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

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Key words: Syzygium cymosum, Antioxidant, DPPH, Brine shrimp cytotoxicity assay

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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 IC50 value in DPPH method was 64.99079 μg/ml. The extract showed concomitant increase in reducing power with the increase of concentration of the extract. In case of brine shrimp cytotoxicity assay, LC50 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.

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°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. 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 Na₂CO₃ (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, r² =0.988).

**Determination of Flavonoid content:**
Aluminum chloride colorimetric method was used for flavonoids determination. 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. 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°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. 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. 10 mg of extract in 1 mL 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°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. 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). 500 μL of solution was taken in test tubes each containing 500 μl of simulated seawater.

Stock solution having the concentration 1 mg/mL was obtained by adding 9 mL 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 radicals, quenching singlet and triplet oxygen or decomposing peroxides. 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. 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.

**Flavonoid 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 \(y = 0.005x - 0.028\) 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 stoichiometrically depending...
on the number of electrons taken up. Fig. 1 shows the amount of each extract needed for 50% inhibition (IC50). IC50 value of S.Cymosum was found to be 64.99079µg/ml whereas Ascorbic acid showed the value of 1.16 µg/ml.

![Image: Comparative DPPH Scavenging Activity]

**Reducing Power Assessment:**
The antioxidant activity has been reported to be concomitant with the development of reducing power. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. 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.

![Image: 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

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration of samples (µg/ml)</th>
<th>Log C</th>
<th>% Mortality</th>
<th>Corrected % Mortality</th>
<th>LC50 (µg/ml)</th>
<th>LC90 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bark part of methanolic extract of S.Cymosum</td>
<td>25</td>
<td>1.39794</td>
<td>60</td>
<td>55.55</td>
<td>307</td>
<td>1406</td>
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<tr>
<td></td>
<td>50</td>
<td>1.69897</td>
<td>40</td>
<td>33.33</td>
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<tr>
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<td>100</td>
<td>2</td>
<td>30</td>
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<td>400</td>
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<td>800</td>
<td>2.90309</td>
<td>70</td>
<td>66.66</td>
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</tbody>
</table>

**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.

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