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Research Letter

Antioxidant activity of the ethanolic extracts of leaves, stems and fruits of *Solanum nigrum*

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ABSTRACT: **Background:** *Solanum nigrum* Linn. (Solanaceae) has been extensively used in traditional medicine in Bangladesh and other parts of the world to cure liver disorders, chronic skin ailments (psoriasis and ringworm), inflammatory conditions, painful periods, fevers, diarrhea, eye diseases etc. **Materials and Methods:** Crude ethanolic extracts of leaves, stems and fruits of *Solanum nigrum* were prepared and evaluated for antioxidant activity by two different *in vitro* methods. **Results:** All the prepared extracts have antioxidant potential. However, leaf extracts showed highest antioxidant activity irrespective of the method used. Gallic acid equivalent phenolic compounds content as well as quercetin equivalent flavonoids content were highest in the leaf extract of the *S. nigrum* and that could be the reason behind the highest antioxidant activity of leaf extract. **Discussion:** As leaf extract showed the highest antioxidant activity among all extracts, it might be investigated further for isolation of antioxidant principles.

KEY WORDS: *Solanum nigrum,* DPPH, antioxidant activity, flavonoid content and phenolic compound content.

INTRODUCTION

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS.[1-2] Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress cause depletion of immune system antioxidants, change in gene expression and induce abnormality in proteins. Oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxides to non-radical forms and functions as natural antioxidants in human body. Due to depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be necessary.[3-5]

Synthetic antioxidants used commercially such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and gallic acid esters are supposed to create or aggravate negative health consequences. Besides, these synthetic antioxidants also demonstrate low solubility problem.[6] Traditionally used medicinal plants are already exploited commercially either as antioxidant additives or as a nutritional supplement. But there is still a demand to find more information concerning the antioxidant potential of medicinal plants.

Solanum nigrum Linn. (Solanaceae) is commonly known as 'Black nightshade.' The plant has been extensively used in traditional medicine in Bangladesh and other parts of the world to cure liver disorders, chronic skin ailments (psoriasis and ringworm), inflammatory conditions, painful periods, fevers, diarrhea, eye diseases etc.[7] The phytochemical studies revealed the plant contains glycoalkaloids (solanine, solamargine, solanigrine and solasodine), steroidal glycosides (β-solamargine, solasonine and α,β- solansodamine), steroidal saponins (diosgenin), steroidal genin (gitogenin), tannin and polyphenolic compounds. Mature fruits are low in alkaloid (solanine) content.[8-10] The fruit of *S. nigrum* is reported to have antiulcer, antioxidant and antitumor promoting agent in rats.[10-12] The fruit of *S. nigrum* has been reported in the ancient Indian medicinal literature with beneficial effects in inflammation, tuberculosis, diuretics etc.[13] In this study attempt has been taken to investigate *in vitro* antioxidant property of the ethanolic extracts of the different parts of *S. nigrum*.

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METHODS

Plant Material

S. nigrum was collected from Mymenshing, Bangladesh and identified by Mrs. Bushra Khan, Senior Scientific Officer, Bangladesh National Herbarium, Dhaka.

Extraction

The plants (as whole) were collected in fresh condition and washed to remove dirt and then leaves, stems and fruits were separated from the plants. All portions were dried separately under the sun followed by in an oven at a temperature < 50 °C until they became suitable for grinding. Each dried part was grounded to a powder separatley using a grinder. The powdered plant materials were submerged into sufficient volume of ethanol in an air-tight flat bottomed container for seven days for extraction, with occasional shaking and stirring. The extracts were then filtered and dried on an electrical water bath. The dried extracts were stored in respective air tight vials in a freezer until further use.

Drugs and Chemicals

1,1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, quercetin and gallic acid were purchased from Sigma Chemical Co. (MO, USA). Folin-Ciocalteu reagent (FCR) was bought from Merck, Germany. All other chemicals and reagents were of analytical grade.

Determination of Total Phenol

Total phenols were determined by Folin Ciocalteu reagent.[14] A diluted extract of each portion (0.5 mL of 1:10 g/mL) or Gallic acid (std. phenolic compound) was mixed with Folin-Ciocalteu reagent (5 mL, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 mL, 1 M). The mixtures were allowed to stand for 15 minutes and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/L solutions of Gallic acid in methanol:water (50:50, v/v). Total phenol values of the respective extract was expressed in terms of Gallic acid equivalent (GAE) mg/g of dry mass of the extract.

Determination of Flavonoid content

Aluminum chloride colorimetric method was used for flavonoids determination.[15] Each portion of the extracts (0.5 mL of 1:10 g/mL) in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. It was kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 μg/mL in methanol.

Determination of Total Antioxidant Capacity

It is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex at acidic pH.[16] The antioxidant capacity is expressed as ascorbic acid equivalent (AAE). Sample extract (0.3 mL) was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm against blank. Total antioxidant capacity of the extract was measured from the regression equation prepared from the concentration versus optical density of ascorbic acid.

DPPH Scavenging Activity

DPPH scavenging activity of the *S. nigrum* was measured by the method developed by Manzocco et al.[17] The sample extract (0.2 mL) was diluted with methanol and 2 mL of DPPH solution (0.5 mM) was added. After 30 min, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated from the measured absorbance data. Ascorbic acid was used as a reference or standard antioxidant in this assay method.

RESULTS AND DISCUSSION

Total Phenolic Compound Assay

The content of total phenolic compounds in the ethanolic plant extracts was determined using the Folin-Ciocalteu assay[14] and the results are shown in Table 1 along with the results reported by others on similar work.

Absolute value of phenolic compounds content in the extract may depend on solvent to plant material ratio, time of extraction and other factors and hence same plant material may lead to different content of phenolic compounds. This fact is evident from the results (Table 1) of phenolic compounds content in leaf and fruit extracts of *S. nigrum* reported by us and others.

However, it is important to notice here that except with the extract of chloroform, phenolic compounds content was found high in the leaf compared to that of fruit irrespective of solvent used (Table 1) and the phenolic compounds content in leaf to fruit varied from 2:1 to 4:1, which is more meaningful than individual absolute value. In our case it was approximately 2:1 (Table 1). Very low dielectric constant of chloroform might have somehow modulated the extraction of phenolic compounds in it and thus showed different result from the extracts prepared with the solvents of about 4 to 7 times high dielectric

constant (Table 1). Except us, no one yet reported the phenolic compounds content in the stem extract of *S. nigrum* and one gram of its dried extract was found to contain 0.53 mg GAE phenolic compound which was much lower than either of the leaf and fruit extract. The decreasing order of phenolic compounds content in the studied parts of *S. nigrum* was leaf > fruit > stem.

It is an established fact that plants that are rich in polyphenolic compounds, such as phenolic acids, flavonoids, carotenoids, tocopherols and tannins possess outstanding antioxidant activities.[19] Some studies have shown positive correlation between increased dietary intake of natural antioxidants and reduced coronary heart disease and cancer mortality, as well as longer life expectancy.[20-21] This study suggests that phenolics are important components of *S. nigrum* and that can be suggested for the treatment of diseases related to oxidative stress.

Flavanoid Content Assay

Flavonoid content was calculated from the regression equation ($y= 0.01x + 0.034$, $R^2= 0.970$) of the calibration curve and is expressed as Quercetin equivalents (QE). The flavonoid content in ethanolic leaf, stem and fruit extract of *S. nigrum* is given in Table 2. Earlier report showed that aqueous boiling water leaf extract of *S. nigrum* contained 2.34 mg of catechin equivalent (CE) in one gram of dried extract, which is very similar to our result and it might be real or accidental coincidence.[22] As per our knowledge, no other report on the content of flavonoid in the studied parts of *S. nigrum* was available in literature during this work and the preparation of manuscript. However, it is important to note here that, like phenolic compounds content in the ethanolic leaf extract (Table 1), flavonoid content was also the highest in the same extract (Table 2) among the parts of *S. nigrum* studied.

Flavonoids are known to exhibit free radical scavenging, inhibition of hydrolytic and oxidative enzymes, anti-inflammatory action. Epidemiological studies have shown that flavonoid intake is inversely related to mortality from coronary heart disease and the incidence of heart attacks. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity[23] through scavenging or chelating process.[24] It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. *S. nigrum,* specially its leaf, having high content of flavonoid may thus contribute to human nutrition and health.

Total Antioxidant Capacity Assay

The total antioxidant capacities of the ethanolic extracts of *S. nigrum* were determined from the calibration curve ($y = 0.008x- 0.114$, $R^2 = 0.986$) established by ascorbic acid standard at 695 nm. Ascorbic acid equivalent (AAE) antioxidant activity of leaf, stem and fruit extract of *S. nigrum* is shown in Table 3.

Folin-Ciocalteu assay or total flavonoid content assay determines only the amount of total phenol or total flavonoid respectively in the extract under investigation but does not necessarily represent the total antioxidant activity of the constituents present in the extract. Because the constituents present in the extract other than phenol

Table 1: Gallic acid equivalent (GAE) phenolic compounds content in the extracts of different parts of *S. nigrum*

Solvent Used for extraction	Dielectric constant of solvent	Plant part	GAE Phenolic compounds content per gram of dried extract	Reference
Chloroform	4.81	Leaf	5.01 mg	[18]
Chloroform	4.81	Fruit	5.01 mg	[18]
Acetone	20.7	Leaf	1.1 mg	[18]
Acetone	20.7	Fruit	0.4 mg	[18]
Ethanol	24.5	Leaf	2.6 mg	[Present study]
Ethanol	24.5	Stem	0.53 mg	[Present study]
Ethanol	24.5	Fruit	1.47 mg	[Present study]
Methanol	32.7	Leaf	0.4 mg	[18]
Methanol	32.7	Fruit	0.1 mg	[18]

Table 2: Quercetin equivalent (QE) or catechin equivalent (CE) flavonoid content in the extracts of different parts of *S. nigrum*

Solvent Used	Dielectric constant of solvent	Plant part	Flavonoid content per gram of dried extract QE/CE		Reference
			QE	CE	
Boiling water	80.1	Leaf		2.34 mg	[22]
Ethanol	24.5	Leaf	2.55 mg		Present study
Ethanol	24.5	Stem	1.32 mg		Present study
Ethanol	24.5	Fruit	0.62 mg		Present study

Table 3: Antioxidant activity of the extracts of different parts of *S. nigrum*

Antioxidant activity	Extract		
	Leaf	Stem	Fruit
Total antioxidant capacity (in terms of AAE) per gram of dried extract	10.27 mg	10.21 mg	8.24 mg
DPPH Scavenging activity (in terms of IC_{50}) per mL of solution (5 µg/ µl) of extract	120.22 µg	301.99 µg	194.98 µg

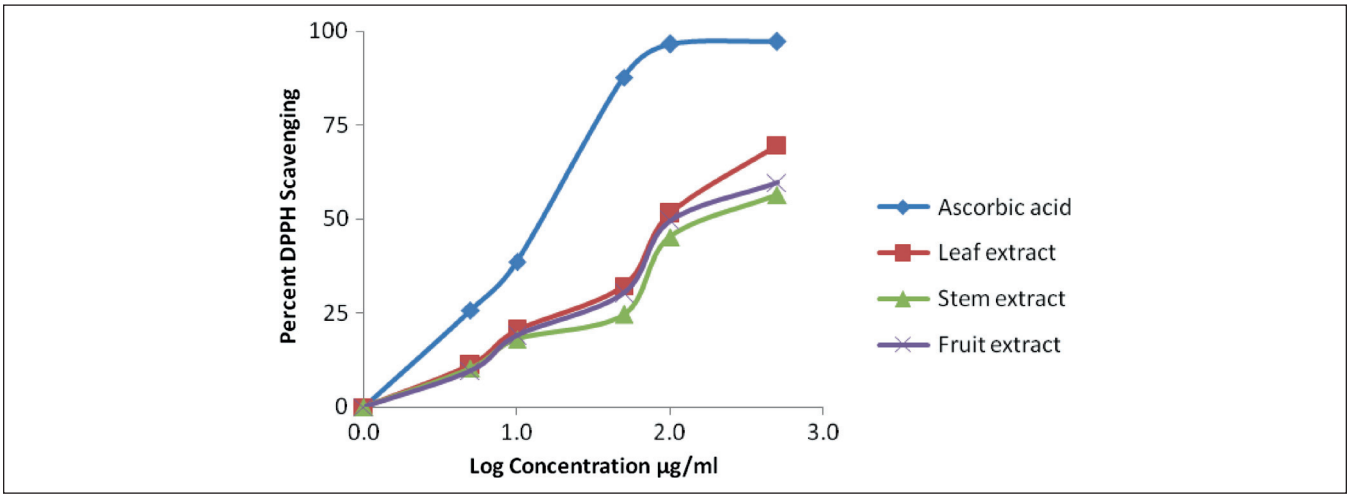

Figure 1: DPPH scavenging activity of the extracts of different parts of *S. nigrum*

and flavonoid might be responsible for the antioxidant action too. Therefore, result of total antioxidant capacity assay is more important for the determination of antioxidant property of an extract. From the present study, it was observed that each of the studied part of *S. nigrum* is potent in antioxidant activity and it was highest with the leaf extract. And numerically it was not much different from the other parts of *S. nigrum* studied. However, the decreasing order of total antioxidant capacity was: Leaves ≈Stems >Fruits. We were unable to compare our results with other works as there was no report on such important study.

DPPH Scavenging Activity

DPPH free radical scavenging method is an easy, rapid and sensitive way to screen the antioxidant activity of a specific compound or plant extracts.[25] Figure 1 shows the amount of each extract needed for 50% inhibition (IC_{50}) or scavenging of DPPH free radical. The highest radical scavenging activity was showed by leaf extract with IC_{50} = 120.22 µg/mL. IC_{50} values for other parts of *S. nigrum* are presented in Table 3. Ascorbic acid solution (5 µg/µl), a standard antioxidant showed the IC_{50} value as14.45 µg per milliliter of ascorbic acid solution.

IC_{50} value in terms of AAE of the methanolic extract of the leaf and fruit extract was reported to be 100 µg and 110 µg respectively per mL of solution of extract[18] which are comparable to our observation (120.22 µg). Irrespective of the study method, leaf extract showed the highest antioxidant activity, which might be due to the highest content of phenolic compounds as well as flavonoids in that (i.e. leaf) as we showed earlier on the basis of the findings of this study.

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