EVALUATION OF CATECHU EXTRACTS FROM ACACIA CATECHU COLLECTED FROM CENTRAL INDIA FOR ANTIDIABETIC ACTIVITY

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ABSTRACT

Catechu, obtained from Acacia catechu heartwood is one of the important substances having various pharmacological and therapeutic activities. During the present study, the heartwood of Acacia catechu was collected from the forests of Jabalpur, Central India and the decoction of heartwood (crude catechu) was partitioned with water, ethanol and ethyl acetate. The aqueous extract was rich in tannins, while flavonoids partitioned with ethanol primarily. The ethanolic extract of catechu showed slower glucose migration (73.9% reduction) across semipermeable membrane, as well as slowed the starch assimilation by inhibiting 67.8% of α-amylase activity, as compared to acarbose (87.2%). Ethyl acetate extract was also effective as antidiabetic agent.

INTRODUCTION

India’s ancient legacy describes the uses of local flora for the treatment of various diseases, as well as their uses as therapeutic agents to prevent such illnesses and up keeping of good health. Acacia catechu is one of those plants, described extensively in various ancient “Materia Medica” available in different languages and in different parts of India, as well as in other countries[1]. The modern scientific evidences also strengthen the ancient believes.

Catechu or “Kattha” in Hindi, is prepared from the decoction heart wood of Acacia catechu, and is one of the main ingredients in “Paan”. Rich in tannins, the catechu also contains various flavonoids, primarily quercetin, quercitrin, catechin, and epicatechin. These various biological active phytochemicals make it a suitable astringent and therapeutic agent with antioxidant, antipyretic, antimicrobial and hepatoprotective activities[2].

However, the modern scientific data is still lacking in coping with the variations arising due to climatic and geographical differences in plant growth conditions, and hence, a generalized statement related to the potency of any plant or phytochemical profile is often ambiguous, and may be misleading to the local medicine practitioners and manufacturers, resulting in drugs of low potency. In order to counter these ambiguities, it is essential to investigate the therapeutic properties of locally available plants[3].

Acacia catechu is native to Central India and is one of the prominent trees found in forests of Central India. The present study is aimed in identifying the antidiabetic activities of Catechu, isolated from the local trees of Acacia catechu in Central India. The antidiabetic activity is performed as hypoglycaemic potential of the catechu extract under in vitro conditions.

MATERIALS AND METHODS

Isolation of catechu

The catechu was obtained using a decoction of heartwood of Acacia catechu. The heartwood was obtained from Acacia catechu tree bark from local forests of Jabalpur, Central India. The heartwood was chipped to small pieces and decoction was prepared by boiling these chips in water for several hours. The decoction was allowed to stand for several hours, and the sediment was collected as a thick slurry, dried and used as crude catechu for further experiments.

Preparation of catechu extract
The dried catechu (10 g) was pulverized, and defatted using extraction with hexane in a Soxhlet extractor for few hours. The residue was extracted first with water (using cold percolation for 48 h), followed by ethanol and ethyl acetate (using Soxhlet extractor). All the extracts were concentrated up to the concentration of 1 mg ml⁻¹.

**Antidiabetic activity**

**Glucose mobilization test**

The *in vitro* glucose mobilization test was performed as per the method of Barapatre et al.⁴ with slight modifications. The mobilization of glucose through a dialysis membrane without (control) or with different extracts of catechu was monitored. The dialysis membrane (4 nm pore size, HiMedia, India) was filled with 1 ml of 1.5 mM D-glucose with 1 ml solvent (controls) or 1 ml catechu extracts (treatments). The membrane was sealed and allowed to dialyze the content into 100 ml distilled water containing 0.1 M NaCl, under constant stirring on a magnetic stirrer for 3 h. After 3 h, the concentration of glucose was measured in dialyzed water using dinitro salicylic acid (DNS) method. The absorbance (A_{540}) of the reaction mixtures was converted to amount of glucose (µg ml⁻¹) using a standard calibration of glucose prepared using the same method.

**α-amylase inhibition assay**

The inhibition of α-amylase by catechu extracts was measured as antidiabetic activity as per the method of Eleazu et al.⁵. A solution (500 µl) of α-amylase (500 µg ml⁻¹, 10,000 units, SRL, India) prepared in 0.1 M phosphate buffer, pH 6.8, having 0.05 M NaCl, was incubated with 500 µl of solvents (controls) or with same amount of catechu extracts for 10 min at 37°C. The reaction was started by adding 500 µl of 1% starch solution (prepared in 0.1 M phosphate buffer, pH 6.8, having 0.05 M NaCl). The reaction mixture was again incubated for 10 min at 37°C, and stopped by adding 1 ml of DNS reagent, and boiling the tubes for 10 min. after colling the tubes, the absorbance was measured at 540 nm using a spectrophotometer (EI, India) against individual blanks, in which enzyme solution was replaced with same amount of buffer. Acarbose (100 µg ml⁻¹) served as a positive control. The percent inhibition of the α-amylase activity was calculated by the following formula: % inhibition = (A_c-A_t/A_c) x 100

(Rate of glucose mobilization test was performed as per the method of Eleazu et al.⁵. Table 2 shows that without any extract, the glucose migrated to the outside reservoir was 52.1 ± 2.1 µg ml⁻¹, highest percent of reduction of glucose migration was observed with ethanolic extract which reduced the migration up to 73.9%, followed by ethyl acetate extract, which showed 40.1% reduction. The aqueous extract reduced this migration only by 17.1%.

**DISCUSSION**

**Acacia catechu**, is a highly valued medicinal tree, mainly for its heartwood. The decoction of heartwood is a rich source of various polyphenols, including tannins, catechin and epicatechin and flavonoids⁶. It is a common procedure to partition these biologically active phytochemicals with solvents of varying polarity, so that role of different phytochemicals.

### RESULTS

The decoction of *Acacia catechu* heart wood resulted in crude catechu, which was extracted with water, ethanol and ethyl acetate solvents. The phytochemical distribution among solvents shows that the crude decoction has good amounts of tannins, flavonoids, terpenoids with detectable quantities of saponins, glycosides and alkaloids. While tannins remained in aqueous extract, flavonoids and terpenoids were distributed in ethanol and to some extent in ethyl acetate (Table 1).

### Table 1 Distribution of major phytochemicals in heartwood decoction obtained from *Acacia catechu* of Central India among different solvents.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Terpenoids</th>
<th>Saponins</th>
<th>Glycosides</th>
<th>Alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decoction</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The extracts were investigated for their property to reduce migration of glucose through semi permeable membrane *in vitro*. Table 2 shows that without any extract, the glucose migrated to the outside reservoir was 52.1 ± 2.1 µg ml⁻¹, highest percent of reduction of glucose migration was observed with ethanolic extract which reduced the migration up to 73.9%, followed by ethyl acetate extract, which showed 40.1% reduction. The aqueous extract reduced this migration only by 17.1%.

### Table 2 Reduction rate of *in vitro* glucose migration without (control) or with different extracts of catechu from *Acacia catechu* of Central India. Data are presented as mean ± SD (n=3)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>Amount of D-glucose in water (µg ml⁻¹)</th>
<th>Inhibition rate of glucose migration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>52.1 ± 2.1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Aqueous extract</td>
<td>43.2 ± 3.1</td>
<td>17.1</td>
</tr>
<tr>
<td>3</td>
<td>Ethanolic extract</td>
<td>13.6 ± 2.3</td>
<td>73.9</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl acetate extract</td>
<td>31.2 ± 3.6</td>
<td>40.1</td>
</tr>
</tbody>
</table>

The inhibition of starch digestibility by α-amylase was also recorded as a potential antidiabetic activity. Acarbose, the positive control could inhibit 87.2 ± 5.2% inhibition of α-amylase activity. Among catechu extracts, maximum α-amylase inhibitory activity was shown by ethanolic extract (67.8 ± 8.4%), followed by ethyl acetate extract (43.2 ± 5.2). Aqueous extract could inhibit only 38.1 ± 3.8% activity of α-amylase (Fig 1).
compositions can be studied for different physiological and pharmacological activities.

Among various phytochemicals, flavonoids are known to be bioactive antioxidant substances, apart from their usual antioxidant activities\[3\]. Our results demonstrated that flavonoids rich ethanolic extract was mainly responsible for the antidiabetic activity in vitro. Since, flavonoids could primarily be partitioned with ethanol, followed by ethyl acetate, the results followed in the same way. Sarfraz et al.\[6\] also showed that flavonoids are better extracted with ethanol. Further, the antidiabetic action of flavonoids rich ethanolic fraction in our study is well supported by the findings of Jadhav and Puchchakayala\[9\] who showed that major flavonoids, i.e. boswellic acid, ellagic acid, quercetin etc can induce hypoglycemic effects on streptozotocin-nicotinamide induced type 2 diabetic rats.

Diabetes, specially type II diabetes, is a common syndrome, characterized by higher level of blood glucose. Since, there may be many reasons for elevated blood glucose levels, a single drug therapy often does not work well\[10\]. The traditional medicine system in India, as well as of other countries hence suggest use of various plants to combat diabetes. The idea behind is to use a combination of phytochemicals to combat diabetes at various levels. One of the prominent counterattack strategy is to block α-amylase. The inhibitors of α-amylase block or at least slow the starch hydrolysis by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltrirose and other simple sugars\[11\]. Further, the slow migration of glucose through semipermeable membrane helps in reducing the glucose deposition in tissues, further helping to prevent adverse effects of diabetes. Our study demonstrates the catechu from Acacia catechu of Central India is rich in flavonoids and can help in treatment of diabetes mellitus.

References


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