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Preliminary Pharmacognosy Evaluation of The Bark of *Acacia Catechu* (Wild.)

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ABSTRACT: -

Khadira (*Acacia catechu* Wild, family: Fabaceae and subfamily: Mimosoideae), is considered as one of the most potent medicine used for various skin diseases in Ayurveda. It is widely used herb in Indian traditional system of medicine. The sample of *Acacia catechu* (Wild.) bark (*Khadira Tvaka*) collected from market. The diagnostic characters of bark of this plant include astringent and bitterin taste, odourless, light brown or brownin colour, Tracheid, Fiber, Calcium oxalate crystals and Starch grains. Physicochemical studies revealed moisture content (2.88%), pH value (5.6), alcohol (3.00%) and water soluble extractive value (3.09%), total ash (1.24%), acid insoluble (0.34%) and water soluble ash (0.89%). Preliminary analysis of various functional groups revealed the presence of carbohydrate, alkaloids, amino acids, protein, saponin, phenolic compound, tannins and Thin Layer Chromatography (TLC) etc. The information generated by this particular study will provide relevant Pharmacognostical and physicochemical data needed for proper identification, authentication, purity, safety and efficacy of the drug.

Keywords: *Acacia catechu* (Wild.), *Khadira*, *Tvaka*, Pharmacognosy.

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INTRODUCTION:

Acacia catechu (Wild.), belonging to family Fabaceae and subfamily Mimosoideae is distributed throughout forests of India, but very common in Sub – Himalayan tract of Punjab to Assam ascending to 1200 m, Peninsular region, particularly in drier parts, Madhya Pradesh, Maharashtra, Gujarat, Bihar, Rajasthan, Tamil Nadu and Eastern slopes of Western Ghats. *Acacia catechu* (Wild.) is known as *Khair* locally and Hindi, *Kherio baval* in Gujarati, *Karingali* in Malayalam, *Kaderi* in Marathi and Cutch tree in English etc.[1]

The historical evidence of *Khadira* is traced from *Vedic* period, *Samhita* period and ancient *Nighantu* period to current modern texts. *Khadira* is a very famous skin benefiting *Ayurvedic* drug. The drug is used in *Dantya* (improves quality of teeth's), *Kandughna* (relieves itching), *Kasaghna* (useful in cough), *Medoghna* (useful in obesity), *Krimighna* (useful in worm infestation), *Mehaghna* (useful in diabetes), *Jvaraghna* (antipyretic), *Shvitraghna* (useful in leukoderma), *Shothahara* (anti-inflammatory), *Amahara* (relieves *aam)* , *Panduhara* (useful in anemia) and *Kushthahara* (useful in skin diseases).[2] *Khadira* has *Tikta - Kashaya Rasa*(pungent-astringent), *Laghu–Ruksha Guna* (light-rough), *Shita Virya,* (cold) *Katu Vipaka, Kushthaghna Prabhava* and

Kaphapittashamaka Karma etc.[3] and attributed *Ruchivardhaka, Stambhana, Shonitasthapana, Mutrasangrahana, Kushthaghna, Kandughna* and *Vranaropaka* (wound healer) properties. Major chemical constituents of *Acacia catechu* (Wild.) are catechin, epicatechin, apicatechingallate, procatechinic acid, tannin, alkaloids quercetin and kaempferol, porifera sterol glucosides, (+)-afzelechin gums are also present in minor quantity.[4] In *Bhavprakash Nighantu* two varieties are mentioned *Khadira* (*Acacia catechu* Wild.) and *Swetakhadira* (*Acacia suma* Buch. – Ham.).[5] In *Dhanvanatari Nighantu* two varieties are described *Khadira* (*Acacia catechu* Wild.) and *Somavalka* (*Acacia suma* Kurz.).[6] In *Raja Nighantu* three types are mentioned *Khadira* (*Acacia catechu* Wild.), *Swetasara* (*Mimosa sama* Robx.) and *Raktakhadira* (Red *Mimosa sama* Robx.).[7] Authentication of the *Acacia catechu* (Wild.) bark (*Khadira Tvaka*) on macroscopic and microscopic level is the need of hour because various drugs are added as adulteration and substitute in market samples of *Khadira Tvaka* (skin). This, study is aimed for the same.

MATERIALS AND METHODS:

Microscopic, physicochemical and phytochemical study including quantitative analysis of *Acacia catechu* (Wild.) bark (*Khadira Tvaka*) was done to determine the diagnostic features for the identification and standardization of intact and powdered drug.

Collection of sample:

Acacia catechu (Wild.) bark (*Khadira Tvaka*) was purchased from market (Shri ram Herbals) Jaipur district, Rajasthan state. The crude drug was identified and authenticated by CSIR - National Institute of science Communication and Information Resources, New Delhi - 110012 (NISCAIR), vide reference number NISCAIR/RHMD/Consult/2019/3487-88-3 as *Acacia catechu* (Wild.) and belong to family Fabaceae and subfamily Mimosoideae. After identifying the plant, bark of *Khadira* were powdered, labelled, packed and subjected for organoleptic and other analytic studies.

Moisture Content[8]: Moisture content was determined by placing weighed sample of 5 g of drug in oven at 105º for 5 hours, and calculated weight of sample for every 30 minute, until the weight of the sample came out to be constant, no variation of weight was recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before weighing.

pH[9]: Immerse pre calibrated electrode of pH meter in 5 % w/v solution of sample and note down vale of pH.

Extractive values Aqueous /Alcoholic[10]:

5 g coarsely powdered air dried drug was macerated with 100 ml of Distilled Water/Alcohol of the specified strength in a closed flask for twenty-four hours. It was then continuously shaken for six hours using rotary shaker and allowed to stand for eighteen hours. The content was filtered using filter paper. The filtrate was transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then, the dish was kept in oven at 105º, to constant weight and weigh. The percentage of Aqueous/Alcohol-soluble extractive was calculated with reference to the air-dried drug.

Ash value[11]:

Total Ash: - Weighed accurately 2 g of the air-dried drug in a silica dish and incinerated at a temperature not exceeding 450ºC until free from carbon. Then, cooled and weighed. Percentage of ash value was calculated on the basis of air - dried drug.

Acid Insoluble Ash: - Boiled the total ash with 25 ml of 2M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible or on an ash less filter paper, washed with hot water, ignite, cool in a desiccator and weighed. Calculate the percentage of acid - insoluble ash with reference to the air - dried drug.

Water – Soluble Ash: - Boiled the total ash for 5 minutes with 25 ml of water; collected the insoluble matter in a Gooch's Crucible or on an ash less filter paper, Washed with hot water and ignite for 15 minutes at a temperature not exceeding 450ºC. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represented the water – soluble ash. Calculate the percentage of water – soluble ash with reference to the air - dried drug.

Qualitative analysis of Photochemical (Primary and Secondary Metabolites):

Name of Test	Procedure	Observation	Result
Carbohydrate			
Molish's test	2 ml Test Solution + 2 ml Molisch's reagent & shake carefully + 1ml. of conc. H_2SO_4 Wait for one 1 minute.	A Purple colour ring at the junction of the two layers	Carbohydrate present
Benedict's test	4 ml Test solution + 1 ml Benedict's solution + ▲	Formation of green, yellow, orange, red or brown colour in order of increasing concentrations of simple sugar in the test solution, due to formation of cuprous oxide.	Reducing sugars present
Fehling's test	Fehling A 1 ml + Fehling B 1 ml + 2 ml Test solution + ▲	Brick Red ppt.	Generally used for reducing sugars

Alkaloids			
Dragendorff's test	2 ml test Solution + 2 ml Dragendorff's reagent	Orange precipitate	Alkaloids present
Wagner's test	Test solution + few drops of Wagner's reagent	Reddish - brown precipitate	Alkaloids present
Hager's test	Test solution + Hager's reagent	Orange yellow precipitate	Alkaloids present
Amino acids			
Ninhydrin test	Test solution + Ninhydrin + ▲	Characteristic deep blue or pale yellow colour	Presence of alpha-amino acids and proteins containing free amino groups.
Protein			
Biuret test	Test solution + 1 ml of 4% NaOH solution + 1 drop of 1% solution of $CuSo_4$.	Development of violet or pink colour	Presence of proteins.
Xanthoproteic test	Test sample + 2 ml of water + 0.5 ml of conc. HNO_3	Development of yellow colour	Presence of proteins.
Millon's test	Test solution + 2-3 ml of Millons reagent were added.	White precipitate slowly turning to pink	Presence of proteins.
Saponin			
Foam test	Test solution + water+ shake	A stable, characteristic honeycomb like froth	Presence of saponins.
Glycosides			
Borntragor's test	1 ml Benzene + 0.5 ml Dil. NH_4 Sol. + Test Solution	Formation of reddish pink colour.	Presence of anthrax Quinone glycosides
Phenolic compound			
Phenolic test	Test Solution + ▲ + 2 ml of $FeCl_3$ sol.	Formation of green and blue colour.	Presence of phenols
Steroids			
Salkowaski reacton	Test Solution + 2 ml of chloroform + 2 ml of conc. H_2SO_4& shake for few minutes	Development of red colour	Presence of steroids.
Tannins			
$FeCl_3$ test	Test Solution + 5 % solution of $FeCl_3$ in 90 % alcohol	Appearance of dark green or deep blue colour	Presence of tannins.
Lead acetate test	Test Solution + 10 percent w/v solution of basic lead acetate in distilled water	Development of precipitate	Presence of tannins.
Pot. Dichromate test	Test Solution + Potassium dichromate Solution	Appearance of dark colour	Presence of tannins.

Thin Layer Chromatography (TLC): -

Chromatography plates: - T.L.C. plate coated with 0.25 mm layer of silica gel 60 F_{254} **Activation of pre-coated Silica gel 60 F_{254}: -**Plates were dried in hot oven at 105^0 C for one and half hour.

Preparation of mobile solution: - Toluene: Ethyl acetate: Formic acid (5:4:1)

Preparation of test solution: - 4 g powdered drugs were extracted with 100 ml of ethanol (90%) in a Soxhlet's apparatus consecutively three times. Extract was filtered and concentrated to 10 ml.

Sample application: - Samples were applied with the help of capillary 1 (one) cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1 (one) cm below the top of the T.L.C. plate.

Visualization: - Anisaldehydesulphuric acid spray.

R_f Value: -Measured and recorded the distance of each spot from the point of its application and calculated R_f value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

OBSERVATIONS AND RESULTS:

The different pharmacognosy parameters were studied and evaluated in order to standardize the drug. The results of pharmacognosy parameters i.e. microscopic study, physicochemical parameters, phytochemical analysis and TLC have b een cited in below.

Macroscopic study of *Acacia catechu* (Wild.) bark (*Khadira Tvaka*): -

Fig. No. 1. *Acacia catechu* (Wild.) bark (*Khadira Tvaka*)

Table No. 1. Macroscopic examination of *Acacia catechu* (Wild.) bark (*Khadira Tvaka*)

S. No.	Observed	*Acacia catechu* (Wild.) bark (*KhadiraTvaka*)
1.	Colour	Light brown or brown
2.	Odour	Odourless
3.	Taste	Astringent and bitter

Powder microscopic study of *Acacia catechu* (Wild.) bark (*Khadira Tvaka*): -

In powder microscopy, structure like Tracheid, Fiber, Calcium oxalate crystals and Starch grains were seen.

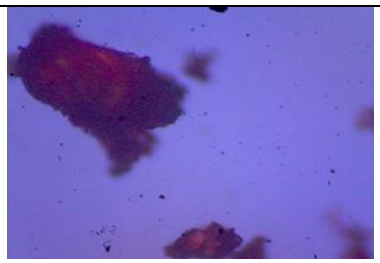
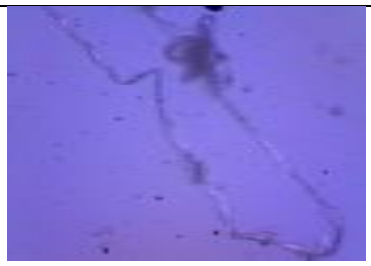
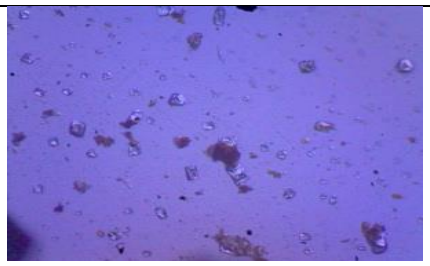
		
Tracheid	**Fiber**	**Calcium Oxalate and Starch Grain**

Fig. No. 2. Powder microscopic characteristic of *Acacia catechu* (Wild.) bark (*Khadira Tvaka*)

Physicochemical study:

In this study, moisture content, pH, extractive value (alcohol and water soluble extractive value) and ash values (total ash, acid insoluble ash and water soluble ash) were determined

.

Table No. 2. Physiochemical analysis of *Acacia catechu* (Wild.) bark (*Khadira Tvaka*) powder

S. No.	Physiochemical Standards (Bark)	Results % w/w
1.	Moisture content	2.88%
2.	pH value	5.6
3.	Water soluble extractive value	3.09%
4.	Alcohol soluble extractive value	3.00%
5.	Total ash	1.24 %
6.	Acid insoluble ash	0.34 %
7.	Water soluble ash	0.89 %

Phytochemical analysis:

Phytochemical are nutritive plant chemicals that have protective or disease preventive properties. A plant cell produces two types of metabolites- primary metabolites involved directly in growth and metabolism (carbohydrates, lipids and proteins etc.) and secondary metabolites not involved in metabolic activity (alkaloids, phenols and sterols etc.) but act as defence chemicals. The preliminary phytochemical investigations of aqueous and alcohol extract of *Acacia catechu* (Wild.) bark (*Khadira Tvaka*) were performed which reveals the presence of carbohydrates, alkaloids, amino acids, saponin, glycosides, steroids and tannins.

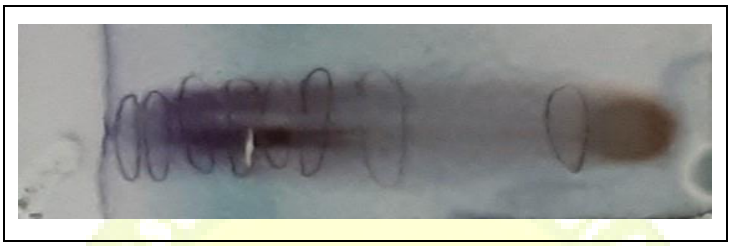
Table No. 3. Phytochemical analysis of *Acacia catechu* (Wild.) bark (*KhadiraTvaka*) powder

Name of test	*Acacia catechu* (Wild.) bark (*Khadira Tvaka*)	
	Aq.	Al.
(+ve) = Positive and (-ve) = Negative		
Carbohydrate test		
Molish test	+ve	+ve
Benedict test	+ve	+ve
Fehling test	+ve	+ve
Alkaloids test		
Dragendorff test	-ve	+ve
Wagner's test	-ve	-ve
Hager's test	-ve	-ve
Amino acids		
Ninhydrin test	+ve	+ve
Proteins		
Biuret test	-ve	-ve
Xanthoproteic test	+ve	+ve
Millon's test	+ve	+ve
Saponin		
Foam test	+ve	-ve
Glycosides		
Borntragor's test	-ve	+ve
Phenolic compound		
Phenolic test	+ve	+ve
Steroids		
Salkowaski reaction	-ve	-ve
Tannins		
$FeCl_3$ test	-ve	+ve
Lead acetate test	+ve	+ve
Pot. Dichromate test	-ve	+ve

Table No. 4. Thin Layer Chromatography of *Acacia catechu* (Wild.) bark (*Khadira Tvaka*) powder

Sample	*Acacia catechu* (Wild.)
R_f value	0.11, 0.45, 0.59, 0.65, 0.72, 0.81, 0.88, 0.95

Fig.No. 3. Thin Layer Chromatography of *Acacia catechu* (Wild.) bark (*Khadira Tvaka*) powder



DISCUSSION:

Acacia catechu (Wild.) bark (*Khadira Tvaka*) is astringent and bitter in taste, odorless and light brown or brown in color. Powder microscopic study of bark powder of *Acacia catechu* (Wild.) revealed Tracheid, Fiber, Calcium oxalate crystals and Starch grains after observation under microscope. Loss on drying is a water holding property of test substance. Moisture content and pH value was found to be 2.88% and 5.6. Extractive value is directly relative to strength or potency of drug which estimates in different solvents. Water soluble extractive value and alcoholic extractive value in sample were found 3.09% and 3.0%. Ash value is the indicator of the presence of inorganic and earthy matter in the plant. The higher ash value is suggestive of thermo – non labile / heat stable or inorganic constituents. The total ash value in sample was 1.24%. The acid insoluble content which indicates the presence of siliceous matter and heavy metals in sample found 0.34%. Water soluble ash estimates the inorganic water soluble salt was found 0.89%in sample. Qualitative analysis of inorganic matter showed the presence of carbohydrate, alkaloid, amino acid, protein, saponin, glycosides, phenolic compound and tannin in *Acacia catechu* (Wild.) bark powder. Thin layer chromatography establishes the phytochemical fingerprint profiling in drug for identity.

CONCLUSION:

Acacia catechu (Wild.) is a well-known *Ayurveda* plant. After performing the work, it was found that the phytochemical screening confirmed the presence of various phytochemical constituents such as carbohydrates, amino acid, protein, tannin, phenolic compound, saponin, glycoside and alkaloid. Different physicochemical parameters such as loss on drying value, pH value, water and alcohol soluble extract value, total ash, acid insoluble ash, water soluble ash, and R_f value were observed. These values can be useful to detect purity, safety and efficacy of the drug. All studied standardization parameters like pharmaco-gnostical, phytochemical and physicochemical analysis provide the knowledge in the identification and authentication of *Acacia catechu* (Wild.) bark (*Khadira Tvaka*).

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Conflict of Interest: Nil

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