Phytochemical Screening and In Vitro Determination of Antioxidant Potential of Methanolic Extract of Stereospermum Chelonoides


INTRODUCTION

Reactive species are generated in living systems through various metabolic processes and environmental stresses. These are free radicals and mainly reactive oxygen species (ROS). Increased level of ROS can damage structure of biomolecules and modify their functions and lead to cellular dysfunction and even cell death. The cumulative effect of increased ROS can increase oxidative stress in systemic level and it is manifested in the form of a variety of health problems such as cancer, age related disease and cardiovascular diseases (Grune et al, 2001; Noguchi & Niki, 2000). Cellular ROS are regulated by interplay of complex antioxidant machineries in living systems. However, when the generation of the active oxygen-free radical is overgrown many degenerative diseases, such as brain dysfunction, cancer, heart diseases, age-related degenerative conditions, declination of the immune system, cancer, coronary arteriosclerosis, ageing processes, carcinogenesis, gastric ulcer and DNA damage arise (Grzegorczyk et al, 2007; Kumaran and Joel, 2007; Shen et al, 2010; Kannan et al, 2010; Prakash et al, 2007). Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Thus, interest in natural antioxidants has increased considerably. Therefore, it is essential to develop and utilize effective natural antioxidants so that they can protect the human body from free radicals. In continuation of our efforts to verify the efficacy of traditional medicine (Taksim et al, 2012, Taksim et al, 2013) we have collected Steriospermum chelonoides DC based on the ethnopharmacological information. Steriospermum chelonoides. (Family: Bignoniaceae) locally known as “Paruli” is a medium size deciduous tree commonly available in Indian Subcontinent. S. chelonoides is widely used in traditional medicine.

ABSTRACT

Plant derived phytochemicals consisting of phenols and flavonoids possess antioxidant properties, eventually renders a lucrative tool to scavenge reactive oxygen species (ROS). Current study evaluates the preliminary phytochemical screening and antioxidant activity of methanolic extract of bark and leaf of Stereospermum chelonoides. Thus, various in vitro assay strategies were implemented to evaluate antioxidant potential of Stereospermum chelonoides, using DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging assay, ferric reducing antioxidant power (FRAP), total antioxidant capacity, determination of total phenol and flavonoid contents. Preliminary phytochemical study revealed the presence of alkaloid, carbohydrate, glycoside, flavonoid, steroid and tannin. In DPPH radical scavenging assay, the fraction showed significant antioxidant activities in the assay compared to the reference ascorbic acid in a dose dependent manner. The IC_{50} value of the crude methanol extract of bark and leaf was 53.99±3.25 µg/mL and 84.73±0.02 µg/mL, respectively, whereas IC_{50} value for the reference ascorbic acid was 14.56±0.24 µg/mL. Moreover, profound total antioxidant activity was observed for bark (309.88±1.03 mg/g equivalent to ascorbic acid) as well as leaf (147.09±1.79 mg/g equivalent to ascorbic acid) at 200 µg/mL extract concentration. Furthermore, extract showed good reducing power capability in both bark and leaf fraction. Total phenol content for the bark was 574.82 mg/g equivalent to gallic acid and for leaf was 189.86 mg/g. For bark, the total flavonoid content was found 55.82 mg/g equivalent to quercetin and for leaf was 14.56±0.24 µg/mL. Moreover, profound total antioxidant activity was observed for bark (309.88±1.03 mg/g equivalent to ascorbic acid) as well as leaf (147.09±1.79 mg/g equivalent to ascorbic acid) at 200 µg/mL extract concentration. Furthermore, extract showed good reducing power capability in both bark and leaf fraction. Total phenol content for the bark was 574.82 mg/g equivalent to gallic acid and for leaf was 189.86 mg/g. For bark, the total flavonoid content was found 55.82 mg/g equivalent to quercetin and for leaf was 14.56±0.24 µg/mL.

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The flower of the plant is considered in treating bilious diarrhea and burning sensation. The fruit is also taken with honey to control hiccups (Rao et al, 1968). Leaf juice when boiled with oil, cures diseases of the ear, teeth and rheumatism. Bark has antibacterial and anti-tuberculosis properties.

Aerial part extract is effective against lymphocytic leukemia. Pill prepared from the leaf of this plant is also used to treat tuberculosis (Mohammad et al, 2006). S. chelonoïdes is used to treat sprain.

Moreover, it contains lapachol (2-hydroxy-3-(3-methyl-2-butetyl)-1,4-napthaquinone), dinatin (4,5,7-tri hydroxyl-6-methoxyflavon), dinatin-7-gluconurisi and beta-sitosterol . In addition, two new quinines have been isolated namely stereochenol A, sterechenol B along with naphthoquinones, sterekunthal B and sterequinone C. (Mohammad et al, 2006). The present research was aimed to investigate the phytochemical constituents and antioxidant activities of S. chelonoïdes. To evaluate the antioxidant potential of methanolic extract of bark and leaves of Stereospermum Chelonoïdes, several in vitro assay strategies have been implemented vz. DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging assay, ferric reducing antioxidant power (FRAP), total antioxidant capacity and determination of total phenolic and flavonoid content to reveal the effectiveness of this plant as a functional food as well as in medicine.

MATERIALS AND METHODS

Chemicals

DPPH (1, 1-diphenyl, 2-picrylhydrazyl) was purchased from Sigma Chemical Co. (USA), Potassium Ferricyanide [K₃Fe(CN)₆] from Loba Chemie Pvt. Ltd. (Mumbai, India), Ascorbic acid from SD Fine Chem. Ltd. (Biosar, India), and Folin ciocalteu’s reagent (FCR), Gallic acid (C₇H₆O₅), Quercetin were purchased from E. Merck (Darmstadt, Germany).

All other chemicals and solvents for extractions were of analytical grade. All UV-Vis measurements were recorded on a Shimadzu UV-1601 (Kyoto, Japan) spectrophotometer.

Plant material

Stems and leaves of Stereospermum chelonoïdes were collected from Jahangirnagar University campus, Savar, Dhaka in June, 2011. Bark was separated carefully from the stem. Then, barks and leaves of the plant were thoroughly washed with water and dried using dryer.

Preparation of plant extract

Powdered dried barks and leaves (100 g each) were macerated with 70 % methanol (500 mL) with occasional stirring at 25 ± 2°C for 3 days. The extract was then filtered using a Buchner funnel and a sterilized cotton filter.

The solvent was completely removed by rotary evaporator and approximately 20 g and 30 g methanolic extract of barks and leaves were obtained, respectively. These crude extract was subjected to antioxidative potential assays.

Preliminary phytochemical screening

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. Phytochemical screenings of the extracts were performed using the following reagents and chemicals; alkaloids with Dragendroff’s reagents, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions and saponins with ability to produce stable foam and steroids with Libermann- Burchard reagent. Gum was tested using Molish reagent and concentrated sulfuric acid; reducing sugars with Benedict’s reagent. These were identified by characteristic color changes using standard procedures by Ghani A, 2005.

Tests for antioxidant activity

DPPH free radical scavenging activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, were determined by the method described by Braca et al, 2001. Plant extract (0.1 mL) was added to 3 mL of a 0.004 % ethanol solution of DPPH. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated from [(A₀-Aₙ)/ A₀] ×100, where A₀ is the absorbance of the control (DPPH solution) and Aₙ is the absorbance of the extract/standard. The inhibition curves were prepared and IC₅₀ values were calculated.

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power was determined according to the method previously described by Oyaizu, 1986. According to this method, the reduction of Fe³⁺ to Fe²⁺ is determined by measuring the absorbance of Perl’s Prussian blue complex. Briefly, different concentrations of extracts (5-200 µg) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 mL, 1 %). The mixture was incubated at 50°C for 20 min. An aliquot (2.5 mL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1 %) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the reference.

Determination of total antioxidant capacity

The antioxidant activity of the extracts were evaluated by the phosphomolybdenum method according to the procedure describe by Prieto et al, 1999. A 0.3 mL extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer (UV-visible spectrophotometer, Shimadzu, 1601) against blank after cooling at room temperature. Methanol...
Determination of total phenol content

The total phenolic content of plant extracts were determined using Folin–Ciocalteu reagent (Yu L et al, 2002). Plant extract (100 µL) was mixed with 500 µL of the Folin–Ciocalteu reagent and 1.5 mL of 20 % sodium carbonate. The mixture was shaken thoroughly and made up to 10 mL using distilled water. The mixture was allowed to stand for 2 hour. Then the absorbance at 765 nm was determined. These data were used to estimate the phenolic contents using a standard curve obtained from various concentration of gallic acid.

Determination of total flavonoid content

The content of flavonoids compounds in the extracts were determined by the method described by Chang et al, 2002. 1.0 mL of extract was mixed with methanol (3 mL), aluminium chloride (0.2 mL, 10 %), potassium acetate (0.2 mL, 1 M) and distilled water (5.6 mL) and incubated the mixture for 30 min at room temperature. Then the absorbance was measured at 415 nm against blank. Methanol (1 mL) in the place of extract was used as the blank and Quercetin was used as the standard solution. All determinations were carried out in triplicates. The amount of flavonoids in plant extracts in quercetin equivalents (QE) was calculated by the following formula: \( X = (A × m0)/(A_o × m) \), where \( X \) is the flavonoid content, mg/mg plant extract in QE, \( A \) is the absorption of plant extract solution, \( A_o \) is the absorption of standard rutin solution, \( m \) is the weight of plant extract in mg and \( m0 \) is the weight of quercetin in the solution in mg.

Statistical analysis

The results were expressed as mean ± standard deviation (SD) from triplicate experiments and evaluated with the analysis of student’s t-test. Differences were considered significant at a level of P<0.05. IC\(_{50}\) was calculated using SigmaPlot 11.0 software.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of various bioactive components like alkaloid, carbohydrate, glycoside, flavonoid, steroid and tannin in both fractions(Table 1).

DPPH radical scavenging activity

As can be seen from Fig.1, in the DPPH radical scavenging assay both extracts exhibited a concentration-dependent antiradical activity by inhibiting DPPH⁰ radical. Ascorbic acid, which is a well known antioxidant, showed higher degree of free radical-scavenging activity than that of the plant extract at each concentration points. The IC\(_{50}\) value of the crude methanol extract of bark and leaf was 53.99 ± 3.25 µg/mL and 84.73 ± 4.02 µg/mL, respectively, whereas IC\(_{50}\) value for the reference ascorbic acid was 14.56 ± 0.24 µg/mL. The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants (Kumarasamy Y et al, 2007). The method is based on the reduction of ethanolic DPPH⁺ solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by reaction. The extract was able to reduce DPPH radical (visible deep purple color) to the yellow-coloured diphenylpicrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds(e.g. hydroquinone, pyrogallol, gallic acid), and aromatic amines (e.g. p-phenylene diamine, p-aminophenol), reduce and decolorise 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability (Blois MS, 1958). Therefore, one of the possible mechanisms of the good antioxidant activity of the extracts might be the resultant of containing good amount of phenolic compounds in both bark and leaf, which shows antioxidant activity due to their redox properties, plays an important role in absorbing and neutralizing free radicals, quenching single and triple oxygen or decomposing peroxide.

Ferric reducing antioxidant power (FRAP)

Fig. 2 shows the reducing power capabilities of the plant extract compared to ascorbic acid. The extract displayed good reducing power which was found to rise with increasing concentrations of the extract. In reducing power assays, the presence of antioxidants in the seeds can reduce the oxidized form of iron (Fe³⁺) to its reduced form (Fe²⁺) by donating an electron. Thus, it can be assumed that the presence of reductants (i.e. antioxidants) in S. chelonoides extracts causes the reduction of the Fe³⁺/ferricyanide complex to the ferrous form. Therefore, the Fe²⁺ complex can be monitored by measuring the formation of Perl’s Prussian blue at 700 nm. A higher absorbance indicates greater reducing power ability (Gordon, 1990). In addition, bark extract showed better reducing capacity than the leaf extract.

Determination of total antioxidant capacity

The total antioxidant capacity of the bark and leaf extract of the S. chelonoides are given in Table 2. Significant amount of total antioxidant activity was obtained from the bark extract (309.88±1.03 mg/g equivalent to ascorbic acid) as well as leaf extract (147.09±1.03 mg/g equivalent to ascorbic acid) at 200 µg/mL extract concentration. The phosphomolybdenum method was based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts (Prieto et al, 1999). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid.
Determination of total phenol content

Several reports have conclusively shown close relationship between total phenolic content and antioxidative activity of the fruits and vegetables. Phenolic compounds, as natural antioxidants exhibit therapeutic potential in multiple diseases including cardiovascular disease, aging and cancer (Vinson JA et al., 1998).

Moreover, the antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Uritani et al., 1994). However, as can be seen from Table 2, the bark and leaf extract of the S. chelonoides was found to contain higher amount of phenolics, 574.82 mg/g and 189.86 mg/g equivalent to gallic acid, for bark and leaf extract, respectively using folin-ciocalteau method. As the exact chemical nature of the folin-ciocalteu reagent is not known, but it is believed to contain heteropolyphosphotunstate molybdates. Sequences of reversible 1 or 2 electron reduction reactions lead to blue species, possibly PMoW_{11}O_{40} (Yu L et al., 2002).

Determination of total flavonoid content

Flavonoids, the main class of polyphenols in plants, are known to be antioxidants and free radical scavengers having the basic structure of diphenylpyrans. The antioxidative activities of flavonoids are multifaceted. Flavonoids possess phenolic hydrogens responsible for the radical scavenging activity. It has been reported that the O-dihydroxyl (catechol) structure in the B ring is the obvious radical target site for all flavonoids. The additional presence of both 3 and 5-hydroxyl groups is responsible for maximal radical scavenging potentials and strongest radical absorption (Ko et al., 1998).

Flavonoids can exhibit their antioxidant activity in several ways: (i) Radical scavenging activity toward either reactive species (e.g. reactive oxygen species: ROS) such as ‘OH, O₂\^ - , O₂^- or toward lipid peroxidizing radicals such as R’, RO\^ , and ROO\^ radical scavenging action generally proceeds via hydrogen atom transfer or electron donation; (ii) prevention of the transition metal-catalyzed production of reactive species (i.e. via Fenton-type reactions) through metal chelation; (iii) interaction with other antioxidants (such as cooperative actions), localization, and
mobility of the antioxidant at the microenvironment (Bombardelli et al., 1993). However, total flavonoid content of S. cheloneoides extracts are shown in Table 2. The results were exhibited as quercetin equivalent of flavonoids per gm of extracts of the sample. For bark and leaf extract, the total flavonoid content were found to be 55.82 mg/g and 49.44 mg/g equivalent to quercetin respectively. These results suggested that the antioxidant activities of S. cheloneoides might be due to its flavonoid content.

CONCLUSION

Although the bark has greater phenol and flavonoid contents than leaf but both the bark and leaf shows promising result along with total antioxidant capacity which may explore the promising features of Styreospermum cheloneoides in near future in various degenerative diseases associated with oxidative stress like cancer, hypertension, diabetes, hyperlipidemia, inflammations etc. Presence of various secondary metabolites like alkaloid, tannin, flavonoid, saponin etc may also make it a plant of choice to isolate compounds to treat diarrhoea bacterial and fungal infection and so on. But further scientific in vivo and in vitro studies are required to finally determine its potentials in all these fields.

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REFERENCES


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