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Research Article

PHARMACOGNOSTICAL STUDIES ON *ACACIA CATECHU* WILLD AND IDENTIFICATION OF ANTIOXIDANT PRINCIPLES

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ABSTRACT

Acacia catechu Willd. (Fam – Mimosaceae; Eng – Cutch tree; Mal – Karingali; Sans – Khadira) is an important medicinal plant used in Ayurveda for so many diseases and commonly for mother and child healthcare. The plant is a moderate sized, deciduous tree up to 3m high and distributed throughout the Sub-Himalayan tract of Punjab to Assam ascending to 1200m. In the present paper, a detailed pharmacognostic study on the heartwood of *Acacia catechu* based on its physicochemical, macroscopic, and microscopic and biochemical features was carried out. The drug is used as a medicine for several common ailments like skin diseases, ulcers, diabetes etc. These medicinal properties may be due to their antioxidant properties. Hence a preliminary study on the antioxidant activity of the alcohol extract was carried out by evaluating the free radical scavenging activity by the method of DPPH radical scavenging assay. The extract gave very good radical scavenging activity comparable with that of ascorbic acid which was used as the standard. Since flavonoids are reported to be strong antioxidants, an attempt was also made to estimate the flavonoids present in the alcoholic extract of *Acacia catechu*. The detection and estimation of flavonoids were carried out by HPLC method using Binary gradient UFLC system equipped with C_{18} column and UV/Vis detector. These studies on *Acacia catechu* are being reported for the first time.

Keywords: *Acacia catechu*, pharmacognostic study, antioxidant activity, DPPH radical scavenging, HPLC

INTRODUCTION

Acacia catechu Willd. (Fam: Mimosaceae, English -Cutch tree, Hindi - Khair, Malayalam - Karingali, Sanskrit – Khadira) is widely used in Ayurveda for many diseases and mainly for skin diseases[1]. Most of the people in Kerala use boiled Khadira water (karingali water) for drinking purpose. The heartwood of Khadira is used in melancholia, conjunctivits, haemaptysis, catarrah, cough, pruritus, leprosy, leucoderma, skin diseases, helminthiasis, norexia, diarrhea, dysentery, foul ulcers and wounds, haemoptysis, haematemesis, haemorrages, fever, anaemia, diabetes and pharyngodynia. There are a number of ayurvedic taila (oil) formulations which contain Khadira as one of the active ingredients[2].

The important chemical constituents reported in the heartwood are catechin, catechutannic acid, epicatechin, catechin tetramer, dicatechin, gallocatechin, kaempferol, taxifolin, isorhamnetin, (+) afzelechinn, L-arabinose, D-galactose, D-rhamnose and aldobiuronic acid[3]. The medicinal properties of *Acacia catechu* may be due to the antioxidant properties of these constituents. The objective of the present study includes the pharmacognostic diagnosis, evaluation of antioxidant properties and detection and estimation of flavonoids.

MATERIALS AND METHODS

Plant material

The heart wood of *Acacia catechu* was procured from the vicinity of Thiruvananthapuram and identified with the help of "Flora of the Presidency of Madras"[4].

Pharmacognostic studies

Pharmacognostic studies include macroscopic, microscopic, biochemical, physico-chemical, and preliminary phytochemical studies. For microscopic studies, free hand sections were taken, stained in safranin, mounted in glycerin and photographs were taken. For the biochemical analysis, the free hand sections were stained with Sudan black (for lipids), 5% Toluidine blue (for lignins), Iodine-potassium iodide (for starch) and Coomassie brilliant blue (for proteins)[5]. For chemical analysis, the fresh heartwood of *Acacia catechu* was dried in shade, cut and crushed. This sample was used for all experimental purposes. The physico-chemical parameters like determination of moisture content, percentage extractives in different solvents, ash content, acid insoluble ash, water soluble ash, solubility in water and alcohol, loss on drying at 110ºC, volatile oil, fibre content etc. were determined by standard methods[6,7]. The ash obtained was subjected to inorganic qualitative analysis. The fluorescence characters of the plant material in different solvents were observed using visible, short UV and long UV light[8]. The preliminary phytochemical tests were carried out using alcohol extract of the plant material by standard methods[9]. The quantitative analysis of sugar was carried out by Fehling`s solution method. All the reagents used were of GPR grade.

Antioxidant studies

The cut and crushed plant material (20 g) was extracted in hot ethyl alcohol by Soxhlet extraction method. The extract was concentrated and the solvent was evaporated off. A part of the residue was redissolved in ethyl alcohol and used for qualitative and thin layer chromatographic (TLC) studies. Another part of the residue was redissolved in water and used for the antioxidant studies. The qualitative antioxidant capacity was analysed by Dot-blot assay[10] and quantitative analysis by DPPH (1, 1, - diphenyl-2-picryl hydrazyl) radical scavenging assay with ascorbic acid as standard[11]. For quantitative analysis, different concentrations of the plant extract were prepared (1 µM to 500 µM). To find out how many antioxidant compounds are present in the extract, TLC was conducted by applying 10µl of the extract on Merck silica gel F_{254} plates. The plates were developed with the chloroform: ethyl acetate: formic acid (5:4:1) (CEF) as eluent system and sprayed with 0.4mM DPPH solution in methanol. Number of spots developed was noted.

Detection and estimation of flavonids

Identification and estimation of the flavonoids – catechin, rutin and isorhamnetin were done by HPLC method using Binary gradient UFLC system equipped with C_{18} column and UV/Vis detector. The quantifications of the three flavonoids were carried out by standard addition method. Sample preparation, apparatus and conditions for HPLC were according to the method of Zu *et al.*[12]. The experiment was conducted at 279 nm for catechin, 265 nm for rutin and 368 nm for isorhamnetin. Catechin was procured from Sigma Chemical Company, USA. Rutin and isorhamnetin were obtained from Ayurveda Research Institute for Mother and Child Health Care, Poojapura, Thiruvananthapuram.

RESULTS AND DISCUSSION

Macroscopic and Microscopic features

Acacia catechu Willd. (Figure-1) is a moderately sized deciduous tree, up to 3m high. The leaves are pinnate with a pair of recurved prickles at the base of the rachis. Flowers are pale yellow in cylindrical spikes. Pods are glabrous oblong. The plant is distributed throughout the Sub – Himalayan tract of Punjab to Assam ascending to 1200m., Peninsular region - particularly in drier parts, Madhya Pradesh, Maharashtra, Gujarat, Bihar, Rajasthan and Tamil Nadu[13]. The anatomical features of heartwood are shown in the Figure-2. The biochemical analysis revealed that the deposition of lipid and lignin was high in the vascular region compared to the deposition of starch and protein. (Figures 3-6).

Fig. 1: Habit

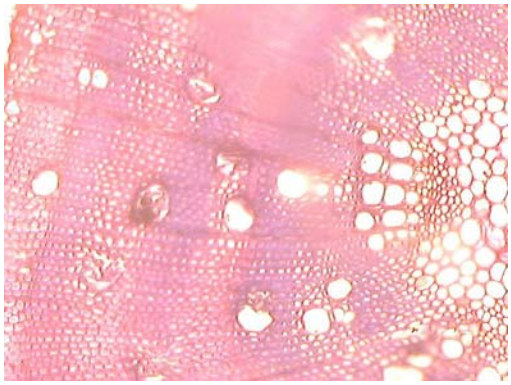


Fig. 2: TS of heartwood enlarged

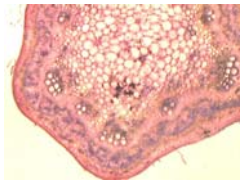


Fig. 3: Lipid

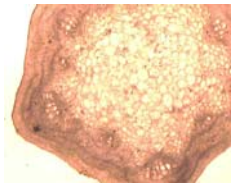


Fig. 4: Lignin

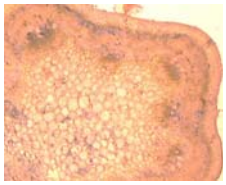


Fig. 5: Protein

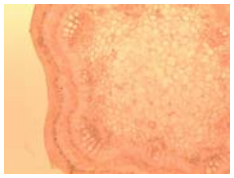


Fig. 6: Starch

Fig. 3-6: Localisation of Lipid, Lignin, Protein and Starch

Physico-chemical parameters

The physico-chemical parameters are given in Table 1. These values are in agreement with the earlier reports[14]. The ash obtained from the drug was tested for inorganic radicals and the tests for CO_3^{2-}, PO_4^{3-}, SO_4^{2-}, Al^{3+}, Ca^{2+},Mg^{2+} and K^+ were found to be positive. The preliminary phytochemical studies showed the presence of catechins, flavones, tannins and sugars. The total sugar content and reducing sugar content are given in Table 1.

Table 1: Analysis of physico-chemical parameters of *Acacia catechu*

Sl.No.	Tests	Results % (range)
1	Loss on drying at 105˚C	9.00-11.00 %
2	Total ash	1.16-2.00 %
3	Acid insoluble ash	0.20-0.4.00 %
4	Water soluble ash	0.09-0.25 %
5	Water soluble extractives	23.00-25.00 %
6	Alcohol soluble extractives	19.00-21.00%
7	pH of water extract	6.02-6.04
8	Volatile oil	Nil
9	Fibre content	49.00-53.00 %
10	Swelling index	4.00-5.00 ml/gm
11	Foaming index	<100
12	Total sugar	1.20-1.90 %
13	Reducing sugar	0.70-1.50 %

The details of observations recorded with respect to behaviour of different solvent extracts under visible and fluorescent light at 254nm & 366nm are given in Table 2.

Table 2: Fluorescence behaviour of different extracts of *Acacia catechu*

Sl.No.	Extractives	Visible light	Short UV	Long UV
1	Petroleum ether	Brown	Greenish brown	Greenish brown
2	Benzene	Colourless	Light yellow	Colourless
3	Acetone	Colourless	Light yellow	Colourless
4	Ethyl acetate	Light blue	Light yellow	Light blue
5	Ethyl alcohol	Light Brown	Bluish brown	Brown
6	Methyl alcohol	Brown	Yellowish brown	Bluish brown
7	Distilled water	Light Brown	Light brown	Brown

Antioxidant properties

The results of dot-blot assay showed yellow coloured spots when sprayed with DPPH solution (Figure-7) showing the plant extract to be antioxidant. When the plates were sprayed with DPPH solution in methanol (0.4mM), the regions where substances with antioxidant capacity occurred stained yellow in the purple back ground.

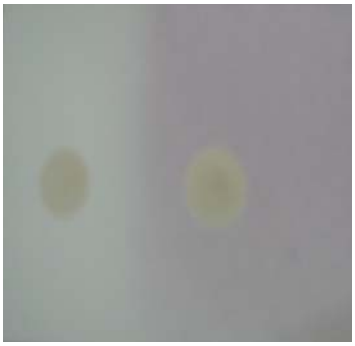


Fig.7: Dot blot assay

The TLC of the alcoholic plant extract conducted using CEF (chloroform-ethyl acetate -formic acid, 5:4:1) as mobile phase and DPPH as spray reagent, gave six major spots with yellow colour indicating that there are at least six antioxidant constituents in the extract. The more intense the yellow colour, the greater the antioxidant activity (Figure-8).

Radical scavenging activity was estimated by DPPH assay and the results were compared with that of ascorbic acid. Ascorbic acid at 66.12µM could scavenge half of DPPH (IC_{50}) when reacted for 30 minutes. IC_{50} value of the plant extract was found to be 61.72µM which is comparable with the values obtained for ascorbic acid (Figure-9).

HPLC studies revealed that the plant extract was rich in catechin (3.30%, Figure-10), rutin (1.51%, Figure-11) and isorhamnetin (1.22%, Figure-12). The retention times (R_t) observed for authentic samples of catechin, rutin and isorhamnetin, and those from *A. catechu* are given in Table 3.

Fig. 8: TLC of alcoholic plant extract

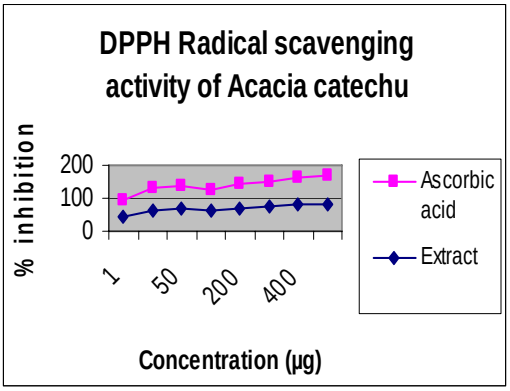


Fig. 9: Radical scavenging activity

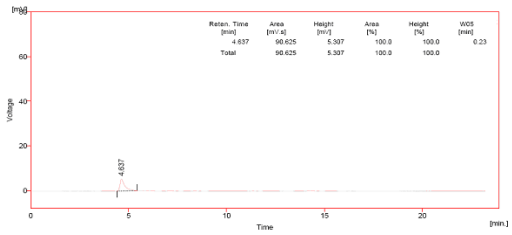
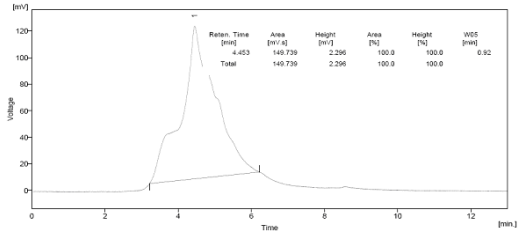


Fig. 10: (a) HPLC of Catechin (standard)



(b) Catechin in *A.catechu*

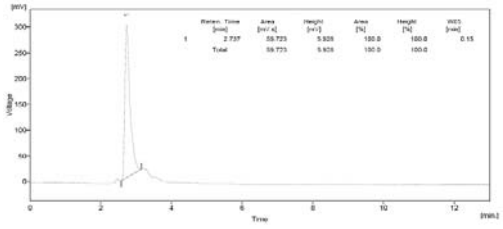
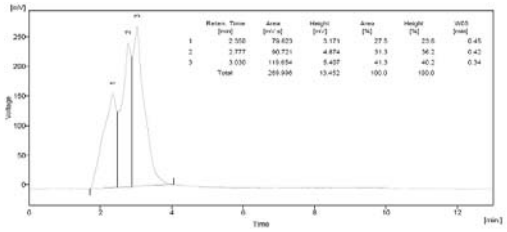


Fig. 11: (a) HPLC of Rutin (standard)



(b) Rutin in *A.catechu*

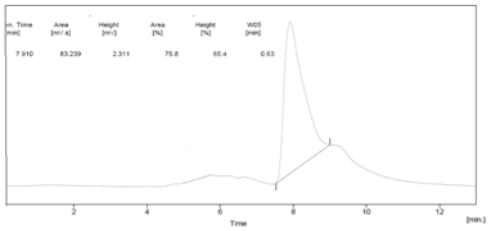
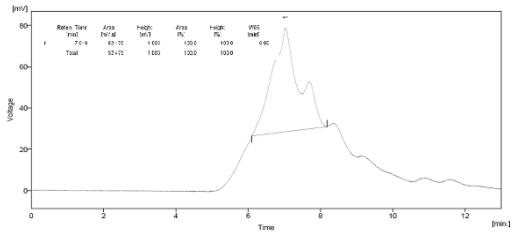


Fig. 12: (a) HPLC of Isorhamnetin (standard)



(b) Isorhamnetin in *A.catechu*

Table 3: R_t values of HPLC analysis

S No.	Compound	Retention time (Authentic sample)	Retention time (plant extract)
1	Catechin	4.637	4.453
2	Rutin	2.737	2.777
3	Isorhamnetin	7.960	7.670

The above chemical and botanical details are specific features of the heartwood of *Acacia catechu* Willd. which depend on its physical properties and chemical constitution. The dot-blot assay, TLC study and the DPPH assay showed that the plant extract is a highly effective antioxidant. Catechin, rutin and isorhamnetin are reported as free radical scavengers and these compounds largely contribute to the bio-potency of *Acacia catechu.*

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