**Introduction**

Plants have been considered ideal and excellent sources of drugs, and thus many of the present medicines are derivatives of them. The drugs obtained from herbal plants are being used significantly all over the world in the past few years as they have fewer side effects and are derived naturally (Modak et al., 2007). Even the WHO recognizes the importance of medicinal plants in healthcare and thus has recommended the assessment and study of the efficacy of plants in conditions where we lack safe modern drugs. India is a land with immense biodiversity and the growing biopharmaceutical world, especially in India, has encouraged research on medicinal plants found in these areas. Diabetes has attained state of pestilence in the present century and it is estimated that the number of people affected by it is going to go on rising (Arya et al., 2012). The oral drugs have many disadvantages like high cost, weight gain, increase risk of hypoglycemia, gastrointestinal side effects, rare lactic acidosis, upper respiratory tract infection, inflammation of the pancreas and issue of liver toxicity (Beatriz et al., 2001). The shift to herbal medicine is thus necessary due to their seeming effectiveness, affordability, safety, and acceptability, with minimal side effects, and relatively low cost. The management of diabetes is focused towards reducing the post prandial blood sugar by delaying or inhibiting the activities of two key enzyme i.e., α amylase and α glycosidase which are responsible for carbohydrate metabolism in the body and glucose absorption (Gulati et al., 2012). There have been number of reports on the presence of polyphenols in plants which help scavenge off free radicals and act as good anti oxidants. Also there have been many studies on the various biochemical pathways that give rise to ROS and lead to oxidative stress on the body leading to diseases (Srinivasan et al., 2007). Thus some of the desirable anti diabetic...
properties in plants are ± amylase inhibitor activity, good phenolic content and Anti oxidant capacities to scavenge the free radicals.

MATERIALS AND METHODS

Material

The plant materials (Andrographis paniculata (Kalmegh), Apium graveolans (Celery) and Butea monosperma (Palash) 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 were stored in polythene bags at 4°C until further use. 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 Ciocelteu 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:

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

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 1ml 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 \( \pm \)-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 α-amylase inhibition was calculated by the following equations:

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

**RESULTS AND DISCUSSION**

**Total Phenol Content**

Polyphenols have been said to be important phytochemicals with significant antioxidant capacities and other important medicinal characteristics. Total phenolics content was determined by the FC method and the standard curve was calibrated using Gallic acid. (Fig 1) A regression equation was got from the standard curve and the amount of Gallic acid in the plant samples was calculated from the regression equation:

\[ y = 0.004x, \quad R^2 = 0.995 \]

The results were determined in terms of mg Gallic Acid Equivalent per gram of plant material. (Fig 2) shows the comparison of the total phenolics of the 3 plant extracts.

Among the four solvents, methanol and water yielded more amount of phenols compared to butanol and ethyl acetate (Table 1). Among the three plants tested for total phenol content, the methanolic and aqueous extract of *Apium graveolens* yielded high amounts. The phenol content of *Andrographis paniculata* varied from 7.112 to 21.737 mg GAE/g plant material. *Butea monosperma* showed varied phenol content from 12.84 to 25.40 mg GAE/g plant material. Similarly, the phenol content of *Apium graveolens* varied from 18.21 to 49.77 mg GAE/g plant material. Among the plants tested, *Apium graveolens* is the promising source of phenols.

**Antioxidant Activity**

Natural antioxidants present in plants are responsible for inhibition or prevention of 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

**Table 1: Total phenolic (expressed as gallic acid equivalents) content from the extracts of Andrographis Paniculata, Apium graveolens and Butea monosperma**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Andrographis paniculata (mg/g)</th>
<th>Apium graveolens (mg/g)</th>
<th>Butea monosperma (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>15.24</td>
<td>41.24</td>
<td>19.246</td>
</tr>
<tr>
<td>Methanol</td>
<td>21.737</td>
<td>49.77</td>
<td>25.40</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>7.112</td>
<td>18.21</td>
<td>12.84</td>
</tr>
</tbody>
</table>

**Table 2: Antioxidant activity of extracts of Andrographis Paniculata, Apium graveolens and Butea monosperma and L-Ascorbic acid at different concentrations and their IC\textsubscript{50} Values**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Andrographis paniculata- IC\textsubscript{50} (µg/ml)</th>
<th>Apium graveolens- IC\textsubscript{50} (µg/ml)</th>
<th>Butea monosperma- IC\textsubscript{50} (µg/ml)</th>
<th>L-Ascorbic acid- IC\textsubscript{50} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>19.824</td>
<td>82.24</td>
<td>36.246</td>
<td>17.10</td>
</tr>
<tr>
<td>Butanol</td>
<td>36.45</td>
<td>164.45</td>
<td>58.12</td>
<td>27.14</td>
</tr>
<tr>
<td>Methanol</td>
<td>25.737</td>
<td>68.77</td>
<td>27.40</td>
<td>15.60</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>79.20</td>
<td>178.21</td>
<td>73.84</td>
<td>42.21</td>
</tr>
</tbody>
</table>
scavenging activity. DPPH is widely used chemical compound for free radical-scavenging assessments due to its ease and convenience. In the present study, the extracts of all the three plants were found to be effective scavengers against DPPH radical. The $I_{50}$ values were calculated and compared with L- ascorbic acid (Table 2). The $I_{50}$ values were in the range of 11-20 µg/ml and the extract of Apium graveolens showed $I_{50}$ of 14.4µg/ml which indicated highest DPPH radical scavenging activity, while the weakest scavenger was the extract of Andrographis paniculata (19.8 µg/mL).

The aqueous extract along with methanol, butanol and ethyl acetate extracts showed a concentration dependent antiradical activity by scavenging DPPH radical with $I_{50}$ value of 19.824, 25.737, 36.45 and 79.20 µg/ml, respectively for Andrographis paniculata, 82.24, 68.77, 164.45 and 178.21 µg/ml, respectively for

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Andrographis paniculata- Scavenging activity(%)</th>
<th>Apium graveolens- Scavenging activity(%)</th>
<th>Butea monosperma- Scavenging activity(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>61.36</td>
<td>85.31</td>
<td>74.2</td>
</tr>
<tr>
<td>Butanol</td>
<td>38.45</td>
<td>53.22</td>
<td>28.12</td>
</tr>
<tr>
<td>Methanol</td>
<td>79.21</td>
<td>95.12</td>
<td>88.6</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>47.40</td>
<td>45.26</td>
<td>31.1</td>
</tr>
</tbody>
</table>

Table 3: Free radical-scavenging activity of the extracts from Andrographis Paniculata, Apium graveolens and Butea monosperma at 250 µg/ml concentration by DPPH method

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Andrographis paniculata- Inhibition (%)</th>
<th>Apium graveolens- Inhibition (%)</th>
<th>Butea monosperma - Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:20</td>
<td>0</td>
<td>15.15</td>
<td>94.11</td>
</tr>
<tr>
<td>1:40</td>
<td>0</td>
<td>3.03</td>
<td>66.66</td>
</tr>
<tr>
<td>1:50</td>
<td>0</td>
<td>0</td>
<td>50.98</td>
</tr>
<tr>
<td>1:60</td>
<td>0</td>
<td>0</td>
<td>49.01</td>
</tr>
<tr>
<td>1:80</td>
<td>0</td>
<td>0</td>
<td>47.50</td>
</tr>
</tbody>
</table>

Apium graveolens and 27.40, 36.246, 58.12 and 73.84 µg/ml, respectively for Butea monosperma. The methanol and aqueous extracts were found to be more potent compared to butanol and ethyl acetate extracts.

The DPPH radical was scavenged by antioxidants through the donation of hydrogen, forming the reduced DPPH-H+. the colour changed from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517nm. It was found that the methanol and aqueous extracts of Andrographis paniculata, Apium graveolens and Butea monosperma (79.21, 61.36, 95.12, 85.31, 88.6 and 74.2%, respectively) showed more scavenging activity (Table 3) than butanol and ethyl extracts.

Alpha amylase inhibition 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. Among the three plant extracts studied, alpha amylase inhibition was observed in the extracts of Butea monosperma and Apium graveolens (Table 4, Figure 1).

**CONCLUSION**

In the present study, the total phenolic content, antioxidant activity and amylase inhibitory activity of Andrographis Paniculata, Apium graveolens and Butea monosperma has been studied. The methanol and water are best solvents to extract more amounts of phenolic contents from all the three plants. Among the three plants studied, Apium graveolens is the potent source of phenolic content, antioxidant and anti-diabetic activity.

**REFERENCES**


25. Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z and Ercisli S., Total phenol content,


