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Phytochemical Screening - Antibacterial, Antioxidant and Enzyme Inhibitory Activity of certain Medicinal Plants

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Abstract

Plants have been used in traditional medicine for several thousand years. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Plants can be used to treat various metabolic and genetic disorders like diabetes, cancer, cystic fibrosis and so on. Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both. One of the effective methods to control diabetes is to inhibit the activity of alpha amylase enzyme which is responsible for the breakdown of starch, thus slowing down the carbohydrate metabolism. The present study aims at finding the antihyperglycemic activity of few traditionalmedicinal plants. The plants that are chosen for the study are: *Butea monosperma* and *Nanrdostachys jatamansi.* Soxhlet extraction experiments were performed to estimate the protein, sugar, flavonoid and phenol levels, their antibacterial activity is estimated using disc diffusion method and the antioxidant activity is estimated using the standard DPPH method. The plants have been tested for their alpha amylase enzyme inhibitory activity. The results showed that the plant extracts had good potential for the management of hyperglycemia and the related condition of oxidative stress.

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Introduction

Numerous medicinal plants were identified and are being used throughout human history [1]. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Specially, plants growing at high altitude in Himalayan pastures are time-honored sources of health and general well-being of local inhabitants. Herbal medicines do not differ greatly from conventional drugs in terms of how they work. Ethno botany (the study of traditional human uses of plants) is recognized as an effective way to discover future medicines .The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care.

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia with disturbances of carbohydrate, lipid and protein metabolism resulting from defect in insulin secretion, insulin action or both. According to the recent data the prevalence of diabetes is on the rise from 143 million persons to 300 million persons by the year 2025. Among various therapeutic approaches to cure diabetes, lowering postprandial hyperglycemia is one such approach. One of the effective methods to control diabetes is to inhibit the activity of alpha amylase enzyme which is responsible for the breakdown of starch to more simple sugars (dextrin, maltotriose, maltose and glucose).

This is contributed by alpha amylase inhibitors, which delays the glucose absorption rate thereby maintaining the serum blood glucose in hyperglycemic individuals. In the present study two plants were chosen to test their alpha amylase inhibitory activity. *Butea monosperma*and *Nardostachys jatamansi* are commonly used by traditional healers for curing various ailments including diabetes. Availability of these plants is easier and these are known for the presence of phytochemicals which have high therapeutic value. Hence we have chosen them for our study.

Materials and Methods

The alpha amylase enzyme and all other required chemicals and solvents were purchased from Himedia.

Collection and processing of plant material

The two plants used in our study are: *Butea monosperma* (bark) and *Nardostachys jatamansi* (rhizome). Jatamansipowder was procured from Baidhyanath ayurvedic stores. The forest fire barkwas collected from Landscape Garden, Osmania University, and Hyderabad.

Extraction of plant material

25 grams of plant material is weighed and is extracted using Soxhlet apparatus. The solvent used is ethanol andextraction was performed for a period of six hours. The obtained extract is air dried and stored in air tight containers for further study.

Preliminary phytochemical screening

The plant material is qualitatively tested for the presence of various phytochemicals present using the standard protocols.

Total flavonoid estimation

The flavonoid estimation is done using the standard aluminum chloride method [2, 3]. A sample of 0.5mlwas taken and is made up to a volume of 1ml with solvent and 4ml of distilled water, 0.3ml of 5% $NaNO_2$ are added. The whole mixture is incubated for 5 min at room temperature. Then 0.3 ml of 10% $AlCl_3$ solution, 2 ml of 1M NaOH solution, and 2.4 ml of distilled water are added. Optical Density is measured at 510nm. The standard is prepared using Quercetin.

Total phenol estimation

Phenol is estimated using folins method [4]. 0.5ml of sample is taken and made it to a volume of 1ml with solventand thenadded 2.5ml of 10 times diluted folin's reagent and 2 ml of 7.5% sodium carbonate solution. The whole mixture isincubated at room temperature for 30min and measured the OD at 760 nm. The standard is prepared using Gallic acid.

Total sugar estimation

Sugar estimation is done using DNS method. Taken 1.5ml of sample and made it to a volume of 3ml with distilled water, added 3ml of DNS reagent. Placed it on a boiling water bath for 15 min and measured the absorbance at 520nm. The standard is prepared using glucose.

Total protein estimation

The protein content is estimated using Biuret method. Standard is prepared using bovine serum albumin.

Antibacterial activity estimation

The antibacterial activity of the plant is tested using the disc diffusion assay. In this method the test microbe is grown in nutrient broth using spread plate technique.

The filter paper disc is dipped in the plant extract and then placed on the petri plates containing test microorganism. In the control plates we place water dipped discs instead of plant extracts. These plates are incubated for 24 hrs at 37◦C in incubator in inverted position. The clear zones of inhibition are observed around the disc area indicating the bacterial growth inhibition by the plant extract. The zone of inhibition is noted down in mm. The test microbes used are: *E-Coli, Staphylococcus aureus, Pseudomonas putida*and Bacillus subtilis.

Antioxidant activity estimation using DPPH radical scavenging activity

Different sample volumes of extract are added to 5 ml of 0.004% DPPH in ethanol and incubated at room temperature for 30 min and measured the OD at 517 nm. A decrease in the OD value with the increase in the quantity of plant extract indicates an increase in the antioxidant activity. [5, 6, 7].

In vitro alpha amylase inhibition assay

Taken different volumes of plant samples and added 125µlit of alpha amylase enzyme solution.Preincubation of the tubes was held at room temp for 5 min.Later, added 125µlit of starch solution to the tubes and the tubes are incubated at room temperature for 5 min.Now added 250microlitres of DNS reagent to the tubes and the tubes are placed on boiling water bath for ten minutes. Cooledthe tubes to room temp, and added 5 ml of distilled water to each of the tubes. Noted down the OD at 520nm.The inhibition is calculated for different plant sample volumes using the following formula.

% inhibition= [(OD of control- OD of test)/ (OD of control)]*100[8, 9, 10].

Results and Discussion

The preliminary phytochemical screening results are tabulated in table 1. The plant having the highest component of each of the biomolecules is tabulated in table 2. The figures 1 and 2 represent the linear relationship between volume of sample and % inhibitions for DPPH assay and alpha amylase inhibition assay respectively. Both the plants possessed good radical scavenging activity. Plants haveeven shown good inhibition percentage of the alpha amylase enzyme. The table 3 shows the IC-50 values calculated from the graphs plotted between %inhibition and sample volume for each of the plants. *Butea monosperma* showed 50% radical scavenging activity and 50% alpha amylase inhibition activity at very low volume of plant sample when compared to *Nardostachys jatamansi*.

Thus, it could be stated that *Butea monosperma*possess better antioxidant and enzyme inhibitory activity than that of *Nardostachys jatamansi*. When compared with the standard Quercetin, both the plants had higher 50% radical scavenging activity than that of Quercetin. In the alpha amylase inhibition assay, *Butea monosperma* showed 50% inhibiton at a lower volume than that of Quercetin. Thus it could be stated that *Butea monosperma* possess better enzyme inhibitory activity than that of standard Quercetin.

Both the plants have shown good antibacterial activity by inhibiting the growth of test microbes. *Nardostachys jatamansi* showed higher antibacterial activity than that of *Butea monosperma*. It resulted in higher zone of inhibition and its data is represented in table 5.The control plates did not show any zone of inhibition which confirms that the plant extracts are alone responsible for the antimicrobial activity.

S.NO	Test method	Phytochemical screened	*Butea monosperma*	*Nardostachys jatamansi*
1	Mayers test	Alkaloids	Positive	Negative
2	Ferric chloride test	Glycosides	Positive	Positive
3	Chloroform test	Terpenoids and steroids	Negative	Positive
4	Ferric chloride test	Tannins	Positive	Positive
5	Fehlings solution test	Reducing sugars	Positive	Positive
6	Foam test	Saponins	Negative	Positive
7	Sodium hydroxide test	Quinones	Positive	Positive

Table 1: Qualitative analysis of phytochemicals present in plants

Sl no	Component present	*Butea monosperma*	*Nardostachys jatamansi*
1	Protein	0.49mg/ml	0.39mg/ml
2	Sugars	225µgm/ml	110µgm/ml
3	Flavonoid	55µgm/ml	58µg/ml
4	Phenols	95µgm/ml	100µg/ml

Table 2: Comparative results of two plants

Table 3: Highest content of biomolecules type present in each plant

S.NO	Component present in highest quantity	Plant name
1	Protiens	*Butea monosperma*
2	Sugars	*Butea monosperma*
3	Flavanoids	*Nardostachys jatamansi*
4	Phenols	*Nardostachys jatamansi*
5	DPPH assay IC-50 value	*Butea monosperma*
6	Alpha amylase inhibition assay IC-50 value	*Butea monosperma*

Table 4: The zone of inhibition obtained for two plants

S.NO	Plant name	Zone of inhibition in mm			
		E-coli	***Staphylococcus***	***Pseudomonas***	***Bacillus***
1	*Butea monosperma*	22	12	10	15
2	*Nardostachys jatamansi*	30	18	27	24

Table 5: Ic-50 values calculated from the graphs plotted between %inhibition and sample volume for each of the plants and for quercetin standard

S.NO	Activity	***Butea monosperma***	*Nardostachys jatamansi*	Quercetin(standard)
1	IC-50 value for DPPH assay	102.43µlit	547.64 µlit	85.22 µlit
2	IC-50 value for alpha amylase assay	104.84 µlit	126.103 µlit	127.21 µlit

Fig.1. Linear relationship between volume of sample and % inhibition for DPPH assay

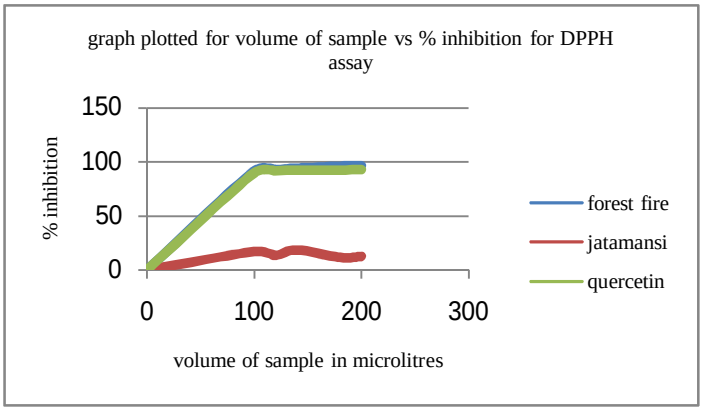


Fig. 2. Linear relationship between volume of sample and % inhibition for alpha amylase inhibition assay.

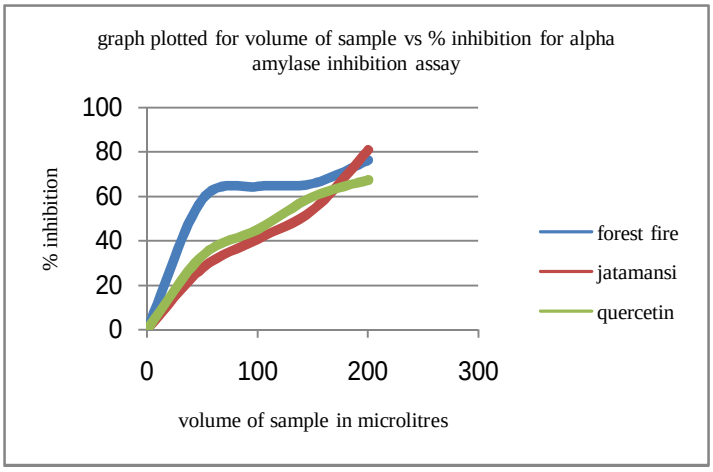
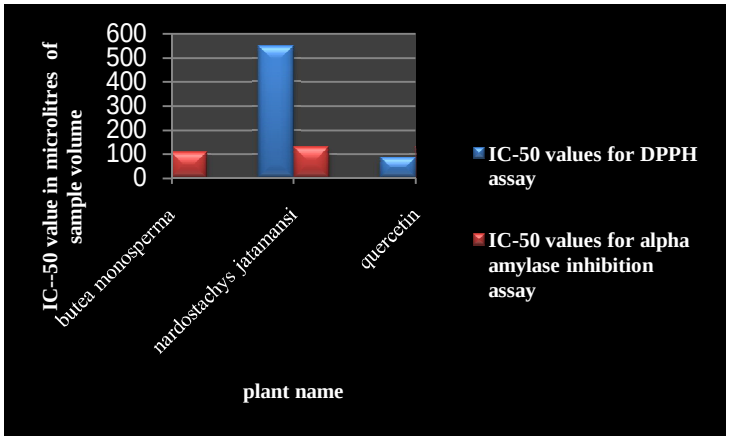


Fig.3. Comparison of IC-50 values of two plants with standard quercetin



Conclusion

It can be concluded from the present work that the plants selected are possessing good antioxidant activity which could be helpful in managing oxidative stress which is seen in most of the type II diabetes patients.

Though synthetic drugs like acarbose and maglitol are potential inhibitors of alpha amylase they possess side effects like bloating, flatulence and abdominal discomfort. Under such conditions these natural products provide good remedy. These alpha amylase inhibitors are called as starch blockers since they prevent or slow down the absorption of starch by blocking the enzyme active sites, thus inhibiting starchhydrolysis. This will help in maintaining blood glucose levels under hyperglycemic conditions.

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