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Antiproliferative activity of *Solanum anguivi* against cancer cell lines

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ABSTRACT

In the present study, the metabolites present in the leaves of *Solanum anguivi* was extracted using ethyl acetate. HEPG-2 and MCF-7 cell lines were collected from NCCS. The ethyl acetate extract of the *Solanum anguivi* was analyzed for its antiproliferative activity against the cell lines using MTT assay at various concentrations. The cell viability was reduced upto 50% at the concentration of 0.625 mg/ml for HEP G2 cell line and 1.25 mg/ml MCF-7 cell line. The DNA fragmentation assay was performed to identify the DNA fragmenting pattern before and after treatment with plant extract. The present study clearly illustrated the ethyl acetate extract of *Solanum anguivi* possesses the anticancer activity and further characterization was necessary know its activity at molecular level.

Key words: *Solanum anguivi*, Cell lines, MTT assay, DNA fragmentation.

INTRODUCTION

Cancer is considered as the second leading cause for death worldwide. Cancer can be characterized by the failure in the regulation of tissue growth results in the uncontrolled multiplication of the normal cells to form tumors which in further invades into nearby parts of the body [1]. The cancer can be caused by both carcinogenic factors and also hereditary. The cancer is considered as a preventable disease since the majority of the cancer was caused by the environmental (including lifestyle) factors [2].

Currently, Chemotherapy and Radiotherapy treatments were followed for the treatment of various cancers, but are found to be having limited survivability and possess various side effects [3]. Hence there is an urge for the development of new anticancer drug for its treatment and prevention. Natural products from plants play a dominant role in the discovery of such new drugs. It has been estimated that about 60% of approved drugs were of natural origin [4].

Solanum anguivi is a non tuberous and widely distributed plant possesses various medicinal properties. Mostly, the plant prefers to grow in humid temperature and commonly found as weed in gardens [5]. The dried powders of the fruits were used in the medication for Blood pressure [6]. The actual mechanism between the flavanoids and the antitumor effect is not clearly understood. But, it was assumed that the cytotoxic action was done by activation of various enzymes involved in the metabolic pathways [7].

The cancer cell lines were used as the invitro model for the study of human hepatocytes [8]. In the present study, the cytotoxic HEPG-2 (a human liver carcinoma cell line) and MCF-7 (breast cancer cell line) cell lines were selected for analyzing the antiproliferative activity of *Solanum anguivi* leaf ethyl acetate extract. These cells were found to be suitable models to study the intracellular trafficking since because of their high degree of morphological and functional differentiation [9].

The present study aimed at the analysis of antiproliferative activity of the ethyl acetate extract of *Solanum anguivi* towards the HEPG-2 and MCF-7 cell lines and their DNA fragmentation profile analysis.

MATERIALS AND METHODS

Collection and culturing of cell lines

The cytotoxic HEPG-2 and MCF-7 cell lines were collected from the National Centre for Cell Sciences (NCCS), Pune. The cells were maintained in Minimal Essential Media (MEM; HiMedia) supplemented with 10% Fetal bovine serum (FBS), penicillin (100 µg/ml), and streptomycin (100 µg/ml) in CO₂ incubator at 37°C.

Extraction of metabolites from *Solanum anguivi*

The healthy leaves were collected from *Solanum anguivi* plants and were washed with tap water thoroughly, rinsed with distilled water and shade dried (for three days) until they completely dried. Then they were cut into small pieces and ground into powder using pestle and mortar and stored at room temperature. The leaf samples were extracted with ethyl acetate 1:10 (w/v) and kept under shaking condition at 100 rpm over night. The extracts were collected and repeated the above extraction procedures for thrice and collected maximum extracts. The samples were condensed and concentrated for further analysis.

Antiproliferative activity of *Solanum anguivi* on HEPG-2 and MCF-7 cell lines

The antiproliferative activity of the ethyl acetate extract of *Solanum anguivi* against HEPG-2 and MCF-7 cell lines was determined by methylthiazolyl diphenyl- tetrazolium bromide (MTT) assay [10]. In a 24-well plate (Costar Corning, Rochester, NY), the cells were allowed to grow for 48 hours to reach confluence. The cells were then incubated in the presence of various concentrations of the *Solanum anguivi* ethyl acetate extract in 0.1% DMSO for 48h at 37°C. After removal of the plant extract solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide cells (MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC₅₀) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of HEPG-2 and MCF-7 were expressed as the % cell viability, using the following formula:

$$\% \text{ cell viability} = (\text{A}_{570} \text{ of treated cells} / \text{A}_{570} \text{ of control cells}) \times 100$$

DNA Fragmentation

For the DNA fragmentation assay [11], 0.5 ml of the cell suspension was centrifuged and the pellet was collected and dissolved in TTE (Tris-Taps-EDTA) buffer with vigorous shaking. The mixture was centrifuged and the supernatant was transferred carefully to separate tubes marked as T. To the pellet added 0.5 ml of TTE buffer, 0.5 ml of Ice-cold 1M NaCl and 0.7 ml of ice-cold isopropanol. The mixture was incubated overnight at -20°C to enhance precipitation. The content was centrifuged further to obtain pellet and was purified by repeated wash with 0.5 ml of 70% ice cold ethanol. The final pellet obtained was air dried and dissolved in 20-50µl of TE (Tris-EDTA) buffer. The fragmentation patterns of both the HEPG-2 and MCF-7 cell lines were analyzed and compared with their control.

RESULTS AND DISCUSSION

Cancer is considered as the serious health problem worldwide and various natural compounds from the plants were analyzed for their anticancer effects by scientists. Medicinal plants represent a vast potential resource for anticancer compounds. Various medicinal plants reported to having antiproliferative activity so far were: *Azadirachta indica* (Neem), *Tinospora cordifolia* (Guduchi), *Triticum aestivum* (Wheatgrass), *Aloe barbadensis* (Aloe vera), *Ocimum sanctum* (Tulsi), etc [12]

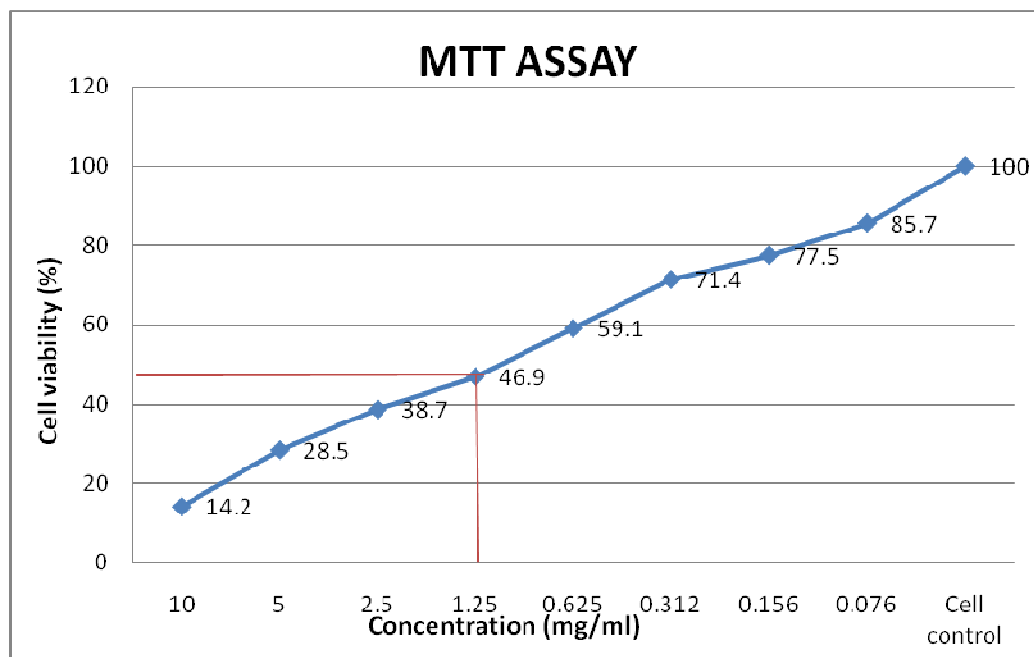


Fig. 1 Anticancer effect of *Solanum anguivi* ethyl acetate extract on HEp G2 cell line

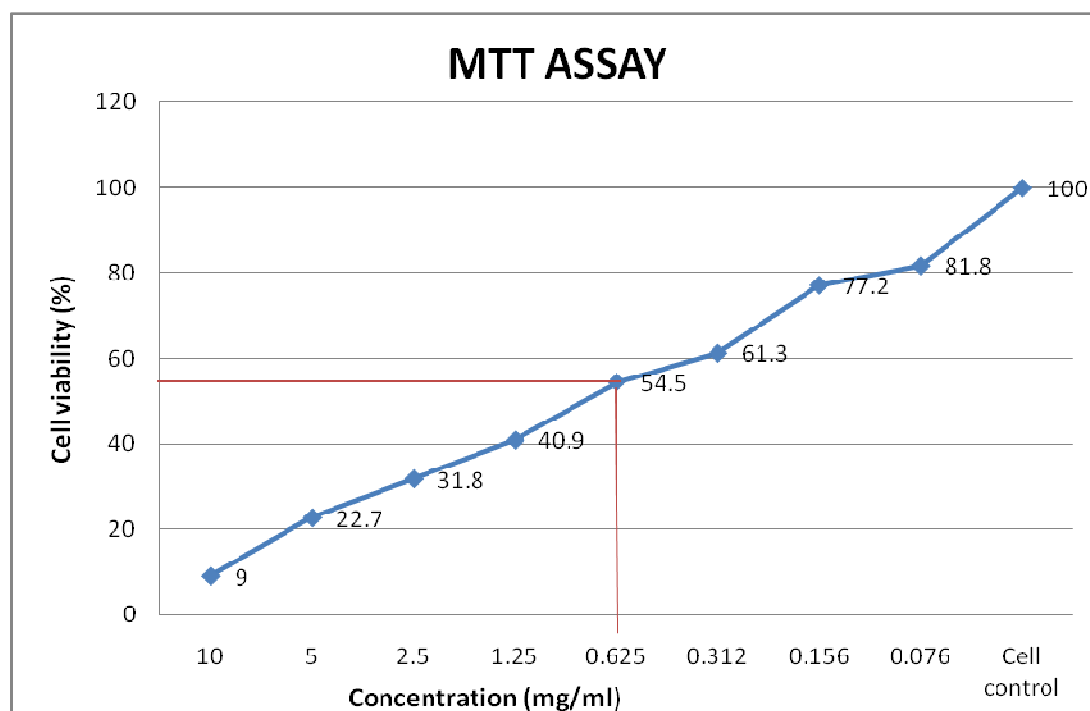


Fig. 2 Anticancer effect of *Solanum anguivi* ethyl acetate extract on MCF-7 cell line

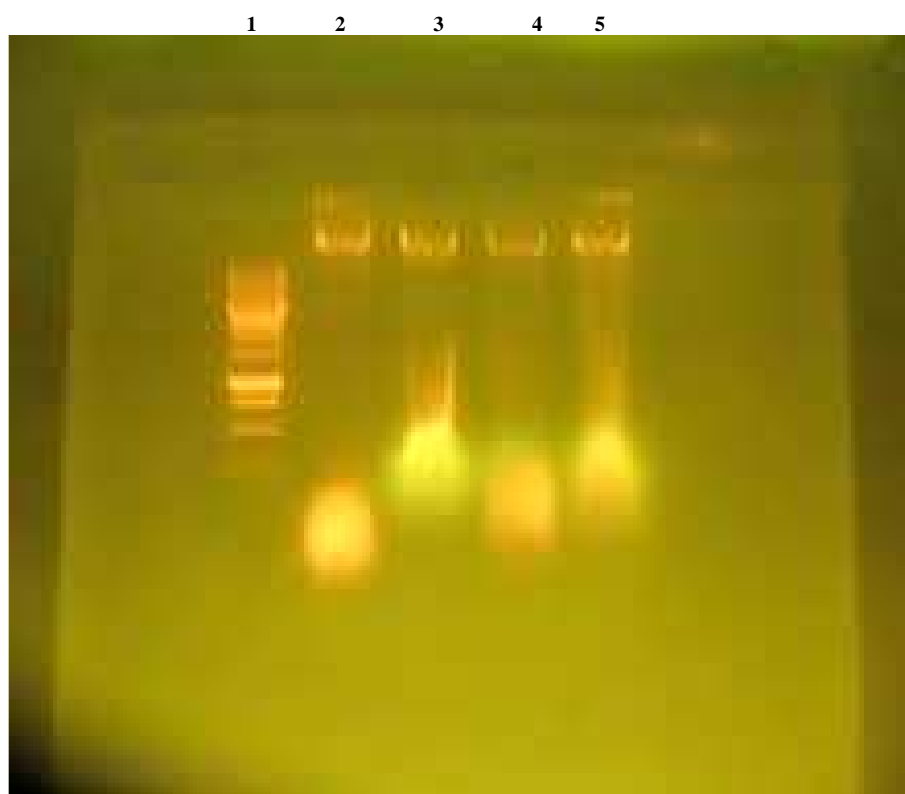
Medicinal plants continue to play a central role in the healthcare system of large proportions of the world's population. Medicinal plants are the most exclusive source of life saving drugs for the majority of the world's population. Several species of the Solanaceae family are currently used for the treatment of various infections and skin disorders. Several reports were available for the antiproliferative activity of Solanaceae family on several

human cancer cell lines [7]. Hence, in the present study also, the antiproliferative activity of the *Solanum anguivi* on cancer cell lines was analyzed.

The cytotoxic cells were grown under controlled conditions, outside of their natural environment. The antiproliferative activity of the ethyl acetate extract was subjected for MTT assay. In this assay, cell death and cell viability was estimated. The crude extract reduced the cell viability upto 50% at the concentration of 0.625 mg/ml for HEp G2 cell line (Fig. 1) and 1.25 mg/ml MCF-7 (Fig. 2) cell line which is very low and the inhibition was time and dose dependent manner. It indicates the *Solanum anguivi* ethyl acetate extract has better antiproliferative activity towards HEp G2 cell line at minimum concentration. The various compounds such as vitamins, carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, enzymes, minerals etc. might be responsible for the antiproliferative activity of such medicinal plants [13].

In the DNA fragmentation assay, the crude extract induced a very low fragmentation in the case of HEP G2 cell lines (Fig. 3) and the moderate fragmentation was observed in the MCF-7 cell lines (Fig. 4). The decrease in the concentration of plant extract leads to the increase in the DNA fragments. The concentration of the plant extract if indirectly proportional to the degree of fragmentation of MCF 7 cell lines. The DNA fragmentation was done for understanding the deactivation pattern of the genes codes for the enzymes involved in Cancer.

Fig. 3 DNA Fragmentation on HEpG-2 cell line



Lane 1: Marker 1000bp ladder

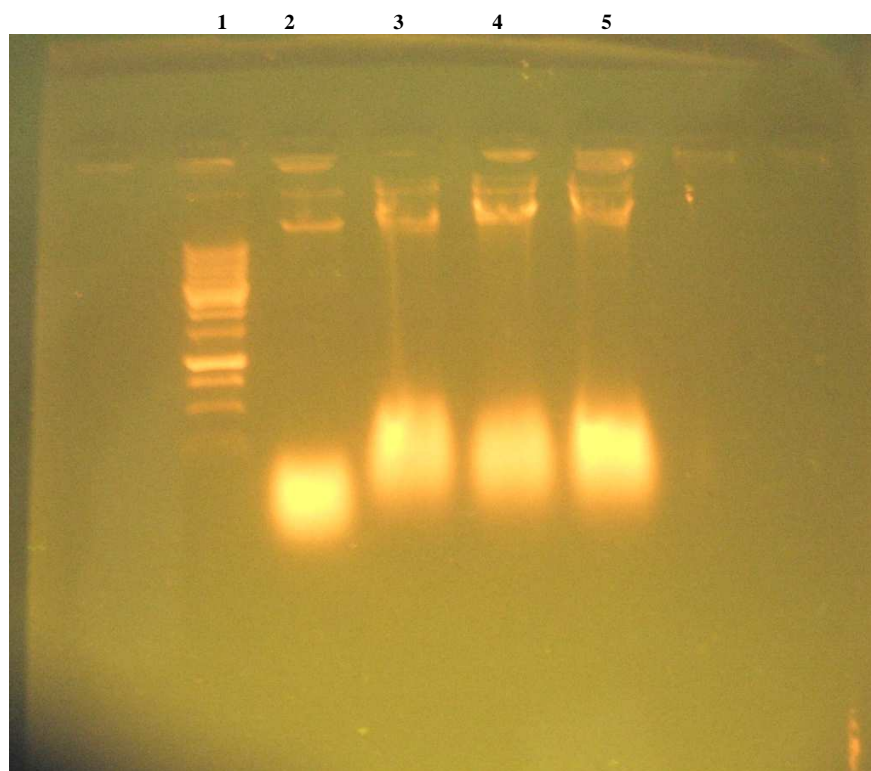
Lane 2: Control

Lane 3: Con: 2.5mg/ml

Lane 4: Con: 1.25mg/ml

Lane 5: Con: 0.625mg/ml

Fig. 4 DNA Fragmentation on MCF-7 cell line



Lane 1: Marker 1000bp ladder
Lane 2: Control
Lane 3: Con: 1.25mg/ml
Lane 4: Con: 0.625mg/ml
Lane 5: Con: 0.312mg/ml

The addition of TTE buffer in the DNA fragmentation assay helps in the disruption of nuclear structure and release of fragmented chromatin from the nuclei. The histones present in the DNA were removed by the addition of NaCl salt.

CONCLUSION

It is well known that the Indian traditional medicinal plants has now led to several therapeutically and useful preparation and compounds, which generates enough encouragements among the scientists in exploring more information about these medicinal plants. Development of such modern drug from the medicinal plants should be emphasized for the treatment of various diseases including cancer.

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