Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 3, 2014

Original Article

FREE RADICAL SCAVENGING POTENTIAL OF THE *SOLANUM SURATTENSE* BURM F.: AN IMPORTANT MEDICINAL PLANT

ANKITA YADAV*, RICHA BHARDWAJ, R. A. SHARMA

Plant Physiology and Biochemistry Laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India.
Email: yadanki22@gmail.com

Received: 03 Oct 2013, Revised and Accepted: 17 Apr 2014

ABSTRACT

Objective: To evaluate the DPPH free radical scavenging activity of methanolic extract of different plant parts (stems, leaves and fruits) of *S. surattense*.

Methods: Methanolic extracts were tested for DPPH free radical scavenging activity, further total phenolic content (TPC), total flavonoidal content (TFC) and ascorbic acid content of *S.surattense* were analyzed through standard methods and their corresponding effect on antioxidant activity of the plant was assessed through correlation analysis.

Results: The highest TPC (25.91 ± 0.803 mg gallic acid equivalents/g dry weight), TFC (17.7 ± 2.36 mg quercetin equivalent/g dry weight) and ascorbic acid content (0.746 ± 0.025 mg/gdw) were found in crude methanolic extract of leaves. The leaves also showed strong antioxidant activity. The highest radical scavenging effect was observed in leaves of *S. surattense* with IC_{50} = 22.936 ± 2.685µg/ml.

Conclusion: Owning to these results, the *S. surattense* has the potential to serve as the source of alternative natural antioxidants and can be used as a medicine against the diseases caused by free radicals.

Keywords: *Solanum surattense,* DPPH radical scavenging activity, Polyphenols, Flavonoids.

INTRODUCTION

Plants have been used for several years as a source of traditional medicine to treat various diseases and conditions [1]. *Solanum surattense* (Solanaceae) is commonly known as Yellow Berried Nightshade and grows wild as a perennial herbaceous weed in different regions of Indo-Pakistan subcontinent up to very high altitudes. Different parts of this plant are used in the treatment of the diseases like fever, asthma, bronchitis, laxative**,** tuberculosis, kidney disorder, cough, constipation, tooth ache, sore throat, rheumatism and gonorrhoea. It has also been investigated for antioxidant activity, antipyretic activity[2], antiulcer activity, antimicrobial activity (anti-inflammatory activity, anthelmintic [3,4]. Leaves juice with few seed of black pepper is a very useful remedy in joint pain.

The innate defence of human body is not enough for severe oxidative stress that further has been associated with cancer, ageing, inflammation, neurodegenerative diseases, hypertension, arthrosclerosis etc. Over production of various forms of activated species, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) and non-free radical species is considered to be the main contributor to oxidative stress. Epidemiological data, as well as in vitro studies, strongly suggest that plants containing phytochemicals with antioxidant potential have strong protective effects against major disease risks caused due to oxidative stress [5]. The protective action of those plants has been attributed to the presence of antioxidants, especially polyphenolic compounds and antioxidant vitamins, including vitamin C, vitamin E, β carotenes, phenolic acids, phytoestrogen, phytate, tocophenols, benzoic acids, folic acid etc[6,7].

The antioxidative activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decreasing peroxides [8]. Ascorbic acid is a redox catalyst which can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide [9]. In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the redox enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants. Ascorbic acid is present at high levels in all parts of plants and can reach concentrations of 20 millimolar in chloroplasts [10]. Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized according to chemical structure.

Ascorbic acid is the principal biologically active form but *L*-dehydroascorbic acid, an oxidation product; it functions in collagen formation, absorption of inorganic iron, reduction of plasma cholesterol level, inhibition of nitrosoamine formation, enhancement of the immune system, and reaction with singlet oxygen and other free radicals. As an antioxidant, it reportedly reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer [11].Due to the beneficial effect antioxidant activity of plant extracts on physiological activity of human cells and their potential to replace synthetic antioxidants that are used in foodstuffs [12], the present study was designed to evaluate the antioxidant activity of methanol extracts of leaves, stems and fruits of *S. surattense* by using widely accepted free radical scavenging model system viz DPPH and its correlations with total phenolic, total flavonoidal and ascorbic acid contents as till now not much attention has been made towards this aspect of the concerned plant.

MATERIALS AND METHODS

Plant material

The different plant parts (stems, leaves and fruits) of *S.surattense* were collected in the month of November-December from the Jaipur-Delhi highway. It was washed with tap water, dried at room temperature 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 of botany.

Chemicals

Chemical like 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma (St. Louis, Missouri, USA). All the chemicals used were of analytical grade and purchased from Himedia from Hi-media Laboratory Pvt. Ltd. Mumbai, India.

Total Phenolic and Flavonoidal Content

Plant Extraction

2gm each of the dry material (leaves, stems and fruits) was extracted with 25ml of methanol at room temperature for 48 hours, filtered

through Whatman paper no 1 filter paper, stored and used for quantification.

Total Phenolic Content

Total phenolic compound contents were determined by the Folin-Ciocalteau method [13,14,15,16]. 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 20-200µg/ml (R^2=0.987). Total phenol values are expressed in terms of Gallic acid equivalent (mg/ g of dry mass), which is a common reference compound. Total phenolic content can be calculated from the formula:

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

Where,

T=Total Phenolic concentration

C= Concentration of gallic acid from caliberation curve (µg/ml)

V= Volume of extract (ml)

M= Wt. of methanol plant extract

Total Flavonoidal Content

Total flavonoid content was determined by using aluminium chloride colorimetric method ($AlCl_3$) according to the known method [17,18] with slight modifications using quercetin as standard. 1ml of test material was added to 10ml volumetric flask containing 4ml of water. To above mixture, 0.3ml of 5% $NaNO_2$ was added. After 5mins, 0.3ml of 10% $AlCl_3$ was added. After 6min, 2ml of 1M NaOH was added and the total volume was made up to 10ml with distilled water. Then the solutions were mixed well and absorbance was measured against blank at 510nm. The standard curve was prepared using the standard solution of Quercetin in methanol in the range 0.5- 5.0mg/ml (R^2=0.991). Total flavanoidal content of the extracts was expressed in milligram of quercetin equivalents/gdw. Total flavanoidal content can be calculated from the formula:

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

Where,

T=Total flavanoidal concentration

C= Concentration of quercetin from caliberation curve (mg/ml)

V= Volume of extract (ml)

M= Wt of methanol plant extract

Ascorbic acid Content

Extraction and Quantification

Each of the fresh experimental material was homogenized thoroughly with 10 ml of acetate buffer (ph 4.8) and centrifuged (1200 rpm, 20min). The supernatants were separately collected, out of which 1 ml was measured to other test tube; From the stock solution of ascorbic acid (10mg/100ml in 4% TCA), varied concentrations (0.01 to 0.09 mg/ml) were also prepared in different test tube, 4ml of 4% trichloroacetic acid (TCA) was then added to all test tubes, left overnight and later, centrifuged [19]. To the supernatant of each sample, 1ml of the colour reagent (prepared by mixing 90ml of 2.2% 2,4 dinitrophenylhydrazine in 10N H_2SO_4, 5ml of 5% thiourea and 5 ml of 0.6% CuSO4 solution) was added and incubated at 57^{0}C for 45min. Later, on cooling 7 ml of 65% was H_2SO_4 added to each mixture, brought to the room temperature and the ODs were measured at 540 nm in spectrophotometer against a blank. A regression curve was computed between the optical density and the concentration of standard ascorbic acid, which followed beer's law.

DPPH Radical Scavenging Activity

Plant extraction

10 gm each of the plant material was soxhlet extracted with methanol for 24 hours. The extract was filtered with Whatman filter paper no 1 and the crude extract was concentrated to dryness in a rotary flash evaporator under reduced pressure and controlled temperature (40–50^{0}C). The extract was preserved in vacuum desiccators for subsequent use in antioxidant assay.

Assay

The antioxidant activities were determined using 1, 1,diphenyl-2-picryhydrazyl (DPPH) as a free radical. Experiments were initiated by preparing a 0.25mM solution of DPPH and 1mg/ml solution of different plant parts extracts (stock) in methanol. To the methanolic solutions of DPPH an equal volume of the extract dissolved in methanol was added at various concentrations. An equal amount of alcohol was added to the control. The setup was left at dark in room temperature and the absorption was monitored after 20 minutes. Ascorbic acid was used as a control. Experiment was performed in triplicate [20,21]. A control reaction was carried out without the test sample. Absorbance values were corrected for radicals decay using blank solution. Ascorbic acid was used as a standard. The inhibitory effect of DPPH was calculated according to the following formula:

% Inhibition = $[1 - (Abs_{SAMPLE} / Abs_{CONTROL})] \times 100$

Linear graph of concentration Vs percentage inhibition was prepared and IC_{50} values were calculated. The antioxidant activity of each sample was expressed in terms of IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve [22,23,24].

Statistical analysis: Experimental results are expressed as means ± standard deviation (SD). All measurements were replicated three times. 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 DPPH radical scavenging assay 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 total phenolic, flavonoid and ascorbic acid contents of the methanolic extracts of different plant parts of *S.surttense* were considerable (Table 1). Total phenols and flavonoids were expressed in terms of gallic acid equivalent (GAE) and Quercetin equivalent (QE) per gram of the dry weight basis. The results revealed that total phenolics, flavonoids and ascorbic acid content vary among different plant parts. The leaves methanolic extracts showed highest phenolic content (25.91 GAE/gdw) and decreases in the order stem (5.879 GAE/gdw), fruits (4.975 GAE/gdw), whereas the total flavonoid content was found to be highest in the leaves extracts (17.7 QE/gdw) when compared to that of the fruits (5.208 QE/gdw), stems (3.129 QE/gdw), the highest ascorbic acid content was found to be in leaves(0.746 mg/gdw) and lowest in stems(0.261 mg/gdw). The DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. Table 2 shows the IC_{50} (µg/ml) values of methanolic extracts of various plant parts for free radical scavenging activity by DPPH(Figure1,2,3). *S. surattense* showed dose-dependent DPPH radicals scavenging activity. The decrease in absorbance of DPPH caused by antioxidants is due to the reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation [25]. It was observed that the leaves extracts exhibited the highest radical-scavenging activity (lowest IC_{50} value= 22.936 µg/ml). The elevated DPPH radical scavenging ability of the leaves extracts can be contributed to the presence of high contents of phenolic, flavonoids and ascorbic acid content. It is likely that the activity of extracts is due to these compounds as polyphenolic compounds are known to have antioxidant activity [26,27]. This activity is believed to be

mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [28]. *S. surattense* exhibited effective antioxidant activity and was better than standard (IC_{50} value= 72µg/ml). Previously also plant has been investigated for antioxidant activity [29,30].

Table 1: Total Phenolic, Flavonoidal and Ascorbic acid content in different plant parts of *Solanum surattense*

Plant Part	Total Phenolic Content (mg GAE/gdw)	Total Flavonoidal Content (mg QE/gdw)	Ascorbic acid(mg/gdw)
Leaves	25.91 ± 0.803	17.7 ± 2.36	0.746 ± 0.025
Stems	5.879 ±0.978	3.129 ± 0.069	0.261 ± 0.046
Fruits	4.975 ± 0.463	5.208 ± 0.721	0.662 ± 0.054

Table 2: The IC_{50} values of different plant parts of *S.surattense* of DPPH radical scavenging assay (µg/ml)

Plant Part	IC_{50} values(µg/ml)
Leaves	22.936 ± 2.685
Stems	357.14 ± 3.11
Fruits	231.57 ± 4.275
Standard(Ascorbic acid)	72.0

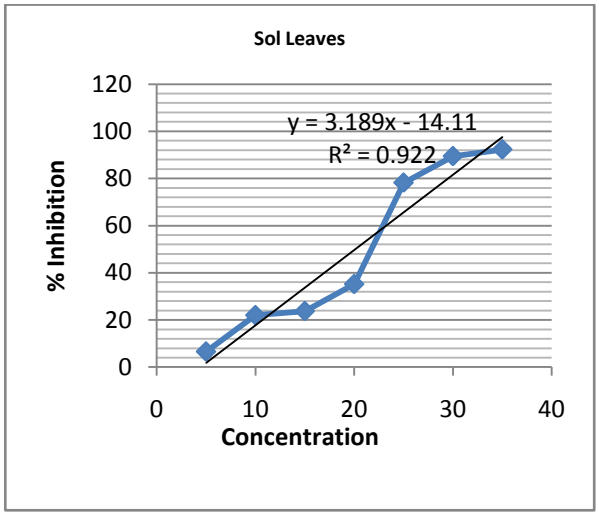

Fig 1: DPPH Scavenger Assay of the. methanol extract of leaves of *S.surattense* methanol extract of stems of *S.surattense*

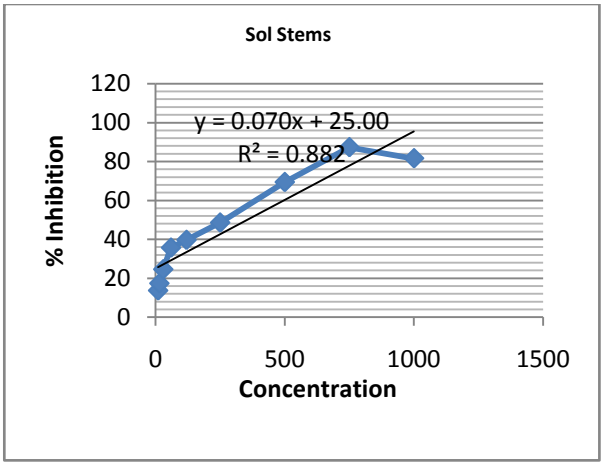

Fig. 2: DPPH Scavenger Assay of the, methanol extract of leaves of *S.surattense* methanol extract of stems of *S. surattense*

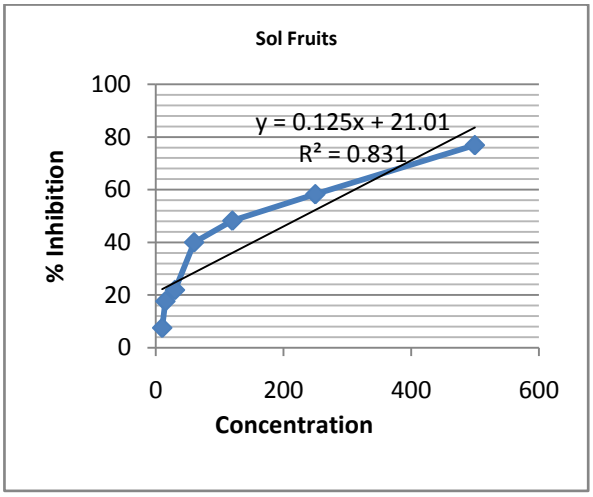

Fig. 3: DPPH Scavenger Assay of the methanol extract of fruits of *S.surattense*

The correlation between percentage inhibitions levels of DPPH and the total phenolics, total flavonoids, ascorbic acid content are shown in Table 3. High correlation between TPC, TFC and Ascorbic acid content was observed in case of leaves, but this was negative in case of stems and fruits. Further the negative correlation between TPC, TFC and antioxidant activity suggested that it could be related to other antioxidant compounds contained in the plants[31].

Table 3: Correlations between the IC_{50} values of DPPH assay, phenolic and flavonoids content, Ascorbic acid content of *S. surattense*

Plant part	Correlations		
	TPC and DPPH	TFC and DPPH	AA and DPPH
Leaves	0.938	0.929	0.986
Stems	0.309	0.544	-0.368
Fruits	0.449	-0.860	0.758

Stems show good correlation with the flavonoids compared to phenols that shows that flavonoids act through scavenging or chelating process. The antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups especially of 3', 4'-orthodihydroxy group on the B cycle and the 4-carbonyle group on the C cycle. The presence of 3-OH and 5-OHgroups on the C cycle are also relevant to antioxidant activity. In the absence of the o-dihydroxystructure in ring B, a catechol structure in ring A can compensate for flavonoid antioxidant activity.

CONCLUSION

Determination of the natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy. In the present study, the total phenolic, flavonoidal, ascorbic acid content and antioxidant activity of methanolic extracts of different plant parts was measured in *S.surattense*. The study reveals the potential of methanolic extracts of plant to scavenge the harmful free radicals. The maximum activity was recorded in leaves which can be attributed to high polyphenolic and ascorbic acid content which is also proved by good correlation between them. Nevertheless, a comprehensive investigation of the bioactive compounds affecting the antioxidant capacity is needed to explain the high free radical scavenging activity of these leaves. Well proved potent antioxidant activity *of S. surattense* in the present study, strongly emphasize that it can be used as an accessible source of natural antioxidants with potential to provide protection against free radicals induced damage to biomolecules.

ACKNOWLEDGMENT

The corresponding author is grateful to the UGC for providing financial support as meritorious student fellowship file no. F. 4-1/2006 (BSR)/7-219/2009(BSR).

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