

EVALUATION OF *IN VITRO* CYTOTOXIC ACTIVITY OF CHLOROFORM EXTRACT OF *SIDA ACUTA* BURM.F.LAKSHMI KANTA KANTHAL^{1*}, KAUSIK BHAR², PAGIDIGUMMULA RAVALI³

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ABSTRACT

Objective: The aim of the present work was to investigate the *in vitro* cytotoxic activity of chloroform extract of *Sida acuta* Burm.f.

Methods: The *in vitro* cytotoxic activity of chloroform extract was performed by 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide assay method against A-431 (human epidermoid carcinoma) and HeLa (human cervical cancer) cell lines.

Results: The various concentrations of crude chloroform extract (1000, 500, 250, 125, and 62.5 µg/ml concentration) of *S. acuta* whole plant were performed for cytotoxic activity. Effect of inhibition of cell growth showed significantly cytotoxic against A-431 cell lines (human epidermoid carcinoma) with an inhibit cell growth by 50% (IC₅₀) of 375±0.00 and HeLa cell lines (human cervix carcinoma) with an IC₅₀ of 610.00±2.5. The results obtained from the study indicate significant cytotoxic activity. The result of anticancer activity study in cell lines of the extract indicates that *S. acuta* has anticancer activity against A-431-human epidermoid carcinoma and HeLa-human cervical cancer cell lines.

Conclusion: The present study concluded that the chloroform extract of *S. acuta* Burm.f. possess potent cytotoxic activity.

Keywords: *Sida acuta* Burm.f., Chloroform extract, Cytotoxic activity, Inhibit cell growth by 50%.

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INTRODUCTION

Medicinal plants continue to be widely practiced on many accounts. In particular, higher plants have been the source of medicinal agents since the earliest times, and today, they continue to play dominant role in the primary health care of about 80% of the world's population [1]. Natural products and medicinal agents are also an essential feature in the health-care system of the remaining 20% of the population residing mainly in developed countries with more than 50% of all drugs in clinical use having a natural product origin [2]. Herbal drug is still the strength of about 75-80% of the world population. Herbalism is the ancient form of healthcare known to humans [3]. It was a fundamental part for the improvement of the modern society. Herbal drugs are used in therapeutics to prevent and treat diseases and ailments or to support health and healing [4].

In various countries, herbal medicines may also include by habitual, natural organic, or inorganic active constituents which are not of plant resource. Herbal drug is a principal component in habitual medicine and a general constituent in Ayurvedic, Homeopathic, Naturopathic, and other medication systems. Herbs are generally considered as safe because they belong to innate sources [5]. This research provides a widespread scheme of herbal medicines and anticipates to elucidate the therapeutic efficacy of different herbal medicines, undesirable drug reactions, drug interactions, standardization, and stability testing of herbal medicines, pharmacovigilance, and dogmatic status of herbal medicines [6]. About 120 plant-derived drugs commonly used in many countries. 74% were discovered as a result of chemical studies directed by the isolation of the active constituents of plants, which are used in traditional medicine [7].

Several classes of anticancer agents have been developed and many of them are from natural origin. However, a major problem in the use of these

agents in cancer treatment is the undesirable side effects produced as a result of non-tumor specificity and multiple-drug resistance. Therefore, in cancer research, traditional medicine has aroused renewed interest in the search for safe, potent, and selective anticancer compounds [8].

METHODS

Plant material

The whole plant of *Sida acuta* was collected from the local areas of Tallarevu (M), Korangi (V), East Godavari Dist., Andhra Pradesh. Identification and authentication of the plant was done by Mr. P. Venu, Additional Director, Botanical Survey of India, Deccan regional Centre, Hyderabad - 500 048. A specimen voucher has been deposited there.

Preparation of extracts

The whole plant parts of *S. acuta* were shade dried at room temperature and powdered and passed through 60 mesh size sieves. Accurately weight 180 g of powdered plant parts and extracted with chloroform solvent (800 ml) using cold maceration method. Thus, obtained extract was filtered using Whatman filter paper grade no. 1, followed by concentration of the filtrate. The extract (1.9 g) was shifted to a bottle having screw cap that is previously sterilized and kept in refrigerator by labeling for further use.

Preliminary phytochemical screening

The chloroform extract was tested for carbohydrates, protein and amino acids, alkaloids, glycosides, flavonoids, tannins, steroids, and saponins [9-18].

Cytotoxic activity

Chemicals

The chemicals used in *in vitro* cytotoxic activity are 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), fetal bovine

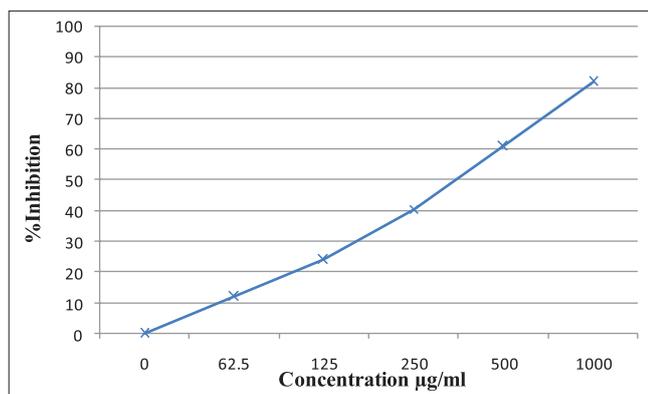


Fig. 1: Cytotoxic activity of *Sida acuta* Burm.f. on A-431 cell line

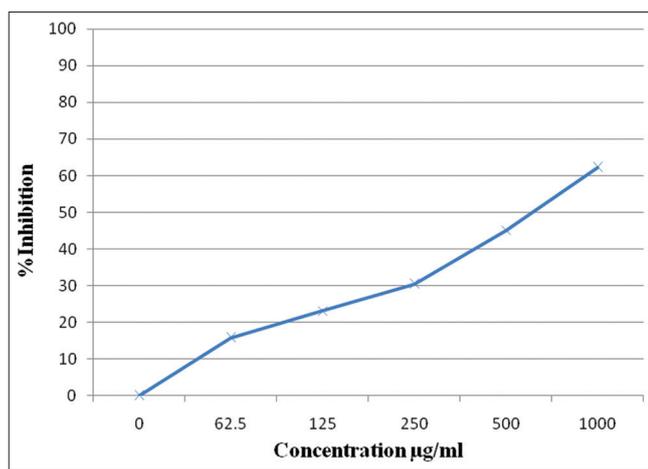


Fig. 2: Cytotoxic activity of *Sida acuta* Burm.f. on HeLa cell line

serum (FBS), phosphate-buffered saline (PBS), Dulbecco's Modified Eagle's medium (DMEM), and trypsin which were obtained from Sigma Aldrich Co., St Louis, USA. Ethylenediaminetetraacetic acid (EDTA), glucose, and antibiotics were obtained from Hi Media Laboratories Ltd., Mumbai. Dimethyl sulfoxide (DMSO) and propanol were obtained from E Merck Ltd., Mumbai, India.

Cell lines and culture medium

A-431-human epidermoid carcinoma and HeLa-human cervical cancer cell lines were procured from National Centre for Cell Sciences, Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated FBS, penicillin (100 IU/ml), streptomycin (100 µg/ml), and amphotericin B (5 µg/ml) in a humidified atmosphere of 5% CO₂ at 37°C until confluent. The cells were dissociated with trypsin phosphate versene glucose solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm² culture flasks, and all experiments were carried out in 96 microtiter plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of test solutions

For cytotoxicity studies, weighed test extracts were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two-fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT assay

The *in vitro* anticancer activity was determined using MTT assay to measure cell viability.

The monolayer cell culture was trypsinized and the cell count was attuned to 1.0×10⁵ cells/ml using DMEM containing 10% FBS.

Every well of the 96-well microtiter plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hrs, when a partial monolayer was produced, the supernatant was flicked off, the monolayer was washed once with medium, and 100 µl of different test concentrations of extracts was added on to the partial monolayer in microtiter plates. The plates were then incubated at 37°C for 3 days in 5% CO₂ atmosphere, microscopic examination was carried out, and observations were noted in every 24 hrs interval. After 72 hrs, the sample solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were lightly shaken and incubated for 3 hrs at 37°C in 5% CO₂ atmosphere. The supernatant was removed, 100 µl of propanol was added, and the plates were smoothly shaken to solubilize the formed formazan. The absorbance was calculated using a microplate reader at a wavelength of 540 nm [10]. The percentage growth inhibition (PGI) was calculated using the following formula and concentration of test sample needed to inhibit cell growth by 50% (IC₅₀) was generated from the dose-response curves for each cell line.

% Growth inhibition = 100 - [(mean occyte diameter (OD) of individual test group/mean OD of control group) × 100].

RESULTS AND DISCUSSION

The preliminary phytochemical study revealed that chloroform extract of *S. acuta* whole plant contains carbohydrates, alkaloids, saponins, fixed oils, tannins, and flavonoids.

In vitro cytotoxic activity of *S. acuta* chloroform extract for the concentrations, 62.5, 125, 250, 500, 1000 µg/ml against A-431-human epidermoid carcinoma and HeLa-human cervical cancer cell lines was studied using MTT assay. There was a gradual less increase in the value of PGI as the concentration of the *S. acuta* extract was increased against A-431-human epidermoid carcinoma and HeLa-human cervical cancer cell lines (Figs. 1 and 2). Effect of inhibition of cell growth showed significantly cytotoxic against A-431 cell lines (human epidermoid carcinoma) with an IC₅₀ of 375±0.00 and HeLa cell lines (human cervix carcinoma) with an IC₅₀ of 610.00±2.5. The result of cytotoxic activity study in cell lines of the extract indicates that *S. acuta* has anticancer activity against A-431-human epidermoid carcinoma and HeLa-human cervical cancer cell lines.

CONCLUSION

The present plant *S. acuta* can be considered as an important source of natural products that have potent cytotoxic activity due to the presence of different phytochemical constituents. Future scope demands that there is a need for the isolation of the constituents responsible for the pharmacological action and to screen the exact mechanism of action for the curative purpose.

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