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An *In vitro* studies on indigenous ayurvedic plants, having hypoglycemic activity

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ABSTRACT

Objective: Finding a cure for diseases and infections using herbal medicines are as old as mankind. The present study aimed at investigation of anti-hyperglycemic potential of methanolic extract of some indigenous ayurvedic plants used in Karnataka. **Design setting:** There are a few explicit studies to confirm invitro hypoglycemic activity. Our work is based on the study of some indigenous plants which show inhibitory effect on glucose oxidase and are in use as hypoglycemic agents in traditional system of medicine. **Result:** *Syzygium cumini* , *Trigonella foenum graecum seed*, *Moringa alba leaf*, *Punica granatum peel*, *Emblica officinalis* and *Momordica charantia* possessed highest hypoglycemic activity of varying degree. *S. cumini* and *T. foenum* had shown the better activity in neutral and basic media than others. Whereas, *Alterathera ficoicka* leaf, *T. foenum* and *Momordica charantia* have shown prominent result in acidic media. The result in three different media revealed that, acidic medium shows less prominent hypoglycemic activity as compared to neutral and basic medium. **Conclusion:** S. cumin, *T. foenum* seed and *M. charantia* gave the impression of being prominent candidates for drug targets for diabetes. This may be the first report using invitro approach to prove their antidiabetic properties.

1. Introduction

Finding healing power in plants is an ancient idea[1]. Prior epidemiological studies have shown that the intake of natural antioxidants is allied with reduced risks of several diseases like diabetes[2]. Recently, there is a growing interest in finding natural antioxidants to replace synthetic ones[3,4]. India has a great biodiversity due to its geographical and climatic conditions. It is well known fact that the southern western ghats zone has more than 320 plant species belonging to more than 147 genera[5]. Among these there are more than 100 plant families having medicinal uses. Ethano-pharmacological survey studies have revealed a list of approximately 76 plant species which are traditionally used in Karnataka, India for many ailments[6]. There are a few or almost none studies on these plants that have been recorded. Also, these plants were recommended by the ayurvedic vaidya or natural healers for treatments of diabetic symptoms and its complications. However, there are few, whose antihyperglycemic property have been scientifically proved using in vivo studies such as *Alterathera ficoicka* leaf, *Amaranthus* leaf, *Amaranthus* stem, *Corriandrum* leaf, *Cotus igneous* leaf, *Zea mays* Silk, *Emblica officinalis* (Gooseberry), *Tridex* flower, *Lucas* leaf, *S. cumnii* seed, *Punica granatum Peel* (var dadim), *Moringa Olifera* Leaf , *Moringa Olifera* Stem, *Lucas* stem[1,7,8].

There are several in vivo methods to investigate hypoglycemic activity of these plants. But there are a few specific studies to check their invitro hypoglycemic activity as presented in Khan et al, 2005[9] and Middha et al, 2012[10]. The present methodology could be used to minimize animal sacrifices in the preliminary screening of antihyperglycemic activity of plant species. Neither reviews of literature provide any substantiation on invitro hypoglycemic studies of these indigenous plants nor on which medium or pH does the plant show more healing activity. Therefore, this study

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is designed to observe the inhibition of glucose oxidase, a marker of antihyperglycemic activity of medicinal plants used in Karnataka's traditional system.

2. Materials and Methods

2.1 Chemicals

Glucose oxidase, Glucose, Potasium dihydrogen phosphate, Potasium monohydrogen phosphate, Hydrochloric acid and EDTA were purchased from Merck and Sigma. All chemicals and reagents used were of the highest commercially available purity.

2.2 Plant selection and collection

A direct questionnaire as a way of detection for most common plants that are used in traditional medicine as anti-diabetic was designed and was forwarded directly to traditional medicinal plant markets. The questionnaire raised the question, what are the commonly used anti-diabetic plants? Sample study included 100 traditional herb markets from different locations in north, south, west and east Karnataka.

Punica peel (var *dadim* from Kumaun region), Trigonella foenum seed, Alternathera ficocika leaf, *Amaranthus* viridus leaf, *Amaranthus* viridis stem, Nerium indicum, Cassia auriculata, Creteva nurvala, *Corriandrum* leaf, Costus igneous leaf, Pogomia pinnata, Phyllanthus anarus, *Emblica officinalis*, Leucas leaf, *Moringa Olifera* leaf, *Momordica charantia*, *Syzygium cumini* seed, Tridax flower, *Zea mays* silk, Calatripus giganeta, Trichosanthes dioica, Cassia gluaca, Murraya Koenigii, Ragi, Bajra were selected. For the study, these plants were procured from the local market and natural healers of Bangaluru, Karnataka during summer 2011 and taxonomic identification authentication was done by botany department, MLACW, Bangalore. A specimen copy was deposited in the herbarium of the regional research centre (RRCBI/2137-61).

2.3 Extract Preparation

The collected plant materials were cleaned, shade dried (Temp<40℃). The dried plant material was grounded into a moderately coarse powder using domestic electric grinder. The powdered drug was boiled with sixteen parts of methanol for a period of 15 minutes [11]. It was filtered hot through muslin cloth. The filtrate was evaporated under reduced pressure and dried .The yield of the extract was 2.0-4.76% (w/w).

2.4 Procedure for glucose oxidase method

Determination of glucose using glucose oxidase was determined according to Trinder (1969) [12] method with a slight modification.0.05 ml glucose solution of concentration 20, 40, 60, 80& 100 mg/dl was added in to different test tubes. 0.05 ml of plant extract was then added to each test tube and incubated in dark for 4hrs. Then 5 ml of glucose oxidase enzyme was added in all test tubes and kept for 30 minutes in dark at room temperature. Results were spectrophotometrically (Thermoscientific Multiscan Go) recorded at 546nm. Concentration of glucose was measured as following

Concentration of glucose= (Ak/Au)× C

Where Au = Absorbance of unknown (extracts of plants), Ak = Absorbance of known (standard glucose), C= Concentration of standard glucose

2.5 Statistical Analysis[2]

All samples were tested and analyzed in triplicate. Results were calculated as the mean±SD (standard deviation) for each sample. Statistical analysis was done with one way analysis of variance using Graph pad Prism, Version 4.0 (Graph Pad Software, San Diego, CA, USA). The correlation coefficient (R^2) was used to show correlations. A significant difference was judged to exist at a level of $P < 0.05$.

3. Results

3.1 Plant selections and Frequency

In the present study, glucose utilization was estimated using enzyme glucose oxidase. The selected plants species with a frequency of herbal healer recommendations, more than 21% and above nominated as medicinal plants used for the treatment of diabetes symptoms and its complications are shown in Table 1. The concentration of glucose was checked of selected plants species and standard deviation of each plant sample was calculated individually.

3.2 In vitro Hypoglycemic activity at different pH

In the results, figure 1, 2 & 3 show hypoglycemic activity of the different plants at the different pH 2, 7 and 9 respectively. The extracts in neutral and basic media showed considerable hypoglycaemic activity in comparison to acidic media. At a concentration of 80 μg/ml, Mimordica charantia, Alternaria has the highest antihyperglycemic activity at neutral pH, whereas *Syzygium cumini* and Gooseberry were showing highest activity among all indigenous herbs used

by traditional healers. *Syzygium cumini* had shown the similar pattern of activity at neutral and basic pH, followed by *Punica granatum peel*. The statistical analysis indicated significant difference between hypoglycaemic activity of plant extracts at $P< 0.05$. However, at the acidic pH *Syzygium cumini* had shown the best results in comparison with others. It was followed by *T. foenum*, Leucas leaf and *Moringa Olifera* stems.

Table 1

indicates the plants used by traditional healers for Diabetes and its complication

Plants	% of Plant used by natural healer
Momordica charantia	66%
Syzigium cumnii seed	61%
Punica granatum Peel	49%
Ragi	41%
Costus igneous leaf	34%
Trichosanthes dioica	34%
Gooseberry leaf	33%
Cassia auriculata	32%
Alterathera ficoicka leaf	31%
Bajra	31%
Corriandrum leaf	31%
Nerium indicum	30%
Amaranthus viridus leaf	29%
Cassia gluaca	29%
Calatripus giganeta	28%
Moringa Olifera Leaf	26%
Tridex flower	26%
Phyllanthus anarus	25%
Zea mays Silk	25%
Creteva nurvala	24%
Pogomia pinnata	24%
Lucas leaf	23%
T. foenum seed	23%
Amaranthus viridus stem	22%
Murraya Koenigii	21%

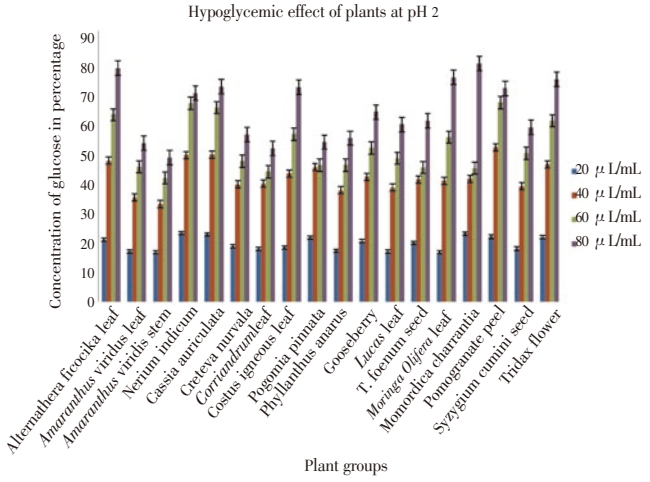

Figure 1: Hypoglycemic activity of different plants at acidic pH (*concentration shown in %)

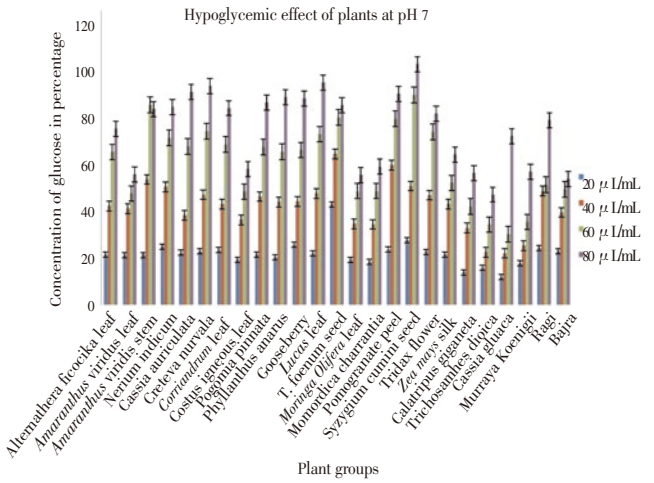

Figure 2: Hypoglycemic activity of different plants at neutral pH. (*concentration shown in %)

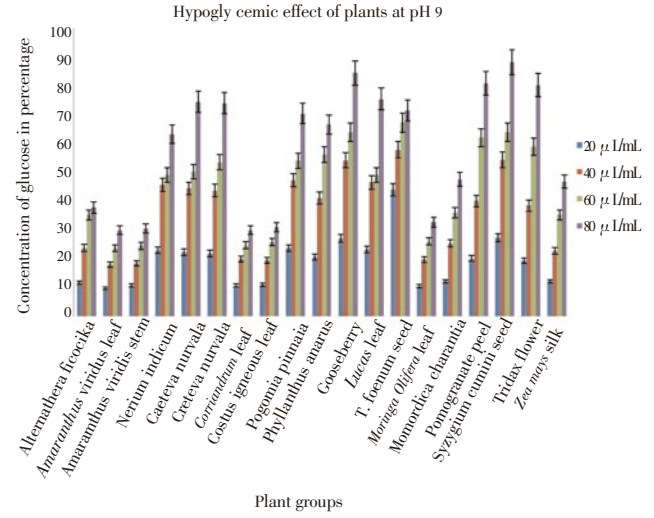

Figure 3: Hypoglycemic activity of different plants at basic pH. (*concentration shown in %)

4. Discussion

The Glucose oxidase method has its advantages because of its specificity and also enables glucose estimation in a complex mixture or an extract. The phytochemical analyses of more than 25 ayurvedic plants of southern Karnataka and dadim (from Kumaun region) were assessed which revealed their hypoglycemic activity. The results indicate the presence of active constituents in the solvents extracted from medicinal plants material. Special attention to these effective medicinal plants will lead us to obtain novel drugs in the management of diabetes mellitus. One of the possible reasons for the strong hypoglycemic activity of Syzgyium cumini could be its adenosine deaminase inhibitory activity, hence reduces glucose levels in hyperglycemic patients [13]. Our studies are in accordance to previous studies [2, 7, 10, 14]. Studies revealed that *M. charantia* repairs damaged

β-cells, increases insulin levels, and also enhance the sensitivity of insulin. It inhibits glucose oxidase and therefore glucose absorption and also suppresses the activity of disaccharides in the intestine [15]. These results confirm the use of *S. cumini* and *M. charantia* in traditional system of medicine to treat diabetes [14, 15]. Since these plants are found to have antihyperglycemic activity. They can be supplemented in diabetes. Further, comprehensive chemical and pharmacological investigations are needed to exploit its relevant therapeutic effect to substantiate its ethano-medical usage.

5. Conclusion

S. cumini , *T. foenum* seed, *P. granatum* peel (dadim) and *Momordica charantia* gave the impression of being a prominent candidates for drug targets for diabetes. This may be the first approach using invitro approaches to prove their antidiabetic properties.

6. Acknowledgements

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Conflict of interest

We declare that we have no conflict of interest.

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