INTRODUCTION

Diabetes is a serious, chronic disease which occurs either when the production of insulin decreases, or when the insulin produced is not effectively used by the body [1]. Gain in the level of glucose inside the body leads many irregular functioning of the organs like blood vessels damage, heart problems, vision problems, kidney function and nerves. In WHO recent report, it is stated that over 400 million people live with diabetes [2]. Diabetes is of two types i.e. Type 1 diabetes which is also known as insulin-dependent diabetes is occurring due to decrease in the production of insulin in the body. Type 2 diabetes which is otherwise known as non insulin-dependent diabetes is characterized as ineffective use of insulin by the body. The most prevalent type in the world is Type 2 diabetes [1]. Earlier Type 2 diabetes was found in adults but now it is also prevalent among children [2]. According to the recent data on the prevalence of diabetes, the number of diabetic patients is going to high 434 million persons to 300 million persons by 2025 [3]. The researcher showed that the gastro retentive microsphere is useful for delivering repaglinide (RG) for the treatment of non-insulin dependent diabetes mellitus (NIDDM) [4].

People for treatment of diabetes have taken advantage of medicinal plants since time immemorial. All the main active compounds present in medicinal plants which are responsible for the treatment of disease and their mode of action are provided in the encyclopedia. Man depends on plants for all of their needs whether it related to health or uses of daily requirement [5]. The secondary metabolites such as alkaloids, steroids and flavonoids etc. which are produced by the plants has been used by pharma industries at solving many of the issues of human beings. As concerns related to the side effect of different available medicine are rising hence there is a continuous need of research and development of new herbal drugs for the treatment of various diseases found in among human [6].

α-amylase is one of the key digestive enzyme which catalyses hydrolysis of starch to maltose in the beginning and at the end convert it to glucose therefore by inhibiting the activity of alpha amylase one can think about the control of diabetes as diabetes is related to increase in glucose level inside body and alpha amylase is responsible for the conversion of starch into many simple sugars like dextrin, maltose and glucose [7]. The activity of alpha amylase is correlated to the elevated postprandial hyperglycemia therefore control of alpha amylase activity becomes significant in the treatment of diabetes [8].

Herbal products which consist of many compounds have been used by the people and which has become the dependable source of remedy for various diseases like diabetes. Hence, the discovery of new potential drugs from medicinal plants with fewer side effects for the treatment of diabetes has to be continued. *Albizia lebbeck* known as *Shirisha in Ayurveda* is an important medicinal plant belonging to the family Fabaceae and subfamily Mimosaceae. It is recognized in the Indian subcontinent for its medicinal uses. Bark is dark brown to greenish black. It is utilized in the Indian traditional system as a folk medicine as well to remedy of several diseases like asthma, arthritis and burns [9]. Antihyperglycaemic and anti diabetic effect of the leaf extracts of *Albizia lebbeck* on alloxan and streptozotocin induced diabetic mice has been reported [10]. Studies related to pharmacological activities on bark of this plant are scarce. Its bark extract has been reported to possess’ antispermatogenic, antiandrogenic activities in male albino rats [11] and also antioxidant potential [12].

Review of literature reveals that there are no reports showing the comparative alpha amylase inhibitory activity of polygon, non-polygon, free flavonoids, bound flavonoids, alkaloid and steroid extract of *Albizia lebbeck* bark. Hence in the present study an attempt was made to evaluate the potential and compare the alpha amylase inhibitory activity of different extracts of *Albizia lebbeck* bark.

MATERIALS AND METHODS

Collection of plant material

Bark of *Albizia lebbeck*, was collected from the eastern region of Rajasthan i.e. Jaipur. Plant was identified by the senior taxonomist of...
Preparation of extracts

**Flavonoid extraction**

Bark of *Albizia lebbeck* was collected; shade dried, finely powdered and Subramanian and Nagarjan method was used for extraction of flavonoids [15]. 100 grams of sample with 80% of hot methanol was soxhlet extracted on a water bath for 24 h and filtered. The filtrate was re-extracted using separating funnel successively with petroleum ether in the first fraction and ethyl ether in the second fraction, and ethyl acetate in the third fraction. As petroleum ether dissolved fatty substances in it, the fraction of it was discarded, whereas fractions of ethyl ether and ethyl acetate were further analyzed for free and bound flavonoids respectively. The ethyl acetate sample was refluxed with 7% H₂SO₄ for two hours so that hydrolysis removed bounded sugars and again in separating funnel the filtrate was refluxed with ethyl acetate. To neutralize the obtained filtrate it was washed with distilled water. Thus, the ethyl ether fraction contains free flavonoids and ethyl acetate fractions contain bound flavonoids were dried in vacuo and weighed. The obtained extracts were stored at 4 °C.

**Alkaloids extraction**

Alkaloids were extracted from bark of *Albizia lebbeck* by well-established method [16]. Hundred grams of sample were extracted in 20 ml methanol after shaking of 15 min. Filtrate obtained was kept for drying and then the residual mass was treated with 1% H₂SO₄ (5 ml) for 2 times. After this, extraction was done in 10 ml chloroform using separating funnel. The chloroform layer which was organic by nature was rejected and the other aqueous layer was basified using 30% NH₄OH of pH=9-10. Again basified layer was extracted in 10 ml chloroform and organic layer of chloroform which was in a lower position was collected in a flask and the step was repeated with fresh chloroform. Extracts was thus obtained was dried in vacuum for further use.

**Steroid extraction**

Steroids were extracted from bark of *Albizia lebbeck* by well-established method [17]. Fine powdered hundred gram of sample of plant bark was extracted in petroleum ether for two to four hours. Then it was filtered and residual mass was treated with 15% ethanolic HCl for four hours. Further it was extracted in ethyl acetate and washed with distilled water to neutralize the extract. To remove the moisture content of the neutral extract it was passed over sodium sulphate and was dried in vacuum.

**Extraction in different polar and non polar solvents**

Powdered bark of *Albizia lebbeck* (20g) was taken in three flasks and water, methanol and petroleum ether were used as solvent. The dried material and solvents were taken in a 1:10 ratio. Those were kept at soxhlet unit for complete one day. Obtained extracts were thus filtered and the filtrate was subjected to dry in vacuum to obtain extract. The residual extract that obtained was stored in a refrigerator at 4 °C in sterile glass bottles.

**In vitro alpha amylase inhibitory assay**

**Starch iodine assay**

Screening of alpha amylase inhibitors were performed using Xiao et al. method in test tubes with slight modifications based on the starch iodine test [18]. The assay mixture was about 120 μl of 0.02M sodium phosphate buffer (pH 6.9), 1.5 ml of salivary alpha amylase and bark extracts at a concentration from 0.5-1.5 mgml⁻¹ (w/v) was incubated at 37 °C for 10 min. After that, soluble 1% starch was added at each reaction mixture and incubated at 37 °C for 15 min. Then 60 μl of 1 M HCl was added to the reaction mixture to stop the enzymatic reaction and immediately 300 μl of iodine reagents was added. If any change in colour was noted and at 620 nm the absorbance was read. The plant extracts was not added to the control reaction showing 100% enzyme activity. Extract control was also included to check if any absorbance produced by bark extract. Thus different colour obtained indicates the presence of starch (dark-blue), absence of starch (yellow) and partially degraded starch (brownish) in the reaction mixture. If inhibitor was present in the extract of plant bark it inhibit the degradation of starch added to the enzyme assay mixture and form a dark-blue colour complex whereas no colour showed the absence of inhibitor.

**3.5-dinitrosalicylic acid assay**

The inhibition assay of bark extract of *Albizia lebbeck* was performed using the chromogenic dinitrosalicylic acid (DNSA) method [19]. The assay mixture consists of 500 μl of 0.02 M sodium phosphate buffer (pH 6.9), 1 ml of salivary alpha amylase and 400 μl extracts at concentrations from 0.5-1.5 mgml⁻¹ were incubated at 37 °C for 10 min. After pre-incubation, 580 μl of 1% starch solution was added to each tube and incubated at 37 °C for 15 min. Using 1.0 ml DNSA reagent the reaction was terminated and tubes were placed in boiling water bath for 5 min. After this, cooled to room temperature and at 540 nm the absorbances were measured. The control did not contain any bark extract represented 100% enzyme activity. Extract control was also included to check if any absorbance produced by bark extract except for the enzyme.

Formula for calculation of the percent inhibition of alpha amylase:

\[
\% \text{ Relative enzyme activity} = \frac{\text{Enzyme activity of test}}{\text{Enzyme activity of control}} \times 100
\]

\[
\text{Percent Inhibition of the \( \alpha \) amylase activity} = 100 - \% \text{ Relative enzyme activity}
\]

**Statistical analysis**

Experiments were performed in triplicates for three different sets and mean±standard error of the mean was used for calculation. Graphpad prism5 software was used for ANOVA, linear regression and statistical difference analysis. The IC₅₀ values were computed.

**RESULTS**

Out of the solvent extracts methanol extract exhibited significant inhibition of alpha amylase activity. Free flavonoids extract of bark showed maximum alpha amylase inhibitory activity as compared to bound flavonoid, alkaloid and steroid extract. Maximum activity of free flavonoids extracts may be due to presence of potential inhibitory compound in extract. Alkaloid, steroid and petroleum ether extracts showed negligible inhibitory activity with insignificant IC₅₀ value.

**Extract with maximum inhibitory effect on the alpha amylase activity**

Free flavonoids extracts (at a concentration 0.5-1.5 mg/ml) showed maximum α-amylase inhibitory activity from 43.50±0.17% to 73.43±0.08% with an IC₅₀ value of 0.6653 mg/ml. At the same concentration methanol, water and bound flavonoids extracts also showed good inhibitory activity i.e. 28.63±0.15% to 37.50±0.29%, 26.67±0.12% to 32.07±0.17% and 26.70±0.15 to 32.67±0.12% with an IC₅₀ values of 7.3621 mg/ml, 22.2844 mg/ml and 41.6869 mg/ml respectively (table 1).

**Extracts with inhibitory effects on the \( \alpha \)-amylase activity**

Petroleum ether, alkaloid and steroid extracts showed minimum inhibitory activity from 2.67±0.19 % to 4.9±0.17%, 1.17±0.07% to 2.23±0.03%, 1.53±0.03% to 2.23±0.03% with an IC₅₀ value of 1000 mg/ml, 16982.365 mg/ml and 16982.365 mg/ml respectively (table 2).

**Statistical analysis**

Experiments were performed in triplicates for three different sets and mean±standard error of the mean was used for calculation. Graphpad prism5 software was used for ANOVA, linear regression and statistical difference analysis. The IC₅₀ values were computed.

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Values are given as mean ± standard error of mean (SEM) (n=3). One way analysis of variance (ANOVA) was used which show significant difference with respect to control (P<0.05).

Table 1: Extract with maximum inhibitory effect

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of extract</th>
<th>Concentration Mg/ml</th>
<th>% inhibition</th>
<th>Regression equation</th>
<th>IC50 value (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Free flavonoid</td>
<td>0.5</td>
<td>43.50±0.17</td>
<td>Y=5.288+1.624X</td>
<td>0.6653</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>59.53±0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>73.43±0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Water</td>
<td>0.5</td>
<td>26.67±0.12</td>
<td>Y=4.499+0.379X</td>
<td>22.2844</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>32.73±0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>32.07±0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>methanol</td>
<td>0.5</td>
<td>26.63±0.15</td>
<td>Y=4.561+0.507X</td>
<td>7.3621</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>32.57±0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>37.50±0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Bound flavonoid</td>
<td>0.5</td>
<td>26.70±0.15</td>
<td>Y=4.451+0.339X</td>
<td>41.6869</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>28.87±0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>32.67±0.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Extract with insignificant inhibitory effect

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of extract</th>
<th>Concentration Mg/ml</th>
<th>% inhibition</th>
<th>Regression equation</th>
<th>IC50 value (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pet ether</td>
<td>0.5</td>
<td>2.67±0.19</td>
<td>Y=3.133+0.622X</td>
<td>16982.365</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>3.33±0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>4.9±0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>alkaloid</td>
<td>0.5</td>
<td>1.17±0.07</td>
<td>Y=2.785+0.524X</td>
<td>16982.365</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>1.83±0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>2.23±0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>steroid</td>
<td>0.5</td>
<td>1.53±0.03</td>
<td>Y=2.785+0.524X</td>
<td>16982.365</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>1.83±0.03</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>2.23±0.03</td>
<td></td>
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</tbody>
</table>

DISCUSSION

Natural products from plants used for the treatment of diabetes. There has been a continuous search for the development of novel and cost effective drugs from natural sources. A part of A. lebbeck for the treatment of various diseases has been used since ancient times. In many previous studies on its use as antimicrobial [20-21], use of seeds in inflammation, skin diseases, leprosy, leucoderma, chronic catarrh, seminal weakness, ophthalmopathy and poisoning has been described [22-23]. The earlier experimental investigations demonstrated that many naturally occurring agents and plant extracts had shown antidiabetic activities [24]. These alpha amylase inhibitors are also called as starch blockers since it prevents or slows the absorption of starch in to the body mainly by blocking the hydrolysis of 1, 4-glycosidic linkages of starch and other oligosaccharides into maltose, maltotriose and other simple sugars [25]. Only few studies focused on the antidiabetic activity of Albizia lebbeck extracts and that too without testing for free flavonoid, bound flavonoids, steroids and alkaloids. Though there are reports available on the antidiabetic activity of Albizia lebbeck but there is no report on antidiabetic activity using salivary alpha amylase of Albizia lebbeck bark. Review of literature showed that very less work has been done on the anti diabetic potential of different bark extract therefore in this study, we compared IC50 value of α-amylase inhibitory activity of steroids, free flavonoids, bound flavonoids and alkaloids extracts isolated from the bark of A. lebbeck with polar and non polar solvent based extracts. The goal of this work was to develop a potential antidiabetic compounds from the crude extracts of Albizia lebbeck bark. The present study confirmed that the free flavonoid bark extract has a higher antidiabetic activity with the IC50 at 0.6653 mg/ml when compared to water, methanol and bound flavonoid bark extracts. The petroleum ether, steroid and alkaloid bark extract showed the very less activity with insignificant IC50 value. To find out the mode of action of bark extracts as alpha amylase enzyme inhibitors, further research is required to be continuous so that to qualify the action of different constituents in the extract. The results of this study directs further researches to evaluate the therapeutic potentialities of free flavonoids, methanol, water and bound flavonoids extracts of bark of A. lebbeck in the management of diabetes either alone or in a combinatorial therapy.

CONCLUSION

Diabetes is among the major health problems worldwide. The current available therapies are not of much use in prevention or reduction of disease. Thus, continuous search for new natural product with fewer side effects is the requirement of the period. Results of selected plant extract showed significant antidiabetic properties. We were able to identify promising medicinal plants which could be used for treatment of diabetes. Further chemical isolation of novel molecules and their mode of action are warranted for the elucidation of the activity. Our results justified the use of investigated plants in the Indian ethnomedicine. The present study showed that the bark extract of selected plant exhibited significant inhibition of alpha amylase activity with less IC50 value. These findings support that a number of investigated plants could be a valuable source of new antidiabetic compounds that can potentially deliver novel mechanisms of actions. Hence the extract may be useful as better therapeutic agent especially for the treatment of diabetes. Further studies are warranted to follow our observations.

ACKNOWLEDGEMENT

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AUTHOR CONTRIBUTION

Principal author: Performed collection of sample, extraction, analysis, interpreted data, wrote the manuscript and acted as corresponding author.

Co-author contribution: Supervised the development of work and helped in the evaluation of the manuscript.

CONFLICT OF INTERESTS

There is no conflict of interest.
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