Anti-oxidant and Anti-hyperglycemic Properties of Methanolic Extracts of Medicinal Plants

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Total phenolic content, DPPH free radical scavenging activity and alpha amylase inhibitory potential was determined for three selected medicinal plants – Gymnema sylvestre, Terminalia arjuna and Tinospora cordifolia. The plant extracts were prepared with methanol. The total phenolic content of methanolic extracts of Gymnema sylvestre, Terminalia arjuna and Tinospora cordifolia were 6.862, 20.862 and 7.987 mg GAE/g plant material respectively. All the three plants showed anti oxidant activities with their IC50 values were 6.862, 20.862 and 7.987 µg/ml compared to IC50 value of the standard L-Ascorbic acid, which was 11.59µg/ml. The extracts of Gymnema sylvestre showed ±- amylase inhibition. Thus the results provided evidence that among the studied plants, Gymnema sylvestre potential sources of natural antioxidant and antidiabetic activity.

Key words: Medicinal plants, total phenols, antioxidant activity, amylase inhibitory activity.

Diabetes mellitus is one of the leading epidemics of the world. It is a group of metabolic disorders with one common manifestation – hyperglycemia. Management of diabetes is a global problem and there are free radicals that leads to oxidative stress which causes diseases associated with diabetes. Antidiabetic medications include sulphonylureas, biguanides, meglitinides and thiazolidinediones. Generally all these drugs are administered orally with the exception of exenatide, liraglutide, insulin and pramlintide. With the progress of the disease, monotherapy fails to act due to which there is a need for multiple combinations of drugs. Each of these drugs have their own side effects, some of which are nausea, hypoglycemia, weight gain, liver diseases, gastrointestinal disorders, mild anorexia, abdominal discomfort, flatulence and diarrhea. Keeping these side effects in mind, there is a need to switch to drugs which are non toxic, have greater efficacy and are cost effective. There are about 800-1000 medicinal plants that have anti-diabetic potential. The aim of this project was to determine the total phenolic content, antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay and ±-amylase inhibitory activity from Gymnema sylvestre, Terminalia arjuna and Tinospora cordifolia.

MATERIALS AND METHODS

The plant materials (Leaves of Gymnema sylvestre and Tinospora cordifolia and the bark of Terminalia arjuna) were purchased from University of Agricultural Sciences, Bangalore, India. The Gallic acid and DPPH were purchased from Sigma chemical company, St. Louise, USA. All other
chemicals and reagents used were of analytical grade.

**Preparation of plant extracts**

The leaves and bark were washed with distilled water. The leaves and bark of the plants were processed according to the method of Ranilla et al (2010). The cleaned leaves and bark were spread on a paper in cleaned, ventilated laboratory and air dried at 25°C for fifteen days. Dried samples were grounded into a powder using a blender and stored in polythene bags at 4°C until further use. The powdered samples were subjected for extraction with water, methanol, butanol and ethyl acetate. The powdered samples were taken in a thimble bag made of blotting paper and whatmann filter paper and loaded into a Soxhlet apparatus with water, methanol, butanol and ethyl acetate as solvents (1:20, w/v). The extracts were then concentrated using rotary vacuum dryer at 50°C. The yield obtained was 6.4, 11.5, 7.2, and 6.7 mg/100g in water, methanol, butanol and ethyl acetate respectively. The dried crude extracts were stored at -4°C in air tight bottles until further use.

**Determination of Total phenolic content**

Total phenolics were estimated by FC method (Spanos et al., 1990). A dilute extract of each plant extract (1.0 ml) or Gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (2.5 ml, 1:10 diluted with distilled water) and aqueous Na2CO3 (2 ml, 2%). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 720 nm. The standard Gallic acid curve in each solvent was prepared (80 µg/ml) and total phenol values were expressed in terms of Gallic acid equivalent (mg/ g of plant material). Total content of phenolic compound was calculated by the equation:

\[ C = \frac{(c \times m)}{V} \]

where, \( C \) = total content of phenolic compound in Gallic acid equivalent, \( c \) = concentration of Gallic acid established from the standard curve (µg/ml), \( m \) = weight of crude plant extract, \( V \) = volume of plant extract.

**Determination of Antioxidant activity**

Antioxidant activity was determined by the DPPH free radical scavenging activity according to the method of Patel et al (2011). Ascorbic acid was used as a reference Standard. 50µl of dilute solutions of different concentrations of the extracts were taken and 100µl of each solvent was added followed by 150µl of DPPH and incubated at room temperature on a Rotary Shaker for 15mins. 3ml of each solvent was added to each of the test tubes and the absorbance was measured at 520nm with respective solvent as blank. Control sample was prepared containing the same volume without any extract and reference was taken as ascorbic acid.

Percentage scavenging of the DPPH free radical was measured by using the following equation:

\[ \text{Scavenging activity} = \frac{(\text{Absorbance of control} - \text{absorbance of sample}) \times 100}{\text{Absorbance of control}} \]

**Determination of alpha amylase inhibitory activity**

\( \alpha \)-Amylase inhibitor activity was determined according to the method of Bernfeld (1955). Alpha amylase was produced from human saliva. 1 ml of enzyme and 1 ml of the diluted plant extract were taken in test tubes and incubated at room temperature for 10 minutes. After incubation 1ml of starch was added as substrate and incubated for 10 minutes. The reaction was arrested using 1 ml of DNS reagent (of 3,5-dinitrosalicylic acid) followed by keeping the test tubes in boiling water bath for 10 min. The residual \( \alpha \)-Amylase activity was determined by measuring the absorbance at 540 nm.

A standard calibration curve was prepared for the maltose taking 1ml of 360-1800 µg/ml dilutions of maltose. The percentage (w/v) of maltose in the reaction wells was calculated from the corrected absorbance of each test and using the equation of the calibration curve. Control incubations, representing 100% enzyme activity were conducted in the same manner replacing the plant extract with distilled water. The percentage of \( \alpha \)-amylase inhibition was calculated by the following equations:

\[ \% \text{Inhibition} = \frac{(\text{Absorbance of control (enzyme) - Absorbance of test}) \times 100}{\text{Absorbance of control}} \]

**Total Phenolic Contents**

Polyphenols have been said to be important phytochemicals with significant antioxidant capacities and other important
medicinal characteristics. Total phenolic content was determined by the FC method and the calibration curve developed using Gallic acid. A regression equation was obtained from the standard curve and the amount of Gallic acid in the plant samples was calculated from the regression equation:

$$y = 0.004x, R^2 = 0.995$$

The results were determined in terms of mg Gallic Acid Equivalent per gram of plant material. (Fig 1-a) shows the comparison of the total phenolics of the 3 extracts. Among the three plants tested for their phenolic content, Terminalia arjuna showed the highest phenol content (20.862 mg GAE/g plant material), whereas Gymnema sylvestere showed the lowest phenol content (~7 mg GAE/g plant material). Similar results were seen by (Shahriar et al., 2012) where the methanolic extract of T.arjuna showed the highest phenol content when compared with other extracts.

Antioxidant capacity

Natural antioxidants present in plants are responsible for inhibiting or preventing the injurious effects of oxidative stress caused by free radicals in the body. Polyphenols present in plants have said to be efficient free radical scavengers. (Khalaf et al., 2008). Thus after determining the total phenol content of the plants, the antioxidant activities of the plants were estimated for their free radical scavenging activity (Patel et al.,2011).

The assay was performed at different concentrations for the standard i.e., L ascorbic acid and the methanolic crude extract of the three plants. Scavenging activity was calculated and the graphs of scavenging activity (%) against concentrations were plotted in each case. In the present study, the extracts of all the three plants were found to be effective scavengers against DPPH radical. The IC$_{50}$ values were calculated and compared with L- ascorbic acid (Table 2, Fig. 1). The IC$_{50}$ values were in the range of 16-34 µg/ml and the extract of Gymnema sylvestere showed IC$_{50}$ of 16.8µg/ml which indicated highest DPPH radical scavenging activity, while the weakest scavenger was the extract of Tinospora cordifolia (33.4 µg/ml). Figure 2 shows the total phenol content and antioxidant capacities of the plants. The extracts do not show any significant correlation between the antioxidant capacity and phenol content. Terminalia arjuna has the highest phenol content with an IC$_{50}$ of 18 µg/ml and Gymnema sylvestere has the lowest phenol content i.e. 6.862 mg GAE/g plant material with an IC$_{50}$ of 16.8 µg/ml which indicated highest DPPH radical scavenging activity, while the weakest scavenger was the extract of Tinospora cordifolia (33.4 µg/ml).

Table 1. Total phenolic (expressed as gallic acid equivalents) content from the extracts of Gymnema sylvestere, Terminalia arjuna and Tinospora cordifolia

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Phenolic content (GAE mg/g plant material)</th>
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<tbody>
<tr>
<td>Gymnema sylvestere</td>
<td>6.862</td>
</tr>
<tr>
<td>Terminalia arjuna</td>
<td>20.862</td>
</tr>
<tr>
<td>Tinospora cordifolia</td>
<td>7.987</td>
</tr>
</tbody>
</table>

Table 2. Antioxidant activity of extracts of Gymnema sylvestere, Terminalia arjuna and Tinospora cordifolia and L-Ascorbic acid at different concentrations and their IC$_{50}$ Values.

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<th>Plant Species</th>
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<tbody>
<tr>
<td>Gymnema sylvestere-IC$_{50}$ (µg/ml)</td>
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</tr>
<tr>
<td>Terminalia arjuna-IC$_{50}$ (µg/ml)</td>
<td>20.862</td>
</tr>
<tr>
<td>Tinospora cordifolia-IC$_{50}$ (µg/ml)</td>
<td>7.987</td>
</tr>
<tr>
<td>L-Ascorbic acid-IC$_{50}$ (µg/ml)</td>
<td>11.59</td>
</tr>
</tbody>
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No correlation between antioxidant capacity and total phenol content could be due to many factors like the antioxidant capacity was not entirely due to the phenol contents, but could be due to presence of other phytochemicals also. Also Folin Ciocalteau method is not a complete indicator.
Fig. 1. Comparison of IC<sub>50</sub> values of the plants.

Fig. 2. DPPH scavenging activity and Total phenol content.

Fig. 3. Comparison of Alpha amylase inhibition of the 3 plants.

of the phenol content and thus there may be other phenol compounds present that contribute to the antioxidant capacities.

**Alpha amylase inhibitory activity**

Alpha amylase is one of the key enzymes that play a role in digestion of starch and glycogen and carbohydrate metabolism. Its inhibition is one of the strategies for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity. It is involved in carbohydrate metabolism and thus
inhibiting it would lead to reduced post prandial blood sugar (Paloma et al. 2012).

The three plant extracts were tested for alpha amylase inhibition. The standard maltose curve was plotted to note the absorbance corresponding to the maltose content. The extracts were then tested for amylase inhibition at different concentrations and the % inhibition was calculated by the amount of maltose present after inhibition of the enzyme and the amount of maltose present in the control i.e. only the enzyme – alpha amylase. Amongst the 3 plants Terminalia arjuna and Tinospora cordifolia did not show amylase inhibition. Gymnema sylvestre showed amylase inhibition (Fig. 3), but the inhibition is not concentration dependant.

CONCLUSION

Highest total phenolic content was seen in Terminalia arjuna (20.862 mg GAE/g of plant material) and lowest was seen in Gymnema sylvestre (6.862 mg GAE/g of plant material). All the plants showed good antioxidant activity. Alpha amylase inhibitory activity was observed only in Gymnema sylvestre. Tinospora cordifolia and Terminalia arjuna did not show alpha amylase inhibition. Among the three selected plants, Gymnema sylvestre showed alpha amylase inhibition and free radical scavenging capacity and thus is a potent sources for anti-diabetic and antioxidant agents.

REFERENCES


