

ANTIPROLIFERATIVE EFFECT OF ETHANOLIC LEAF EXTRACT OF SOLANUM TRILOBATUM ON HEP2 CANCER CELL LINES

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ABSTRACT

Objective: To analyze the antioxidant and antiproliferative potential of *Solanum trilobatum* was evaluated.

Methods: Free radical scavenging potential of the plant was studied by various *in vitro* assays such as DPPH, β Carotene and Frap assay. The antiproliferative activity of *Solanum trilobatum* was analysed by MTT assay.

Result: The ethanol extract is more powerful in scavenging free radicals. The preliminary phytochemical screening of *Solanum trilobatum* revealed the presence of phenolics, carbohydrates alkaloids, flavonoids and tannins in high amount. The antiproliferative potential of the ethanol extract was studied on Hep2 cell lines by MTT assay. The extract had an IC₅₀ value of 100 μ g/mL which showed cell viability of 22.67%. From the result, it is clear that ethanol extract of *Solanum trilobatum* has cytotoxic effect on Hep2 cell lines.

Conclusion: Thus, the study revealed that *Solanum trilobatum* could be considered as a significant source of antioxidant and antiproliferative agents.

Keywords: Antioxidant, antiproliferative, *Solanum trilobatum*, DPPH assay, Hep2 cell line.

INTRODUCTION

It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including of atherosclerosis, ischemic heart disease, aging, diabetes, milletus, cancer, immune suppression, neurodegenerative diseases and others [1]. Traditional medicine is widespread and plants still presents a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several anti-inflammatory, digestive, anti-necrotic, neuro protective, and hepatoprotective drugs have recently been shown to have an antioxidant and/or anti-radical scavenging mechanism as part of their activity [2, 3, 4, 5 and 6]. Oxidation processes are intrinsic in the energy management of all living organisms and are therefore kept under strict control by several cellular mechanisms [7]. However, the excessive production of free radicals and the unbalanced mechanisms of antioxidant protection result in the onset of numerous diseases and accelerate ageing. In the past decade, a number of epidemiological studies have confirmed that intake of exogenous antioxidants is effective in preventing or suppressing such diseases [8, 9]. Several commercially available synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) are currently in use but their possible toxic properties for human health and environment are inevitable [10]. Traditional plant medicines are used throughout the world, for a range of diabetic presentations. *Solanum trilobatum* (Solanaceae), a thorny creeper with bluish violet flowers, more commonly available in Southern India has been used traditionally in Siddha system of medicines, to treat various diseases. Popularly called 'thuthuvali' by the local tribes, villagers and herbalogists in Tamilnadu, this ethnobotanical herb in known to have unique medicinal properties. It has been widely used to treat respiratory disorders, especially bronchial asthma [11]. It has hepatoprotective activity, antibacterial activity, antidiabetic activity and antioxidant properties and can treat several kinds of leprosy. Therefore, the present investigation was undertaken to evaluate the antioxidant and antiproliferative potential of *Solanum trilobatum*.

MATERIALS AND METHODS

Collection of plant material

The leaves of *Solanum trilobatum* were collected from Madhavaram and plant were raised in pots containing soil and farm yard Manure (FYM) IN 1:1 ratio under green house condition at Poonga Biotech Research centre, Chennai forest, Chennai. The collected plant parts were cleaned and shade-dried for 7 d and powdered. Twenty gram of each powdered samples were extracted with 200 mL of methanol for 1 min using ultra Turax mixer(13, 000rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-vator at 40°C to a constant weight and dissolved in methanol solution. The solution was stored at 18°C until use.

Free radical scavenging activity on DPPH

Antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH according to [12].

Sample extracts at various concentrations was taken and the volume was adjusted to 100 μ L with ethanol. Ethanolic solution (5 mL) of DPPH (0.1 mM) was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

$$\% \text{ DPPH activity} = (\text{Control OD} - \text{Sample OD} / \text{Control OD}) \times 100.$$

Determination of antioxidant activity - β -carotene assay

The antioxidant capacity was estimated by thermally induced β -carotene bleaching assay was evaluated according to [13]. A solution of β - carotene was prepared by dissolving 2 mg of β - carotene in 10 ml of chloroform. 2 ml of this solution was pipetted into 100 ml

conical flask after chloroform was removed under vacuum. 40 mg of purified linoleic acid, 400 mg of tween-40 emulsifier and 100 ml of aerated distilled water were added and shaken vigorously. 4.8 ml of offish emulsion were added to test tubes containing 200µl of ethanolic leaf extract of *Solanum trilobatum*. BHT was used as control for comparison. As soon as the emulsion was added to each

tube, zero time absorbance was measured on UV-VIS spectrophotometer at 470nm. The tubes were then placed in water bath at 50°C and the measurement of absorbance was continued until the colour of β-carotene disappeared in the control tube which was devoid of the sample. A blank devoid of β-carotene was also prepared for background correction.

1- (Initial absorbance of the sample - Final absorbance of the sample)

X100

Initial absorbance of control – final absorbance of control

Ferric reducing antioxidant power (FRAP Assay)

The total antioxidant potential of the sample determined by means of ferric reducing ability (FRAP) assay. FRAP solution (3.6 mL) add to distilled water (0.4 mL) and incubated at 37°C for 5 min. Then this solution mixed with certain concentration of the leaf extract (0.5mL) and incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of FeSO₄·7H₂O (0.4, 0.8, 1.2, 1.6, 2.0 mM) were used and the absorbance values were measured as for sample solutions.

Qualitative and quantitative phytochemical screening: The extracts were subjected to preliminary phytochemical screening to identify the presence of phytoconstituents such as alkaloids, flavonoids, saponins, tannins, phenols, glycosides and steroids according to [14].

Estimation of total free phenolics

Total phenolic constituents of plant extracts were estimated by Folin-Ciocalteu's method using Folin-Ciocalteu reagent. The estimation was done spectrometrically at 760 nm and the results were expressed as gallic acid equivalents (GAE) [15].

Estimation of total flavonoids

Aluminium chloride method was employed to quantify the total flavonoid content in the plant extracts. The results were expressed as quercetin equivalents (QE) [16].

Estimation of total alkaloids

Total alkaloid content of the plant extracts was determined according to [17]. Five gram of the sample was filtered and concentrated to one quarter of the original volume on a water bath after treatment with 200 mL of 10% acetic acid in ethanol. Concentrated NH₄OH was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH₄OH, filtered and weighed.

Estimation of total saponins

Powdered sample (20 g) was treated with 100 mL of 20% aqueous ethanol, heated over a hot water bath for 4 h at about 55°C with continuous stirring. The mixture was filtered and the residue re-extracted. The combined extracts were reduced to 40 mL over water bath at about 90°C and the concentrate was transferred into a separating funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 mL of n-butanol was added to the combined extracts and washed twice with 10 mL of 5% aqueous NaCl. The remaining solution was heated in a water bath, dried in an oven to a constant weight and the saponin content was calculated as percentage.

Thin layer chromatography

Preliminary identification of phytochemicals was made by thin layer chromatography (TLC) using silica gel plates (5gm of silica gel dissolved with 90ml of water). The extracts were eluted with chloroform:methanol:water (30:20:4) and the chromatogram was developed by spraying with methanol:sulphuric acid (1:1) and heating to 110°C. Then R_f value was calculated as the ratio of distance travelled by the solute to the distance travelled by the solvent [18].

Anticancer activity

In oncology research and clinical practice many anti-proliferative assays are used in the assessment of cancer types of individual patients. Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay has been described as rapid, simple and reproducible method, widely used in the screening anticancer drugs and to measure the tumor cell proliferation [19, 20 and 21].

RESULT AND DISCUSSION

Radical scavenging activity of leaf extracts

The DPPH radical is widely used to evaluate the free-radical scavenging capacity of antioxidants. From the dose dependent response curve, DPPH radical scavenging activity of different plant extracts of *Solanum trilobatum* was observed. The scavenging activity of ethanol extract reached 98%. The ethanol extract of *Solanum trilobatum* showed good antioxidant and free radical scavenging activity (Figure. 1). Being a stable free radical, the DPPH assay is a simple and rapid method frequently used to evaluate the ability of antioxidants to scavenge free radicals. It gives reliable information concerning the antioxidant ability of the tested compounds to act as free radical scavengers or hydrogen donors. The old electron in DPPH free radicals gives a strong absorption maximum at 517nm. When DPPH becomes paired with hydrogen from a free radical scavenging antioxidant, its purple color fades rapidly to yellow to form reduced DPPH-H [22]. The resulting decolorization is stoichiometric with respect to number of electrons captured.

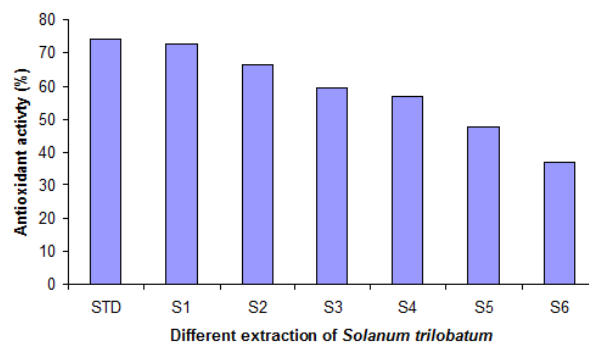


Fig.1: DPPH Radical Scavenging Assay of *Solanum trilobatum* methanol extract.

S1-Methanol, S2-Ethanol, S3-Ethylacetate, S4-Aqueous, S5-Hexane, S6-Dichloromethane

The comparable beta-carotene bleaching rates of control, BHT (Standard) and ethanolic extracts of leaf. The highest antioxidant activity of leaf was found to be 48.87%. The beta carotene bleaching method is one of the most frequently applied methods for determining the total antioxidant property of the extract. In the beta-carotene bleaching assay linoleic acid produces hydroperoxides as free radical during incubation at 50°C and attacks the beta carotene molecules that cause reduction in absorbance at 470nm. Beta carotene in the systems undergoes rapid discoloration in the absence of antioxidant and vice versa in its presence. The presence of different antioxidants can delay the extent of beta carotene bleaching by neutralizing the linoleate free radical and other free radical system. Thus, degradation rate of beta carotene-linoleate

depends on the antioxidant activity of the extracts. The result showed the control had substantial and rapid oxidation of beta-carotene. Accordingly, the absorbance decreased rapidly in the sample without antioxidant, while the sample extract with the presence of antioxidant retained their colour and also absorbance for a longer time.

In the present study, free radical scavenging activity was determined by the FRAP (Ferric reducing ability of plasma) method, which depends upon the reduction of ferric tripyridyltriazine (Fe(III)-

TPTZ) complex to the ferrous tripyridyltriazine. Fe(II)-(TPTZ) by a reductant at low P_H . Fe(II)-TPTZ has an intensive blue color and can be monitored at 593nm. FRAP values are obtained by comparing the absorbance change at 593nm in test reaction mixtures with those containing range with antioxidant mixtures. The free radical scavenging power of leaf extract of *Solanum trilobatum* increased with increased amount of extract. There is a clear difference between the control and the sample containing the extract. Among all the extracts the ethanol extract was showing maximum antioxidant power (13.7mmol of feso4/100g dry plant equivalents in *Solanum trilobatum*).

Qualitative phytochemical analysis

The preliminary phytochemical screening of *Solanum trilobatum* revealed the presence of phenolics, carbohydrates, alkaloids, flavonoids, saponins, tannins and steroids, in high amounts followed by glycosides, aminoacids and proteins. (Table 1)

Table 1: Phytochemical estimation.

Compound	Result
Alkaloids	+
Carbohydrates	+
Glycosides	+
Saponins	+
Proteins and amino acids	+
Phenolic compounds	+
Steroids	+
Flavonoids	+
Tannins	+

(+) Presence of all phytochemicals

Quantitative phytochemical analysis: The major phytochemicals present in the selected plant extracts were phenols, flavonoids, alkaloids and tannins were quantified. According to the results, suggested with the antioxidant activity of each extract tested. In general, the ethanolic extracts showed higher content of phenols and flavonoids, and likewise higher antioxidant activity. The results of total phenol content, alkaloids, saponins and flavonoids are given in (Table 2).

Table2: Quantitative phytochemical estimation.

Bioactive compound	Result
Total phenols	259.63 μ g GAE/g sample
Alkaloids	1.43 mg/g sample
Saponins	0.116 mg/g sample
Tanins	1.43mg/g dry weight
Flavonoids	64.3 μ g Quercetin equivalent/g

Thin layer chromatography

The chromatogram developed with 10% methanol in chloroform revealed the presence of five major compounds at Rf value of 0.33; 0.48; 0.56; 0.76; 0.86 as visualized under iodine vapour and UV illumination.

Anticancer activity

Since, IC50 value for Hep2 (liver) cell line (100 μ g/ml) methanol leaf extract was found to be effective, the reduction percentage of MTT at 48Hrs also estimated for Hep2 (liver) cells. When incubated with the extract, it induced Cytotoxicity in a significant manner

which implicit the damage to the member integrity of the cell when contributed with control. The cytotoxicity was minimized in the extract treated cells and near normal level was attained at various concentrations (25, 50, 100 and 150 μ g/ml) and maximum effect was found when treated at 100 μ g/ml. From the above results, it was confirmed that *Solanum trilobatum* methanol leaf extract at 100 μ g/ml seems to offer significant protection and maintain the structural integrity of the hepatocellular membrane and this active concentration was followed for further studies. Trypan blue is one of the several stains recommended for use in dye exclusion procedure for viable cell counting. This assay is based on the principle that live cells do not take up blue, where as dead cells do and appear as blue under microscope - depicts the viability of cells by Trypan Blue assay. The viability is measured in terms of percentage was found to decreased 98% in drug treated hepatic cell line. The cell treated with *Solanum trilobatum* methanol extract at various concentrations (25, 50, 100 and 150 μ g/ml) showed protective nature of the extract act against the deleterious effects and the maximum effect was observed at 100 μ g/ml. (figure 3). The extract had an IC50 value of 100 μ g/mL which showed cell viability of 22.67%. (Table 3) From the result, it is clear that ethanol extract of *Solanum trilobatum* has cytotoxic effect on Hep2 cell lines. Cell cycle arrest is a common feature of cells that are undergoing terminal differentiation and defective proliferation [13].

Table: 3% MTT reduction on Hep2 cell line:

S. No	Concentration(μ g)	MTT reduction (%)
1	Control	98.17
2	25	84.12
3	50	71.47
4	100	46.3
5	150	22.67
6	PC	11.13

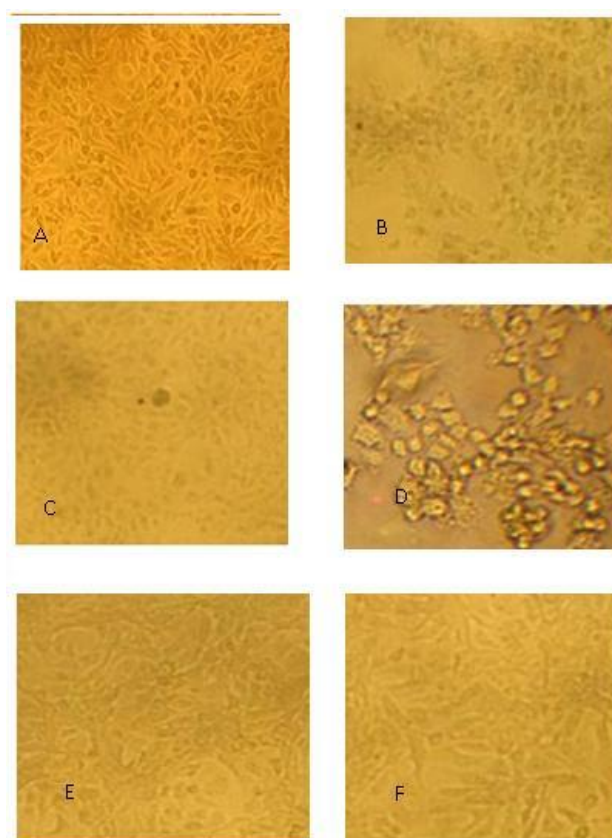


Fig.2: Antiproliferative effect of hep2 cell line.

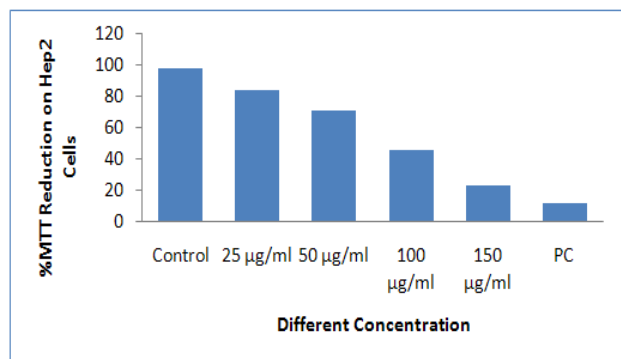


Fig. 3: A: Control cells (Untreated), B: Ethanol extract 25 µg/ml, C: Ethanol extract 50 µg/ml, D: Ethanol extract 100 µg/ml, E: Ethanol extract 150 µg/ml, F: Cyclophosphamide (Positive control) 180 µg/ml.

CONCLUSION

The present investigation suggests that *Solanum trilobatum* possesses significant antioxidant and anti-proliferative potential. Hence, we can conclude that with further mechanistic studies, the plant can be considered as an efficient source of antioxidant and anti-proliferative agents.

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