HPTLC QUANTIFICATION & PHYTOCHEMICAL INVESTIGATION OF ROOTS OF ACACIA CATECHU WILDL

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ABSTRACT: Present work has been undertaken to establish the necessary pharmacognostic standards and phytochemical evaluation of roots of Acacia catechu Willd. Various morphological parameters of fresh as well as shade dried form of the roots were studied. Microscopy shows the presence of medullary rays, starch grains, pith, phloem fibres, cork cells and xylem cells. Physico-chemical constants such as Fluorescence analysis of root powder and extracts, ash values, loss on drying, extractive value, swelling index, percentage extractive values for successive extracts, consistency and color of different extracts under ordinary and UV light were evaluated. Phytochemical screening of total ethanolic and aqueous extracts shows the presence of flavonoids, tannins, saponin glycosides, & reducing sugars. HPTLC was carried out for quantification of quercetin in ethanolic extract of the roots of A. catechu Willd. It was concluded that plant contains various phytochemicals; among these tannins and flavonoids are its main constituents.

INTRODUCTION: Acacia is a genus of shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae. Acacia catechu Willd. (Synonym: Senegalia catechu) common names include Catechu, Cachou and Black Cutch is a deciduous, thorny tree which grows up to 15 m (50 ft) in height. Acacia catechu Willd. is widely 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. It is also found in Eastern slopes of Western Ghats. It is also distributed in Ganjam, Burma, throughout the Konkam, S.M. country & Deccan. Acacia catechu Willd. contains various phytochemicals. The seeds yield oil (3.5%) with neutral lipids, 55%; and polar lipids, 44.5%. The major fatty acids are oleic and linoleic. The leaves contain the trace elements: Cu, 8.90; Fe, 126.08; Mn, 25.31; and Zn, 24.26 ppm on dry matter basis. The chief constituents of the heartwood are catechin and catechutannic acid, acacatechin, epicatechin, catechin tetramer, dicatechin, gallicatechin, gossypin, kaempferol and dihydro derivative, taxifolin, procyanidine, isorhamnetin, (+) afzelchin and flavonoids like quercetin. Yadava RN & Sodhi S (2002) isolated a new flavone glycoside: 5,7,3’,4’-tetrahydroxy-3-methoxyflavone-7-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside from the stem. Catechu...
resin contains catechin, catechu-tannic acid & tannins. Acid hydrolysis of the gum afforded L-arabinose, D-galactose, Dvhamnosne, aldobiouronic acid (6-β-D-glucoronosyl-D-galactose), 6-O-β-D-glucopyranosyluronic acid-D-galactose, 3-O-β-D-galactopyranosyl – D - galactose, 3 – O – β – D - galactopyranosyl (1→3)–O-β-D-galactopyranosyl (1→3)-D-galactopyranose quercetin etc. A. Catechu Willd. plant possess various biological activities like antipyretic, hepatoprotective hypoglycaemic & anti diarrhoeal activity; antimicrobial activity and found effective in the treatment of lepromatous leprosy. The leaves, roots & bark of Acacia catechu Willd. showed potent anti-mycotic activity. The plant is used for hypotensive activity, antifertility activity and immunomodulatory activity.

The present study established the necessary pharmacognostic standards and phytochemical constituents for evaluation of roots of Acacia catechu Willd. because roots are the essential part of plant and contains tannins and flavonoids which shows various therapeutic activity like antioxidants, hepatoprotective, wound healing, etc. Therefore, the present study was carried out to standardize the roots using chemical, botanical and analytical means i.e. HPTLC so that this might be an important tool of identification for herbalists.

MATERIALS AND METHODS:
Plant material and extract preparation:
Roots of Acacia catechu Willd. were collected from the beat of Trilokpur block, Village Bhood under the range of Raipur Rani, Panchkula, after having permission of Forest Department of Raipur Rani, Panchkula. The plant material was identified by Dr. H.B. Singh (Scientist F and Head, Raw Materials Herbarium and Museum, NISCAIR, Delhi) under a voucher specimen number-NISCAIR/RHMD/Consult/-2009-10/1278/82 dated Oct. 1, 2009. The roots were cut into small pieces, then, subjected to shed drying and further crushed to coarsely powder.

The shade dried and powered root was subjected to maceration with different solvents viz. petroleum ether, chloroform, ethyl acetate, ethanol (95%) and finally with water to get respective extracts. All extracts were individually filtered and evaporated to dryness. The dried extracts were weighed and percentage yields were determined respectively and stored in freeze condition for further use.

Pharmacognostical evaluation:

Chemicals and instruments:
Solvents viz. petroleum ether, chloroform, ethyl acetate, ethanol (95%), and reagents viz. phloroglucinol, glycerine, HCl, chloral hydrate and sodium hydroxide were procured from RFCL, Mumbai, India. Photographs of tissue arrangement were taken with Labomed ATC-2000 microscope attached with Sony camera. HPTLC was done using CAMAG HPTLC densitometer.

Macroscopic and microscopic analysis:
The colour, shape, size, odour, fracture and surface texture of dried roots were observed. For microscopic study, thin hand sections were prepared and cleared with chloral hydrate; stained with phloroglucinol and hydrochloric acid, then mounted in glycerin for the identification of various regions. For powder study, powder (sieve no. 60) of dried root was taken, separately treated with phloroglucinol and hydrochloric acid, glycerin, iodine solution, ruthenium red solution, safranin solution. (Fig. 1-4)

Fluorescense study:
The powder material was treated separately with different reagents and exposed to visible and ultraviolet light (Table 2). The Fluorescence nature of different extracts of roots was studied by using a minute quantity of petroleum ether, chloroform, ethyl acetate, ethanol and water extract (Table 3). The extracts were put on the slide and observed under visible and UV light. (Table 2 & 3)

Physicochemical parameters:
Physicochemical parameters adopted to confirm the purity and quality of drug. Total ash, water-soluble ash and acid-soluble ash were determined. Ethanol-soluble, and water-soluble extractive values were determined. Loss on drying and swelling index was also determined. Preliminary phytochemical screening was carried out (Table 4), by using standard methods, to identify the presence of various phytoconstituents. (Table 4)
Quantification of Quercetin by HPTLC:
A HPTLC densitometric method was developed for quantification of quercetin in the ethanolic extract of roots of *Acacia catechu* Willd. (Fig. 5-7) Sample was applied using CAMAG Linomat 5 "unknown" S/N 0.00 (00.00) instrument, with application parameters (Spray gas: Inert gas; Sample solvent type: Methanol; Dosage speed: 150 nl/s; Pre-dosage volume: 0.2 μl) & sequence (Syringe size: 100 μl; Number of tracks: 4; Application position Y: 10.0 mm; Band length: 8.0 mm)

**TABLE 1: SEQUENCE OF APPLICATION OF SAMPLE & STANDARD ON TLC PLATE**

<table>
<thead>
<tr>
<th>No.</th>
<th>Appl. Position</th>
<th>Appl. Volume</th>
<th>Vial#</th>
<th>Sample ID</th>
<th>Active</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.0mm</td>
<td>5.0 μl</td>
<td>1</td>
<td>Sample A</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>29.0mm</td>
<td>10.0 μl</td>
<td>1</td>
<td>Sample A</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>43.0mm</td>
<td>5.0 μl</td>
<td>2</td>
<td>Std-Quercetin</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>57.0mm</td>
<td>10.0 μl</td>
<td>2</td>
<td>Std-Quercetin</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Development of TLC:
TLC was developed in glass tank (Twin Trough Chamber 10x10cm), pre-conditioning mobile phase [Toluene: Ethyl acetate: Methanol (4.4:5:0.6)] and dried at 60 °C using hair dryer for 5 Minutes.

RESULTS AND DISCUSSION:

**Pharmacognostic Studies:**

**a) Morphological studies:** *Acacia catechu* Willd. roots was found reddish brown in colour, disagreeable odour, fibrous and hard to fracture, 5-40 inches length and 0.5-10 cm diameter size, cylindrical shape, fibrous texture, rough touch and also showed the presence of adventitious roots

**b) Microscopical studies:**
Transverse section of roots of *A. catechu* Willd. has a spherical transaction which showed the presence of pith, xylem cells, medullary rays, vessels, epidermis and grains. Powder Microscopy of *A. catechu* Willd. showed the presence of cork cells, fibres & pitted cells.
Fluorescence analysis

The Fluorescence nature of different extracts of roots was observed by using a minute quantity of petroleum ether, chloroform, ethyl acetate, ethanol and water extract under visible and UV light. The dried root powder treated with different chemical reagents viz. 1N NaOH in methanol, 1N NaOH in water, 1N HCl, 50% H₂SO₄, 50% HNO₃, 50% HCl and change in colour was observed under UV light.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Treatment</th>
<th>Visible (400-800nm)</th>
<th>U.V. short (254 nm)</th>
<th>U.V. Long (366 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Powder as such</td>
<td>Light Brown</td>
<td>Light Green</td>
<td>Brown</td>
</tr>
<tr>
<td>2.</td>
<td>1N NaOH in Methanol</td>
<td>Brown</td>
<td>Dark Green</td>
<td>Brown</td>
</tr>
<tr>
<td>3.</td>
<td>1N NaOH in Water</td>
<td>Brown</td>
<td>Yellowish Brown</td>
<td>Brown</td>
</tr>
<tr>
<td>4.</td>
<td>1N HCl</td>
<td>Brown</td>
<td>Yellowish Brown</td>
<td>Dark Brown</td>
</tr>
<tr>
<td>5.</td>
<td>50% HNO₃</td>
<td>Dark Brown</td>
<td>Dark Green</td>
<td>Black</td>
</tr>
<tr>
<td>6.</td>
<td>50% HCl</td>
<td>Brown</td>
<td>Dark Green</td>
<td>Dark Brown</td>
</tr>
<tr>
<td>7.</td>
<td>50% H₂SO₄</td>
<td>Brown</td>
<td>Light Green</td>
<td>Blackish brown</td>
</tr>
</tbody>
</table>
Physicochemical constants parameters

Total ash 1.8% w/w, water-soluble ash 0.8% w/w, acid-insoluble ash 0.3% w/w, ethanol-soluble extractive value 5.94% w/w, water-soluble extractive values 5.46% w/w, loss on drying 10.4% w/w and swelling index Nil were calculated.

Preliminary phytochemical screening of roots revealed the presence of flavonoids, tannins, saponin glycosides, reducing sugars and lipids.

Quantification of Quercetin by HPTLC:

Quantity of Quercetin in ethanolic extract of roots of A. catechu Willd. determined by HPTLC was 2.11% w/w.
CONCLUSION: From the present study, it was concluded that the plant contains tannins and flavonoids in its roots as main constituents and can be used pharmacologically in treatment of diseases. As tannins and flavonoids further contribute to antioxidant activity, antiulcer and hepatoprotective activity; so these beneficial characters of the plant can be best utilized in the form of medicament to treat such ailments. Moreover, there is a scope of manufacturing formulation either alone or in combination with other herbal extracts to prevent or treat various ailments.

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REFERENCES: