

Studies on the Phytochemistry, Spectroscopic Characterization and Antibacterial Efficacy of *Sida cordifolia*(Linn)

Bibhu Prasad Sahu¹, Panchanan Gouda², Chakrapani Patnaik³

Post Graduate Department of Chemistry, Khallikote University, Berhampur- 760 001, Odisha, India

Abstract: *Sida cordifolia*(Linn) is a plant belonging to the family Malvaceae. The present study investigates the phytochemistry, characterization and antibacterial activity of ethanolic extract of *Sida cordifolia*(Linn).The sample was collected from the Khallikote University campus. The collected plant material was shade dried and pulverized. The plant material was studied for phytochemistry, spectroscopic analysis i.e., ¹H NMR, ¹³C NMR, IR, Mass and antibacterial activity. The phytochemical analysis indicates the presence of flavone and glucoside in the plant. The present study provides evidence that the ethanolic extract of *Sida cordifolia*(Linn) contains bioactive compounds that might make the plant a novel anti *V. cholerae* O1drug.

Keywords: *Sida cordifolia*(Linn), Ethanolic extract, Phytochemistry, ¹H NMR, ¹³C NMR, IR, Mass and antibacterial

1. Introduction

Plant Description

Sida cordifolia grows well through the plains of India, especially, in damp climates. The shrub grows up to 0.75 – 1.5 meters in height. The root and the stem are stout and strong. The leaves are heart shaped, 2.5-7 cm long and 2.5-5 cm broad with 7-9 veins. The flowers are small, yellow or white in colour, solitary and axillaries. The fruits are moong-sized, 6-8 mm in diameter. The seeds are called as Bijabanda in Ayurveda, are greyish black in colour and smooth. The plant flowers from August to December and fruiting occurs from October to January [1].



Figure 1: *Sida cordifolia*(Linn)

It is widely distributed along with other species throughout the tropical and sub-tropical plains all over India and Srilanka up to an altitude of 1050 m., growing wild along the roadside. Parts of the plant used are seeds, leaves and roots.

Scientific Classification

Kingdom – Plantae, Division – Angiospermae, Class – Eudicots, Order – Malvales, Family – Malvaceae, Genus – *Sida*, Species - *Sida cordifolia*. In Sanskrit it is called Bala, Bala while locally in Odia it is called Bisvokopari, Badianaula or Suvarna. *Sida cordifolia* has been reported to possess analgesic, anti-inflammatory and hypoglycemic activities as well as hepatoprotective activity [2].

2. Ethnomedicinal Properties of *Sida Cordifolia* L

Therapeutic Uses [2]

The plant has been taken as a whole before flowering. Therefore, the extract of the plant is devoid of the chemical constituents of the flowers and seeds. The Plant is used as an astringent, emollient or aphrodisiac etc. Bark is considered as cooling. It is useful in blood, throat, urinary system related troubles and piles [3].

Traditional Uses

The plant is analgesic, anti-inflammatory and tonic. It affects the central nervous system and provides relief from anxiety. Its extract is consumed to reduce body weight, tones the blood pressure and improves the cardiac irregularity. It is also useful in fever, fits, ophthalmic problems, rheumatism, colic and nervous disorders. It has also been reported to improve sexual strength. *Sida cordifolia* oils are used topically to the sore muscles and sore joints in rheumatism and arthritis with the crushed leaves can be carried out a cataplasm to alleviate local pains and because of its astringent value for the cure of external wounds or imperfections of the skin. Besides it shows antiplaque and antifungal activities.

The bronchodilator value of the vasicinone, vasicine and vasicinol are used to elaborate preparations for the treatment of the bronchial affections. Decoction of the root of bala and ginger is given in intermittent fever attended with cold shivering fits. Root juice is also used to promote healing of wounds. Powder of the root and bark together, is given with

milk and sugar for frequent micturition. Oil prepared from the decoction of root bark mixed with milk and sesame oil, finds application in diseases of the nervous system and is very efficacious in curing facial paralysis and sciatica [4]. According to Ayurveda „Bala“ has more effect on vata dosha. Leaves are cooked and eaten in cases of bleeding piles. Juice of the whole plant pounded with a little water is given in doses of ¼ seers for spermatorrhea, rheumatism and gonorrhoea. Made into paste with juice of palmyra tree, it is applied locally in elephantiasis (Yogaratanakaram).

Pharmacological Activities

Franco *et al.* tested the hydro alcoholic extract of *Sida cordifolia* as a depressant and decreases CNS activity [5]. Additional research appears to confirm that *Sida cordifolia* does not stimulate the CNS as confirmed by Mediros *et al.*[6]. *Sida cordifolia* as a weight loss product is through its hypoglycemic activity. It is also believed that anti-obesity effect is not limited to ephedrine content alone. Use of *Sida cordifolia* as a weight loss product is through its hypoglycaemic (blood sugar lowering) activity and therefore may help to reduce the storage of fat with fat cells [7]. *S. cordifolia* can increase pain tolerance and appears to have anti-inflammatory properties. Anti-inflammatory activity of ethyl acetate and alcohol extracts of both *Sida cordifolia* aerial and root extracts showed dose dependent activity [8]. Kumar *et al* reported the fumaric acid isolated from *S. cordifolia* to be hepatoprotective [9].

Extracts of all the parts of *Sida cordifolia* have effective reducing power and free-radical scavenging activity. The highest antioxidant activity was observed in the root extract

[10] which also exhibited superoxide-scavenging activity and inhibited lipid peroxidation. All these antioxidant properties were concentration dependent. Leaves of *Sida cordifolia* L. have been successfully tested for the acute stomatitis of asthma and nasal congestion and proved to be toxic at high dose [11].

Phytochemical Analysis

Quantities of less than 2% of ephedrine (Fig. 2) and pseudoephedrine found in the leaves of *Sida cordifolia* which is known to stimulate the central nervous system (CNS) and enhance weight loss. Plants with relatively higher amounts of ephedrine are being used in weight loss products. *Sida cordifolia*, with its ephedrine and pseudoephedrine has gained a lot of interest and is now marketed [12].

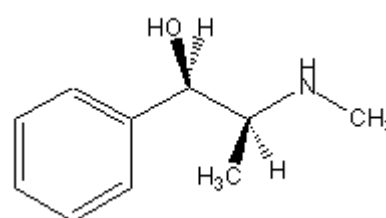


Figure 2: Structure of Ephedrine

Sutradhar R.K. *et al.* isolated [13] (5'-Hydroxymethyl -1'-(1,2,3,9-tetrahydro-pyrrolo [2,1-b]quinazolin-1-yl)-heptan-1-one) (Fig 3) from the aerial parts of *S. cordifolia* Linn. and reported Analgesic and Anti-inflammatory properties.

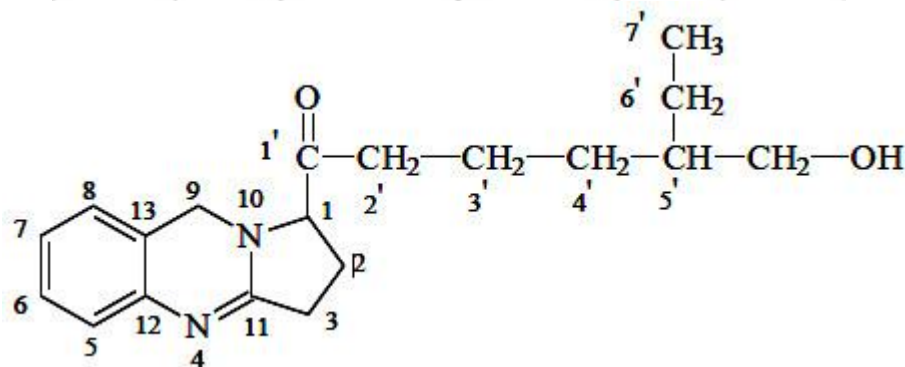


Figure 3

The water extract of the leaves was reported to possess analgesic and anti-inflammatory activities in animal models [14]. The water extract of the whole plant is specially used in the treatment of rheumatism [15]. Earlier phytochemical studies on the roots had shown the presence of ephedrine, vasicinol, vasicinone and N- methyl tryptophan [16],[17],[18]. Traditionally, root juice is also used to promote the healing of wounds [19].

Sida cordifolia actually acts as a depressant and decreases CNS activity [20]. It acts as a weight loss product is through its hypoglycemic activity [21]. Aqueous acetone extracts of *S. acuta* Burn f. and *S. cordifolia* L. contain saponosides, coumarins, steroids, phenolic compounds and alkaloids. In addition, their extracts have showed good antioxidant and anti-inflammatory activities [21]. It was observed that the

level of alkaloids in this plant declines by age [22]. The well-known indoloquinoline alkaloid, cryptolepine is a possible constituent of *S. cordifolia* where it was recently isolated from this plant [23]. In contrast, a previous study reported the absence of cryptolepine in *S.cordifolia* [24], [25]. Alcoholic extract of the whole plant and nodal callus has showed 0.0558 % and 0.5359 % of Ephedrine respectively.

In continuation to our research interests in the phytochemicals present in the flora of the Ganjam district of Odisha and surroundings and their medicinal uses [26], [27], [28] and as metal ion adsorptive biomass [29], the above observations prompted us to explore the isolation and structural characterisation of secondary metabolites from

Sida cordifolia basing on various spectral data and also evaluation of its anti-microbacterial activity etc.

3. Experimental

All the solvents used in this research work viz. ethanol, methanol, benzene, petroleum benzene, chloroform, carbon tetrachloride, ethyl acetate and dichloromethane were of highest purity. Thin layer chromatography (TLC) was carried out using silica gel G for identification and preparative TLC purposes.

Plant material Extraction and isolation of compounds

The whole plant of *Sida cordifolia* weighing 5.0 Kgs was collected locally. The thoroughly water washed and shade dried sample was pulverized into powder form and was extracted with ethanol (95%) by dissolving for two weeks. It was filtered and then concentrated. The residue was again extracted with hot ethanol using Soxhlet apparatus. It was also filtered and concentrated. The purity of both the extracts was checked by TLC. It was found that both contained two spots at equidistance from the base line. Hence, both the extracts were mixed up and distilled in a hot water bath. The residue was dark green in colour. It was then treated with cold water to remove water soluble part. The water insoluble part was dried thoroughly, dissolved in ethanol and filtered. The filtrate was treated with dry sodium sulphate to absorb water. After concentration by distillation over hot water bath it was dried in a desiccator over anhydrous calcium chloride.

The perfectly dried residue was dissolved in dry benzene and slurry was prepared with 60-120 mesh silica gel. The separation of the components was done by column chromatography using 60-120 mesh silica gel and benzene: petroleum benzene (1:9) as the eluent. The purity of the fractions was examined by TLC at a regular interval. Eluents were collected in small receivers and the fractions containing same component were mixed together. After removal of the solvent pure compounds B1 and B2 were recovered. The compound was identified by studying the spectral data. Antibacterial activity of the isolated products B1 from *Sida cordifolia* was performed.

4. Antibacterial Activity

Microorganisms

In vitro antimicrobial activity was examined for chloroform extract of the ethanol isolated compound (B1) from *Sida cordifolia*. Amongst the four microorganisms investigated two Gram negative bacteria were *Escherichia coli* and *Klebsiella pneumonia* while the Gram positive bacteria were *Staphylococcus aureus* and *V. cholerae* O1. Microorganisms were maintained at 4°C on nutrient agar slants.

Antimicrobial Assay

The antimicrobial assay was performed by agar disc diffusion method (Bauer *et al.*, 1966) [30] with the extract. The molten Mueller Hinton Agar (HiMedia) was inoculated with the 100 µl of the inoculum (1 x 10⁸ CfU) and poured into the sterile Petri plates (HiMedia). For agar disc diffusion method, the disc (0.7cm) (Hi-Media) was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented.

5. Results and Discussion

Compounds B1 and B2 extracted from the ethanolic extract of *Sida Cordifolia* have no sharp melting point. Both the compounds were subjected to spectral analysis for their identification.

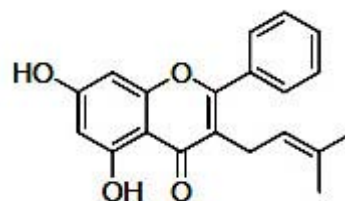
Spectral Analysis of Compound (B1)

Molecular mass of B1 was found to be 350. IR (ν, nujol) cm⁻¹: 2950(C-Hstr), 1420, 1460, 1510(Aromatic), 1680(>C=O). ¹H NMR (CDCl₃); δ PPM : 0.822(s, 3H, -CH₃), 0.827 (s, 3H, -CH₃), 1.009(s, 3H, -OCH₃), 1.219(s, 3H, -OCH₃), 4.218(m, 6H, glucoside), 4.314(m, 6H, glucoside), 2.3(d, J=7Hz, 2H, isoprenyl), 5.35(t, J=7Hz), 7.1(d, J=2Hz, H8), 7.5(d, J=2Hz, H6), 7.3(s, 6H, Ar-H).

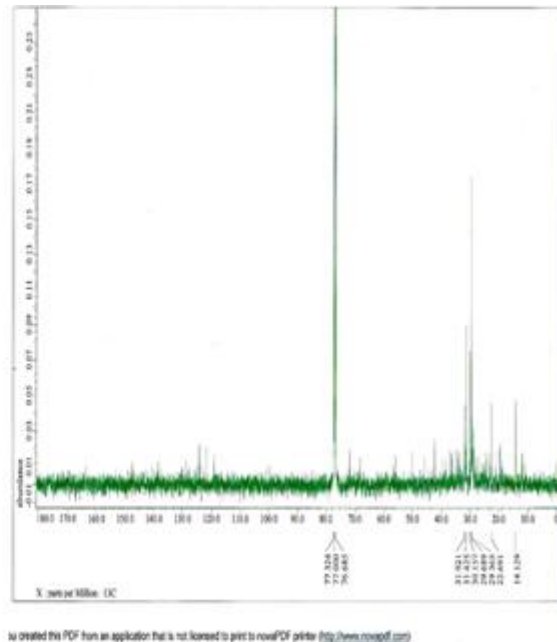
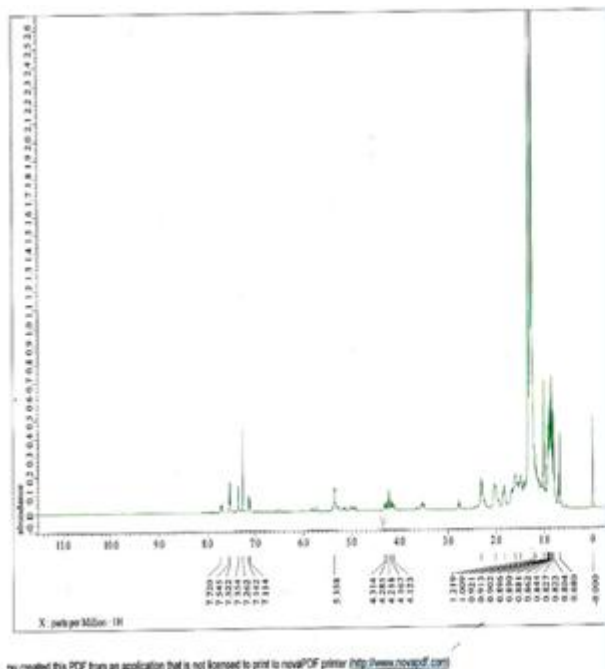
¹³C NMR (CDCl₃); δ PPM : 30.157(-CH₃), 31.425(-CH₃), 31.921(C7), 76.685(-OCH₃), 77.00(-OCH₃), 77.234(-OCH₃), 114(C31), 115(C41), 116(C61), 117(C21), 118(C1*), 119(C51), 121(C7), 122(C6), 123(C8), 124(C9), 125(C10), 147(C8*), 149(C9*), 152(C3), 156(C2), 165(C4).

Mass (m/Z): 78, 102(100%), 156, 179, 238, 350.

Basing on the above spectral studies of the isolated compound B1 from *Sida Cordifolia*, the molecular formula corresponds to C₂₂H₂₂O₄ i.e. 5,7-dihydroxy-3-isoprenyl flavone.

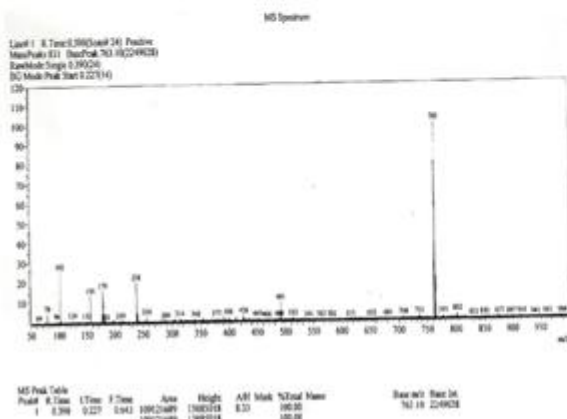


B1: 5,7-dihydroxy-3-isoprenyl flavones



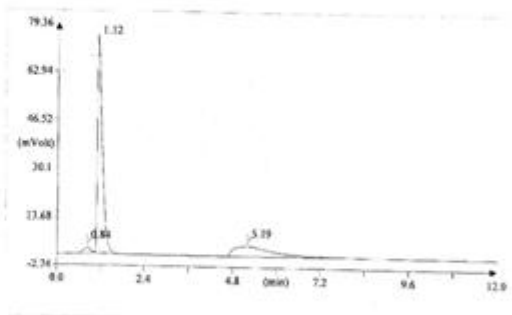
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Element Name	Element %	Ret. Time
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Carbon	79.93	1.12
Hydrogen	12.26	5.19

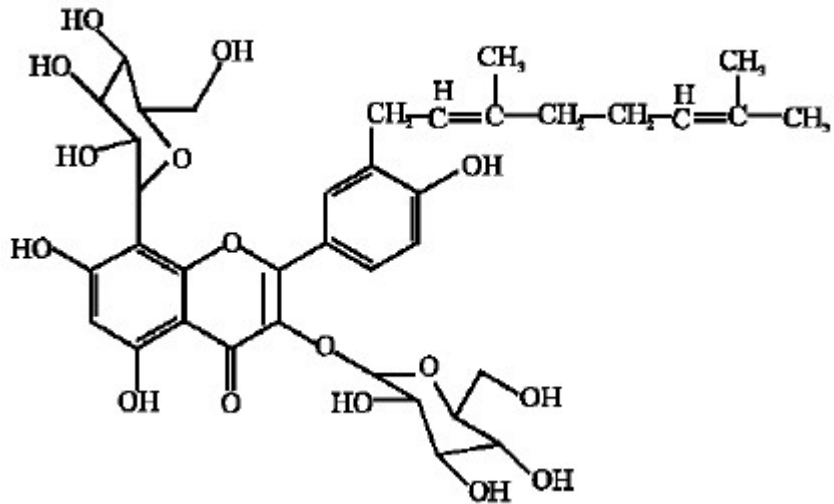
Spectral Analysis of Compound B2

B2 has molecular mass of 732. IR (ν , *nujol*) cm^{-1} : 3585(-OH), 2980(C-Hstr), 1685(>C=O), 1480, 1420, 1390(Aromatic region). Mass (*m/z*): 190, 406(100%), 569, 698, 715, 732.

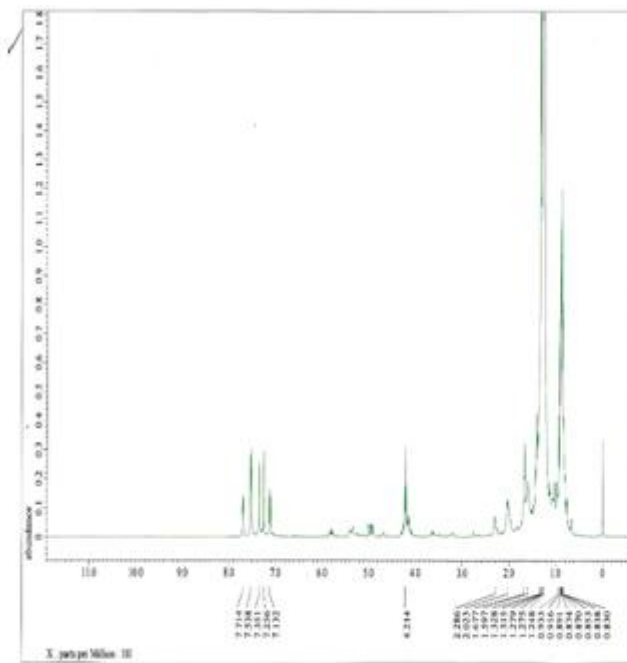
^1H NMR (CDCl_3); δ PPM : 0.916(s, 3H, $-\text{CH}_3$), 1.248 (s, 3H, $-\text{CH}_3$), 4.214(d, 2H, $J=7\text{Hz}$, H_4''), 4.218(t, 1H, $J=7\text{Hz}$, H_3''), 4.214(bm, 6H, H of glucopyranoside), 4.30(d, 2H, $J=7\text{Hz}$, H_7''), 5.358(t, 1H, $J=7\text{Hz}$, H_6''), 7.132(d, 1H, $J=3\text{Hz}$, H_5''), 7.256(s, 1H, H_2''), 7.351(d, 1H, $J=3\text{Hz}$, H_8), 7.714(d, 1H, $J=3\text{Hz}$, H_6).

^{13}C NMR (CDCl_3); δPPM : 14.053($\text{C}1''$), 14.119($\text{C}16''$), 22.681($\text{C}15''$), 22.684($\text{C}4''$), 23.702($\text{C}7''$), 30.357($-\text{CH}_2-$), 30.319($-\text{CH}_2-$ grp.), 31.415($\text{C}2''$), 31.911($\text{C}3''$), 40.129($\text{C}5''$), 41.023($\text{C}6''$), 68.123, 76.676, 70.000, 77.315(C-glucopyranoside at C3 and C7), 119.048(CAr), 123.958(CAr), 124.435(CAr), 128.782(CAr), 130.880(CAr), 147.413(CAr), 167.531(>C=O).

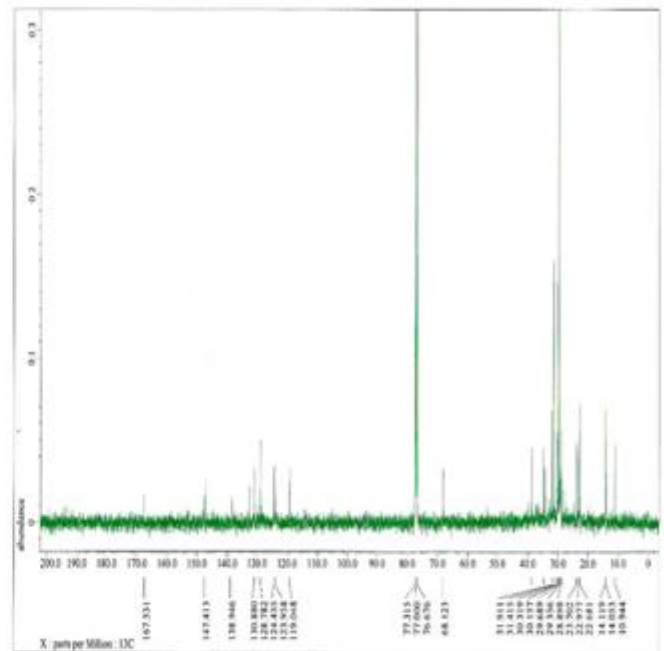
Basing on the above spectral studies of the isolated compound B2 from *Sida cordifolia*, the molecular formula corresponds to $\text{C}_{36}\text{H}_{44}\text{O}_{16}$. The mass, ^1H and ^{13}C NMR spectral data identified B2 as 3'- $(3'',7''$ -dimethyl-2'',6''-octadiene)-8-C- β -D-glucosyl-kaempferol 3-O- β -D-glucoside.



B2: 3'-(3'',7''-dimethyl-2'',6''-octadiene)-8-C-beta-D-glucosyl-kaempferol 3-O-beta-D-glucoside



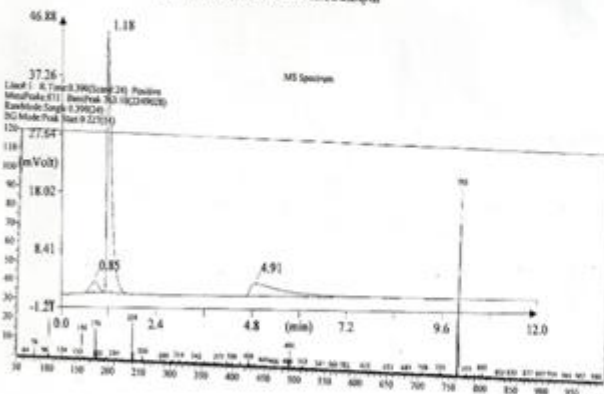
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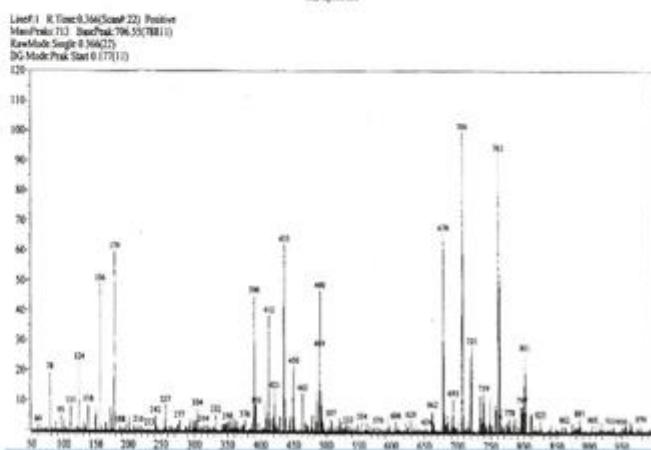
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1	0.85	6.04	0.85			Nitrogen	0.85
2	1.18	77.18	1.18			Carbon	1.18
3	4.91	12.07	4.91			Hydrogen	4.91

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Antimicrobial Susceptibility Test

Antibacterial activity of plant extract B1 (zone of inhibition in mm) studied through Disc Diffusion Method (Bauer et al. 1966) are given in Table- 1.

Table 1: Antibacterial Susceptibility Test of Plant Extract (B1) from *Sida cordifolia*

Microorganism	Gram-positive/ Gram-negative	Zone of Inhibition (mm)
<i>Escherichia coli</i>	Gram -ve	10.66±1.15
<i>Klebsiella pneumonia</i>	Gram -ve	11±1
<i>Staphylococcus aureus</i>	Gram +ve	13±1
<i>V. cholera O1</i>	Gram +ve	30±1

The values represent zone sizes excluding the zones of inhibition of control. Values represent means of 3 experiments.

The presence of antibacterial substances in the higher plants is well established. The traditional healers use primarily water as the solvent but it is found that the plant extracts by ethanol provided more consistent antimicrobial activity compared to those extracted by water. The results of antibacterial activity isolated compound(s) from the plants against the investigated bacterial strains are presented in Table- 1. None of the aqueous extracts produced zones of inhibition in the Kirby-Bauer analysis. This might have resulted from the lack of solubility of the active constituents in aqueous solutions while ethanol extract showed various degrees of antibacterial activity.

6. Interpretation

Over the past few decades emergence of drug resistance has appeared as major threat in the therapeutic field in clinical

management of diseases. However, researchers are also struggling hard to introduce newer drugs to invade the pathogens to keep us free from diseases. In this context in our preliminary experiment, our indigenous organic plant extract B1 is susceptible to *V. cholera O1*, which is equivalent to susceptible zone size of the antibiotic Tetracycline, Doxycycline, Azithromycin and more than ciprofloxacin, norfloxacin and ofloxacin prescribed for cholera patients. However, further characterization is required to determine its MIC value, mechanism, organic structure, in vivo study etc. and have to validate these before application. Further, its broad-spectrum applicability should be studied so that it will yield an attractive impact in therapeutic field.

7. Conclusion

As the higher plants continue to retain their historical significance as important sources of novel compounds useful either directly as medicinal agents or as lead compounds for synthetic/semi synthetic structural modifications/ optimization or biochemical/ pharmacological probes, the plant *Sida cordifolia*. Traditionally it is used as astringent, thermogenic, tonic, and in the treatment of fever, uropathy, arthritis, leucorrhoea, gonorrhoea, hyperdiuresis, rheumatism, spermatorrhea and diarrhoea.

As the chemical investigation of the plant *Sida cordifolia* has not been dealt in deep, phytochemical investigation of this extract in ethanol was undertaken. Investigation of phytoconstituents has been encouraging for its therapeutic action, which can be useful for research and therapeutic activities as analgesic and anti-inflammatory activity as well as easing the handling of tumor and cancer treatment.

The organic extract B1 showed zone of inhibition of 30mm when tested to detect antimicrobial effect against *V. cholerae* O1. This when compared with the standard susceptible drugs prescribe for cholera treatment proves to be a promising candidate for a potential drug in combating cholera; even though further tests are needed to be performed before accepting it as a dependable and marketable drug.

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