

Original Article

CYTOTOXICITY STUDIES OF AN ETHNOBOTANICALLY SELECTED *SOLANUM PUBESCENS* WILLD. AGAINST SELECTED HUMAN CANCER CELL LINES

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Received: 04 Sep 2019, Revised and Accepted: 21 Nov 2019

ABSTRACT

Objective: The present study was aimed to determine the cytotoxicity concentration (CTC₅₀) of different extracts made from the leaf and stem bark of an ethno botanically selected *S. pubescens* against Human liver carcinoma (Hep G2), Human colon carcinoma (CaCo₂) and Human breast cancer (T-47 D) cell lines.

Methods: Ethnobotanical survey was done through interviewing traditional medicinal practitioners then a potential herbal plant was selected after a thorough literature survey and its identity was confirmed. The soxhlet extraction method was adopted using five different solvents from leaf and stem bark powders of the study plant and the CTC₅₀ of all the extracts were determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay method.

Results: Among the different extracts, CTC₅₀ values were significant for stem bark extracts made from methanol (24.51±0.08 µg/ml) against Hep G2, while leaf chloroform extract was promising (57.15±1.75 µg/ml) against CaCo₂ and n-hexane extract of leaf exhibited significant value (20.27±1.52 µg/ml) against T-47 D cancer cell lines.

Conclusion: The major findings of the present study clearly provides evidence that the leaf and stem bark of *S. pubescens* possesses the potential anticancer bioactive compound solasodine.

Keywords: *S. pubescens*, Cytotoxic activity, MTT assay, Hep G2, CaCo₂, T-47 D

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INTRODUCTION

Cancer is all concerning uncontrolled cell growth [1], it is escalating at an alarming rate causing a threat to human life and sustainability. Strategies for predictable cancer cure by chemotherapy and radiation therapy decrease the bulk of tumor cells but a population of cancer stem cells remains [2] and plant-based anticancer drug discovery gain importance due to the limited success of clinical therapies using synthetic anticancer drugs together with their immense side effects [3, 4]. Many plant-derived compounds "phytochemicals" have been acknowledged to have anti-tumor properties, for example, induction of apoptosis and inhibition of cell proliferation resulting in reducing the risk of cancer [5, 6]. Still many of the potential herbs which are used by the traditional medicinal practitioners to treat several diseases are yet to be studied for their cytotoxic studies based on their traditional medicinal use. Some of the villagers in the Chitradurga district used to approach traditional medicinal practitioners for various ailments and get treated. This indicates that documentation of the indigenous knowledge, conservation, domestication and better management of key medicinal plants is necessary to ensure intergenerational benefits from the herbal medicines [7]. Cytotoxic studies of different extracts from the species of *Solanum* like *S. pseudocapsicum* [8], *Solanum nigrum* [9, 10], *S. trilobatum* [3], *S. anguivi* [11], *S. torvum* [12, 13], *S. erianthum* [14] and *S. lycopersicum* [15] were reported. Researches on the selection of medicinal plants for its phytochemical extraction and their cytotoxicity studies should begin with the ethnobotanical survey-based approach with traditional medical practitioner's knowledge. The genus *Solanum* of Solanaceae members found to have rich steroidal glycoalkaloids which are an important group of plant secondary metabolites isolated from more than 350 *Solanum* species [16, 17]. But, comprehensive phytochemicals extraction and their cytotoxic activity studies were not reported in *S. pubescens* Willd. Hence, in the present study, *S. pubescens*, an ethnobotanically important plant was selected for the extraction of phytochemicals and investigation of their

cytotoxicity against Hep G2, CaCo₂ and T-47 D cell lines were made to identify a potent medicinal plant for anticancer drug molecules.

MATERIALS AND METHODS

The ethnobotanical survey, plant material selection and extraction of phytochemicals

To select a herbal medicinal plant with anti-cancerous properties, an ethnobotanical survey of medicinal plants used by the traditional medicinal practitioners was conducted in six taluks of Chitradurga district, Karnataka, India (latitude 14 ° 13' 48" N and longitude 76 ° 24' 0" E) during September 2014 to August 2016. In this survey, a standard questionnaire was framed and used to interview traditional medicinal practitioners [18]. Details of the plants used to cure various diseases were documented and the plants were identified by using valid literature and illustrations [19]. Based on the traditional medicinal use and the research reports, the study plant *S. pubescens* was collected from the study area and the voucher specimen number is IDSGH43. The plant was identified and confirmed by referring Phytographia [20] and further authenticated by Prof. L. Rajanna, Chairperson, Department of Botany, Bangalore University, Bangalore, Karnataka, India. The plant herbarium was deposited and maintained in the department of Botany, Indavara Dodda Siddalinge Gowda Government College, Chikkamagaluru, Karnataka, India (fig. 1).

The matured leaf and stem bark of *S. pubescens* was dried, powdered and soxhlet extractions for each sample were made separately using five different solvents and samples were labelled as leaf ethyl acetate extract, stem bark ethyl acetate extract, leaf n-hexane extract, stem bark n-hexane extract, leaf methanol extract, stem bark methanol extract, leaf hydro alcohol extract, stem bark hydro alcohol extract, leaf chloroform extract and stem bark chloroform extract [21].



Fig. 1: A. Habit of *S. pubescens*, the source of leaf material, B. The stem with bark

Preparation of test solution

For cytotoxicity studies, 10 mg of test substances were separately dissolved and volume was made up with Dulbecco's Modified Eagles Media-High Glucose (DMEM-HG) supplemented with 2% inactivated Fetal Bovine Serum (FBS) to obtain a stock solution of 1 mg/ml for all the cell lines and sterilized by 0.22 μ syringe filtration. Serial two-fold dilutions were prepared from this for carrying out cytotoxic studies.

Chemicals

Ethyl acetate, n-Hexane, Methanol, Chloroform, Dimethyl sulfoxide (DMSO), Propanol were obtained from E Merck Ltd., Mumbai, India. MTT, FBS, Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co. St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai.

Cell line and culture medium

Hep G2, CaCo2 and T-47 D cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in their respective media viz., DMEM-HG supplemented with 10% inactivated FBS, penicillin (100 IU/ml), streptomycin (100 μ g/ml) and amphotericin B (5 μ g/ml) in a humidified atmosphere of 5% CO₂ at 37°C until confluent. The cells were dissociated with Trypsin Phosphate Versene Glucose (TPVG) solution [0.2% trypsin, 0.02% ethylene diamine tetraacetic acid (EDTA), 0.05% glucose in PBS]. The stock cultures were grown in 25 cm² culture flasks and all experiments were carried out in 96 well microtiter plates (Tarsons India Pvt. Ltd., Kolkata, India).

Cytotoxicity studies

In all the cell lines, the monolayer cell culture was trypsinized and the cell count was adjusted to 100,000 cells/ml using respective

media viz., DMEM-HG containing 10% FBS. To each well of the 96 well microtiter plate, 0.1 ml of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, monolayer washed once with medium and 100 μ l of different test concentrations of test substances were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37 °C for 72 h in a 5% CO₂ atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval [22].

Cell viability assay

After 72 h incubation, the drug solutions in the wells were discarded and 50 μ l of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37 °C in a 5% CO₂ atmosphere. The supernatant was removed and 100 μ l of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the standard formula and the concentration of test substances needed to inhibit cell growth by 50% (CTC₅₀) was generated from the dose-response curves for each cell line. DMSO was used as control. Trials were conducted with three replicates for each concentration and data were expressed as mean \pm Standard Deviation (SD).

RESULTS AND DISCUSSION

Selection of *S. pubescens* was accomplished through an ethnobotanical survey, a herbal medicinal plant used by the traditional medicinal practitioners to treat cancer. In the survey, a sum of 190 plant species belonging to 156 genera and 75 families were screened from six taluks of Chitradurga district whereas in the literature cytotoxicity study of *S. pubescens* is not yet reported. Hence, in the present investigation, MTT assay-based cytotoxicity was determined in all the ten different extracts against Hep G2 cell line with the concentrations of test solutions ranging from 1000-7.8 μ g/ml. Best four extracts that exhibited 50% viability of cell lines are 24.51 \pm 0.08, 44.92 \pm 0.04, 48.85 \pm 0.41 and 49.03 \pm 0.17 μ g/ml in stem bark methanol, stem bark hydro alcohol, leaf chloroform and leaf n-hexane extracts, respectively (table 1 and fig. 2 A, B). In the Hep G2 cell line, the cytotoxic study showed that the stem bark is found to be the good source of the anti-cancer drug for human liver carcinoma and the methanol is a suitable solvent to extract the phytochemicals. Further, cytotoxic studies on CaCo2 and T-47 D cell lines exhibited the CTC₅₀ values 57.15 \pm 1.75 and 20.27 \pm 1.52 μ g/ml respectively and both the fractions were extracted using chloroform from leaves of the study plant. The present study findings indicate that the leaf should be the source of herbal raw material to investigate the anticancer drugs against human colon and breast cancer (table 1 and fig. 2 C-F).

Table 1: Cytotoxic properties of leaf and stem bark extracts of *S. pubescens* against HepG2, CaCo2, T-47 D cell lines

| S. No. | Name of the solvent used | Cytotoxicity concentration (μ g/ml) | | | | | |
|--------|--------------------------|--|-------------------|------------------|------------------|------------------|-------------------|
| | | Hep G2 | | CaCo2 | | T-47 D | |
| | | Leaf | Stem bark | Leaf | Stem bark | Leaf | Stem bark |
| 1. | Ethyl acetate | 159.73 \pm 2.01 | 187.88 \pm 0.86 | ND | ND | ND | ND |
| 2. | n-hexane | 49.03 \pm 0.17 | >1000 | 61.03 \pm 0.12 | ND | 26.67 \pm 1.70 | ND |
| 3. | Methanol | 116.21 \pm 0.58 | 24.51 \pm 0.08 | ND | 83.68 \pm 0.33 | ND | 112.29 \pm 4.28 |
| 4. | Hydo alcohol | 105.40 \pm 0.13 | 44.92 \pm 0.04 | ND | 77.50 \pm 0.62 | ND | 94.93 \pm 0.55 |
| 5. | Chloroform | 48.85 \pm 0.41 | 184.55 \pm 0.66 | 57.15 \pm 1.75 | ND | 20.27 \pm 1.52 | ND |

*CTC₅₀ expressed as mean \pm SD, n=3, ND=Not detected

The ethnobotanical survey in the study area revealed the traditional knowledge from medicinal practitioners about the preferential use of medicinal plants for the treatment of various diseases and *S. pubescens* for cancer. According to WHO (World Health Organisation), cancer is a leading cause of death worldwide, accounting for an estimated 9.6 million deaths in

2018 and some of the most common causes of cancer death are cancers of colorectal (8,62,000 deaths), liver (7,82,000 deaths) and breast (6,27,000 deaths). The limited success of allopathic anticancer drugs and their massive side effects led to the move towards the development of plant-based anticancer drug discovery [3, 4].

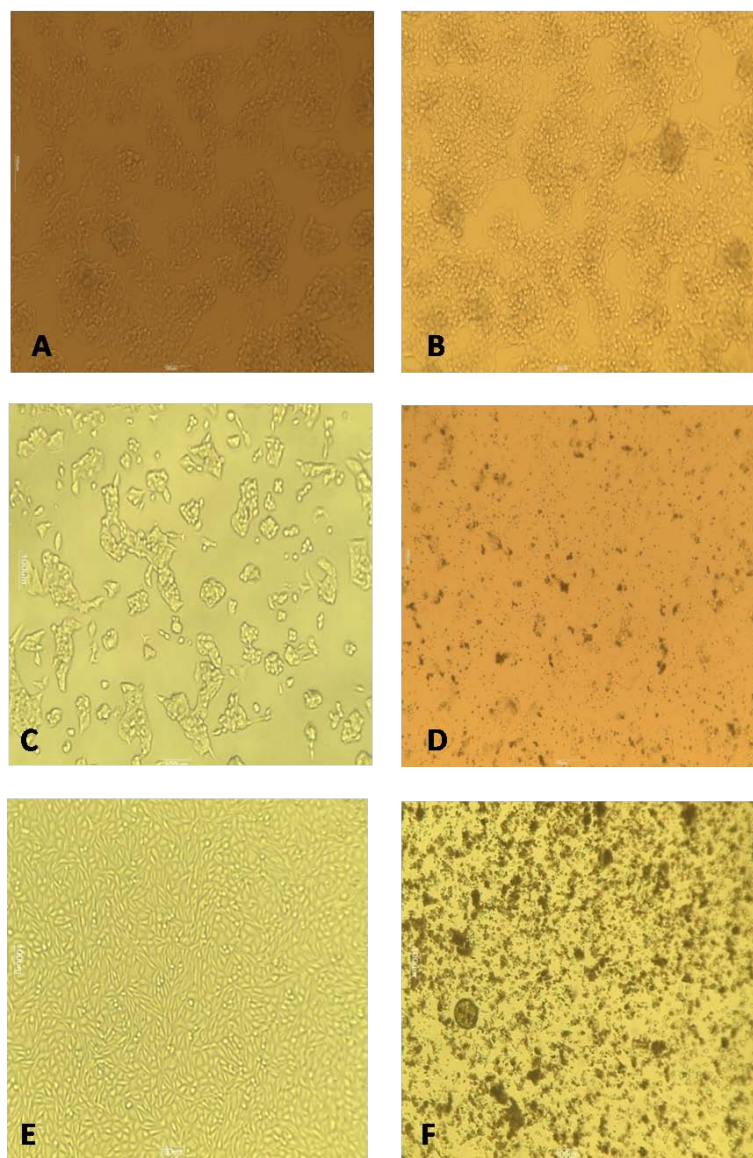


Fig. 2: A-F. Shows the cytotoxic inhibitory effect on cell viability by the different extracts made from leaf and stem bark of *S. pubescens* on different human cancer cell lines. A-Control for HepG2, B-stem bark hydro alcohol 7.8 $\mu\text{g/ml}$ (CTC_{50} 44.92 \pm 0.04 $\mu\text{g/ml}$), C-Control for CaCo2 cell line, D-leaf chloroform 15.6 $\mu\text{g/ml}$ (CTC_{50} 57.15 \pm 1.75 $\mu\text{g/ml}$), E-Control for T-47D cell line, F-leaf chloroform 15.6 $\mu\text{g/ml}$ (CTC_{50} 20.27 \pm 1.52 $\mu\text{g/ml}$)

Over the past decades, there has been a resurgence of interest in the investigation of herbs as a source of potential anticancer drugs. Investigations on solasodine and its concentration in *S. pubescens* were made and found that the leaf and stem bark are the right sources of raw material for the cancer drug material [21]. In the present study, all the ten extracts were tested for their cytotoxic activity and the extracts like stem bark methanol, stem bark hydro alcohol, leaf chloroform and leaf n-hexane are noteworthy as they could exhibit promising anti-proliferative effects and the CTC_{50} values are quite evident (24.51 \pm 0.08, 44.92 \pm 0.04, 48.85 \pm 0.41 and 49.03 \pm 0.17 $\mu\text{g/ml}$) on Hep G2 cell line (table 1 and fig. 2 A, B). These results authenticate that the stem bark and leaf are found to be a good source of herbal drug raw material against human liver carcinoma. Liver cancer is a progressively increasing tumor with high mortality, not responsive to the cytotoxic agents used in chemotherapy and existing effective therapies have harmful side effects [23, 24]. Several *Solanum* species were extensively screened for its potential cytotoxic and anti-proliferative activity of phytochemicals extracted from the leaf, stem, fruit, seed and aerial parts against Hep G2 [3, 8, 9, 11, 14, 23] and found that the $\text{CTC}_{50}/\text{IC}_{50}$ values reported are from 7.04 \pm 1.08 to 225 \pm 15.2 $\mu\text{g/ml}$

with the concentration ranging from 5 to 1000 $\mu\text{g/ml}$. In the present study, different extracts' test solutions concentrations used were 7.8 to 1000 $\mu\text{g/ml}$ and the CTC_{50} values are between 24.51 \pm 0.08 and 49.03 \pm 0.17 $\mu\text{g/ml}$. In the Hep G2 cell line, the cytotoxicity result findings conclude that the methanolic extracts from stem bark induced a significant 50% antiproliferative effect.

Further, the extracts were evaluated for cytotoxic studies on CaCo2 and T-47 D cell lines and the significant CTC_{50} values recorded are 57.15 \pm 1.75 and 20.27 \pm 1.52 $\mu\text{g/ml}$ respectively and both the fractions were extracted using chloroform from leaves of the study plant (table 1 and fig. 2 C-F). Similar cytotoxic activity reports were found from the fruit extracts of *S. aculeastum* [25] and leaf extracts of *S. nigrum* [10] and the $\text{CTC}_{50}/\text{IC}_{50}$ values are 24.40 \pm 1.13 $\mu\text{g/ml}$ for CaCo2 and 0.948 mg/ml for Human Colorectal Carcinoma cell lines, respectively. Also, a report on cell viability assay of leaf and fruit extracts from *S. erianthum* on human breast cancer cell line (MCF-7) was found [14]. In *S. pubescens* Willd., research reports have been made on pharmacological studies like antidiabetic activity [26], anti-inflammatory activity [27], anti-diarrheal activity [28], antinociceptive activity [29], anticonvulsant and sedative effects

[30] and antibacterial activity [31, 32] but reports on anticancer activity of phytochemical extracts from its leaf and stem bark extracts are not accessible. Hence, the present study was focused on the extraction of phytochemicals using five different solvents, tested their potent cytotoxic activity on human liver, colon and breast cancer cell lines and revealed the suitable solvents and plant parts that substantiate the herbal drug encounter for cancer therapy from *S. pubescens*. A thought-provoking fact was found reported [33] on solasodine and other related alkaloids that trigger against the cancer cell and death by the lysosomal-mitochondrial mediated apoptosis pathway and the death receptor-mediated apoptosis pathway. Furthermore, the carbohydrate moiety of these steroidal alkaloids has anti-metastatic properties and plays a key role in expressing antineoplastic actions and also a class of clinically important multi-drug-resistance with cancer cells. Antiproliferative and cytotoxic effects of phytochemicals extracted from leaf of *S. trilobatum* were reported from a locally available plant Madhavaram, Tamil Nadu, India, its vital that such locally available plants belong to Solanaceae are rich in bioactive compounds for many diseases and hence their therapeutic potential need to be thoroughly investigated [34, 35].

CONCLUSION

Cytotoxicity studies of leaf and stem bark extracts of *S. pubescens* Willd. on Hep G2, CaCo2 and T-47 D cell lines are a first of its kind report, the findings have inferred that the stem bark (methanolic extract) is found to be the source of raw material for anti-cancer drug for human liver carcinoma, the leaf (chloroform extract) is against both human colon and breast cancer and it is evident that solasodine is the vital anti-carcinogenic compound as reported in our previous investigation. Moreover, the solasodine of *S. pubescens* Willd. is a major source of anti-proliferative compounds that is to be further investigated on the line of isolation, purification, characterization and testing its individual anti-carcinogenic potential at the molecular level.

ACKNOWLEDGEMENT

The authors are thankful to the P. G. Department of Botany, I.D. S. G. Government College, Chikkamagaluru, Karnataka State, India for providing the facilities for performing the extraction work.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

The first author has planned the research work, designed and carried out all the experiments, data compilations, statistical analysis, and preparation of the manuscript under the guidance of the second author. The second author has made critical observations in the manuscript and developed the final publishable format.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest

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