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Free Radical-scavenging Potential of Methanol Extracts of *Solanum surattense*

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ABSTRACT

Natural products from plants provide unlimited opportunities for discovery of new drugs. The present study was aimed to evaluate the total phenolic content, total flavonoids content and their influence on different antioxidant activities. The antioxidant activity was evaluated by Ferric Reducing Ability of Plasma (FRAP), peroxidase assay, ABTS (2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium) and lipid peroxidation assay (LPO). The maximum total phenolic and flavonoids content was observed in leaves, 25.91±0.8035 mg GAE/g DW and 17.7±2.36 mg QE/g DW, respectively. Fruits showed the maximum ABTS activity (IC_{50} = 53.97±5.64 mM L^{-1} g^{-1}) while leaves showed maximum peroxidase (0.472±0.02 mM min^{-1} g^{-1} DW), LPO activity (36.21±4.21 MDA g^{-1} DW). The ethyl acetate extract of leaves showed maximum FRAP activity in leaves (529.67±5.5 mM L^{-1} g^{-1} DW) compared to methanol and hexane extracts. These results clearly indicate that *S. surattense* is effective against free radical mediated diseases.

Key words: *S. surattense*, free radical scavenging activity, TPC, TFC, ABTS, FRAP

INTRODUCTION

Solanaceae is one of the most economically and medicinally important families of angiosperms. About 1500 species of *Solanum* that are present in the world, most are distributed in the tropical and sub-tropical areas, with a small number in the temperate areas (Edmonds and Chweya, 1997). *Solanum xanthocarpum*, commonly known as Yellow Berried Nightshade is a spiny diffuse green perennial herbaceous weed, woody at the base, 2-3 m in height and found mostly in roadsides and waste lands (Anonymous, 1998). Its roots are one of the constituents of well known Ayurvedic preparation "Dasmula Ashva (Khare, 1995). It has been used traditionally for curing various ailments like cough, fever, rheumatism, laxative, tuberculosis, kidney disorder, constipation, asthma, bronchitis, tooth ache, sore throat and gonorrhoea (Kiritikar and Basu, 1994). It has also been reported for antianaphylactic activities and antiandrogenic activities (Smit *et al*., 1995).

Oxygen is the absolute requirement of life, as it provides adequate energy production but it can also be very toxic. Oxygen exerts its toxic properties primarily through the direct or indirect formation of free radicals, defined as, "Any species capable of independent existence that contain one or more unpaired electrons". Free radicals are highly reactive and therefore short lived and

destructive to their surroundings. Our body can defend itself against free radicals but only upto a certain limit (Lobo *et al*., 2008). A healthy body has mechanisms to handle the normal loads of free radicals. The oxidative damages could be retarded by endogenous defense sytems such as catalase, superoxide dismutase but these systems are not completely efficient (Poon *et al*., 2004). However, this ability can be severely compromised by our lifestyles. It also declines with age. *In vivo*, the potentially damaging effects of free radicals is opposed by a network of antioxidants. When these antioxidants are overwhelmed, oxidative damage can occur to proteins, lipids, carbohydrates and nucleic acids. Numerous diseases are associated with reduced antioxidant defense and increased oxidative stress (McCord, 2000) likeways atherosclerosis, ischemic/perfusion, ageing, diabetes mellitus, cancer, immuno suppression, neuro degenerative diseases, idiopathic pulmonary fibrosis, asthma, chronic obstructive pulmonary disease, acute respiratory distress syndrome (Shubha *et al*., 2013; Sharma *et al*., 2013). A variety of plant preparations have been mentioned in Ayurveda and other indigenous systems of medicine which are claimed to be useful in scavenging the free radicals. The protective action of the plants has been attributed to the presence of antioxidants, especially polyphenolic compounds and antioxidant vitamins, including vitamin C, vitamin E, β-carotenes, phytoestrogen, phytate, phenolic acids, folic acid, tocophenols, benzoic acids, etc. (Yadav *et al*., 2013).

The present investigation has been carried out to shed light on the efficiency of different plant part extracts of *S. surettense* in scavenging the free radicals. The findings from this study may add to the overall value of the medicinal potential of the herb.

MATERIAL AND METHODS

Plant material: The different plant parts (leaves, stems and fruits) of *S. surettense* were collected in the month of November to December from the Jaipur-Delhi highway. It was washed with tap water; air dried at room temperature to constant weights and ground to fine powder. The plant was botanically identified and authenticated in the Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India and a voucher specimen (No. RUBL20878) was deposited at the herbarium, Department of Botany, University of Rajasthan, Jaipur.

Chemicals: Chemical like 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ascorbic acid, quercetin, gallic acid, potassium persulphate was obtained from Sigma (St. Louis, Missouri, USA). Folin-Ciocalteus's phenol reagent, sodium carbonate, hydrogen peroxide, pyrogallol, sodium dihydrogen phosphate, dihydrogen hydrogen phosphate were from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used were of analytical grade.

Total phenolic and flavonoidal content

Plant extraction: Two gram each of the dry material was extracted with 25 mL of methanol at room temperature for 48 h, filtered through Whatman No. 1 filter paper, stored and used for quantification.

Total phenolic content: Total phenolic compound contents were determined by the Folin-Ciocalteau reagent by using method of McDonald *et al*. (2001), Ebrahimzadeh *et al*. (2008a, b) and Nabavi *et al*. (2008). The extract samples (0.5 mL; 1: 10 diluted) were mixed with Folin Ciocalteu reagent (5 mL, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 mL, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols

were determined by colorimetric method at 765 nm. The standard curve was prepared using the standard solution of gallic acid in methanol in the range 20-200 μg mL^{-1} (R^2 = 0.987). Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of dry mass), which is a common reference compound. Total phenolic content can be calculated from the following equation:

$$T = \frac{C.V}{M}$$

where, T is total phenolic concentration, C is concentration of gallic acid from caliberation curve (μg mL^{-1}), V is volume of extract (mL), M is wt. of methanolic plant extract.

Total flavonoidal content: Total flavonoid content was determined by using aluminium chloride colorimetric method ($AlCl_3$) according to the known method (Dewanto *et al*., 2002; Sakanaka *et al*., 2005) with slight modifications using quercetin as standard. One milliliter of test material was added to 10 mL volumetric flask containing 4 mL of water. To above mixture, 0.3 mL of 5% $NaNO_2$ was added. After 5 min, 0.3 mL of 10% $AlCl_3$ was added. After 6 min, 2 mL of 1 M NaOH was added and the total volume was made up to 10 mL with distilled water. Then the solutions were mixed well and absorbance was measured against blank at 510 nm. The standard curve was prepared using the standard solution of quercetin in methanol in the range 0.5-5.0 mg mL^{-1} (R^2 = 0.991). Total flavonoidal content of the extracts was expressed in milligram of quercetin equivalents per gram of dry weight. Total flavonoidal content can be calculated from the following equation:

$$T = \frac{C.V}{M}$$

where, T is total flavonoidal concentration, C is concentration of quercetin from caliberation curve (mg mL^{-1}), V is volume of extract (mL), M is wt. of methanolic plant extract.

Determination of antioxidant activity

Reducing ability (FRAP assay): The determination of the total antioxidant activity (FRAP assay) in the extract is a modified method of (Benzie and Strain, 1996). The stock solutions included 300 mM acetate buffer (0.3 M acetic acid and 0.3 M sodium acetate), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM ferric chloride($FeCl_3.6H_2O$) solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ and 2.5 mL $FeCl_3.6H_2O$. The temperature of the solution was raised to 37°C before use. Plant extracts (100 μL each of methanolic ethyl acetate and hexane) were allowed to react with 2900 μL of the FRAP solution for 30 min in the dark condition. Readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 100 and 1000 μM $FeSO_4$. Results are expressed in mM Fe (II)/g dry mass.

Peroxidase assay: The method of assay measures the oxidation of pyrogallol to purpurogallin by peroxidase when catalyzed by peroxidise at 420 nm and at 20°C. Plant sample (200 mg) was homogenized with 10 mL of phosphate buffer and refrigerated centrifuged at 10000 rpm for 20 min. The clear supernatant was taken as the enzyme extract. The activity was assayed after the method of Chance and Maehley (1955) with following modifications. About 2.4 mL of phosphate

buffer, 0.3 mL pyrogallol (50 μM) and 0.2 mL of H_2O_2 (30%) were added. The amount of purpurogallin formed was determined by taking the absorbence at 420 nm immediately after adding 0.1 mL enzyme extract. The extinction coefficient of 2.8/mM/cm was used in calculating the enzyme activity that was expressed in terms of millimole per minute per gram dry weight.

Lipid Peroxidation Assay (LPO): The LPO activity was calculated using the protocol of Heath and Packer (1968). About 0.5 g of dry material was homogenized with 10 mL of 0.1% (w/v) Trichloroacetic acid (TCA). The homogenate was centrifuged for 5 min (15000 g, 4°C). Supernatant was collected and 1 mL of supernatant was mixed with 4 mL of 0.5% (w/v) TBA (Thiobarbituric acid) in 20% (w/v) TCA and then incubated in water bath at 95°C for 30 min. Reaction was quickly ended by incubating on an ice bath. In case the solution is not clear, centrifuge at 10000 g for 10 min and the absorbance was measured at 532 and 600 nm. The OD_{600} values were subtracted from MDA-TBA complex values at 532 nm and MDA concentration was calculated using the Lambert-Beer law with an extinction coefficient εM = 155/mM/cm. Results were presented as μM MDA/g.

ABTS radical scavenging assay: To determine ABTS radical scavenging assay, the method of Re *et al.* (1999) was adopted. The stock solutions included 0.002 M ABTS solution and 0.07 M potassium persulphate solution. The working solution was then prepared by mixing the 25 mL of ABTS stock and 0.1 mL of potassium persulphate stock and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing ABTS solution with ethanol to obtain an absorbance of 0.706±0.001 U at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1 mL) at varying concentration were allowed to react with 3 mL of the ABTS solution and the absorbance was taken at 734 nm after 6 min using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of BHT and percentage inhibition calculated as:

$$\text{Inhibition}(\%)=\left[1-\frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right]\times 100$$

where, $Abs_{control}$ is the absorbance of ABTS radical + methanol, Abs_{sample} is the absorbance of ABTS radical + sample extract /standard.

Statistical analysis: Experimental results are expressed as Means±Standard Deviation (SD). All measurements were replicated three times. The IC_{50} values were also calculated by linear regression analysis. Experiments results were further analyzed for Pearson correlation coefficient (r) between total phenolic, flavanoid and different antioxidant assay systems using the Microsoft Excel 2007 software and two way analysis of variance (ANOVA) was applied to investigate the differences among means by using software. The values were considered to be significantly different at $p<0.05$.

RESULTS AND DISCUSSION

The biological properties of plants are considered as an evaluation of the medicinal and nutritional values of medicinal plants (Kumbhare *et al.*, 2012). Free radicals are known to play a definite role in a wide variety of pathological manifestations. The therapeutic benefit of medicinal plants is often attributed to their antioxidant property (Nayak and Pinto Pereira, 2006). Phenolic

Table 1: Total phenolic, flavonoidal and ascorbic acid content in different parts of *S. surattense*

Plant part	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
Leaves	25.91±0.8030	17.7±2.36000
Stems	5.879±0.978	3.129±0.069
Fruits	4.975±0.463	5.208±0.721

TPC: Total phenalic contents and TFC: Total flaconoidal contents in mg quercetin equivalent/g dry weight

Table 2: Total antioxidant (FRAP) activity of methanol, ethyl acetate and hexane extracts of different plant parts of *S. surattense* (mM L^{-1} g^{-1} DW)

Plant part	Methanol extract	Ethyl acetate extract	Hexane extract
Leaves	443.67±4.72	529.67±5.50	31.17±2.02
Stems	143.67±8.08	99.67±1.53	29.93±1.75
Fruits	120.67±4.16	47.27±2.61	12.89±2.07

Table 3: Peroxidase activity in different plant parts of *S. surattense* (mM min^{-1} g^{-1} DW)

Plant part	Methanolic extract
Leaves	0.472±0.02
Stems	0.125±.010
Fruits	0.513±0.02

compounds are known to contribute in plant defence mechanisms to counteract Reactive Oxygen Species (ROS) in order to survive and prevent molecular damage. In our present study, S.surattense leaf possessed highest total phenolic contents (25.91±0.803 mg GAE/g DW) and total flavonoid contents (17.7±2.36 mg quercetin equivalent/g DW) while lowest total phenolic contents (4.975±0.463 GAE/g DW) was recorded in fruits and total flavonoid contents (3.129±0.069 mg quercetin equivalent/g DW) in stems (Table 1). The scavenging or chelating activity of flavonoids has been reported to be responsible for antioxidant activities of plants (Kessler *et al*., 2003; Ghimire *et al*., 2011). It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers.

As shown in Table 2, there were large variations in ferric reducing antioxidant power among the different extracts indicated that extraction solvent significantly influenced *S. surattense* reducing power. The FRAP values for the methanol extracts were significantly higher than that of ethyl acetate and hexane fractions, but the ethyl acetate fraction of leaves showed the maximum activity (529.67±5.5 mM L^{-1} g^{-1} DW). The significant differences in the FRAP activity of the extracts may be attributed to different solvents used. The FRAP assay evaluates the ability of a substance to reduce Fe^{3+} to Fe^{2+}, which is measured by the formation of a coloured complex with TPTZ that can be read spectrophotometrically at 593 nm. The presence of reluctant is the basis for their reductive capacity, which exhibits their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom (Singh and Rajini, 2004).

In present investigation, fruits showed maximum peroxidase activity (0.513±0.02 mM min^{-1} g^{-1} DW) and while the stems showed the least (0.125±0.01 mM min^{-1} g^{-1} DW) (Table 3). Peroxidases catalyze the reduction of hydrogen peroxide (H_2O_2) to water, which is a common end product of oxidative metabolism, rendering it harmless. An increase in peroxidase activity has been associated with environmental stresses on plants (Rabe and Kreeb, 1979; Van Assche *et al*., 1988). This analysis, in conjunction with other plant physiological and toxicological techniques, will be used to assess the impact of

Table 4: Lipid peroxidation assay (LPO) activity in different plant parts of *S. surattense* (mM min^{-1} g^{-1} DW)

Plant part	Methanolic extract
Leaves	36.21±4.21
Stems	11.33±1.00
Fruits	17.61±0.63

Table 5: ABTS activity (IC_{50}) in different plant parts of *S. surattense* (mM min^{-1} g^{-1} DW)

Plant part	Methanol extract
Leaves	426.72±11.88
Stems	93.69±6.290
Fruits	53.97±5.640

Table 6: Pearson's correlation coefficient of total phenolic and total flavonoid of methanolic extracts of different plant parts of *S. surattense* and determination of ABTS, LPO and peroxidase antioxidant activity

Activity	Total phenol	Total flavanoid
Peroxidase		
Leaves	0.780	-0.996
Stems	-0.993	-0.968
Fruits	-0.697	0.199
LPO		
Leaves	-0.742	0.779
Stems	-0.552	0.658
Fruits	0.516	0.027
ABTS		
Leaves	-0.199	-0.529
Stems	-0.995	0.974
Fruits	-0.835	0.405

contaminants on plants. In present investigation, leaves have showed maximum LPO activity (36.21±4.21 mM min^{-1} g^{-1} DW) while stems showed the least (11.33±1.0 mM min^{-1} g^{-1} DW) (Table 4). Flavanoids and flavanols are two polyphenolic compounds that play an important role in stabilizing lipid oxidation and are associated with antioxidant activity (Yen *et al*., 1993). The high LPO activity of leaves can be associated with high amount of TPC and TFC present in the leaves of *S. surattense*.

The methanolic fruits extracts of *S. surettense* were fast and effective scavengers of the ABTS radical. It exhibited potent scavenging effects against ABTS with an IC_{50} value of 53.97±5.64 mM min^{-1} g^{-1} DW (Table 5). The ABTS assay is based on the inhibition of the absorbance of radical cation, $ABTS^{+}$ (bluegreen chromogen), which is generated by the oxidation of ABTS with potassium persulfate, its reduction is measured spectrophotometrically at 734 nm, by hydrogen donating antioxidants and coloured radical is again converted back to colourless radical. The inhibition was found to be concentration dependent. Antioxidant activity of these plants extract has been reported *in vivo* by Gupta *et al*. (2011), Sampathkumar (2012) and Kumar *et al*. (2012).

The correlation coefficients of TPC, TFC and different antioxidant activities are depicted in (Table 6). Pearson correlation coefficient is considered positively high if $0.65 \leq `r \geq 0.97$. The highest positive correlation between TPC and peroxidase activity was observed in leaves ($r = 0.780$) but there was poor correlation between TFC and peroxidase activity. Leaves have showed highest positive correlation between TFC and LPO activity ($r = 0.779$), this explains that the high amount of phenols and flavonoids in leaves may be responsible for the peroxidase and LPO activity,

respectively. Stems have showed highest positive correlation between TFC and ABTS activity (r = 0.974) and highest negative correlation between TPC and ABTS (r = -0.995), this explains that ABTS activity is independent of phenolic content of the plant. Further, the negative correlation between TPC, TFC and different antioxidant activity assay models suggested that it could be related to other antioxidant compounds contained in the plants (Goswami *et al*., 2014).

CONCLUSION

From the results, it can be concluded that methanol extracts of *S. surattense* possess potent antioxidant activity and can be used as a source of natural antioxidants for medicinal uses against cancer, ageing, autoimmune diseases, diabetes and other related to free radicals, thus replacing the synthetic ones. The methanol extracts of plant exhibited different levels of antioxidant activity in all models studied. Further investigation of individual compounds with their *in vivo* antioxidant activities and different antioxidant mechanisms is needed.

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