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Research Article

IN VITRO ANTIOXIDANT AND ANTICANCER ACTIVITIES OF SEED EXTRACT OF SOLANUM *VIRGINIANUM*

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ABSTRACT

The aim of the present study is to evaluate the antioxidant and anticancer activities of seed extract of *Solanum Virginianum*. The antioxidant activity was assessed using DPPH scavenging assay while the anticancer property of the *Solanum virginanum* in swiss albino mice against Dalton Ascites Lymphoma (DAL).Tumor was induced in mice by intraperitoneal inoculation of Dalton Ascites Lymphoma cells (1 x 10^5 cells / mouse).The seed extract was found to possess significant antioxidant and anticancer activities.

Keywords: Antioxidant, Antitumor, Dalton Ascites Lymphoma, *Solanum Virgininum*

INTRODUCTION

Therapeutic plants have been used to treat disease in all over the globe. In fact, plants have some bioactive molecules. These molecules are a rich source of the variety of medicines. Medicinal plants have played a significant role in drug development programs in the pharmaceutical science [1]. Therapeutic plants have a lot of organic compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols, and flavonoids. In general, conventional medicines obtained from plant products are safer than their synthetic counterparts [2, 3]Oxidation is necessary for many living organisms for the production of energy. Antioxidant presence in fruits, vegetables, and beverages play an important role in the maintenance of health and prevention of disease. Antioxidant acts as a radical scavenger and is capable of protecting the human body from diseases such as cancer, rheumatoid arthritis, and arteriosclerosis as well as in degenerative processes linked to aging. Antioxidants are synthesized in the body and can also be extracted from the food such as fruits, vegetables, seeds, nuts, and oils. Medicinal plants have attracted great deal of public and scientific attention because of their anticarcinogenic and many other pharmacological effects. [(4-5)] Cancer is a very dangerous disease in humans, and presently there is a significant scientific innovation of new anticancer drugs from natural products[6].Lymphoma is one form of cancer that affects the immune system (white blood cells).

The Solanaceae is a huge plant family with 96 genera and 2,300 species, about one half of which belongs to the genus Solanum. *Solanum* is one of the biggest genera of flowering plants[7]. Solanum Virginianum L. is a very itchy perennial herb. In Ayurvedic, this medicinal plant is used in the preparation of a variety of medicines. It is employed in the treatment of epilepsy, pain relieving,headache, hair falls, bronchial asthma, skin problems, cough, and other diseases[8]. Literature survey revealed not much work has been done to find out the antioxidant and anticancer activities of Solanum virginianum. In the present work, it is aimed to find out antioxidant and anticancer activities of seed extract of *Solanum virgininum.*

MATERIALS AND METHODS

Chemicals and Instruments

α-α diphen yl β picryl hydrazide (DPPH),Ascorbic acid were purchased from Aldrich Merck chemical and other analytical grade chemicals were purchased. The UV/VIS Spectrophotometer(Elico, UV 3000+), Microcentrifuge (Tarsons, Kolkata) were used.

2.1. Plant materials:The fresh fruits with seeds of *Solanum Virginianum* were collected from Salem, Tamil Nadu in India and authenticated from Tamilnadu Agricultural University, Coimbatore. Freshly collected material washed with water and dried Seed materials (60g) were refluxed in methanol by using Soxhlet apparatus (60-80^{0}C). The extract was concentrated under reduced pressure in a rotary evaporator and dried using a high vacuum pump. The crude extract was dissolved in ethanol to required concentration and subject to a qualitative test for identification of various active constituents.

Phytochemical screening:Phytochemical screening of the seed extract of *Solanum Virginianum* was carried out by using the standard procedure. Seed extract was subjected to preliminary phytochemical screening for the presence of alkaloids, tannins, flavonoids, saponins, terpenoids, phenolic compounds, Proteins and carboxylic acids. [9-10]

FT-IR Analysis

A little powder of seed extract was mixed with KBr salt. The FT-IR spectra of seed extract of solanum virginianum were recorded on Perkin-Elmer RX I spectrometer with KBr pellet and their functional groups were identified in the region 4000-400 cm^{-1}.

DPPH-Antioxidant assay

DPPH radical scavenging activity : It is one of the rapid methods to evaluate the antioxidant. The DPPH is stable free radicals that have been generally accepted as free radical scavenging activities of antioxidants. Seed extract to scavenge 1, 1-diphenyl-2-picryhydrazyl (DPPH) free radicals was assessed from the standard procedure.[11-12]. The stock solution of the extract was made in methanol to prepare the concentration of 1 mg/ml. Dilutions were prepared to attain concentrations of 10, 20, 30, 40,50 and 60µg/ml. 1 ml of each diluted solutions were mixed with 3 ml of methanol solution of DPPH. After 30 minutes of incubation at room temperature, the reduction of the DPPH free radical was measured with the evaluation of the absorbance at 517 nm by UV-Visible Spectrophotometer. Absorption of the blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control. Ascorbic acid was used as the standard. The experiment was carried out in triplicate, and the % inhibition was calculated from the following equation (1) and IC_{50} values were estimated from the % inhibition versus concentration plot.

$$\% \textit{ inhibition} = \frac{\textit{Control } \textbf{Absorbance} - \textit{Sample } \textbf{Absorbance}}{\textit{Control } \textbf{Absorbance}} \textbf{ X 100 --------- (1)}$$

Anticancer Activity

Tumor cell line:

Dalton's Ascites Lymphoma (DAL) cells were obtained through Amala Cancer Research Institute, Thrissur, in Kerala. DAL cells were maintained by weekly intraperitoneal (i.p.) inoculation of 1×10^6 cells/mouse.

In-vitro short term cytotoxicity assay

Trypan blue exclusion method: The tumor cells were aspirated from the peritoneal cavity of tumor-bearing mice were washed thrice with normal saline and checked for viability. The cell suspension ($1x10^6$ cells in 0.1 ml) was added to tubes containing various concentrations of the extract (10, 25, 50, 100 and 200 µg/ml) and the volume was made up 1 ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. These assay mixtures were incubated for 3 hours at 37^0C and percent of dead cells were evaluated by trypan blue exclusion method. The viability of the cells was then determined using the following formula[13]

% viability = [(total cells-dead cells) ×100]/total cells

% cytotoxicity =100-(%viability)

RESULTS AND DISCUSSION

Phytochemical screening: The methanolic extract of *Solanum Virgininum* seed were tested for different phyto constituents like Alkaloids, flavanoids, steroids, terpenoids, carbohydrates, tannin and protein by using standard procedure and methanol extract was found to contain more number of phytoconstituents like alkaloids, flavanoids, tannin, and phenols. The results are given in table.1

FT-IR Analysis:The FT-IR spectrum was used to identify the functional groups of the compounds in *Solanum virgininum* based on the values in the region of infrared radiation**.** The FT-IR spectrum of the seed extract of *Solanum virgininum* is shown in figure-1. It is concluded that the peak value at $1385cm^{-1}$ corresponding to CH_3, the values at 3276 cm^{-1} and 2923 cm^{-1} are related to CH Aliphatic. The value at 1725 cm^{-1} corresponds to the carbonyl group, and value at 1600.5 cm^{-1} indicates the presence of (C=N). A very broad peak in the region at 3276 cm^{-1} indicates presence the of OH group in acid.

Table 1: Result of preliminary phytochemical analysis of SolanumVirgininum

S.No	Phytochemical Components	Test	Results
1	Alkaloids	Dragendorff's test	+
		Mayer's test	
		Wagner's test	
2	Flavanoids	Ammonia test	+
		Magnesiun ribbon test	
		Alkaline reagent test	
3	Steroids	Liebermann's Burchart test	-
		Liebermann's test	
4	Terpenoids	Salkowaski reagent test	-
5	Carbohydrates	Benedict's test	-
		Molisch's test	
		Fehling's test	
6	Tannins	Gelatin test	+
		Lead acetate solution	
		Ferric chloride solution	
7	Phenols	Ferric chloride test	+
8	Saponin	Foam test	-
9	Protein	Biuret test	-
		Xanthoproteic test	

+ Indicates the presence of the constituents; - Indicates the absence of the constituents

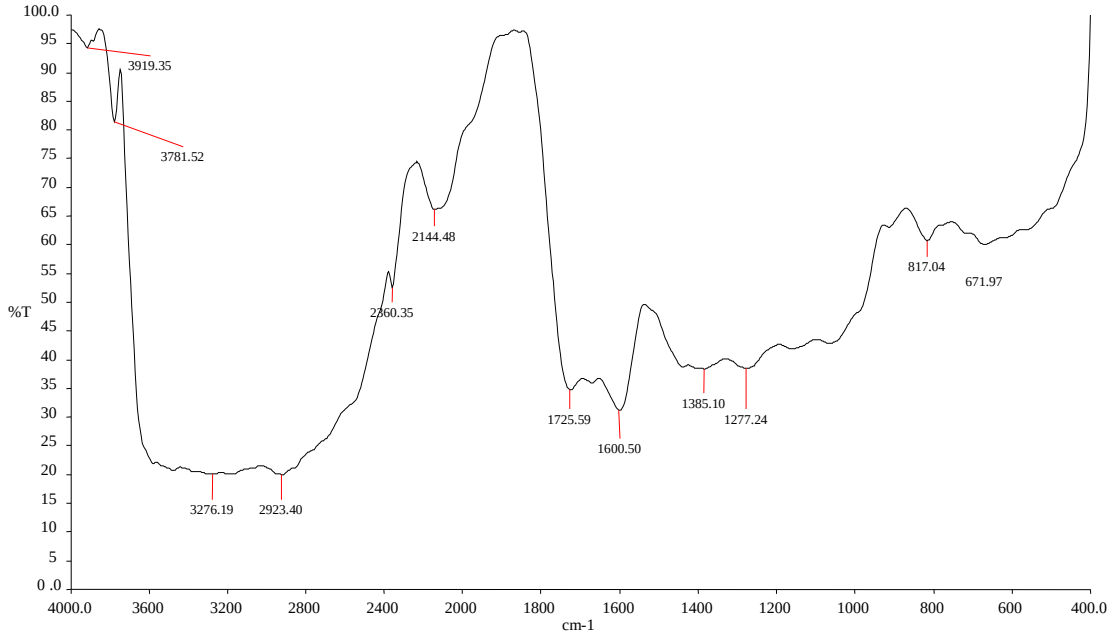

Figure 1- FT-IR Spectrum of seed extract of *Solanum virgininum*

Evaluation of Antioxidant Activity

Flavanoids and tannin are phenolic compounds acts as a primary antioxidant. Since these compounds were found to be present in this extract, it might be responsible for the potent antioxidant property of seed extract of the plant.

DPPH is a free radical, purple in color. DPPH reacts with suitable reducing agents reduced it becomes the yellow colored and then electrons become paired off while solution loses their color stoichiometrically with number of electrons taken up[14-15].From this, the DPPH radical scavenging activity of methanol seed extract of *Solanum Virgininum* was detected and compared with standard ascorbic acid and the result is given in figure-2. The percentage inhibition at various concentrations (10-60µg/ml) of seed extract as well as standard Ascorbic acid (10-60µg/ml) was calculated using the graph shown in Fig 2.The IC_{50} values for seed extract was calculated from graph, and it was found to be 35µg/ml.

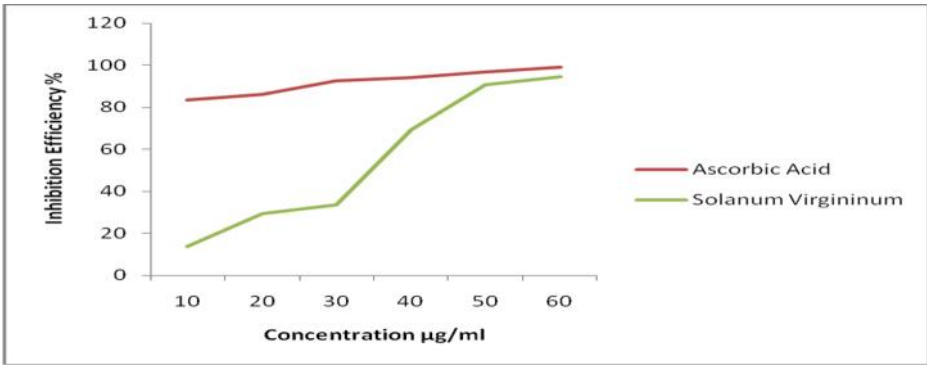

Fig.2.DPPH radical scavenging activities of the methanol seed extract *Solanum Virgininum*

Evaluation of anticancer activity

Cancer is a second largest disease in the world requires a multi- dimensional approach to its treatment, control, and prevention. Researchers are interested in the past few decades due to certain discoveries about antioxidant and the number of anticancer agents like vinca alkaloids, taxanes, podophyllotoxin, camptothecin and its derivatives. Particularly, solanum virginium plant reported as an important medicinal plant used in folk medicine to treat various ailments. Based on this information it is decided to investigate its anticancer effect against DAL induced cell and Ascitic tumor condition. In-vitro short term cytotoxicity assays by DAL cells against seed extract of Solanum *Virgininum* is shown in table-2. The graph was plotted the percentage of cytotoxicity and concentration of seed extract of *solonum virgininum* and shown in figure-3. From the graph, it could be concluded that seed extract of *Solanum virginium* showed significant cytotoxic activity against the tested cells Dalton's Ascites Lymphoma**.** The concentration of drug needed to inhibit the cell values is generated from the dose- response curve for the cell line. Dalton's ascites lymphoma is more cytotoxic and possesses anticancer activity.

Table: 2 In-vitro short term cytotoxicity assays by DAL cells against Seed extract of *Solanum Virgininum*

Drug concentration µg/ml	Percentage of cell death %	IC_{50} value µg/ml
200	100	10
100	100	
50	80.81	
20	65.30	
10	50.75	

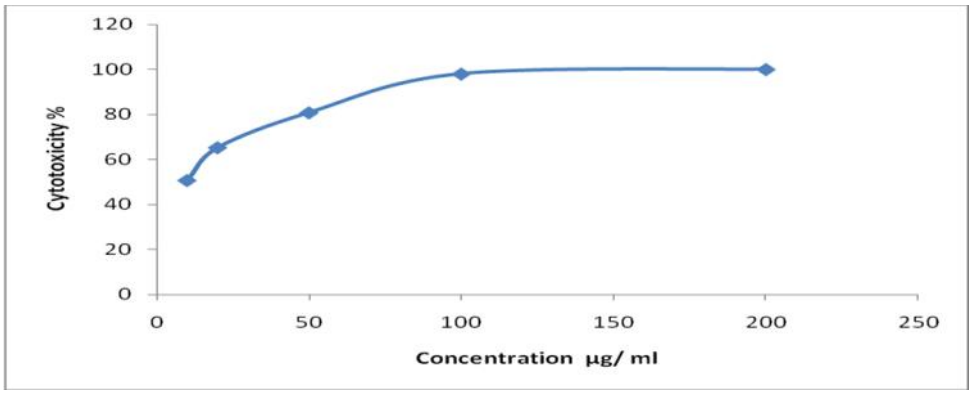

Figure 3: Percentage Cytotoxicity of seed extract of *Solanum Virgininum*

CONCLUSION:

It could be concluded that the seed extract of *Solanum Virgininum* showed the antioxidant and anticancer activities. Probably, the presence of flavonoids and phenolic compounds showed significant antioxidant activity and this activity may be responsible for its anticancer activity.

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