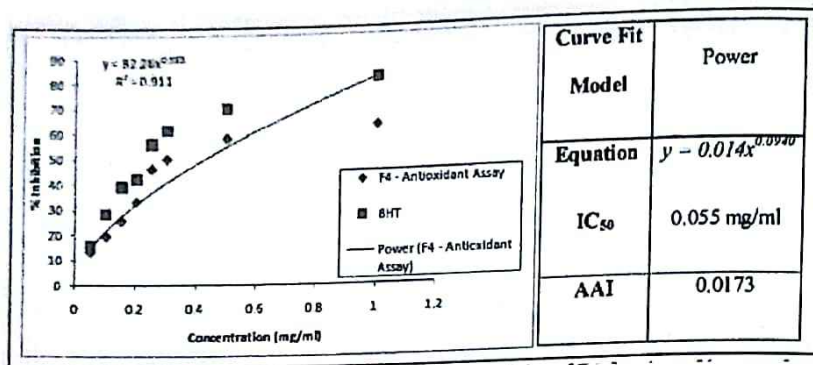


Fig. 3. Graphical representation of the antioxidant activity of F_4 fraction of leaves of *Musa paradisiaca*. Tabulated : Curve fit model, Equation, IC₅₀ and Antioxidant Activity Index (AAI) of the antioxidant activity of F_4 fraction.

depending upon the number of compounds the fraction holds. Finally, the chromatographed plates are put in iodine chamber or observed under UV to detect fluorescent spots.

Biochemical analysis

The biochemical parameters of a plant extract helps asserting its bioactivity and prediction of functional groups. The role of the fraction on plant defence or fragrance or strong taste can be holistically be concluded by observing the biochemical composition (Shad et al. 2013). In this study we have worked on three primary biochemical parameters.

Total Phenol Content (TPC)

The total phenolic content was determined following Folin-Ciocalteu method (Baba and Malik 2015) using 0.1 ml of the extract with a concentration range of 0.05–0.3 mg/ml of the leaf leachate. The extracts were mixed with Folin–Ciocalteu reagent (Phosphomolybdate and Phosphotungstate) and sodium carbonate (Na_2CO_3) which forms an alkaline solution. A blue chromophore of Folin–Ciocalteu complex is formed following incubation for 30 mins at room temperature. The change of color was measured in spectrophotometer with absorbance reading at 765 nm. Gallic acid in the same concentration as the sample was used as positive control. The total phenol content was expressed as GAE in milligram per gram of

dry material using the calibration curve, where X was the absorbance and Y was GAE (mg/g).

Total Flavonoid Content (TFC)

Flavonoid estimation was carried out following the method (Agati et al. 2012). The preferred concentration range for the leaf leachates were 0.2–1.2 mg/ml with 0.1 ml of the extract. Later the extract was added with 1.2 ml distilled water, 0.12 ml of 5% sodium nitrite (NaNO_2) with uniform intermixing. Following incubation for 5 minutes at 25°C temperatures the nitrication is enabled in acid labile medium using 0.12 ml of 10% AlCl_3 solution. The tubes were further incubated at room temperature for 5 minutes and added with 0.8 ml of 1 mm sodium hydroxide (NaOH) solution and 1.16 ml of distilled water. The absorbance was measured at 510 nm. Methodically, quercetin in the same concentration as the sample was used as positive control. Total flavonoids content as calculated as quercetin (mg/g) using the calibration curve, where X was the absorbance and Y was QE (mg/g).

Total Tannin Content (TTC)

The total tannins content using tannic acid as standard curve. Briefly, 200 μl of extracts of 0.05–0.3 mg/ml was mixed with 200 μl of ferric ammonium citrate (0.35%) prepared freshly and 200 μl of ammonia (0.8%). The change of color of Iron–Tannate complex was measured at 525 nm and expressed as TAE mg of per gram of extracts or fractions.

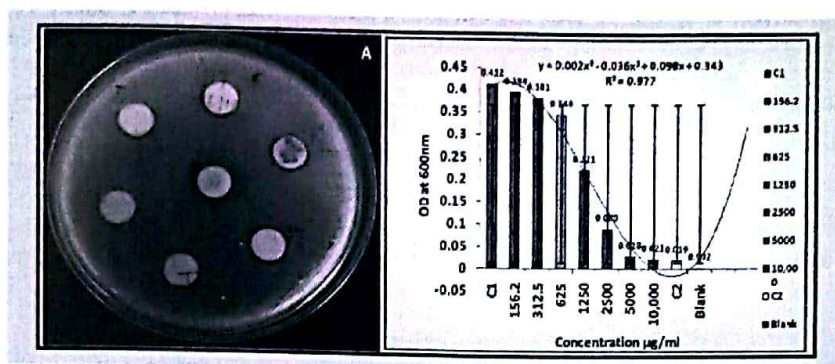


Fig. 4. A : Disc diffusion assay against *E. tarda* ; B : Graphical representation MIC and MBC of *E. tarda*.

Antioxidant assay

DPPH radical scavenging activity

The free radical scavenging activity of extracts and fractions for the radical DPPH was measured as described (Gulcin 2012). Freshly prepared DPPH solution 0.004% (w/v) in methanol was prepared and 3.9 ml of this solution was mixed with 0.1 ml of extract in methanol containing different concentration range (0.05–1 mg/ml) of the extract. After an incubation of 30 minutes, the absorbance was measured at 517 nm using spectrophotometer. The DPPH solution was assured with an absorbance of 0.50–0.60 at 517 nm. Butylated Hydroxy Toluene (BHT) in the same concentration as the sample was used as positive control. The capability to scavenge the DPPH radical was calculated using the following equation :

$$\text{DPPH radical scavenging activity (\%)} = \left\{ \frac{A_c - A_s}{A_c} \right\} \times 100$$

Where A_c is the absorbance of the blank reaction and A_s is the absorbance in presence of the sample of the extracts. IC_{50} which defines the concentration of the plant extract that's needed to scavenge 50% of the radical present was calculated by the following equation.

$$IC_{50} = \left\{ \frac{\text{Percentage inhibition}}{\text{Concentration of the sample}} \right\} \times 50$$

A second parameter for the antioxidant activity (Arteaga Figueroa et al. 2014) is expressed as the an-

tioxidant activity index (AAI) which is formulated as :

$$\text{AAI} = \frac{\text{Final concentration of DPPH } (\mu\text{g.ml}^{-1})}{\text{in the control sample} / IC_{50} \mu\text{g.ml}^{-1}}$$

Microbiological screening

The plant fractions were subjected to antimicrobial assay against *Edwardsiella tarda* and *Streptococcus aureus*. The bacterial strains were confirmed using 16S rRNA sequencing at molecular level. [Primers- 16S univ 5'-GAG TTT GAT CCT GGC TCA G-3' 27f, 5'-TAC GGT TAC CTT GTT ACG AC-3' 1492r]. Single pure colony was confirmed in R²A medium. Antimicrobial activities of different extracts were

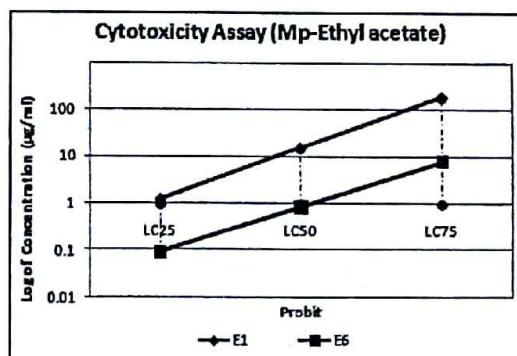


Fig. 5. Graphical representation of LC_{25} , LC_{50} and LC_{75} against log of concentration for *M. paradiasiaca* at 1 h, 6 h and 12 h exposure time of brine shrimp lethality assay of Ethyl Acetate fraction.

Table 2. Statistical analysis of antioxidant assay and cytotoxicity assay ; Test of normality : Shapiro Wilk and Kolmogorov-Smirnov test and probit analysis of F_4 (Ethyl acetate fraction) of *M. paradisiaca* on brine shrimp lethality at 1 h and 6 h exposure time.

Model summary							
		R	R. square	Adjusted R square		Std. error of the estimate	
		0.953	0.908	0.893		0.179	
The independent variable is concentration							
Anova							
		Sum of squares	df	Mean square	F	Sig.	
Regression		1.907	1	1.907	59.449	0.000	
Residual		0.192	6	0.032			
Total		2.099	7				
The independent variable is concentration							
Correlations							
		percentage of inhibition		concentration		BHT as positive control	
percentage of inhibition	Pearson correlation	1		0.925**		0.948**	
	Sig. (2-tailed)			0.001		0.000	
	N	8		8		8	
Concentration	Pearson correlation	0.925**		1		0.872**	
	Sig. (2-tailed)	0.001				0.005	
	N	8		8		8	
BHT as positive control	Pearson correlation	0.948**		0.872**		1	
	Sig. (2-tailed)	0.000		0.005			
	N	8		8		8	
**Correlation is significant at the 0.01 level (2-tailed).							
Tests of normality							
		Kolmogorov-Smimov ^a			Shapiro Wilk		
Time	1 h- <i>Musa</i>	Statistic	df	Sig.	Statistic	df	Sig.
Mortality	1	0.192	5	0.200*	0.949	5	0.730
*. This is a lower bound of the true significance.							
a. Lilliefors Significance Correction							
Tests of normality							
		Kolmogorov-Smimov ^a			Shapiro Wilk		
Time	6 h- <i>Musa</i>	Statistic	df	Sig.	Statistic	df	Sig.
Mortality	6	0.223	5	0.200*	0.879	5	0.304
*. This is a lower bound of the true significance.							
a. Lilliefors Significance Correction							

Table 2. Continued.

Time 1 h	Parameter	Parameter estimates				95% Confidence interval	
		Estimate	Std. error	Z	Sig.	Lower bound	Upper bound
PROBIT*	Concentration	0.620	0.054	11.533	0.000	0.515	0.726
	intercept	-0.730	0.107	-	6.809	0.000	-
.837	-	.622					

a. PROBIT model $PROBIT(p) = Intercept + B \times (Covariates \times \text{are transformed using the base 10.000 logarithm})$

Chi-Square tests

Parameter	Chi-Square	df	Sig.
PROBIT Pearson goodness-of-fit test	9.336	3	0.025*

- a. Since the significance level is less than .150, a heterogeneity factor is used in the calculation of confidence limits.
 b. Statistics based on individual cases differ from statistics based on aggregated cases.

Parameter estimates

Time 6 h	Parameter	Parameter estimates				95% Confidence Interval	
		Estimate	Std. error	Z	Sig.	Lower bound	Upper bound
PROBIT*	Concentration	0.699	0.83	8.453	0.000	0.537	0.861
	Intercept	0.051	0.111	0.460	0.646	0.060	0.162

- a. PROBIT model $PROBIT(p) = Intercept + B \times (Covariates \times \text{are transformed using the base 10.000 logarithm})$

Chi-Square tests

Parameter	Chi-Square	df*	Sig.
PROBIT Pearson goodness-of-fit test	3.518	3	0.318*

- a. Since the significance level is greater than, 150, no heterogeneity factor is used in the calculation of confidence limits.
 b. Statistics based on individual cases differ from statistics based on aggregated cases.

evaluated by the agar well diffusion method with little modification and Minimum inhibitory concentration (Verma et al. 2012).

Sample and media preparation

The sample was concentrated to dryness and re-suspended in 1% DMSO. A dilution range of 1000 µg/ml to 1 µg/ml ($T_1 - T_6$) was prepared with 1% DMSO as control. The 18 h old bacterial culture grown in Muller Hilton Broth (MHB) was transferred to 0.85% saline solution and the turbidity adjusted to 0.5 McFarland using a turbidimeter to ensure 5×10^8 CFU/ml. Barium

sulfate was used as standard. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined by serial broth dilution assay (Coulidiati et al. 2011).

Disc diffusion method

The disc diffusion method was used to determine the antibacterial activity. The Muller Hilton Agar (MHA) plates were swabbed (sterile cotton swabs) with 50 µl of 0.5 McFarland adjusted culture. The plate extracts were adsorbed in the 10 mm cellulose sterile disc with 20 µl of sample placed in the plate and

allowed to stand in room temperature for 45 minutes along with the control plates. The plates were incubated at 35°C for 36–48 h. The diameter of the inhibition zone (mm) was measured.

Minimal Inhibitory Concentration (MIC) and Minimal Bacterial Concentration (MBC)

Broth dilution method was preferred to perform the MIC assay. The dilutions were chosen depending on the results of the disc diffusion assay (Sadeghian et al. 2011). 1 ml of broth was transferred in all the nine tubes ranging from 10000 µg/ml to 156 µg/ml of the extract and two controls one without the inoculum and the other without extract was maintained for experimental assurance. 20 µl of the inoculums was transferred from saline solution to Muller Hilton Broth (MHB) for clear reading and incubated for fifteen minutes. The extract and 5 µl of the inoculums was added all the eight tubes. The tubes were incubated in 35°C for 18 h and the OD reading was recorded at 600 nm with broth as the blank (Coulidiati et al. 2011).

Cytotoxicity assay

Brine Shrimp lethality Test (BLT)

The safety of the applicable plant sample in an ecosystem is assured by *in vitro* eco-toxicity assay brine shrimps (*Artemia salina*) are small crustaceans which are capable of laying dormant eggs also known as cysts. Brine shrimp lethality test is a quantal toxicity assay useful and significant especially when the target ecosystem is a continuous resource variable-water. We have tested our experimental compounds on the nauplii stage of the shrimps (Ghosh et al. 2015). The BLT procedure is simple, rapid and above all requires a very small amount of the compound. Brine shrimp (*Artemia salina*) cysts were obtained from Jadavpur University, Kolkata West Bengal. The plant sample was diluted for five concentration range from 10⁴ ppm – 1 ppm with DMSO as control. The eggs were hatched in artificial sea water prepared by 38% of sea salt with salinity 25–32 ppt. The light intensity of 1200 lumen with a partition for dark (covered) and light effects for a period of 36 h and pH of 7.5–8.0 was used. Once hatched the nauplii were attracted towards light. 10 shrimps were added in each of the dilutions.

After 24 h the mortality of the shrimps were recorded in each dilutions (Syahmi et al. 2010). The PHR (Percent Hatching Rate) was approximately 92%. The % mortality of hatched nauplii were converted to probit and plotted against concentration to obtain the LC₅₀ and LC₇₅ to assess the toxicity curve of the sample.

Results and Discussion

Extraction and isolation of active fraction

The solvents/solvent systems used for column chromatography and the procured quantity are tabulated in Table 1. TLC was done with three different solvent systems. (a) Pet ether: Chloroform :: 5:5; (b) Hexane: Ethyl acetate :: 7:3 and (c) Acetone : Methanol:: (9.9:0.1. Rf of approx. 0.74 was calculated.

Biochemical screening

Total Phenol Content (TPC)

Phenol content was expressed as Gallic acid equivalents (GAE) in mg/g dry weight. The experimental data exhibits (Fig. 2) there was no proportionate increase along the concentration gradient with a sudden fall at 0.15 mg/ml with 5.44 GAE mg/g and at 0.20 mg/ml with 5.41 GAE with the highest noted at 0.3 mg/ml with 12.85 GAE mg/g of (0.05–0.3 mg/ml) extracts concentration. The result is validated with the coefficient of determination ($R^2 = 0.963$) which reads the data as best fit along the regression line.

Total Flavonoid Content (TFC)

The total flavonoid content was expressed as Quercetin equivalents (QE) in mg/g fresh weight. The flavonoid content showed a consistent data (Fig. 1) evenly throughout the concentration range. It increased throughly with raise in concentration and 1.2 mg/ml extract had the highest flavonoid content with 6.91 mg/gm QE. Presence of flavonoid may result as alkaloids and glycosides in the plant extract. The R^2 value 0.935 was found to be significant and defines the goodness of fit of the data along the regression line.

Total Tannin Content (TTC)

Tannic acid equivalents (TAE) in mg/g dry weight is preferred as a yard stick for the progress of tannin. The highest tannin content was recorded at 0.3 mg/ml of the leaf extract with 2.74 mg/g TAE (Fig. 2). The strength of the response variables and the model was found to be significant with $R^2 = 0.959$. Considering the above results, F_3 and F_4 fractions were further worked upon for antioxidant assay and bioactivity.

Antioxidant activity

DPPH Radical Scavenging Assay

DPPH assay (Fig. 3) was performed to evaluate the antioxidant activity. Following a similar trend with phenols, the highest was recorded at 0.3 mg/ml. The positive control BHT recorded the highest percent inhibition as 35.6 at 0.3 mg/ml concentration.

Microbiological screening

The diameter of the zone of inhibition is tabulated below. The ethyl acetate fraction of *M. paradisiaca* showed appreciable inhibition on *Edwardsiella tarda* (Fig. 4) but showed very negligible impact on *Streptococcus aureus*. Hence MIC and MBC (Fig. 4) was proceeded only with *E. tarda*. 1250 μg was found to be the MIC because turbidity appeared till 625 μg OD was taken at 600 nm. Following which four concentrations 1250 μg , 2500 μg , 5000 μg , 10,000 μg were plated and kept in incubator at 37°C for 24 h / 48 h. 5000 μg was found to be the MBC as no growth appeared at 5000 μg and 10,000 μg .

Cytotoxicity assay

Brine Shrimp lethality Test

M. paradisiaca showed uniformity with concentration dependent mortality. However graphical re-presentation showed the 1 h exposure time to be a better fit model than 6 h time interval. The LC_{50} for 6 h was 0.845 $\mu\text{g}/\text{ml}$ and that of 1 h was 15.005 $\mu\text{g}/\text{ml}$. Nevertheless the extract showed a toxicity effect beyond 6 h. This suggested that the fraction could contain cytotoxicity compounds (Fig. 5).

Statistical analysis

The statistical analysis of the antioxidant assay and brine shrimp lethality test is evaluated to evaluate the experimental data considered for conclusive comparison by IBM SPSS 20. The relationship graph for % scavenging distributed through a range a concentration is statistically evaluated below where the shrunken R^2 read at 0.908 with standard error at 0.179 consequently the accuracy of prediction with the regression model is high with significant difference between the group means. There is significant correlation between scavenging activity of the plant sample and the positive control following concentration incline (Table 2). For cytotoxicity test, the normal distribution of the dependent variable is displayed by the stem and leaf model of Shapiro-Wilk significance test of 0.730 and 0.304 for 1 h and 6 h respectively ($p > 0.05$) which confirmed its normal distribution. Henceforth, probit analysis for normal distribution was persuaded. However chi-square test with $p < 0.05$ displayed the 1 h exposure time to be a better curve fit model than 6 h time exposure (Table 2).

The use of chemicals in aquaculture is a very common practise which not only devastates the aquatic health but also accounts for major diseases upon consumption. By virtue of plant based products this chemical could be substituted (Alnamer et al. 2013) in terms of antibacterial for fish and other disinfectants (Volpatti et al. 2014). The ethyl acetate fraction of *M. paradisiaca* contains phenol and antioxidants. The fraction showed inhibitory effect against *E. tarda*, causative for emphysematous putrefactive disease of catfish. The graphs for biochemical and antimicrobial activity (Najiah et al. 2011) follow a non-monotonic power model of data. The compound displays threshold toxicity at 6 h exposure with $LC_{50} < 1 \mu\text{g}/\text{ml}$ beyond which there is complete mortality.

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OP – 04

***Vallisneria spiralis* - Rapid aquatic colonizers to invitro anti dandruff dermatitis and broad spectrum bacteriocide**

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Abstract

Allelopathy is usually regarded as a terrestrial science and little attention has been paid to the allelochemicals involved in aquatic autotrophs. In addition to allelopathy being a key factor for explaining macrophyte assemblages, community structure and the dynamics of the populations within the aqua ecosystem, the pharmaceutical evaluation of the macrophytes is an emerging aspect. In this context, *Vallisneria spiralis* (Hydrocharitaceae) is a perennial stoloniferous species and a key ecological community in freshwater ecosystem. It is commonly used as aquarium plant and inhabits both in lentic and lotic environment of tropical and sub-tropical regions worldwide. Being rapid colonizers of aquatic ecosystem, literature study states the root of macrophytes can excrete special function groups that can absorb, filter and precipitate chemical compound of water and through the auxiliary function of the microorganism, the macrophyte can get the result of purifying water quality. Considering the significance of the roots of *Vallisneria*, in our current study we have extracted the root exudates of *Vallisneria* which has been purified by liquid-liquid extraction process and further crystallized and each fraction subjected to biochemical analysis viz; phenols, flavonoids and tannins followed by antioxidant and antimicrobial activity. The inductive inferences of the various assays showed the methanolic fraction of *Vallisneria* to be a potent tool to ecological management and a natural source containing antidandruff compounds against *Malassezia globosa*- the dandruff causing dermatitis fungus and also bacteriocidal against broad spectrum *Escherichia coli*.

OP-11

Prospecting of *Terminalia travencorensis* Wt. & Arn. for Antiradical efficiency

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Abstract

Resistance of virulent/ pathogenic organisms has once again escalated human reliance & faith on traditional therapies. Focusing this, our work was aimed to explore antiradical efficiency of *Terminalia travencorensis* Wt. & Arn. - Endemic to Idukki, Kerala. Chemical kinetics and mechanism of antioxidant activity is explained by DPPH free radical method.

Keywords: Antiradical efficiency, *Terminalia travencorensis* Wt. & Arn., DPPH free radical method, Idukki-

OP-12

Bioactive Phyto-compounds: facet of Green Chemistry - A review

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Abstract

Producing bioactive compounds seems to be convention than the exception. These natural compounds exert a wide spectrum of effects from acute deadly to intense curative. In India the status of plant bioactivity search and further implication in most cases, is a two faced aspect- bioavailability without convincing scientific confirmation on the contrary. Emphasis is given on some of the phyto-compounds we came across. The 80% ethanol extract of the leaf leachates of *Vallisneria spiralis* L. were found (mol wt. m/z 359) with fungicidal trait against *Trichophyton glabrum* - the dandruff causing dermatitis fungus. The foliar extract of *Musa paradisiaca* (banana) was tested upon on its ethyl acetate extract which showed antibacterial activity against facultative anaerobe catfish pathogen *Edwardsiella tarda*. The aqueous fraction of *Ipomea aquatic* is presently worked upon which has also shown antidandruff properties. Examples of *Eclipta alba* can also be cited for the methanol extract of its root showing vehement microbial inhibition against *Escherichia coli*, *Streptococcus aureus* and *Pseudomonas aeruginosa*. Preliminary phyto-chemical analysis showed the presence of phenols and tannins in most active compounds. Traditional knowledge and smart strategies of isolation, extraction and elucidation of phyto-compounds facilitate discovery of new drug candidates and development of rational synergistic botanical formulations with standardized quality control parameters.

Keywords: Phyto-compounds; *Vallisneria spiralis*; *Musa paradisiaca*; *Eclipta alba*; dandruff; antimicrobial, root extracts and quality control

PP - 18

Studying Ft-Ir Analysis for Curative Traits Of Aquatic Macrophytes - A review

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Abstract

Plant natural product chemistry have ensued numerous pharmaceutical intermediates or drug precursors which are further utilized for the production of synthetic drugs. However lack of quantitative and qualitative tool for incessantly monitoring the potency of the plant drugs, hinders application of formulated herbal products to a greater extend. Molecular structure is the major theme in chemistry and is especially important in organic chemistry. A molecule absorbs a unique set of InfraRed light frequencies and as such every molecule will have its own characteristic spectrum. These are the characteristic function group bands that assert a character to any phyto-compound. The application of FT-IR spectroscopy in crude medicine authentication and quality evaluation is to be emphasized as it is the most convenient tool to sort medicinal plants holistically at molecular level. I have tried to exemplifying the fact with two wetland macrophytes from my work viz; *Vallisneria spiralis* and *Ipomea aquatica*. The sample is to be prepared in 70% ethanol followed by ultrasonic treatment and percolation. Once the IR spectrum is received, the two regions are to studied *functional group region* (4000cm^{-1} to 1450cm^{-1}) and *Finger print region* (1450cm^{-1} to 500cm^{-1}). Finally, the bands of spectra could be interpretative by scanning the sample tablet immediately.

Key Words: FT-IR; Functional group; Finger print region; macrophytes; medicinal property.

Toxicity assessment of polar and non-polar phyto-chemicals: brine shrimp lethality assay

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ENV 23

The toxicity of phyto-chemicals is an important determiner for its deliberate utilization in an ecosystem. In this study, the bioactive allelochemicals extracted and column purified from three aquatic macrophytes, exhibiting in-vitro antimicrobial trait against fish pathogens, is assayed for brine shrimp (*Artemia salina*) toxicity test. The relative mortality of the freshly hatched nauplii in brine solution containing phyto-chemicals in the concentration range $1\mu\text{g/ml}$ to $10^4\mu\text{g/ml}$ is statistically plotted to obtain the lethal concentrations LC_{25} , LC_{50} and LC_{75} . In addition to lethality, the percent hatching rate in each concentration is also considered as a bench mark keeping all other hatching factor constant. The readings for every 6hr until 24hrs are measured in which the 18hr phenomenon is seen prominent for changing trend line of the graph. The non-polar fractions viz; Hexane and Pet Ether fraction are found to be most toxic and polar fractions viz; methanol and aqueous fractions are least. The most toxic fraction was that of ethyl acetate fraction of *Vallisneria spiralis* at 0hrs interval with LC_{50} at $0.407\mu\text{g/ml}$ and least toxic of that of aqueous fraction of *Ipomoea aquatica* of $LC_{50} < 2 \times 10^4\mu\text{g/ml}$. The pet ether fraction of *Cyperus rotundus* initially showed low toxicity with LC_{50} $14195\mu\text{g/ml}$ at 1 hr interval but displayed acute toxicity at latter intervals with LC_{50} $36.03\mu\text{g/ml}$ at 24 hrs interval explaining the time taken by the carrier solvent to blend the non-polar fraction with the brine solution. The binomial probit regression analysis showing the converging trend of non-polar fractions and diverging trend of polar fractions for LC_{25} , LC_{50} & LC_{75} , with increasing time interval, restricts unprocessed release of non-polar fraction in the aquatic ecosystem. Conclusively, the brine shrimp lethality assay can be stated to be a technically simple and scientifically feasible method to resolve the critical toxicity component of bio-chemicals.

Keywords: Brine Shrimp, LC50, Non-polar, Phyto-chemicals, Polar and Toxicity

Relative vulnerability of the flathead grey mullet *Mugil cephalus* to climate variation along the south-east coast of India

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ENV 24

The flathead grey mullet *Mugil cephalus* is a benthopelagic catadromous euryhaline fish that can live in marine, brackishwater and freshwater environments across tropical, sub-tropical and temperate zones. It is a commercially valued table-fish of aquaculture significance and forms a local fishery in Tamil Nadu on the south-east coast of India. Its wide distribution and high tolerance to salinity regimes makes it a good candidate for the study of climate change impacts. Vulnerability assessments provide useful information on the innate behaviour of a species to a changing climate and helps to understand whether the species would be positively or negatively impacted by a series of climate change events that act in tandem with other external pressures like fishing, pollution etc. We assessed the relative vulnerability of the *M. cephalus* using environmental, biological and fishery-related criteria, and assigning scores to the performance of the species against its likely exposure to environmental changes and fishing pressure along the south-east coast of India, as well as to adaptability based on its eco-biological traits. Within a Vulnerability Matrix of Low (score < 1.0), Medium (score = 1.0-1.5) and High (score > 1.5), *M. cephalus* was found to be of medium vulnerability, with a Vulnerability Index of 1.1. The study indicates the need for precautionary management interventions in the fishery of the species as well as promoting its production through aquaculture in order reduce stress on its natural population along this coast.

Keywords: Adaptability, Climate change, Impact, *Mugil cephalus*, Vulnerability

Inhibition of Fish Pathogenic Bacteria By Plant Hydrocarbons

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Abstract

Bio-hydrocarbons serve as secondary metabolite in plants and microorganisms which are a major constituent of fat and oil based compounds. They serve various vital purposes in metabolic pathway of the producing organisms and auxiliary purposes in the outer environment as dissemination of pleasant fragrance. The partially purified petroleum ether extract of the dried leaves of *Vallisneria spiralis* L., has been found to be rich in cyclo-alkanes, cyclo-alkenes and acyclic olefins which not only serves as aromasource but also shows profound antibacterial activity against disease causing bacteria *Aeromonas hydrophila* and *Pseudomonas putida* in *Pangasius hypophthalmus*. The bacterial identification was done by 16S rRNA sequencing. With MIC < 100 µg/ml, the fraction also showed a strong free radical scavenging activity with IC50 32.4 µg/ml. The fraction was analyzed with Gas Chromatography Mass Spectrometry (GC-MS). The analysis shows the major constituents as Nonadecene (C19H38; MW - 266) with Retention time (RT) 20.432mins comprising almost 100% area of the chromatogram followed by α-Tridecene (C13H26; MW - 182) with RT 16.07mins comprising 99.4% and 5-cyclohexyl eicosane (C26H52; MW - 364) with RT 11.409 mins comprising almost 25.4% area of the chromatogram. Literature survey interprets Tridecene to be an acyclic olefin found in coconut flesh. 5-cyclohexyl eicosane and Nonadecene are accredited for their sweet odour which can be witnessed once the shade dried leaves of *V. spiralis* are subjected to grinding.

Key Words : Hydrocarbons; Fish pathogens; *Vallisneria spiralis*; *Pangasius hypophthalmus* and GC-MS.



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