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Short Communication

Cytotoxic and antioxidant properties of four plants belonging to the genus *Solanum*

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SUMMARY

The aim of the study was to evaluate *in vitro* antioxidant and cytotoxic activities of the methanolic extracts of leaves of *Solanum sisymbrifolium, Solanum anguivi multiflora, Solanum barbisetum* and *Solanum jasminoides.* In the *in vitro* antioxidant screening using ABTS [2,2′-azino-bis (3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt] method, the methanol extracts of *Solanum jasminoides, Solanum barbisetum* and *Solanum anguivi multiflora* exhibited potent antioxidant activity with IC_{50} values 31.25 ± 0.35, 40.33 ± 0.57 and 54.33 ± 0.57 μg/ml, respectively. *Solanum barbisetum* also showed potent activity in DPPH [1,1-diphenyl-2-picryl hydrazyl] method with an IC_{50} value of 55.33 ± 1.66 μg/ml. In the cytotoxicity studies, the methanol extract of *Solanum barbisetum* exhibited moderate activity against Vero, HEp-2, HeLa and A-549 cell lines with IC_{50} values in the range of 83.30 - 127.30 μg/ml. *Solanum anguivi multiflora* extract also showed moderate activity against Hep-2 cell line with IC_{50} value of 80.13 μg/ml. *Solanum barbisetum* possessing both the activities requires further investigation.

Keywords: *Solanum*; Cytotoxicity; Free radical

INTRODUCTION

The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. A large number of plants belonging to the genus *Solanum* are being used traditionally against several diseases (Anonymous, 1989). *Solanum* is reportedly the largest genus evaluated for anticancer potential (Pandey, 2002). Reports indicate that steroidal alkaloids from *Solanum* species possess strong cytotoxic and antitumor properties (Ikeda *et al*., 2003). *S. nigrum* (Mohamed *et al*., 2007), *S. trilobatum* (Mohanan and Devi 1996, Shahjahan *et al*., 2004), *S. pseudocapsicum* (Vijayan *et al*., 2002) etc., are known to possess these properties. Several plants, *S. nigrum* (Mohamed *et al*., 2007), *S. incanum* (Mohamed *et al*., 2007), *S. tuberosum* (Andre *et al*., 2007), *S. trilobatum* (Shahjahan *et al*., 2005) and *S. lyratum* (Wei *et al*., 2006) are known to exhibit strong antioxidant properties. Experimental evidence suggests that free radicals and reactive oxygen species (ROS) can be involved in a high number of diseases including cancer (Halliwell and Gutteridge, 1999). Hence, currently the reduction of endogenous and exogenous sources of oxidative stress is potentially the most important means of preventing oxygen free radical related cancer. Many plants are known to possess strong anticancer

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properties along with potent antioxidant properties (Natesan *et al.,* 2007). It is significant that over 60% of currently used anticancer agents are derived in one way or another from natural sources (Cragg and Newman, 2005). The Nilgris is one of the important biosphere reserves of India. The present study was carried out to screen four plants belonging to the genus *Solanum* available in The Nilgiris for their *in vitro* cytotoxic and antioxidant properties using standard methods. A literature survey indicated that, these properties were so far not carried out on these plants.

MATERIALS AND METHODS

Collection and authentication

The leaves of *Solanum sisymbrifolium* Lam, *Solanum anguivi multiflora* Lam variety multiflora, *Solanum barbisetum* Nees and *Solanum jasminoides* Paxt (Solanaceae) were collected during August 2003 from the forests of The Nilgiris, Tamil Nadu, and authenticated by Dr. S. Rajan, Medicinal Plants Survey and Collection Unit, Government Arts College, Ootacamund, India.

Chemicals

DPPH (2,2-diphenyl-1-picryl hydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt), MTT (3-(4, 5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide) and SRB (Sulphorhodamine B) were obtained from Sigma Chemicals Co., St. Louis, USA. Ascorbic acid, NBT (Nitro blue tetrazolium) and BHA (Butylated hydroxy anisole) were obtained from SD Fine Chemicals Ltd., Mumbai, India. 2-Deoxy-D-ribose was obtained from Himedia Laboratories Pvt. Ltd., Mumbai. Naphthyl ethylene diamine dihydrochloride was obtained from Roch - Light Ltd., Suffolk, England. All the chemicals used were of analytical grade.

Cell lines

Vero (African green monkey kidney, Pasteur Institute of India, Coonoor), HEp-2 (Laryngeal epithelial carcinoma human, National Cell Science Center, Pune), HeLa (Epithelial human cervix cancer, V. P. Chest Institute, New Delhi) and A-549 (Small cell lung carcinoma, Christian Medical College, Vellore) cells were used.

Extraction

The powdered leaves (190 g) of all the plants were extracted separately with methanol (2 L) in Soxhlet extractor for 18-20 h. The extracts were concentrated to dryness in a rotary evaporator under reduced pressure and controlled temperature (40-50^{o}C). The nature and yields of the extracts are shown in Table 1. All the extracts were preserved in a refrigerator at 4^{o}C till further use.

Preparation of test and standard solutions

All the extracts and three known antioxidants

Table 1. *In vitro* antioxidant activity of the methanol extracts of four *Solanum* plants

Extract/Standard	% Yield	Nature of the extract	IC_{50} values ± S.E. μg/ml* by methods				
			DPPH	Nitric oxide	H_2O_2	Alkaline DMSO	ABTS
Solanum anguivi var. multiflora	5.16	Greenish	208.55 ± 5.60	305.00 ± 20.00	150.62 ± 6.86	165.32 ± 6.53	54.33 ± 0.57
Solanum barbisetum	5.50	Black green	55.33 ± 1.66	330.00 ± 20.00	95.65 ± 5.36	195.36 ± 5.23	40.33 ± 0.57
Solanum jasminoides	4.71	Greenish	95.00 ± 4.50	142.14 ± 12.86	65.86 ± 5.36	102.36 ± 6.36	31.25 ± 0.35
Solanum sisymbrifolium	3.50	Green	406.66 ± 59.55	671.00 ± 36.36	101.53 ± 5.63	125.53 ± 9.36	100.00 ± 1.65
Ascorbic acid		--	2.69 ± 0.05	ND	ND	>1000	11.25 ± 0.49
Rutin		--	3.19 ± 0.10	65.4 ± 2.5	36.6 ± 0.2	>1000	0.51 ± 0.01

*Average of three determinations, in the de-oxy ribose and p-NDA methods, all the extracts showed IC_{50} values > 1,000 μg/ml.

ascorbic acid, rutin and BHA were dissolved in freshly distilled DMSO separately and used for the *in vitro* antioxidant testing using different methods, except the hydrogen peroxide method. For the hydrogen peroxide method, the extracts and the standards were dissolved in distilled methanol and used. The stock solutions were serially diluted with the respective solvents to obtain the lower dilutions. For cytotoxicity studies each extract was separately dissolved in freshly distilled DMSO and volume was made up to 10 ml with DMEM (Dulbecco's Modified Eagle's medium), pH 7.4, supplemented with 2% inactivated new born calf serum (maintenance medium, PAA Laboratories, Austria) to obtain a stock solution of 1 mg/ml concentration, sterilized by filtration and stored at -20°C till use. Serial two fold dilutions were made using maintenance medium from the stock solutions.

***In vitro* antioxidant activity**

All the extracts were tested for *in vitro* antioxidant activity using several standard methods. The final concentration of the extract and standard solutions used were 1,000, 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 µg/ml. The absorbance was measured spectrophotometrically against the corresponding blank solution. The percentage inhibition was calculated by using the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{\text{OD control - OD sample}}{\text{OD control}} \times 100$$

IC_{50}, which is the concentration of the sample required to scavenge 50% of free radicals was calculated.

DPPH radical scavenging method

The antioxidant activity of various extracts of *Solanum* species and the standard compounds were assessed on the basis of radical scavenging effect on the DPPH stable free radical (Hwang *et al*., 2001). The extract or standard solution (10 µl) was added to DPPH in methanol solution (200 µl) in a 96-well microtitre plate (Tarsons Products (P) Ltd., Kolkata, India). After incubation at 37°C for 30 min, the absorbance of each solution was determined at 490 nm using an ELISA microtitre plate reader (Bio Rad Laboratories Inc, California, USA, Model 550). The corresponding blank readings were also taken and the remaining DPPH was calculated.

Nitric oxide radical inhibition assay

Nitric oxide is a free radical and scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Marcocci *et al*., 1994). Nitric oxide was generated from sodium nitroprusside and measured by the modified Griess Ilosvog reaction (Garrat, 1964; Badami *et al*., 2003).

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (1 ml) and extracts or standard solutions (1 ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was removed, 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was mixed and allowed to stand for 5 min for completing diazotisation, and 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min. A pink coloured chromophore was formed in diffused light. The absorbance was measured at 540 nm against the corresponding blank solutions in microtitre plates using ELISA reader.

Scavenging of hydrogen peroxide

Various concentrations of the extracts or standards in methanol (1 ml) were added to 2 ml of hydrogen peroxide solution (20 mM) in PBS (phosphate buffered saline, pH 7.4). The absorbance was measured at 230 nm after 10 min (Guddadarangavvanahally *et al*., 2004).

Scavenging of superoxide radical by alkaline DMSO method

Superoxide was generated according to the

alkaline DMSO method (Elizabeth and Rao, 1990). The reduction of NBT by super oxide was determined in the presence and absence of extracts. To the reaction mixture containing 0.1 ml of NBT (1 mg/ml) and 0.3 ml of extract or standard in DMSO, added 1 ml of alkaline DMSO (1 ml, 1% water, 5 mM NaOH) to give a final volume of 1.4 ml and the absorbance was measured at 560 nm.

Scavenging of ABTS radical cation

The principle behind the technique involves the reaction between ABTS and potassium persulphate to produce the ABTS radical cation, a blue-green chromogen. In the presence of the antioxidant-reductant, the colored radical cation is converted back to colourless ABTS, the absorbance of which is measured at 734 nm (Re *et al*., 1999). To 0.2 ml of various concentrations of the extracts or standards, added 1.0 ml of distilled DMSO and 0.16 ml of ABTS solution. Absorbance was measured spectrophotometrically, after 20 min at 734 nm.

Scavenging of hydroxyl radical by deoxyribose method

The sugar deoxyribose (2-deoxy-D-ribose) is degraded on exposure to hydroxyl radicals, generated by irradiation or by Fenton systems. If the resulting complex mixture of products is heated under acid conditions, MDA (malondialdehyde) is formed and may be detected by its ability to react with TBA (thiobarbituric acid) to form a pink chromogen (Barry *et al*., 1987).

To the reaction mixture containing deoxyribose (3 mM, 0.2 ml), ferric chloride (0.1 mM, 0.2 ml), EDTA (0.1 mM, 0.2 ml), ascorbic acid (0.1 mM, 0.2 ml) and hydrogen peroxide (2 mM, 0.2 ml) in phosphate buffer, pH 7.4 (20 mM), were added 0.2 ml of various concentrations of extract or standard in DMSO to give a total volume of 1.2 ml. The solutions were then incubated for 30 min at 37°C. After incubation, ice-cold trichloroacetic acid (0.2 ml, 15% w/v) and thiobarbituric acid (0.2 ml, 1% w/v), in 0.25 N hydrochloric acid were added. The reaction mixture was kept in a boiling water bath for 30 min, cooled and absorbance was measured at 532 nm.

Scavenging of hydroxyl radical by P-NDA method

Hydroxyl radical scavenging is measured by the inhibition of p-NDA bleaching. Hydroxyl radicals generated through Fenton reaction can bleach p-NDA specifically. Scavenging activity was measured by the extent of inhibition of bleaching in the presence and absence of the extract solutions (Elizabeth and Rao, 1990).

To a solution mixture containing ferric chloride (0.1 mM, 0.5 ml), EDTA (0.1 mM, 0.5 ml), ascorbic acid (0.1 mM, 0.5 ml), hydrogen peroxide (2 mM, 0.5 ml) and p-NDA (0.01 mM, 0.5 ml) in phosphate buffer pH 7.4 (20 mM), were added various concentrations of extract or standard in distilled DMSO (0.5 ml), to give a final volume of 3 ml. Absorbance was measured at 440 nm.

***In vitro* cytotoxic activity**

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 ml of different sample concentrations were added to the cells in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were recorded every 24 h. After 72 h, the extract solutions in the wells were discarded and the cellular viability was determined by using MTT and SRB assays (Francis and Rita, 1986; Skehan *et al*., 1990).

RESULTS AND DISCUSSION

In the *in vitro* antioxidant screening using ABTS

method, the methanol extracts of *Solanum jasminoides*, *Solanum barbisetum* and *Solanum anguivi multiflora* exhibited potent antioxidant activity with IC_{50} values 31.25 ± 0.35, 40.33 ± 0.57 and 54.33 ± 0.57 μg/ml, respectively (Table 1). *Solanum barbisetum* also showed potent activity in DPPH method with an IC_{50} value of 55.33 ± 1.66 μg/ml. All the extracts exhibited moderate antioxidant activity in hydrogen peroxide and alkaline DMSO methods. In the nitric oxide method, *Solanum jasminosides* showed moderate and the other extracts weak activities. Among the four methanol extracts, *Solanum jasminoides* was found to be more potent in all the five methods tested. However, the standard ascorbic acid and rutin showed highly potent antioxidant activity with very low IC_{50} values in all the methods tested.

In the cytotoxicity studies, the methanol extract of *Solanum barbisetum* exhibited moderate activity against all the four cell lines tested with IC_{50} values in the range of 83.30 - 127.30 μg/ml (Table 2). *Solanum anguivi multiflora* extract also showed moderate activity against Hep-2 cell line with IC_{50} value of 80.13 mg/ml. The remaining extracts showed weak cytotoxicity against all the cell lines.

Reactive oxygen species have been implicated in initiating, accompanying or causing pathogenesis of many diseases (Srivastava and Mital, 2005). Therefore, increased antioxidant concentration at the cellular level could provide considerable protection against ROS. Most of the known antioxidants are derived from plants, probably due to their increased capacity to defend themselves from various sources of stress. Strong evidence indicates that ROS plays an important role in the initiation as well as promotion of carcinogenesis (Srivastava and Mital, 2005). Many cancer chemopreventive agents possess antioxidant potentials. A large number of plants belonging to the genus *Solanum* are known to possess strong antioxidant and antitumor properties (Shahjahan *et al.*, 2005; Wei *et al.*, 2006; Andre *et al.*, 2007; Mohamed *et al.*, 2007). Hence, in the present study four plants of this genus were screened for *in vitro* antioxidant and cytotoxic properties.

Potent antioxidant activity was observed for the methanol extract of *Solanum barbisetum* in ABTS and DPPH methods. *Solanum jasminoides* and *Solanum anguivi multiflora* exhibited potent activity in ABTS method and moderate activity in other methods. Among the extracts, *Solanum jasminoides*

Table 2. Cytotoxic activity of the methanol extracts of four *Solanum* plants

Extract	Cell line	CTC_{50} ± S.E. mg/ml by*		
		MTT assay	SRB assay	Average CTC_{50}
Solanum anguivi var. multiflora	VERO	109.37 ± 1.20	150.30 ± 2.30	129.83
	HeLa	137.50 ± 1.05	160.25 ± 3.30	148.87
	HEp-2	84.37 ± 0.60	75.89 ± 0.90	80.13
	A-549	125.50 ± 1.90	160.56 ± 1.20	143.03
Solanum barbisetum	VERO	109.00 ± 2.60	145.60 ± 4.69	127.30
	HeLa	93.75 ± 1.30	120.60 ± 0.50	107.17
	HEp-2	81.25 ± 1.50	110.05 ± 1.20	95.65
	A-549	76.56 ± 0.90	90.05 ± 0.80	83.30
Solanum jasminoides	VERO	221.6 ± 2.10	250.60 ± 1.20	236.10
	HeLa	185.60 ± 1.80	220.65 ± 2.60	203.12
	HEp-2	201.70 ± 2.10	250.60 ± 1.90	226.15
	A-549	146.85 ± 1.20	158.09 ± 1.10	152.47
Solanum sisymbrifolium	VERO	453.12 ± 4.53	390.65 ± 2.60	421.88
	HeLa	521.70 ± 4.97	625.03 ± 5.30	573.36
	HEp-2	472.80 ± 3.90	450.65 ± 5.60	461.72
	A-549	609.20 ± 5.80	570.80 ± 6.80	590.00

*Average of three determinations.

showed low IC_{50} values in most of the methods indicating its potent antioxidant nature. In the cytotoxicity studies, moderate activity was observed for the methanol extract of *Solanum barbisetum* against all the four cell lines tested and for *Solanum anguivi multiflora* extract against Hep-2 cell line. Preliminary phytochemical examination of the four extracts indicated the presence of alkaloids, flavonoids, glycosides, phenolics, steroids and triterpenoids. Steroidal alkaloids from Solanaceous plants are known to exhibit potent cytotoxic properties (Ikeda *et al*., 2003) and phenolic compounds including flavanoids and tannins possess strong antioxidant activity (Tripathi *et al*., 1996). Hence, the observed cytotoxicity and antioxidant activities in the present study may be due to the presence of such compounds in the extracts. *Solanum barbisetum* possessing both the activities merit further investigation in animal models and isolation of its active constituents.

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