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RESEARCH ARTICLE

PHARMACOGNOSTIC AND CHROMATOGRAPHIC PROFILING OF KHADIRA (*ACACIA CATECHU* (LINN.F.) WILLD).

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Abstract

Introduction: Khadira is an Ayurvedic plant drug extensively used in Ayurvedic formulations. The source plant of Khadira, *Acacia catechu* (Linn.f.) Willd, is a moderate sized tree commonly growing in dry climatic conditions. Its heartwood is used for preparing Ayurvedic medicines. Ascertaining the identity, genuineness and purity of herbal drugs has an important role in the maintenance of the quality of Ayurvedic formulations. Hence the present work is taken up with an objective to standardize the heartwood of Khadira with detailed pharmacognostical and preliminary phytochemical standards.

Methods: Genuine heart wood of *Acacia catechu* (Linn.f.) Willd (Khadira) was collected from botanical garden of Government Ayurveda College, Poojappura, Thiruvananthapuram and shade dried. Macroscopic, physicochemical, chromatographic and heavy metal analysis was carried out as per ICMR procedures.

Result: Heart wood is light red to brownish red in colour, hard and even textured. On physicochemical evaluation water soluble extractive value was 6.1 + 0.047 & alcohol soluble extractive value was 1.5 + 0.032. Heavy metals copper, cadmium, iron and lead level was found to be 0.1323+ 0.004, 0.1346 + 0.012, 7.888+ 0.061 & 0.0685 + 0.043 respectively. HPTLC was carried out with Toluene: Ethyl acetate: Formic acid as solvent system and 3 spots were obtained.

Conclusion: The results obtained in this study may help in standardization, identification and further research in *Acacia catechu* (Linn.f.) Willd (Khadira).

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Introduction:-

Khadira is an Ayurvedic plant drug extensively used in Ayurvedic formulations. According to *Caraka Samhitha* (an ancient Indian medical literature), *Khadira* is grouped under *Kushaghna* (that alleviates skin diseases) and *Udarda prasamana varga* (that which relieves urticaria) based on its pharmacological properties. It is the herbal drug which is described as a drug of choice in the treatment of *kushta* (skin diseases) in classical Ayurvedic text books¹. The source plant of *Khadira* is *Acacia catechu* (Linn.f.) Willd. *Acacia catechu* (Linn.f.) Willd (*Khadira*) is a moderate sized tree mainly found in western ghats and Himalayan region². It has an antimicrobial action and works as a mouth freshener diminishing bad breath. The drug is also observed to be an important ingredient in the formulations

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described in the treatment of skin diseases and oral diseases.

Khadira (*Acacia catechu* (Linn.f.) Willd) is a drug widely used in the pharmaceutical industry. It is an ingredient of several ayurvedic formulations like *Khadirarishtam*, *Kanakabindvarishtam*, *Katakakhadiradi kashayam*, *Musalikhadiradi Kashayam* etc³. *Ascertaining the identity, genuineness and purity of herbal drugs has an important role in the maintenance of the quality of Ayurvedic formulations.* This indicates the need to assess the genuineness of *Khadira* through pharmacognostical and phytochemical evaluation.

The objective of the present study is to evaluate the preliminary pharmacognostical phyto-chemical characters of the drug.

Materials And Methods:-

Sample collection- The sample drug *Khadira* (*Acacia catechu* (Linn.f.) Willd) was collected from herbal garden at Pharmacognosy Unit, Govt. Ayurveda College, Thiruvananthapuram and the sample was authenticated by Botanist, Drug Standardisation Unit, Govt. Ayurveda College Thiruvananthapuram, Kerala.

Study settings

Drug Standardisation Unit, Govt. Ayurveda College Thiruvananthapuram, Kerala.

1. Macroscopic evaluation- Fresh log of wood of *Acacia catechu* (Linn.f.) Willd was collected. The bark and the sapwood were removed and the heartwood was collected, shade dried and stored. The heartwood of *Acacia catechu* (Linn.f.) Willd was subjected to organoleptic evaluation and the observed macroscopic characters were recorded.

2. Microscopic evaluation- Microscopic evaluation is used for studying the histological features of transverse and longitudinal section of heartwood of *Acacia catechu* (Linn.f.) Willd. A portion of the heartwood was selected, soaked in water overnight and enough number of sections were taken. Transverse and longitudinal sections were prepared and stained as per standard procedure⁴. The sections were carefully transferred to a petri dish containing water and few thin sections that floated in water were selected. A stained section was carefully transferred on a clean glass slide using thin brush. With the help of forceps and needle a clean cover slip was gently placed over the section. Excess water was wiped with a blotting paper and the slide was placed on the stage of a digital microscope (Olympus digital- CS41, Japan, with CCD camera) for histological examination and direct images were taken.

3. Preliminary physical and phytochemical evaluation

Preliminary physicochemical and phytochemical analysis of genuine sample of the study were conducted. Physicochemical analysis included the evaluation of parameters like foreign matter, moisture content, ash values, fibre content, volatile oil content, sugar content and different extractive values, were done. It also included the qualitative tests for the detection of phenols, steroids, alkaloids, flavonoids, tannins and glycosides. The procedures were done as per procedures mentioned in ICMR⁵ and Ayurvedic Pharmacopoeia of India⁶.

Heavy metal analysis by Atomic Absorption Spectroscopy and chromatographic techniques like TLC, HPTLC were also conducted.

Results And Discussion:-

The organoleptic evaluation and the microscopy of heartwood of *Acacia catechu* were conducted and the observations were noted. The data was analysed and results were interpreted from it.

1. Macroscopic evaluation

The observed macroscopical characters of heart wood of *Acacia catechu* (*Khadira*) are given in Table 1.

Table no.1 Macroscopic evaluation of heart wood of *Acacia catechu*

Characters	Heart wood of <i>Acacia catechu</i>
External surface	even textured, hard
Colour	Light red to brownish red in colour
Odour	Characteristic
Taste	Astringent

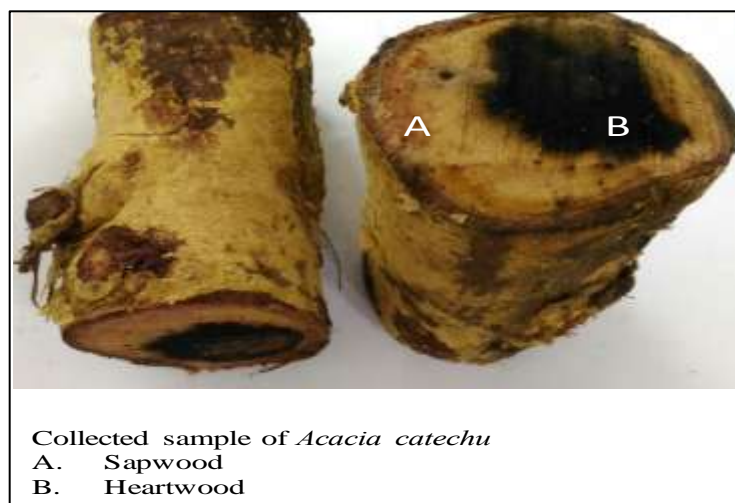


Fig 1:- Collected sample of *Acacia catechu*

II. Microscopic evaluation

The microscopic evaluation of *Acacia catechu* was carried out. Transverse section of heart wood showed vessels occurring isolated or in small groups. Vessels were often blocked with tyloses. Biseriate to multi seriate medullary rays were seen. Medullary rays were seen as vertically running linear bands. Multiseriate medullary rays and pitted border vessels were seen.

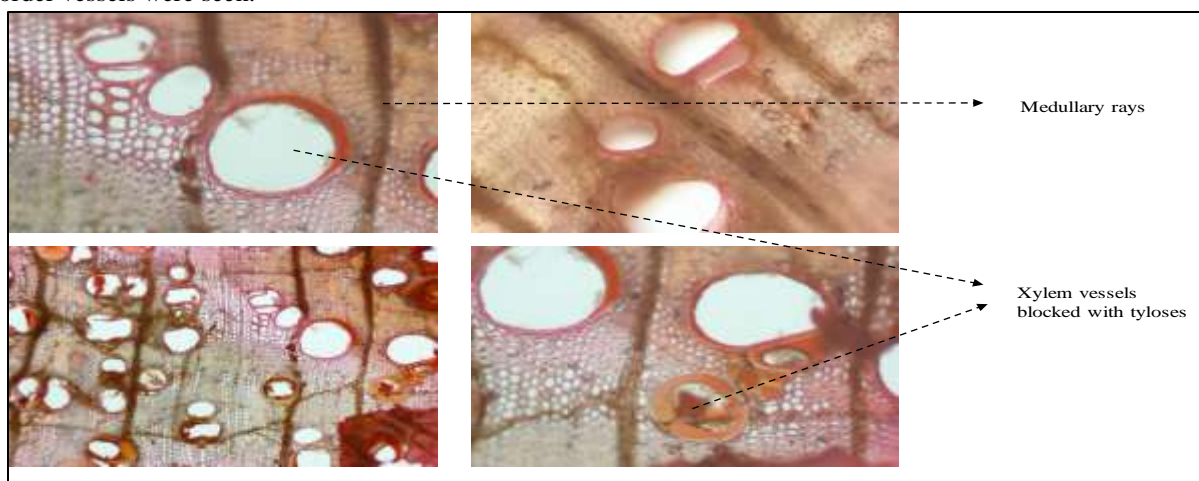


Figure 2:- Transverse section of heartwood of *Khadira*



Figure 3:- Longitudinal section of heartwood of *Khadira*

Results of preliminary physicochemical evaluation

The screening of physical and phytochemical parameters of *Acacia catechu* were done as per the standard procedures. Physical characters like moisture content, total ash, acid insoluble ash, water extractive and alcohol soluble extractives were analysed.

Table No.2 Preliminary physicochemical analysis

SI No	Name of the experiment	<i>Acacia catechu</i>
1	Foreign matter (%)	Nil
2	Moisture content (%)	10 +/- 0.335
3	Volatile oil (%)	Nil
4	Total ash (%)	1.9 +/-0.217
5	Acid insoluble ash (%)	1.1 +/-0.012
6	Water soluble extractive (%)	6.1 +/-0.047
7	Alcohol soluble extractive (%)	1.5 +/-0.032
8	Fibre content (%)	45.24 +/-1.56
9	Sugar content	
	Reducing sugar (%)	0.93 +/-0.204
	Total sugar (%)	1.20 +/-0.276

IV. Results of preliminary phytochemical evaluation

The ethanolic extractive obtained was subjected to qualitative analysis for identification of various plant constituents like steroids, phenols, alkaloids, flavonoids, tannins and results were summarized in Table No:3. The presence of various phytochemicals determines the pharmacological action and therapeutic potential of that plant. The preliminary phytochemical screening of heartwood showed the presence of different phytoconstituent groups such as steroid, flavonoid, saponin, alkaloid and tannin.

Table 3:- The results of qualitative chemical evaluation of alcoholic extract

SI no	Chemical constituent	<i>Acacia catechu</i>
1	Steroid	-
2	Flavonoid	++
3	Phenol	+
4	Alkaloid	+
5	Tannin	++
6	Saponin	+

V. Results of TLC and HPTLC

The spots obtained in TLC gives an idea about the plant constituents. The best separation was achieved using Toluene: Ethyl acetate: Formic acid in the ratio 6:3:1. The plates were first viewed through UV-fluorescence viewing cabinet (365 nm) and the Rf values of the spots were noted (Table No: 4). HPTLC was done and the HPTLC profile was shown in figure 4 and its 3D scan profile was shown in figure 5.

Table 4:- Rf values of spots obtained in Chromatography

Solvent system with ratio	Sample	No of spots	Rf value
Toluene:Ethyl acetate: Formic acid. 6:3:1	Ethanolic extract of <i>Acacia catechu</i>	3	0.84
			0.75
			0.13

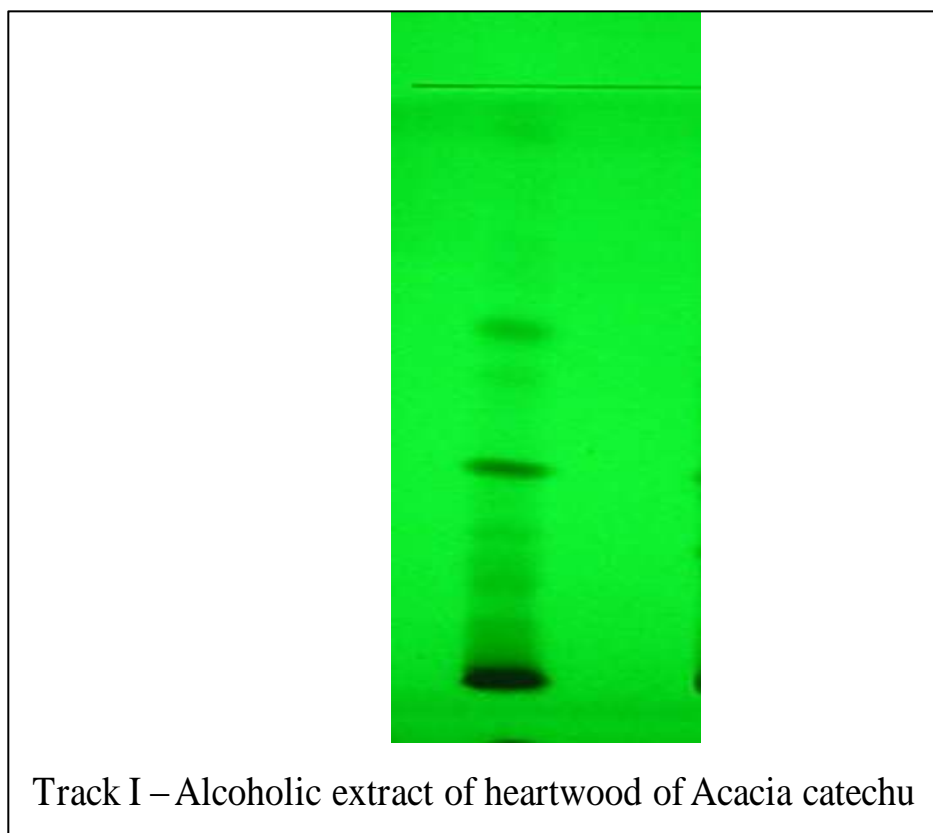


Figure 4:- HPTLC of heartwood of *A catechu* with solvent system Toluene:Ethyl acetate: Formic acid 6:3:1

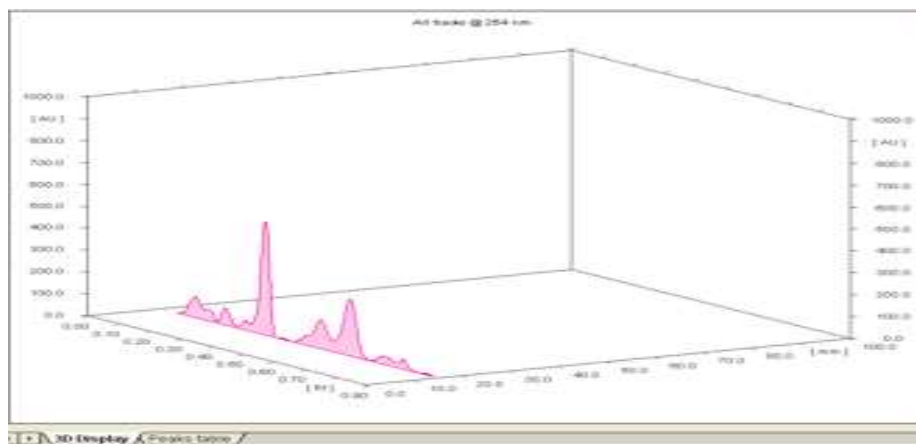


Figure 5: 3D scan profile of HPTLC of heartwood of *A catechu* with solvent system Toluene: Ethyl acetate: Formic acid 6:3:1 at 254 nm

VI. Results of Atomic Absorption Spectroscopy

Atomic absorption spectroscopy is used in the determination of heavy metal elements and some non-metal elements in atomic state and the results are shown in Table No: 5. Four heavy metals- copper, cadmium, iron and lead contents were analysed and found within permissible limits. Hence the drug is not contaminated by heavy metals and can be used safely for internal administration.

Table 6:- Atomic absorption spectroscopy

Samples	Heavy metal concentration (in ppm)			
	Cu	Cd	Fe	Pb
Heartwood of <i>A catechu</i>	0.1323 +/- 0.004	0.1346 +/- 0.012	7.8888 +/- 0.061	0.0685 +/- 0.043

Conclusion:-

Macroscopy and microscopy along with the preliminary phytochemical evaluation of heartwood of *A catechu* confirmed the identity, quality and purity of the plant. On physico chemical analysis the heartwood was found with water soluble extractive value higher than alcohol soluble extractive value (6 % and 1.5 % respectively). The total ash value was 2.9 %. It showed high fibre content (45.24%) The preliminary phytochemical screening showed the presence of different phytoconstituent groups such as flavonoids, saponins, alkaloids and tannins. AAS evidenced that the drug is not contaminated by heavy metals and can be used safely for internal administration. Here the observations and results obtained are useful for further pharmacological and therapeutical evaluation.

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