Research Article

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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 (IC_{50}-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 IC_{50} (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 2025[1-2]. 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 absorption[3]. 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 implements[6]. The plant is reported for wide range of activities like antiviral[7], antimicrobial[8], antifeedant activity[9] etc., Some other species of *Melia* were reported to have incriminated properties and chemical constituents[10-13]. The antidiabetic activity of the fruits has been well established[14]. 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 30°C, 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

determined by Folin-Ciocalteu assay[15]. Results were expressed as gallic acid equivalent in mg/g of extract.

Flavonoid estimation

The flavanoid content of the extracts were processed according to the method of Markham[16] and absorbance was measured at 415 nm. Using rutin as standard the linearity was obtained and the results were expressed in rutin equilvalent in mg/g of the extract.

Antioxidant activity

Nitric oxide radical scavenging method

All the extracts and the standard (Ascorbic acid) were dissolved and made up with DMSO (100µg/ml) and from the stock solution various concentrations were prepared including standard. To each, 1 ml of sodium nitroprusside was added. All the preparations were incubated at 37°C for 2.5 hours. After incubation 1 ml of Griess reagent was added and made up to the mark with phosphate buffer. All the solution mixtures acquired pink color whose absorbance was measured at 546nm. The percentage inhibition of Nitric oxide radical scavenging activity was calculated and IC_{50} are given in Table 3.

Antidiabetic 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 1ml of each dilution 1ml of *amylase* solution was added and incubated at 25°C for 30 minutes. From the above 1ml mixture, 1ml of starch solution was added and incubated at 25°C for 3 minutes. Then 1ml of color reagent (3, 5-Dinitro salicylic acid, potassium tartarate in 2ml NaOH and distilled water) 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 540nm.

Then the percentage inhibition of α-*amylase* was also assessed and reported as IC_{50} in Table 3.

Table 1: Results of Percentage yield, Phenolic and Flavonoids content

Name of the Extract	Percentage yield w/w	Total Phenolic content (%) (mg/g) of Gallic acid	Total Flavonoid content (%) mg/g of Gallic acid
Hexane	0.98	83.5±0.13	103±1.05
Petroleum ether	3.15	90.8±1.67	55±1.13
Acetone	1.03	88±0.98	110±1.09
Ethanol	1.62	99.02±1.67	175±1.56
Water	3.99	77.3±1.69	36±1.45

Table 2: Results of Preliminary Phytochemicals Analysis

Phytochemicals constituents	n-Hexane extract	Petroleum Ether extract	Acetone extract	Ethanol extract	Water extract
Steroids	+	+	-	-	-
Phytosterols	+	+	-	-	-
Triterpenoids	-	-	-	-	-
Saponins	-	-	-	-	+
Flavonoids	-	-	+	+	+
Tannins	-	-	-	+	-
Protein and amino acids	-	-	-	-	+
Carbohydrates	-	-	+	+	+
Alkaloids	-		-	+	+
Glycosides	-	-	+	+	+
Fat and fixed oil	+	+	-	-	-
Essential oil	-	+	-	-	-

Table 3: *In vitro* antioxidant and *α-amylase* inhibitory activity

Name of the extract	IC_{50} value (µg/ml) Nitric oxide scavenging activity	IC_{50} value (µg/ml) *α-amylase* Inhibitor activity
Hexane	31.33±0.16	47.18±0.18
Petroleum ether	46.77±0.28	59.03±0.27
Acetone	72.44±0.58	40.28±0.62
Ethanol	16.89±0.88	24.82±0.32
Water	77.72±0.78	43.26±0.37
Standard Ascorbic acid	50.25±0.68	-
Acarbose	-	32.08±0.94

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 species leading to oxidative stress. It is found that *Melia dubia* had an ability to protect against oxidative stress effect and also improve glucose metabolism. The present study reveals that the high phenol and flavonoids content of selected plant may have attributed to the greatest combined antidiabetic and antioxidant activities. This significant role may be due to the presence of phenolic group and flavonoids compound which may have acted upon the biological targets involved in Type II diabetes and oxidative stress.

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