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ANTI-PROLIFERATIVE ACTIVITY OF *SIDA CORDATA*

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ABSTRACT

Cancer therapy without any adverse effect is still a challenge in current scenario. Hence a wide search of remedies to treat cancer is being in progress. The aim of this study is to evaluate the anti-proliferative effect of the whole plant *Sida cordata* against KB cell lines. Ethyl acetate, ethanol and aqueous extracts of the whole plant *Sida cordata* at different concentrations (1000, 500, 250, 125, 62.5, 31.2, 15.6 and 7.8 µg/mL) were subjected to cytotoxic study. The anti-proliferative activity of various extracts were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The Ethanolic extract has shown good cytotoxic effect against KB cell line when compared with the ethyl acetate and aqueous extracts. The current study reveals that the whole plant *Sida cordata* can be used as a plant of interest to perform further anti-cancer studies at higher levels and it may fulfill various needs required in the current scenario of cancer treatment.

KEYWORDS: *Sida cordata*, Phytoconstituents, MTT assay, KB cell lines, Anti-cancer activity.

INTRODUCTION

Sida cordata is a traditional plant of South East Asia and possess various biological activities such as free radical scavenging activity, hepato protective activity, anthelmintic activity, membrane stabilizing activity, anti-diabetic activity etc. It is used traditionally to treat piles, wounds and believed to rejuvenate liver diseases. This study was conducted to analyze the anti-cancer activity of whole plant *Sida cordata* against the KB cell lines. KB cells are the human oral carcinoma cells widely used to study the effects of various constituents against

oral carcinoma.[1] This study is an attempt to test the efficacy of the whole plant *Sida cordata* in inhibiting the growth of oral cancer cells in the *in vitro* conditions.

METHODS AND MATERIALS

I. Collection of plant material

The whole plant *Sida cordata* Burm.f was collected from the Chokkampatti village in Tirunelveli district of Tamilnadu during the month of August 2017. The plant was authenticated by the botanist Mr.V.Chelladurai, Research officer- Botany [Scientist-C (Retired)], Central Council of Research in Ayurveda and Siddha, Govt. of India.

II. Extraction of plant material

The entire plant was washed, dried in shade till dry and pulverized to coarse powder. The pulverized plant material was then extracted using various solvents using Soxhlet apparatus. The solvents used were ethyl acetate, ethanol and distilled water. 500 ml of each solvent was used to extract the active constituents present in 50 grams of the crude drug.

The aqueous extract was made by simple maceration process where 50 grams of the crude drug was soaked in a conical flask in a mixture of 500 ml of distilled water and 30 ml of chloroform (acts as preservative) for a period of 7 days. The container was sealed and kept inside the dark chamber at room temperature. It was agitated continuously at regular intervals. Then the soaked plant material was placed in a linen cloth and squeezed to collect the aqueous extract.

The collected ethyl acetate, ethanolic and aqueous extracts were then concentrated by evaporating them with gentle heat and the residue was stored in air tight containers in 4^{o}C.

Evaluation of *in-vitro* anti-cancer activity

Cell line and culture

Human oral carcinoma KB cell lines were obtained from National Centre for Cell Science (NCCS), Pune. The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37°C.

MTT assay[1,2]

KB cells (1×10^5/well) were plated in 24-well plates and incubated at 37^0C with 5% CO_2. After the cell line reaches the confluence, various concentrations of the samples were added

and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4). 100µl of 5 mg/ml 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide solution (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC_{50}) was determined graphically. The percentage cell viability was calculated using the following formula:

% cell viability = [A_{570} of treated cells / A_{570} of control cells] × 100

Graphs are plotted using the percentage of cell viability on Y-axis and concentration of the samples on X-axis. Cell control and sample control were included in each assay to compare the full cell viability assessments.

RESULTS

Cytotoxicity test (MTT assay)

The results of the MTT assay which was conducted to study the cytotoxic effects of ethyl acetate, ethanolic and aqueous extracts of the whole plant *Sida cordata* are given in **Table 1**. The IC_{50} values of different extracts are represented in the figures 1, 2 and 3. Figure 4 shows the microscopic images of the KB cell lines treated with different concentrations of the extracts.

Table 1: Anti-cancer activities of various extracts of *Sida cordata* on KB cell lines.

S. No	Concentration (µg/ml)	Dilution	% Cell viability		
			Ethyl acetate extract	Ethanolic extract	Aqueous extract
1	1000	Neat	23.46	15.57	26.75
2	500	1:1	30.92	22.80	34.86
3	250	1:2	37.17	29.38	42.32
4	125	1:4	43.42	36.40	50.76
5	62.5	1:8	49.56	43.64	58.33
6	31.2	1:16	56.68	50.21	66.77
7	15.6	1:32	63.59	57.67	74.28
8	7.8	1:64	70.61	64.47	82.45
9	Control	-	100	100	100

The IC_{50} value of ethanolic extract is found to be 31.2 µg/ml (Fig 1), ethyl acetate extract is 62.5 µg/ml (Fig 2) and aqueous extract is 125 µg/ml (Fig 3).

From the results of the MTT assay, it is seen that the ethanolic and ethyl acetate extracts have shown anti-proliferative activity against oral carcinoma cell lines (KB cell lines). Agents that have anti-proliferative activity against cancer cell lines are considered potent candidates for having anti-cancer activity.

Phyto-chemical evaluation of the ethanolic, ethyl acetate and aqueous extracts have already been carried out in our laboratory. Standard procedures were followed for this.[3] It was seen that these extracts are rich in flavonoids, tannins, alkaloids, terpenoids, steroids, phenols and glycosides. The Ethanolic extract, in addition, has also shown the presence of Saponins.

Reactive oxygen species (ROS) are highly reactive radicals that have a single unpaired electron in their outermost shell of electrons. Elevated levels of ROS have been detected in almost all cancers. The ROS promote many aspects of tumor development and progression. Tumorigenic events including oncogene activation by mutation of K-ras, metabolic alterations or macrophage infiltration or hypoxia / re-oxygenation processes in tissues can increase intracellular ROS levels and promote tumor formation.[4] Antioxidants play an important role in neutralizing the normal level of oxidative damage caused by these free radicals.[5]

Flavonoids are found to have many biological activities such as antioxidant activity, hepatoprotective activity, anti-Inflammatory activity, anticancer activity etc. Several mechanisms have been proposed for the effect of flavonoids on the initiation and promotion stages of the carcinogenicity including down regulation of mutant p53 protein, cell cycle arrest, tyrosine kinase inhibition, inhibition of heat shock proteins, estrogen receptor binding capacity and inhibition of expression of Ras proteins. Flavonoids mediate their anti-oxidant effects by permitting conjugation between the aromatic rings and the presence of a free 3-OH, by scavenging free radicals and by chelating metal ions.[6,7]

Tannins are effective in the treatment of inflammatory conditions and are also found to have remarkable activity against cancer.[8] The phenolic compounds present in the plants and the sesquiterpene lactones, a class of Terpenoids, are known to possess antioxidant properties.[9,10] Cytotoxicity is the common biological activity of alkaloids. Alkaloids are known to possess vasoconstrictive, analgesic and anti-tumor activities.[11]

Saponins are anti-carcinogenic as they have surface active characters due to their amphiphilic nature. Saponins act against cancer based on their mechanism such as direct cytotoxicity, immune modulatory effects, bile acid binding and normalization of carcinogen induced cell proliferation. Saponins which are steroid or triterpenoid glycosides are proven to have many biological activities such as anti-inflammatory activity.[12] Saponins and glycosides have positive cell membrane permeability properties.[13]

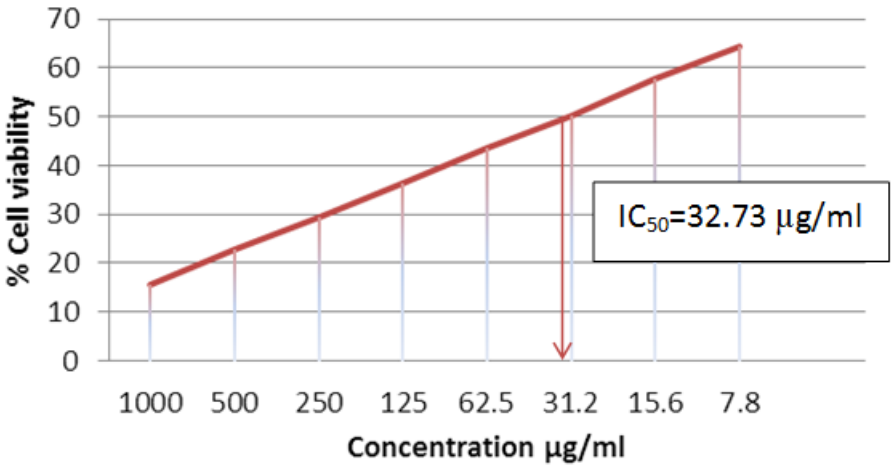


Fig 1: IC_{50} value of Ethanolic extract of *Sida cordata*

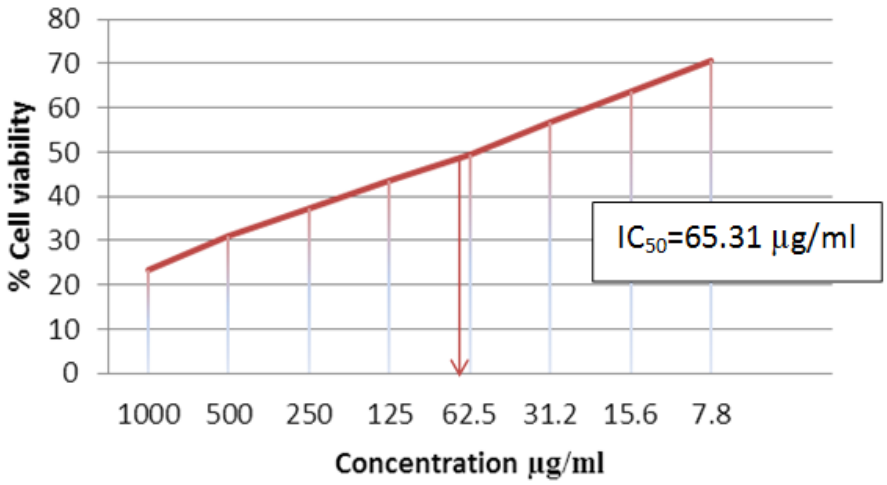


Fig 2: IC_{50} value of Ethyl acetate extract of *Sida cordata*

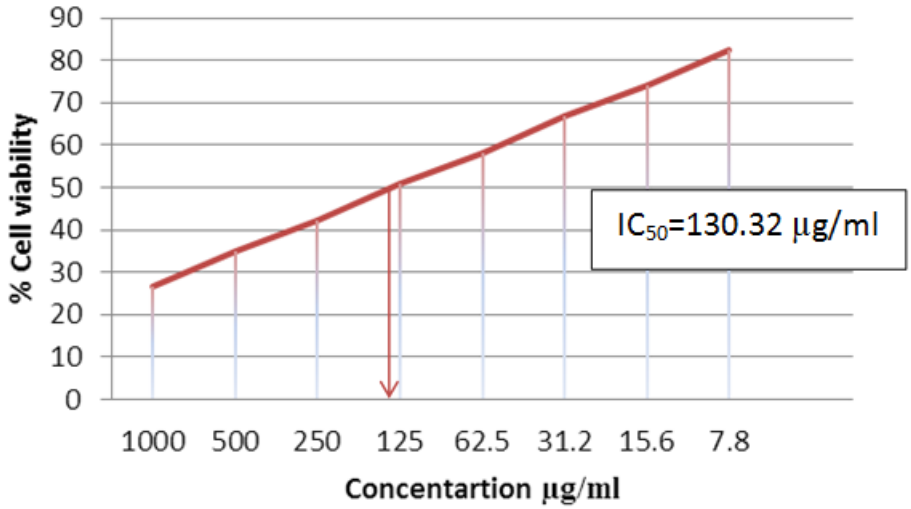
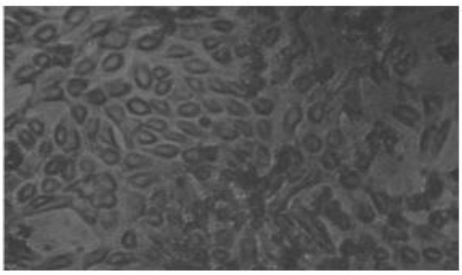
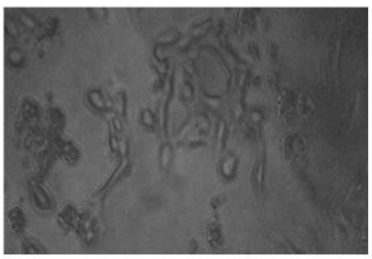


Fig 3: IC_{50} value of Aqueous extract of *Sida cordata*

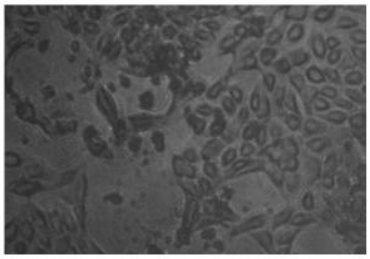
i. Ethanolic extract of *Sida cordata* treated



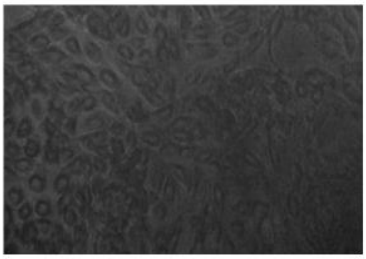
Normal KB cell lines



1000 µg/ml

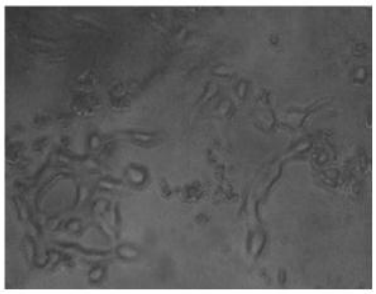


31.2 µg/ml

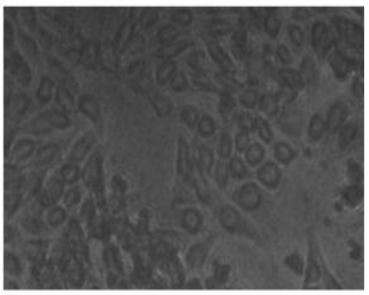


7.8 µg/ml

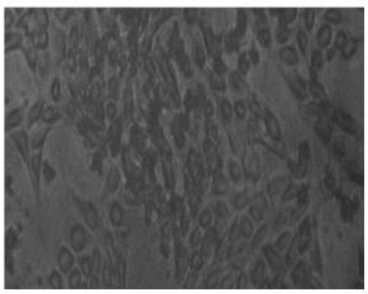
ii. Ethyl acetate extract of *Sida cordata* treated



1000 µg/ml



62.5 µg/ml



7.8 µg/ml

iii. Aqueous extract of *Sida cordata* treated

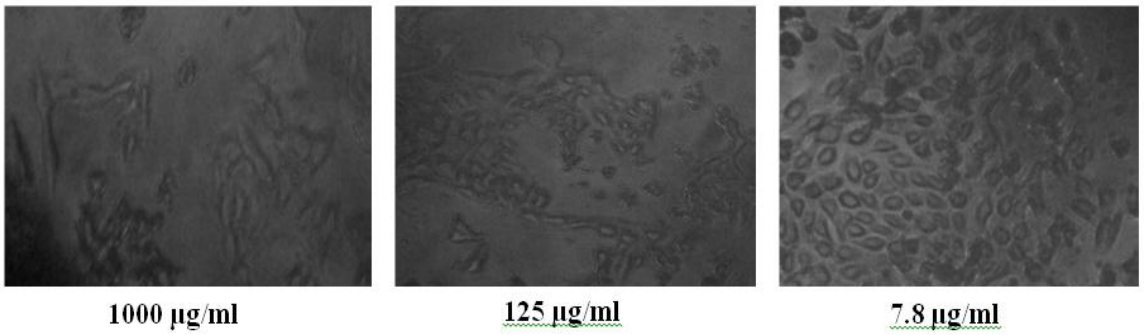


Fig. 4: Density of KB Cells treated with various extracts of *Sida cordata* at various concentrations.

CONCLUSION

From the results of the anti-proliferative studies, it is concluded that the whole plant *Sida cordata* possesses anti-proliferative activity against Human oral epidermoid carcinoma (KB) cell lines. It is quite possible that these extracts, especially ethanolic extract, may also possess significant anti-cancer activity because of its phytochemical profile and better anti-proliferative activity. Hence, further studies are suggested with the ethanolic extract to confirm its anti-cancer activity and to understand the molecular basis of action.

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