IJPSR (2016), Vol. 7, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 September, 2015; received in revised form, 15 December, 2015; accepted, 05 January, 2016; published 01 March, 2016

IN VITRO ANTIOXIDANT ACTIVITIES AND CYTOTOXICITY STUDY OF THE METHANOLIC EXTRACT OF BARKS OF *SYZYGIUM CYMOSUM*

Kakan Sutra Dhar [1], Tania Binte Wahed [*1], A. H. M. Nazmul Hasan [2] and Sonia Binte Wahed [3]

Department of Pharmacy [1], Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.
Department of Pharmacy [2], University of Asia Pacific, Block-A, House-73/C, Road-5/A, Dhanmondi, Dhaka-1209, Bangladesh.
Department of Applied Chemistry and Chemical Engineering [3], Dhaka University, Dhaka- 1000, Bangladesh.

Key words:

Syzygium cymosum, Antioxidant, DPPH, Brine shrimp cytotoxicity assay

Correspondence to Author:
Tania Binte Wahed

Assistant Professor
Department of Pharmacy,
Jahangirnagar, University, Savar,
Dhaka-1342, Bangladesh.

E-mail: tania.wahed@yahoo.com

ABSTRACT: The aim of the study to determine antioxidant and cytotoxic properties of the bark extracts of *Syzygium cymosum* along with phytochemical study for the presence of phytochemical constituents. Total phenolic and flavonoid content of the *S.Cymosum* extracts was determined by using the Folin- Ciocalteu reagent and aluminum chloride (AlCl3) method, respectively. The crude methanolic extract of *S.Cymosum* was studied to evaluate its antioxidant activity by DPPH radical scavenging assay and reducing power assay. Brine Shrimp Lethality Bioassay was done for cytotoxic activity. Bark extracts of *S.Cymosum* have been shown to possess phytoconstituents including carbohydrates, alkaloid, glycosides, steroids, tannins and saponin. The extract was found to contain significant amount of phenol and flavonoid in Folin-Ciocalteau and total flavonoid content assay. Total antioxidant capacity of the extract was estimated to be 505.5±3.535 mg/g of ascorbic acid equivalent. Its IC_{50} value in DPPH method was 64.99079µg/ml. The extract showed concomitant increase in reducing power with the increase of concentration of the extract. In case of brine shrimp cytotoxicity assay, LC_{50} value was obtained to be 307 µg/ml. Data from present results revealed that it act as an antioxidant agent due to its free radical scavenging and it has also cytotoxic activity.

INTRODUCTION: Free radicals are generated during body metabolism, which have been claimed to play an important role in affecting human health by causing several diseases such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases. Exogenous intake of antioxidants can help the body scavenge free radicals effectively [1, 2]. Many polyphenolic compounds, such as phenolic acids, flavonoids, anthocyanidins, and tannins, which possess remarkable antioxidant activities, are rich in plant materials.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(3).1021-25

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (3).1021-25

Some studies have shown the positive correlation of the increased dietary intake of natural antioxidants with the reduced coronary heart disease and cancer mortality, as well as with longer life expectancy [3, 4]. In the search for novel antioxidants, many plant species have been investigated but there is still a demand to find more information concerning the antioxidant potential of plant species [5]. Utilization of these significant sources of natural antioxidant to prevent or improve free radical mediated injury becomes very important. *Syzygium cymosum* of family Myrtaceae was investigated to determine the antioxidant activities in this study [6].

Syzygium cymosum is an evergreen tree is widely distributed in tropical and subtropical area. It is found in India, Singapore, Myanmar and Malacca. Fruit is edible and the wood is used for posts, fuel etc [6].

In order to prove the traditional utilization of *Syzygium cymosum*, this paper was intended to investigate the antioxidant effect of methanolic extract of the barks of *Syzygium cymosum*

MATERIALS AND METHODS:

Plant Materials:

Syzygium cymosum was collected from Sylhet area and identified by taxonomist at the Department of Botany, Jahangirnagar University, Savar, Dhaka.

Extraction:

The barks of the plant were collected in fresh condition. It was sun-dried first and then, dried in an oven at reduced temperature (< 70 ^{0}C) to make suitable for grinding. The powdered plant materials were submerged in sufficient volume of methanol in an air-tight flat bottomed container for seven days, with occasional shaking and stirring. The extracts were then filtered and dried on electrical water bath.

Drugs and Chemicals:

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

Phytochemical tests:

Various phytochemical tests that include Molisch's test for carbohydrates, test for glycosides, Borntrager's test for anthraquinone glycosides, Mayer's reagent; Hager's reagent and Dragendorff's reagent for alkaloids, Frothing test for saponins, Hydrochloric acid test for flavonoids, Salkowski's test for steroids and Ferric chloride test for tannins.

Determination of Total Phenol:

Total phenols were determined by Folin Ciocalteu reagent [7]. A dilute extract of plant extract (0.5 mL of 1:10 g/mL diluted with distilled water) or Gallic acid (Standard 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 are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound. The result was calculated from the regression equation of the calibration curve ($y=0.013x+0.127$, $r^2=0.988$).

Determination of Flavonoid content:

Aluminum chloride colorimetric method was used for flavonoids determination [8]. Each plant 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 remained 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:

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 acid pH [9]. The antioxidant capacity is expressed as ascorbic acid equivalent (AAE). The plant extract (0.3 mL) was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 ^{0}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 *Syzygium cymosum* was measured by the method developed by Manzorro *et al.* [10]. The sample extract (0.2 mL) was diluted with methanol and 2 mL of DPPH solution (0.5 mM) was added. After 30min, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated.

Total Reducing Power Determination: The reducing power of the extract was determined

according to the method of Oyaizu [11]. 10mg of extract in 1mL of distilled water was 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 ^{0}C for 20 minutes. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (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. Gallic acid, Quercetin and Ascorbic acid were used as reference compounds. All the analyses were performed in triplicate and the results were averaged. Increased absorbance of the reaction mixture indicated increasing reducing power.

Cytotoxic study:

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of plant extracts [12]. It was carried out to investigate the cytotoxicity of the extract. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial seawater (prepared by using sea salt 38 g/L and adjusted pH 8.5) under constant aeration for 48h. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a micropipette and placed in each test tube containing 4.5 mL of brine solution. In each experiment, 0.5 mL of the extract was added to 4.5 mL of brine solution and maintained at the ambient room temperature for 24 h and surviving nauplii were counted. For the investigations test solution o the extract was prepared by dissolving 20 mg of the extract in 1 mL of pure dimethyl sulfoxide (DMSO). 500µl of solution was taken in test tubes each containing 500 µl of simulated seawater.

Stock solution having the concentration 1mg/mL was obtained by adding 9mL of simulated sea water in the test tube. A series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. Tests were conducted along with negative control (DMSO treated), and different concentrations (1 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL and 500 µg/mL) of the leaves extract of Syzygium cymosum in a set of two tubes per dose.

RESULT AND DISCUSSION:

Total Phenolic Compound Assay:

The plant phenolics constitute one of the major groups of the compounds acting as primary antioxidants or free radical scavengers. The antioxidant activity of the phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free readicals, quenching singlet and triplet oxygen or decomposing peroxides [13]. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ~1.0 g was daily ingested from a diet rich in vegetables and fruits [14.] The content of total phenolics in the methanolic extracts was determined using the Folin-Ciocalteu assay. The content of phenolics in the extract of *Syzygium cymosum* was 477.00±46 mg/g GAE.

Flavanoid content Assay:

Flavonoids protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. Epidemiological studies have shown that flavonoid intake is inversely related to mortality from coronary heart disease and the incidence of heart attacks. Flavonoid content was calculated from the regression equation of the calibration curve (y = 0.009 - 0.036) and is expressed as Quercetin equivalents (QE). The flavonoid content was 17.00±9.9 mg/g quercetin equivalent in *Syzygium cymosum.*

Total Antioxidant Assay:

The total antioxidant capacities of the methanolic extracts of *S.Cymosum* were determined from the calibration curve (y=.005x-0.028) established by ascorbic acid at 695 nm. Ascorbic acid equivalent of *S.Cymosum* was 505.5±3.535.

DPPH scavenging activity:

DPPH easily accepts an electron or hydride radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colour stoichometrically depending

on the number of electrons taken up. **Fig. 1** shows the amount of each extract needed for 50% inhibition (IC_{50}). IC_{50} value of *S.Cymosum* was found to be 64.99079µg/ml whereas Ascorbic acid showed the value of 1.16 µg/ml.

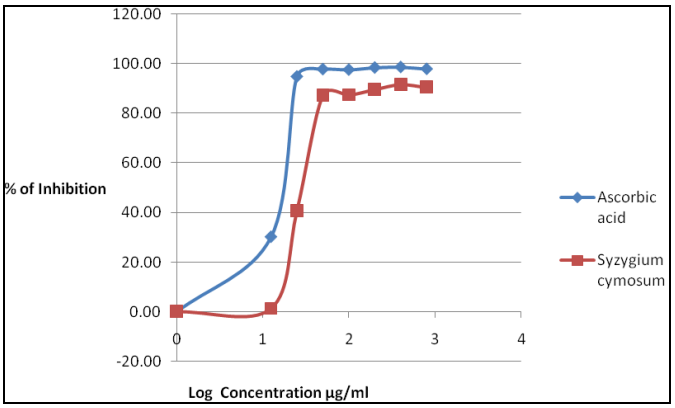

FIG.1: COMPARATIVE DPPH SCAVENGING ACTIVITY

Reducing Power Assessment:

The antioxidant activity has been reported to be concomitant with the development of reducing power [14]. The reducing properties are generally associated with the presence of reductones [15], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [16]. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. **Fig. 2** shows the reductive capabilities of the plant extract compared to ascorbic acid. The extracts were found to display moderate reducing power and the reducing power of the extract was observed to rise as the concentration of the extract gradually increased.

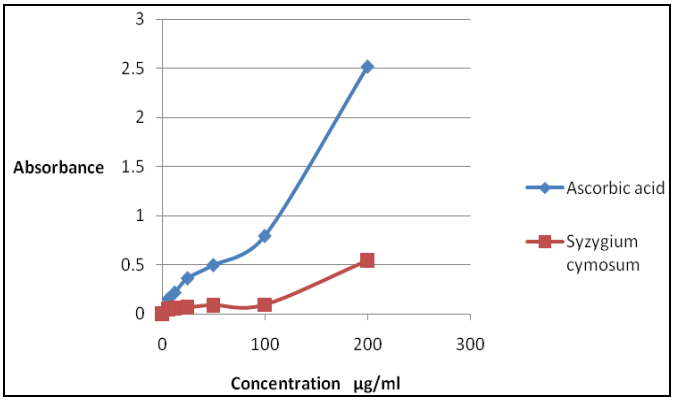

FIG. 2: REDUCING POWER OF THE CRUDE PLANT EXTRACT *SYZYGIUM CYMOSUM*

Cytotoxic Study:

The methanolic extract of *S.Cymosum* was tested for Brine shrimp lethality bioassay using brine shrimp nauplii and DMSO as a solvent. Control was used to see whether DMSO had any effect on brine shrimp lethality. The control group of brine shrimp nauplii with and without DMSO exhibited no mortality. For the extract, the number of nauplii died and percent mortality was counted. The result is shown in the following table

The LC50 value of methanolic extract of *S.Cymosum* bark was 307µg/ml. As apparent from our results it can be revealed that the plant extract exhibited mild cytotoxic effect.

TABLE 1:

Extract	Concentration of samples (µg/ml)	Log C	% Mortality	Corrected % Mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
Bark part of methanolic extract of *S.Cymosum*	25	1.39794	60	55.55	307	1406
	50	1.69897	40	33.33		
	100	2	30	22.22		
	200	2.30103	70	66.66		
	400	2.60206	60	55.55		
	800	2.90309	70	66.66		

CONCLUSION: The methanolic extracts of *Syzygium cymosum* bark exhibited significant antioxidant activity. In the present study, the observed DPPH scavenging activity of the methanolic extracts of bark might be useful for the development of newer and more potent natural antioxidants. The plant extract exhibited mild cytotoxic effect. Further phytochemical and pharmacological studies are also required to use their medicinal and pharmaceutical potentialities.

ACKNOWLEDGEMENTS: Tania Binte Wahed, assistant professor is thankful to Prof. Dr. Md. Rafiquzzaman, Chairman, Department of Pharmacy, Jahangirnagar University for the facilities kindly he provided to execute the research

work presented in the article. The author is also thankful to Kakan SutraDhar, A. H. M. Nazmul Hasan, Sonia Binte Wahed for their knowledge and assistantship during research work.

REFERENCES:

1. Abd Ghafar MF, Prasad NK, Weng KK and Ismail A: Flavonoid, hesperidine, total phenolic contents and antioxidant activities from Citrus species. African Journal of Biotechnology 2010.9(3):326-330.
2. Lien Ai Pham-Huy, Hua He, and Chuong Pham-Huy: Free Radicals, Antioxidants in Disease and Health. Int J Biomed Sci. 2008. Jun; 4(2): 89–96
3. Halliwell B :Dietary polyphenols: good, bad, or indifferent for your health? *Cardiovascular Research*. *Pubmed* 2007,73(2):341–347.
4. Rios ADO, Antunes LMG, Bianchi MDLP: Bixin and lycopene modulation of free radical generation induced by cisplatin-DNA interaction. *Food Chemistry* 2009, 113(4):1113–1118.
5. M. A. Rahman, M. S. Rana, M. M. Zaman, S. A. Uddin, and R. Akter: Antioxidant, Antibacterial and Cytotoxic Activity of the Methanol Extract of *Urtica Crenulata.* J. Sci. Res2010. 2 (1), 169-177.
6. Encyclopedia of Flora and Fauna of Bangladesh published by Asiatic Society of Bangladesh.
7. Folin, O. and Ciocalteu, V: On tyrosine and tryptophane determination in proteins. *Journal of Biol Chem* 1927, 27: 627–650.
8. Chang, C, Yang, M. and Wen, H: Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J.Food Drug Anal.* 2002. 10: 178-182.
9. Prieto, P., Pineda, M. and Aguilar, M.: Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*1999.269: 337- 341
10. Manzorro, L., Anese, M. and Nicoli, M. C: Antioxidant properties of tea extracts as affected by processing. *Lebens-mittel-Wissenschaft Und-Technologie.*1999.31 (7-8): 694-698.
11. Oyaizu, M: Antioxidative activities of products of Browning reaction prepared from Glucosamine. *Jap J of Nutr* 1986 44: 307 – 315
12. Meyer BN, Ferringni NR, Puam JE, Lacobsen LB, Nicols DE and McLaughilin JL: Brine Shrimp: A convenient general bioassay for active constituents. Planta Med1982; 45:31-32.
13. Osawa, T: Novel Natural Antioxidants for utilization in food and biological systems. *In* I, Uritani, V, V, Garica, E.M. Mendoza (Eds.), *Posthavest biochemistry of Plant food-materials in the tropics.* Japan Scientific Press1994. pp. 241-251. Tokyo, Japan.
14. Tanaka, M., Kuie, C.W., Nagashima, Y. and Taguchi, T.: Applications of antioxidative Maillard reaction products from histidine and glucose to sardine products. *Nippon suisan Gakaishi*.1988. 54: 1409-1414.
15. Duh PD, Tu YY and Yen GC: Antioxidant activity of the aqueous extract of harn jyur (Chrysanthemum morifolium Ramat). *Lebensmittel-Wissenschaft and Technologie 1999.*32: 269-277.
16. Gordon MH: The mechanism of antioxidant action in vitro. *In*: BJF Hudson (Ed.), Food antioxidants Elsevier Applied Science1990.London, pp. 1–18.

How to cite this article:
Dhar KS, Wahed TB, Nazmul Hasan AHM and Wahed SB: *In vitro* Antioxidant Activities and Cytotoxicity Study of the Methanolic Extract of Barks of *Syzygium Cymosum*. Int J Pharm Sci Res 2016; 7(3): 1021-25.doi: 10.13040/IJPSR.0975-8232.7(3).1021-25.