

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

Meena Afroze Shanta², Taksim Ahmed^{*1,2}, Mohammad Nasir Uddin^{1,2}, Senjuti Majumder², Md. Solayman Hossain², Md. Sohel Rana².

¹College of Pharmacy, Chosun University, 375 Seosuk-dong, Gwangju 501-759, Republic of Korea.

²Laboratory of natural products research, Department of pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

ARTICLE INFO

Article history:

Received on: 09/01/2013

Revised on: 27/02/2013

Accepted on: 11/03/2013

Available online: 30/03/2013

Key words:

Stereospermum chelonoides, antioxidant potential, DPPH, ferric reducing antioxidant power (FRAP).

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 IC₅₀ 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 IC₅₀ 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 it was 49.44 mg/g.

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 (Grzegorzczak *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 *Stereospermum chelonoides* DC based on the ethnopharmacological information. *Stereospermum 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.

* Corresponding Author

College of Pharmacy, Chosun University, 375 Seosuk-dong,

Gwangju 501-759, Republic of Korea.

Tel.: +82 10 5529 6067

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 anti-bacterial 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. chelonoides* is used to treat sprain.

Moreover, it contains lapachol (2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone), dinatin (4,5,7-tri hydroxyl-6-methoxyflavon), dinatin-7-glucuroniside and beta-sitosterol. In addition, two new quinines have been isolated namely stereochenol A, stereochenol 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. chelonoides*. To evaluate the antioxidant potential of methanolic extract of bark and leaves of *Steriospermum Chelonoides*, several *in vitro* assay strategies have been implemented viz. 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 [$K_3Fe(CN)_6$] from Loba Chemie Pvt. Ltd. (Mumbai, India), Ascorbic acid from SD Fine Chem. Ltd. (Biosar, India), and Folin-ciocalteu's reagent (FCR), Gallic acid ($C_7H_6O_5 \cdot H_2O$), 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 chelonoides* 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 $25 \pm 2^\circ C$ 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 $[(A_0 - A_1) / A_0] \times 100$, where A_0 is the absorbance of the control (DPPH solution) and A_1 is the absorbance of the extract/standard. The inhibition curves were prepared and IC_{50} 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 Fe^{3+} to Fe^{2+} 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 [$K_3Fe(CN)_6$] (2.5 mL, 1 %). The mixture was incubated at $50^\circ C$ 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 $FeCl_3$ (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 $95^\circ C$ 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

(0.3 mL) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

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 μ L) was mixed with 500 μ L 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: $X = (A \times m_0) / (A_0 \times m)$, where X is the flavonoid content, mg/mg plant extract in QE, A is the absorption of plant extract solution, A_0 is the absorption of standard rutin solution, m is the weight of plant extract in mg and m_0 is the weight of quercetin in the solution in mg.

Statistical analysis

The results were expressed as mean \pm 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 DPPH $^{\cdot}$ 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 μ g/mL and 84.73 ± 4.02 μ g/mL, respectively, whereas IC_{50} value for the reference ascorbic acid was 14.56 ± 0.24 μ g/mL. The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (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 $^{\cdot}$ 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 (Fe^{3+}) to its reduced form (Fe^{2+}) 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 Fe^{3+} /ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} 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 ± 1.79 mg/g equivalent to ascorbic acid) at 200 μ g/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.

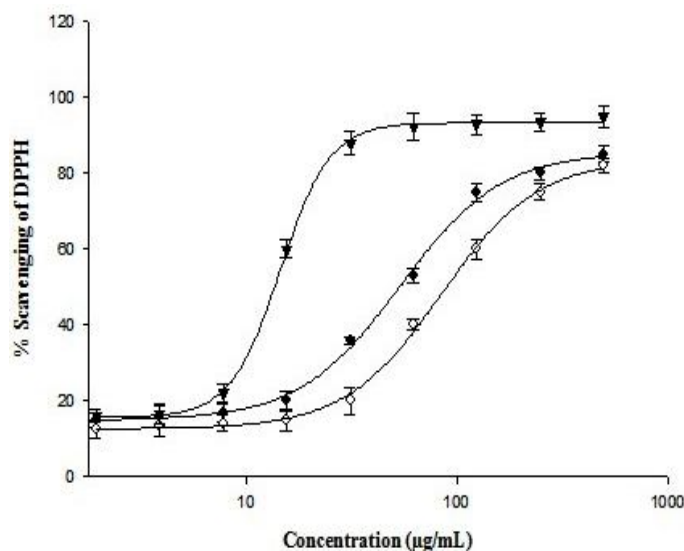


Fig. 1: DPPH radical scavenging activity of methanol extract of (●) bark and (○) leaf of the *Stereospermum chelonoides* along with the standard Ascorbic acid (▼). (Mean \pm SD, n=3). Concentrations are displayed on logarithmic scales.

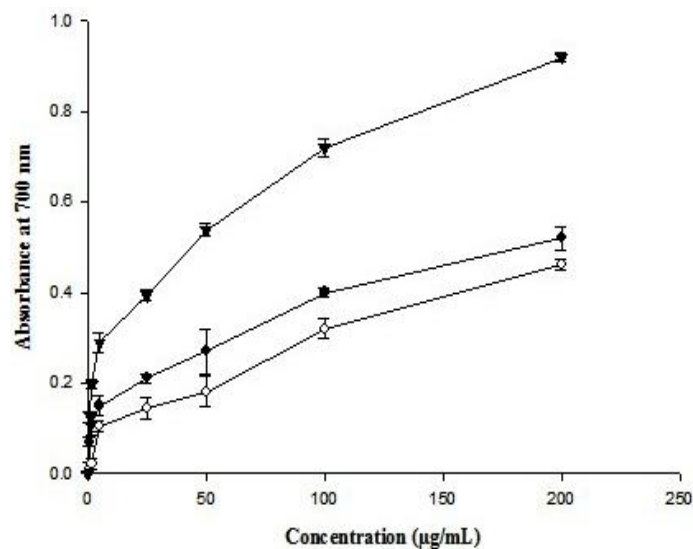


Fig. 2: Reducing power of methanol extract of (●) bark and (○) leaf of the *Stereospermum chelonoides* along with the standard Ascorbic acid (▼). (Mean \pm SD, n=3).

Table 1: Result of phytochemical screening of of methanol extracts of the bark and leaf of *Stereospermum chelonoides*.

Extract	Carbohydrate	Glycoside	Alkaloid	Saponin	Steroid	Flavonoid	Tannin
SCBM	+	-	+	+	+	+	+
SCLM	+	+	+	-	+	+	+

SCBM: *Stereospermum chelonoides* bark methanol, SCLM: *Stereospermum chelonoides* leaf methanol, Values are the average of triplicate experiments and represented as mean \pm SD. ; (+): Present; (-): Absent

Table 2: Total antioxidant capacity, total phenol and total flavonoid contents of methanol extracts of the bark and leaf of *Stereospermum chelonoides*.

Extract	Total antioxidant capacity equivalent to ascorbic acid mg/g plant extract	Total phenol (in mg/g, gallic acid equivalents)	Total flavonoid (in mg/g, quercetin equivalents)
SCBM	309.88 \pm 1.03	574.82 \pm 1.29	55.82 \pm 2.38
SCLM	147.09 \pm 1.79	189.86 \pm 1.56	49.44 \pm 1.35

SCBM: *Stereospermum chelonoides* bark methanol, SCLM: *Stereospermum chelonoides* leaf methanol, Values are the average of triplicate experiments and represented as mean \pm SD.

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-ciocalteu method. As the exact chemical nature of the folin-ciocalteu reagent is not known, but it is believed to contain heteropolymolybdates molybdates. Sequences of reversible 1 or 2 electron reduction reactions lead to blue species, possibly $\text{PMoW}_{11}\text{O}_{40}$ (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 $\cdot\text{OH}$, $\text{O}_2\cdot^-$, $\cdot\text{O}_2$, or toward lipid peroxidizing radicals such as $\text{R}\cdot$, $\text{RO}\cdot$, and $\text{ROO}\cdot$ 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. chelonoides* 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. chelonoides* 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 *Styrospermum chelonoides* 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.

ACKNOWLEDGEMENT

We would like to thank Mr. Md. Ehsanul Haque Mazumder, PhD, School of biomedical science, University of Sydney, Australia, for his valuable suggestions and critical reading of the manuscript.

REFERENCES

- Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature* 1958; 181: 1199–200.
- Bombardelli, E., Morazzoni, P. The Flavonoids: New Perspectives in Biological Activities and Therapeutics. *Chimicaoggi* 1993; 25-28.
- Braca, A., N.D. Tommasi, L.D. Bari, C. Pizza, M. Politi and I. Morelli. Antioxidant principles from *Bauhinia terapotensis*. *J. Natl. Prod.* 2001; 64: 892-5.
- C. Chang, M. Yang and H. Wen. Cheru J. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. *J. Food Drug Anal* 2002; 10: 178-82.
- Ghani A. Practical Phytochemistry. 1st ed. Parash Publishers, Dhaka, Bangladesh (2005) 12-18.
- Grune, T., Shringarpure, R., Sitte, N., & Davies, K. Age-Related changes in protein oxidation and proteolysis in mammalian cells. *Journal of Gerontology* 2001, 56(A): B459–B467.
- Gordon MH. The mechanism of antioxidant action in vitro: In B. J. F. Hudson ed. *Food antioxidants* London: Elsevier Applied Science (1990) 1-18.
- Grzegorzczak I, Matkowski A, Wysokińska H. Antioxidant activity of extracts from in vitro cultures of *Salvia officinalis* L. *Food Chemistry* 2007; 104(2): 536-41.
- Kannan RRR, Arumugam R, Anantharaman P. In vitro antioxidant activities of ethanol extract from *Enhalus acoroides* (L.F.) Royle. *Asian Pacific Journal of Tropical Medicine* 2010; 3(11): 898-901.

Kumaran A, Joel Karunakaran R. In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT - Food Science and Technology* 2007;40(2):344-52.

Kumarasamy Y, Byres M, Cox PJ, Jaspars M, Nahar L, Sarker SD. Screening seeds of some Scottish plants for free-radical scavenging activity. *Phytother. Res.* 2007; 21: 615-621.

M Atiqur Rahman *et al.* Medicinal plants used by *Chakma* tribe in Hill Tracts districts of Bangladesh. *Indian Journal of Traditional Knowledge* 2007, 6(3): 508-517.

Mohammad R. Haque , Khondaker M. Rahman , Magdy N. Iskander , Choudhury M. Hasan, Mohammad A. Rashid. Stereochenols A and B, two quinones from *Stereospermum chelonoides*. *Phytochemistry* 2006, 67: 2663–2665

Noguchi, N., and Niki, E. Phenolic antioxidants: A rationale for design and evaluation of novel antioxidant drug for atherosclerosis. *Free Radical Biology & Medicine* 2000, 28: 1538–1546.

Oyaizu M. Studies on products of browning reactions. Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr* 1986; 44: 307–315.

Prieto, P., Pineda, M., Aguilar, M: Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry* 1999; 269:337–341.

Prakash D, Upadhyay G, Singh BN, Singh HB. Antioxidant and free radical-scavenging activities of seeds and agri-wastes of some varieties of soybean (*Glycine max*). *Food Chemistry* 2007; 104(2): 783-90.

Rao, K., McBride, T.J., Oleson, J.J., 1968. Recognition and evaluation of lapachol as an antitumor agent. *Cancer Res.* 28, 1952–1954.

Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. Antioxidant activity in vitro of the selenium-contained protein from the Se-enriched *Bifidobacterium animalis* 01. *Anaerobe* 2010; 16(4): 380-86.

Taksim Ahmed, Mohammad Nasir Uddin, Shaikh Faisal Ahmed, Arindam Saha, Kaniz Farhana, and Md. Sohel Rana. In vitro evaluation of antioxidant potential of *Artocarpus chama* Buch. fruits . *J. App. Pharm. Sci.* 2012; 2 (10): 075-080.

Taksim Ahmed, Mohammad Nasir Uddin, Md. Kamal Hossain, Md. Imamul Islam, Nizam Uddin, Md. Ehsanul Haque Mazumder. Strategies to evaluate antioxidant and cytotoxic activity of pet ether extract of *Artocarpus chama* Buch. Seed. *J. App. Pharm. Sci.* 2012; 2 (12): 089-095.

Taksim Ahmed, Mohammad Nasir Uddin, Md. Kamal Hossain, Nahid Hasan, Md. Sohel Rana. Evaluation of antioxidant and cytotoxic potential of *Artocarpus chama* buch. Seeds using in vitro models. *Int J Pharm Pharm Sci*, 2013; 5 (1):283-289.

Uritani I, Garcia V. V., Mendoza E. M. T. Postharvest biochemistry of plant food- materials in the tropics. 1st ed. Japan Scientific Societies Press, Tokyo, Japan (1994) 241-251.

Vinson JA, Hao Y, Zubic SK. Food antioxidant quantity and quality in foods: Vegetables. *J. Agric. Food. Chem.* 1998; 46: 3630–3634.

Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M. Free radical scavenging properties of wheat extracts. *J. Agric. Food Chem.* 2002; 50: 1619–1624.

How to cite this article:

Meena Afroze Shanta, Taksim Ahmed, Mohammad Nasir Uddin, Senjuti Majumder, Md. Solayman Hossain, Md. Sohel Rana., Phytochemical Screening and *In Vitro* Determination of Antioxidant Potential of Methanolic Extract of *Styrospermum Chelonoides*. *J App Pharm Sci.* 2013; 3 (03): 117-121.