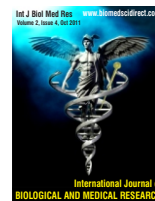


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Original Article

Effect of Bioactive Compounds and its Pharmaceutical Activities of *Sida cordifolia* (Linn.)

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ABSTRACT

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. The general objective of this study shows to identify the bioactive compounds from the *Sida cordifolia* plant and to study the antimicrobial, cytotoxic effect on HeLa cell lines. The GCMS analysis results showed mainly four different compounds such as Vasicinol, Ephedrine, Vasicinone and Hypaphorine in various criteria like retention time and peak observation among the four phytochemical compounds based upon in vivo study. The agar disk diffusion method was used to study the antibacterial activity of *S. cordifolia* extracts against 7 bacterial strains. The Minimum Inhibitory Concentration (MIC) of the plant extracts were tested using two fold agar dilution method at concentrations ranging from 6 to 18 µm/ml. The cytotoxicity results of *S. cordifolia* (L.) extract on HeLa cell lines clearly reflected the treated cell lines are fortunately all the uncontrolled growth has been arrested there is declined level of cancerous cells observed. The methanol extract was found to be an effective against all phytopathogens with low MIC of 6µm/mm and the methanol extract exhibited a higher inhibition activity against *Escherichia coli*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Mycobacterium sp.*, and *Micrococcus variance*, *Pseudomonas aeruginosa* and *B. subtilis*. Meanwhile, the results also indicate the presence of major phytochemical compounds such as vasicinol, Ephedrine, vasicinone, Hypaphorine in the *S. cordifolia* extracts. Hence, the isolation and purification of therapeutic potential compounds from *S. cordifolia* could be used as an effective source against bacterial diseases in human and plants.

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1. Introduction

Sida cordifolia Linn. (Family: Malvaceae) commonly known as berela (Bengali) is herb that is extensively used as a common herbal drug in the Indian subcontinent. The water extract of the leaves was reported to possess analgesic and anti-inflammatory activities in animal models (Gunatilaka et al., 1980). It is used in Ayurvedic medicine. (Pole et al 2006). It has been investigated as an anti-inflammatory (Franzotti et al., 2000) for treating cancer (Jenny et al 2005) and for encouraging liver re-growth. (Silva et al 2006). Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Due to its ephedrine content, it possesses psychostimulant properties, affecting the central nervous system and also the heart (Adam et al 2006). It is also used

as a fat-burning supplement. It is used in Ayurvedic medicine (Sebastian, 2006), it has been investigated as an anti-inflammatory (Franzotti et al., 2000) for treating cancer (Jenny, et al., 2005) and for antibacterial growth Isman et al. (2003; Silva et al., 2006). It has a depressive effect on the central nervous system (Franco et al., 2005). Moreover, previous phytochemical studies on the roots had shown the presence of ephedrine, vasicinol, asicinone and N-methyl tryptophan (Franzotti, 2004). Recent analyses have revealed that ephedrine and pseudoephedrine constitute the major alkaloids from the aerial parts of the plant, which also show traces of sitosterol and palmitic, stearic and hexacosanoic acids. From seed oil sterculic, malvalic and coronaric acids are isolated along with other fatty acids.

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Very recently, Swathy et al., 2010 studied that 50% ethanolic extract of *Sida cordifolia* has got potent antioxidant and antiinflammatory activity and the activity was comparable with the standard drug diphenyl. Since, there no more works has been available this kind of medicinal plants. Hence this research works having designed the following objectives. To identify the responsible secondary metabolites for antibacterial activity of best active plant by performing GCMS analysis. In addition to study the cytotoxic effect of *S. cordifolia* on HeLa Cells.

2. Materials and Methods

2.1. Collection and identification of Experimental plants

The whole plants of *Sida Cordifolia* were collected from Parassala situated in and around the college campus (Malankara Catholic College) Trivandrum District, Kerala. The selected plant was identified by the Herbarium of TBGRI (Tropical Botanical Garden Research Institute, Palode and Trivandrum) and the flora of the presidency of Madras.

2.2. Compound study of crude extract using GCMS

The crude plant extracts were subjected to centrifugation at about 10,000 rpm for about 30 minutes to remove the particulates. The clear supernatant was aspirated using a pipette and transferred into a clean vial and labeled. Then the supernatants were subjected to gas chromatography analysis using a Varian Cp 3,800 model gas chromatography equipped with two flame ionization detectors and connected with two flame ionization detectors and connected with two flame ionization detectors and connected with Cp-ware (Polyethylene glycol) (60m x0.25mm) and Cp-5 (100% dimethyl polysiloxane) capillary column.

The peak area calculations were done by star work station and peak identification by comparison with authentic, wherever available calculations of Kovats Retention index was done. The Kovats index system has been widely used in the analysis of food flavors, pesticides and essential oil analysis. Kovats retention index, (1) is defined and calculated by following equation (Douglas, 2000).

$$I = 100 N + 100 \log'R(N+n) - \log'R(N).$$

Where

t'R (N) = adjusted retention time of n paraffin hydrocarbon of carbon number eluting before solute A.

T'R (N+n) = adjusted retention time of n paraffin hydrocarbon of carbon number (N+n) eluting after solute A.

T'R (A) = adjusted retention time of solute-A.

Mass spectrometry analysis was performed on a Shimadzu GC 17 A QP 5,000 MS coupled with a mass detector, fitted non-polar DB-5 (Diphenyl siloxane) capillary column of length 25 m × 0.25 mm id GC MS operation conditions at initial temperature 60 0 C – 3000C. The injection volume was 0.1µl with helium gas as carrier at the flow rate of 0.6ml per minute. Relative retention times (RRTs) of constituents were determined using c5-c30 straight chain alkanes as standards. Individual constituents of the extract were identified by WILEY and NIST database matching by comparison of mass spectra with published data and by comparison of Mass spectra with published data and by their comparison of their RRTs.

2.3. Statistical analysis

The results were analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Dunnet's test. Values with p<0.05 were considered significant.

3. Results and Discussion

3.1. Bioactive compounds in *S. cordifolia* leaves by GCMS analysis

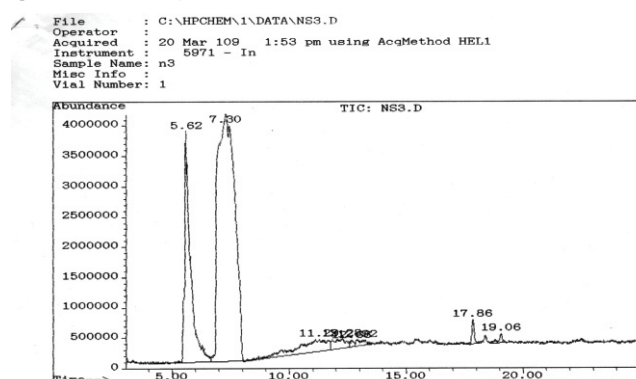
GCMS analysis results showed mainly four different compounds such as Vasicinol, Ephedrine, Vasicinone, Hypaphorine in various criteria like retention time and peak observation among the four phytochemical compounds based upon in vivo type of experimental method expressed maximum retention time 12.8 denoted as Hypaphorine followed by 11.29, 7.30, 5.62 revealed biochemical compounds such as vasicinol, Ephedrine, vasicinone respectively. Though according to area observation the highest level noticed on 68.7 represent bioactive compound was Ephedrine and minimum as well as similar area observed in Hypaphorine, vasicinol. In case of in vitro treated plant possessed max retention time expressed the same in vivo case Hypaphorine and minimum vasicinol 3.73. Furthermore, the area of the peak noticed 72.14 represented Ephedrine compounds also minimum area of the peak obtained on Hypaphorine compounds (Table-1).

Table – 1. GCMS analysis depicted important phytochemical compounds present in the *Sida cordifolia* leaves
Gcms Analysis

Compounds	In vivo		In vitro	
	Retention time	Area	Retention time	Area
Vasicinol	5.62	20.99	3.73	24.90
Ephedrine	7.30	68.27	7.06	72.14
Vasicinone	11.29	3.97	11.11	1.98
Hypaphorine	12.28	3.97	12.96	0.98

From the figure 1 shows two peak higher activity shows the above said compounds such as vasicinol and Ephedrine. Suddenly declined level of secondary metabolic compound observed vasicinone and Hypaphorine of these four compounds maximum well processed bioactive compound is vasicinol. The peak could be identified as Vasicine, when compared with the standard structure and properties were identified with a help of this web address (www.ncbi.nlm.nih).

Fig. 1. GCMS Analysis of Natural Callus Extract of *Sida Cordifolia*



The vasicine compound was more in vitro plant extracts compare with the natural extracts. Its molecular formula was C11H12N2O. Another one peak observed in GCMS chromatogram at 7.06 retention time in cs1 (in vitro extracts) but the same peak was observed at 7.3 retention time in natural extracts. This peak was identified as Ephedrine, when compared with the standard. Its molecular formula was C10H15NO. Some other small peak were also observed at a retention time of 11.1, 12.9, 17.8 and 19.06 compared with the standard it was conformed as vasicinol, hypaphorine, vasicinone and phytosterols.

3.2.Cytotoxicity

To determine the anticancer effect of *S. cordifolia* the extracts were subjected to cytotoxicological studies.

Table-2. Antibacterial Activity of Natural and In Vitro Plant Extract of *S. cordifolia*

Bacteria	Zone of inhibition (µg/mm)		
	C1	C2	N3
<i>Mycobacterium species</i>	18	10	15
<i>Escherichia coil</i>	14	5	12
<i>Bacillus subtilis</i>	13	8	16
<i>Klebsiella pneumoniae</i>	6	7	10
<i>Micrococcus variance</i>	10	9	6
<i>Staphylococcus aureus</i>	8	9	11
<i>Pseudomonas aeruginosa</i>	11	14	13

C1 - NA 1.00mg/l + Kinetin 1.00mg/l

C2 - NA 1.00mg/l + Kinetin 0.5mg/l

N3 - in vivo plant extract of *Sida cordifolia*

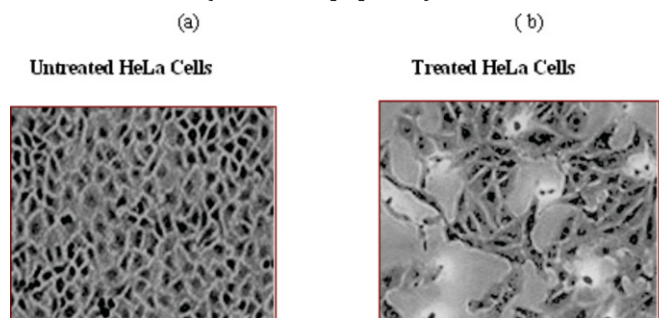
Values are Mean ± SEM (n = 6);

Paw volume is expressed in change of height (in mm) of Hg bath (in parentheses). *p<0.01 compared to control.

3.3.Trypan blue cell viability assay

Two samples were used in this test. One was used as the control (NIC). The plant extract was the test sample (NIT). The tumor cells were viable in the natural sample. The average percentage of viability was 96.33%. But in case of natural extract, most of the cells were dead. The percentage of viability was 30.6% (Table. 3).

Fig. 2. Effect of *S. cordifolia* on HeLa cells Cytotoxicity (Direct contact assay) (a) florescence HeLa cell control, (b) HeLa cells treated (Probable apoptosis) with essential



When compared with control and treated with *S. cordifolia* extracts on the HeLa cells this extract applied with cancer cells visibly observed the following changes initially the cells are scattered with abnormal proliferation and the cells were elongated number of the cell divisions range was decreased when compared with control. Meanwhile, the results of *S. cordifolia* extracts cytotoxicity on HeLa cell lines are shown in Figure 2a and b. It represent the cytotoxicity results of *S. cordifolia* (L.) extract on HeLa cell lines clearly reflected the treated cell lines are fortunately all the uncontrolled growth has been arrested there is declined level of cancerous cells observed. But incase of control cell lines explained clumped aggregated cells were spreaded throughout the HeLa cell lines. The external morphological changes observed using the thymidine fluorescence assay showed slight to higher alterations in cellular components after 96hrs post treatment. Though, a treated cell shows the more prominent growth inhibition and shrinkage of the cells takes place. Furthermore, the inhibitory effect of on HeLa cell lines was mainly influenced with dose-dependent the difference was remarkable starting from 150µgm/mL.

3.4. Lactose dehydrogenase cytotoxicity detection assay

Lactose dehydrogenase Cytotoxicity detection was observed by the absorbancy of cell line culture. The obserbency was higher in control (0.78) the cell used at different levels such as 5000, 10000, 20000 cells/ml. In addition, this table revealed when the lowest concentration of cells through the LDH cytotoxicity assay possessed the more or less similar absorbance values. Though, other two cell concentrations (5000.00 and 20000.00) were remarkably stands for the irregular manner of absorbance value hence, most probably that was very less abnormality (0.18) 20000 cells/ml was showed higher abnormality (0.39). However, among the three different concentrations of cells treated maximum average of cells noted on 20000.00 followed by 10000 and 5000 cells/1ml (Table 3).

Table 3. LDH Cytotoxicity detection assay of *S. cordifolia*

Concentration (µg/ ml)	Blank	5000.00	10000.00	20000.00
Absorbance	0.77	0.16	0.24	0.44
	0.72	0.20	0.25	0.39
	0.84	0.19	0.25	0.35
Average	0.78	0.18	0.25	0.39

Thus, the results of the previous study demonstrated the compounds like (5'-Hydroxymethyl-1'-(1, 2,3, 9-tetrahydro-pyrrolo [2, 1-b] quinazolin-1-yl)-heptan-1- one) has significant analgesic and anti-inflammatory activities. However a more extensive study is necessary to determine exact mechanism(s) of action.

The acetic acid induced writhing test is normally used to evaluate the peripheral analgesic effect of drugs. The response is thought to be mediated by peritoneal mast cells acid sensing ion channels (Voilley, 2004) and the anticancerous activity (Asha and

Bannerjee, 1985; Ramar et al., 2008) A large variety of phytochemicals that have been reported from the natural product research has been proven successfully as anticancerous agents (Ranjit et al., 2008). The methanol extract of this medicinal plant has been indicative effect of probable to regulate the apoptosis. In this result shows the leaf of this experimental plant having highest cytotoxic effect on HeLa cells. This is the first report that *S. cordifolia* leaf possesses cytotoxic activity on HeLa cells (Figure 2b). In previous work, major constituents of its leaves were identified anti-bacterial activity (Newman et al., 2000; Pole, 2006).

This is the similar kind of works have been identified as anticancerous agent. In this work also identified major constituents of leaf that have Cryptonine alkaloid, Dihydro benzophenanthridine (cytotoxic, antitumor alkaloid), as well as constituent that. In this present study proved that was the must be capable of probable apoptosis on Human cervical cancer cell (HeLa) lines. The cytotoxic activity may depend on following bioactive compounds such as vasicinol, Ephedrine, vasicinone, Hypaphorine. These may have cytotoxic effect because most of the alkaloids have been cytotoxic effects (Mohammad et al., 2008; Haque et al., 2000). The absence of normal apoptosis account for uncontrolled cell multiplication and these neoplastic cells have undergone alterations that tend to resist their susceptibility to probable apoptosis. Hence, for aqueous essential oil with cytotoxic activity to be formulated as a potent antitumor agent it should have a direct and selective action on these resistant tumor cervical cancer cells. Apart from this result clearly reveals that *S. cordifolia* leaf consisted bioactive compounds having cytotoxic effect and its profound cellular damage and hence scientifically substantiates the folklore claims. Earlier phytochemical studies on the roots had shown the presence of ephedrine, vasicinol, vasicinone and N-methyl tryptophan (Khan et al., 1989; Kanth and Diwan, (1999). In continuation of our studies on medicinal plants available in Bangladesh for their chemical constituents and biological activities we isolated (5'-Hydroxymethyl -1'-(1,2,1'3,9-tetrahydropyrrolo [2, 1-b] quinazolin-1-yl)-heptan-1-one). Ekramul Islam et al., (2004) and Khatoon et al., 2005 depicted the cytotoxicity and antibacterial activities of crude extracts from the leaves of *Sida rhombifolia* were investigated. This kind of similar experiment were conducted and the results were showed few contradiction that was ethyl acetate extract showed potent cytotoxicity with LC50 values (5.41 ppm) comparable to the reference standard, gallic acid. All the extracts showed weak antibacterial activity against both Gram-positive and Gram-negative test organisms

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