Antioxidant and Type II Diabetes-related Enzyme Inhibition Properties of Few Selected Medicinal Plants

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ABSTRACT

The WHO recognizes the importance of medicinal plants in today's day and age mainly due to the increasing amount of disorders and diseases. Diabetes mellitus is a disorder which is a great cause of concern in the 21st century and has reached a state of pestilence in the present century. The modern oral hypoglycemic agents produce undesirable side effects and oxidative stress and thus there is a need for cost effective and complementary therapies to deal with diabetes mellitus. The aim of this paper is to thus evaluate few selected medicinal plants for their in vitro antioxidant activities and inhibitory potential against key enzymes relevant to hyperglycemia. Total phenolic content, DPPH free radical scavenging activity and alpha amylae inhibitory potential was determined for three selected medicinal plants – *Andrographis paniculata, Apium graveolans, Butea monosperma,* The total phenolic content was in the range 3-9 mg GAE/g plant material and the highest was observed in *Butea monosperma.* All the three plants showed anti oxidant activities with their IC_{50} values ranging between 14-20 μg/ml compared to IC_{50} value of the standard L-Ascorbic acid, which was 11.59μg/ml. The extracts of *Apium graveolans* and *Butea monosperma* showed ±- amylase inhibition*. Butea monosperma* showed the highest inhibition (95%). Thus the results provided evidence that the studied plants are potential sources of natural antioxidant and antidiabetic agents.

Key words: Medicinal Plant, Diabetes, Anti-oxidant, Enzyem inhibtion.

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., ± amylase and ± 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. Louse, 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 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:

$$C= (c*m)/V$$

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:

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

Determination of alpha amylase inhibitory activity

α-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 ±-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:

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

RESULTS AND DISCUSSION

Total Phenol Content

Polyphenols have been said to be important phytochemicals with significant anti oxidant 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:

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

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 methonolic 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 *AndrographisPaniculata, Apium graveolens* and *Butea monosperma*

Solvent	*Andrographis* paniculata(mg/g)	*Apium* graveolens(mg/g)	*Butea* monosperma(mg/g)
Water	15.24	41.24	19.246
Butanol	11.145	19.45	16.12
Methanol	21.737	49.77	25.40
Ethyl acetate	7.112	18.21	12.84

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

Solvent	*Andrographis paniculata*- IC_{50} (µg/ml)	*Apium graveolens*- IC_{50} (µg/ml)	*Butea monosperma* - IC_{50} (µg/ml)	*L-Ascorbic acid*- IC_{50} (µg/ml)
Water	19.824	82.24	36.246	17.10
Butanol	36. 45	164.45	58.12	27.14
Methanol	25.737	68.77	27.40	15.60
Ethyl acetate	79. 20	178.21	73.84	42.21

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 IC_{50} values were calculated and compared with L- ascorbic acid (Table 2). The IC_{50} values were in the range of 11-20 µg/ml and the extract of *Apium graveolens* showed IC_{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 IC_{50} (Table 2) 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 *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.

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

Solvent	***Andrographis paniculata*-Scavenging activity(%)**	***Apiumgraveolens*-Scavenging activity(%)**	***Buteamonosperma*-Scavenging activity(%)**
Water	61.36	85.31	74.2
Butanol	38.45	32.32	28.12
Methanol	79.21	95.12	88.6
Ethyl acetate	47.40	45.26	31.1

Table 4: Amylase inhibition of aqueous extract of *Andrographis Paniculata, Apium* graveolens and Butea monosperma

Concentration	***Andrographis paniculata*- Inhibition (%)**	***Apium graveolens*- Inhibition(%)**	***Butea monosperma*- Inhibition(%)**
1:20	0	15.15	94.11
1:40	0	3.03	66.66
1:50	0	0	50.98
1:60	0	0	49.01
1:80	0	0	47.50

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).*

Butea monosperma showed the highest amylase inhibition (94%). Bases on our study *Butea monosperma* and *Apium graveolens* are potential antidiabetic agents.

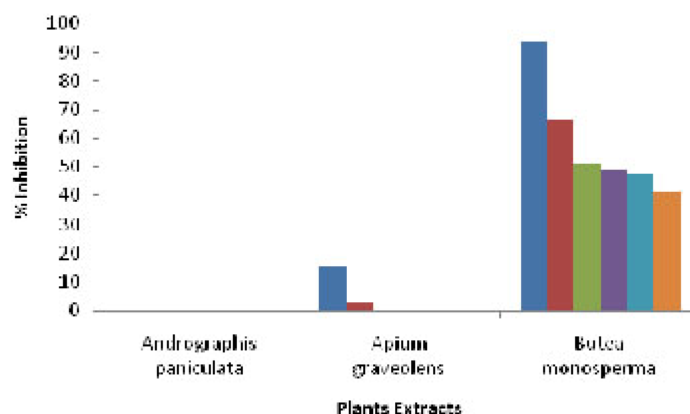

Fig. 1: Amylase inhibition of aqueous extract of *Andrographis Paniculata, Apium graveolens* and *Butea monosperma* at different concentrations

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 antidiabetic activity.

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