PRELIMINARY PHYTOCHEMICAL ANALYSIS AND BIOLOGICAL SCREENING OF EXTRACTS OF LEAVES OF MELIA DUBIA CAV

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ABSTRACT
The traditional knowledge on medicinal plants can lead to the development of drugs and plant based medicine. The leaves of Melia dubia Cav (Meliaceae) are used for the treatment of various ailments. In present study it was screened for antidiabetic and antioxidant activities and also tested for various phytochemical constituents. The phytochemical screening of extracts revealed the presence of alkaloids, carbohydrates, steroids, tannins, flavonoids, saponins and glycosides. Total phenol content and total flavonoid content was performed for all the extracts by standard methods. The extracts were screened for in vitro α-amylase inhibitory activity and the ethanolic extract inhibited α-amylase at much lower concentration (IC50:24.82 µg/ml) than the standard Acarbose. The successive solvent extracts of Melia dubia exhibited excellent antioxidant activity by Nitric oxide radical scavenging method, evidenced by lower IC50 (16.89 µg/ml) value in the ethanolic extract. The results revealed that, ethanolic fraction of Melia dubia Cav which contains highest amount of phenolic and flavonoids compound may be contributing to antioxidant and antidiabetic potential of the plant.

Keywords: Melia dubia, phytochemical screening, phenolic compound, flavonoids, α-amylase, antioxidant.

INTRODUCTION
The increasing price of modern medicine and prevalence of disease have resulted in the demand for discovery of less expensive and potent drugs. Plants are one of such source. Diabetes mellitus is a major health problem nearly affecting 194 million of people worldwide and it may increase to 300 million by 20251-2. Most prevalent are type II diabetes. Prolong treatment with modern drugs produce side effect and also results in drug resistance. WHO in this concern recommends a safe natural antidiabetic drug which either works by insulin mimic action on insulin secreting β-cell or modify glucose absorption3. α-amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to maltose and finally to glucose. Degradation of this dietary starch proceeds rapidly and leads to elevated post prandial hyperglycemia (PPHG). It has been shown that, activity of Human Pancreatic α-amylase (HPA) in the small intestine correlates to an increase in post-prandial glucose levels, the control of which is therefore an important aspect in the treatment of diabetes 4. Within the human body, millions of chemical reaction occurs which require oxygen. Free radicals are compounds generated from normal body chemical process and environmental pollution. They attack the healthy cells DNA and cause them to deteriorate. Antioxidants are compounds that protect cells against the deteriorating effect of reactive oxygen species 5. Therefore the search for safer, specific and effective hypoglycemic agent with antioxidant capacity has continued to be an important area of investigation with natural extracts. Melia dubia cav (Meliaceae) is an indigenous plant possessing several therapeutic properties. It is a deciduous tree found in Sri Lanka, India and Tropical Asia. Its timber is used in making furniture and agricultural implements6. The plant is reported for wide range of activities like antiviral7, antimicrobial8, antieeedent activity9 etc., Some other species of Melia were reported to have incrimented properties and chemical constituents10-13. The antidiabetic activity of the fruits has been well established14. The present study involves preliminary phytochemical screening, antioxidant and antidiabetic evaluation on the leaves of Melia dubia to justify its folklore use.

MATERIALS AND METHODS

Plant collection and identification
The leaves of Melia dubia Cav (PARC/2012/1099) plant was collected from Dehradun and authenticated by P. Jayaram, Director, Plant Anatomy Research Centre, Medicinal Plants Research Unit, Tambaram, Chennai, India.

Extraction
The collected plant material was dried and coarsely powdered, extracted with various solvents like n-hexane, petroleum ether, acetone, ethanol and water by cold maceration method. The plant material was soaked for 72 hours at 30ºC, filtered and to the residue the same solvent was added. This procedure was repeated thrice to obtain maximum extraction. All the filtrates were pooled and evaporated under vacuum in rotary evaporator.

Phytochemical investigation
The preliminary qualitative phytochemical studies were performed for detecting the different constituents such as alkaloids, steroids, tannins etc in all the extracts.

Phenolic content
The total phenolic content of plant extracts were
Nitric oxide radical scavenging method

All the extracts and the standard (Ascorbic acid) were dissolved and made up to 100µg/ml from the stock solution various concentrations were prepared including standard. To each, 1 ml of sodium nitroprusside solution was added and incubated at 25°C for 30 minutes.

To 1 ml of each dilution 1 ml of amylase solution was added and incubated at 25°C for 3 minutes. From the above 1 ml mixture, 1 ml of starch solution was added and placed in 85°C in water bath. Then the mixture was cooled and the generation of maltose was quantified by measuring the absorbance at 540 nm. Then the percentage inhibition of α-amylase was also assessed and reported as IC₅₀ in Table 3.

Antioxidant activity

In vitro α-amylase inhibitory activity

The α-amylase inhibitory activity for all the extracts were determined based on colorimetric method using Acarbose as standard. To 1 ml of each dilution 1 ml of amylase solution was added and incubated at 25°C for 3 minutes. From the above 1 ml mixture, 1 ml of starch solution was added and placed in 85°C in water bath. Then the mixture was cooled and the generation of maltose was quantified by measuring the absorbance at 540 nm. Then the percentage inhibition of α-amylase was also assessed and reported as IC₅₀ in Table 3.

Table 3: Antioxidant activity

<table>
<thead>
<tr>
<th>Name of the extract</th>
<th>IC₅₀ value (µg/ml) Nitrile oxide scavenging activity</th>
<th>IC₅₀ value (µg/ml) α-amylase Inhibitory activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>31.33±0.16</td>
<td>47.18±0.18</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>46.77±0.28</td>
<td>59.03±0.27</td>
</tr>
<tr>
<td>Acetone</td>
<td>72.44±0.58</td>
<td>40.28±0.62</td>
</tr>
<tr>
<td>Ethanol</td>
<td>16.89±0.88</td>
<td>24.82±0.32</td>
</tr>
<tr>
<td>Water</td>
<td>77.72±0.78</td>
<td>43.26±0.37</td>
</tr>
<tr>
<td>Standard Ascorbic acid</td>
<td>50.25±0.68</td>
<td>32.08±0.94</td>
</tr>
<tr>
<td>Acarbose</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are presented as mean ±SD
RESULTS

Phytochemical constituents
The yield of *Melia dubia* leaves using various fraction of solvent are shown in Table 1. The variation in the yield may be due to the polarity of solvents used in the extraction process. Qualitative analysis of phytochemical constituents of different extracts of *Melia dubia* leaves showed the presence of alkaloid, steroid, tannins etc., which are tabulated in Table 2. Total phenol and flavonoid content significantly varied between the various extracts and tabulated in Table 1.

Biological activity
Antioxidant activity by nitric oxide radical scavenging method revealed that the ethanol fraction of *Melia dubia* leaves exhibited better radical scavenging activity with an IC$_{50}$ value of 16.89 (µg/ml). Among the five extracts tested for the leaves of plants, Ethanolic extract of *Melia dubia* has shown high potent α-amylase inhibiting property with IC$_{50}$ value of 24.82 (µg/ml).

DISCUSSION

Hyperglycaemia results in generation of reactive oxygen leaves exhibited.

REFERENCES

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