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Evaluation for substitution of heartwood with small branches of *Acacia catechu* for therapeutic use – A comparative phytochemical approach

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Abstract
Acacia catechu commonly called as Khadira is a medicinal plant and heartwood of which is widely used in Ayurveda for various diseases. Removal of heartwood from trunk of this tree may make this plant weak and susceptible to damage due to which availability of this plant may be difficult in near future. Present study outlines the concept of plant part substitution. Heartwood and small branches of *A. catechu* are compared on the basis of physicochemical analysis, phytochemical analysis, total phenolic contents, total flavonoid contents and high performance thin layer chromatography (HPTLC) to evaluate the possibilities of using small branches instead of heartwood. Results of phytochemical analysis and HPTLC of *n*-hexane, ethyl acetate and ethanol extracts showed many similarities which suggest that small branches may have nearly similar active potency like heartwood and may be used as a substitute of heartwood after comparison and confirmation of same for pharmacological activities.

Keywords: *Acacia catechu*, physicochemical analysis, phytochemical analysis, HPTLC profile

1. Introduction
In developing countries majority of the population depend on traditional system of medicine for their primary health care. The expanding trade in medicinal plants has serious implications on the survival of several plant species, with many under serious threat to become extinct. Over harvesting has also become a threat to the biodiversity in areas all around the world and has expanded to the point that many plant species are on the verge of extinction. Therefore, the management of traditional medicinal plant wealth has become a matter of urgency. In India many commonly used medicinal plants are slow-growing forest trees, with bark, heartwood and underground parts being the parts mainly utilized.

A. catechu (Family: Mimosaceae) commonly called khadira is a most precious medicinal plant widely used in Ayurveda. As per Ayurvedic literature, heartwood of this plant is used in *sotha, kustha, prameha, vrana* [1]. Heartwood is also reported for various pharmacological activities like antioxidant, anti-inflammatory, chemoprotective [2], anti-cancer, cytotoxic potential [3], anti mycotic [4, 5], hepatoprotective, antioxidant [6], protective effect against plasmid DNA strand [7], inhibitor of cyclooxygenase and 5-lipoxygenase to reduce inflammation[8], antihyperglycemic, antinociceptive [9], immunomodulatory [10], antipyretic, antidiarrhoeal, hypoglycemic[11], hypotensive [12] , anthelmintic [13].

Heartwood mainly contains catechin [14-17], epicatechin [15, 17, 18], epicatechin-3-*O*-gallate, epigallocatechin-3-*O*-gallate [15], homo-isocatechin [16], quasiracemate of catechin and epicatechin [17], methyl ether of C_4-C_8 linked tetramer of catechin, 3′-*O*-8 linked dicatechin, 3′,4′,7-tri-*O*-methylcatechin, 3′,4′,5′,5,7-penta-*O*-methylgallocatechin [14], afzelechin, procyanidin, taxifolin, quercetin, isorhamnetin, kaempferol, dihydrokaempferol [18], epicatechol [19].

A strategy which would satisfy the requirements of sustainable harvesting, yet simultaneously provide this plant for primary health care needs, would be the substitution of heartwood with aerial parts of the same plant. Present study is an attempt to evaluate the possibilities of using small branches of *A. catechu* in place of its heartwood. Standard physicochemical parameters of small branches of *A. catechu* have not been prepared yet. So work is also carried out to establish preliminary physicochemical standards of small branches.

Fig 1: Heartwood

Fig 2: Small branches

2. Material and Methods

2.1 Plant material

Heartwood and small branches of *A. catechu* were collected from Gwalior (M.P), India. Identified and authenticated by Shree N.K. Pandey, Research officer (Botany), National Research Institute for Ayurved Siddha Human Resource Development, Aamkho, Gwalior, (M.P).

2.2 Instrumentation

CAMAG HPTLC system (Muttenz, Switzerland) equipped with semi-automatic TLC applicator Linomat IV, twin trough plate development chamber, Win CATS software version 1.4.2 and Hamilton (Reno, Nevada, USA) Syringe (100 µl).

2.3 Material and reagents

All chemicals, reagents and solvents used during the experiments were of analytical grade and HPTLC plates were purchased from E. Merck Pvt. Ltd. (Mumbai, India).

2.4 Physicochemical parameters

Heartwood and small branches were studied for various physicochemical standards like foreign matter, loss on drying at 105 °C, total ash, acid-insoluble ash, alcohol soluble extractive, water soluble extractive and pH of 10% aqueous solution using standard methods [20, 21].

2.5 Preliminary phytochemical screening

n-Hexane, ethyl acetate and ethanol extract of both heartwood and small branches were screened for the presence of phenols, tannins, alkaloids, carbohydrates, saponins, steroids, flavonoids, coumarins, quinone, furanoids and triterpenoids by the standard methods of Harbone [22] and Kokate [23].

2.6 Estimation of total phenolic and flavonoid content

Five grams of each of shade-dried plant material was pulverized into coarse powder and subjected to ethanolic extraction using soxhlet apparatus. Extracts were concentrated to dryness. Dried residues were then dissolved in 100 ml of 95% ethanol. Extracts were used for total phenolic and flavonoid assay.

Total phenolics content was determined by using Folin-ciocalteu assay [24]. An aliquot (1 ml) of extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 µg/ml) was added to a 25 ml volumetric flask, containing 9 ml of distilled water. A reagent blank was prepared using distilled water. One milliliter of Folin-ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. Volume was then made up to the mark. After incubation for 90 min at room temperature, absorbance against reagent blank was determined at 550 nm with an UV/Vis spectrophotometer. Total phenolics content was expressed as mg gallic acid equivalents (GAE).

Total flavonoid content was measured by aluminum chloride colorimetric assay [25]. An aliquot (1 ml) of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) was added to a 10 ml volumetric flask containing 4 ml of distilled water. To the flask, 0.3 ml of 5% $NaNO_2$ was added and after 5 min, 0.3 ml of 10% $AlCl_3$ was added. After 5 min, 2 ml of 1M NaOH was added and the volume was made up to 10 ml with distilled water. Solution was mixed and absorbance was measured against the blank at 510 nm. Total flavonoid content was expressed as mg quercetin equivalents (QUE).

2.7 HPTLC profiles

HPTLC studies were carried out following methods of Sethi [26], Stahl [27] and Wagner *et al* [28]. Heartwood and small branches were powdered coarsely. Ten gram powdered samples of each of heartwood and small branches were accurately weighed and exhaustively extracted by *n*-hexane, ethyl acetate and ethanol (each 100 ml) separately using soxhlet apparatus. Extracts were filtered and concentrated under reduced pressure and made up to10 ml in standard flasks separately.

Mobile phase used for developing the *n*-hexane, ethyl acetate and ethanol extracts of heartwood and small branches was toluene: ethyl acetate (7:3 v/v).

Samples were spotted in form of bands of width 10 mm with a 100 µl Hamilton syringe on aluminum TLC plates pre-coated with Silica gel 60 F_{254} of 0.2 mm thickness with the help of TLC semi-automatic applicator Linomat IV attached to CAMAG HPTLC system, which was programmed through Win CATS software version 1.4.2. 10 µl of each extracts of heartwood and small branches were applied in two tracks as 10 mm bands at a spraying rate of 10 seconds/µl. Track 1 was heartwood and track 2 was small branches for each of extracts applied.

Development of plate up to a migration distance of 80 mm was performed at 27 ± 2 °C with mobile phase for each extracts in a CAMAG HPTLC chamber previously saturated for 30 min. After development the plate was dried at 60 °C in an oven for 5 min and visualized under wavelength 254 nm and 366 nm for ultra violet detection. Developed plate was then dipped in anisaldehyde sulphuric acid reagent for derivatization and dried at 105 °C in hot air oven till color of band appears and visualized under white light. Images were captured by keeping plates in photodocumentation chamber and R_f values were recorded by Win CATS software.

3. Results and Discussion

Physicochemical parameters like foreign matter, loss on drying at 105 ºC, ash values, acid insoluble ash, extractive values and pH are given in Table 1. These data may be useful to pharmaceutical industries for the authentication and batch to batch consistency of the commercial samples. Both the parts of *A. catechu* were found to possess little moisture and hence can be stored at room temperature without fear of spoilage.

Table 1: Physicochemical parameters of heartwood and small branches of *A. catechu*.

S. No.	Parameters	Results	
		Heartwood	Small branches
1.	Foreign matter (% w/w)	Nil	Nil
2.	Loss on drying (% w/w)	9.14	8.33
3.	Total ash (% w/w)	1.23	2.73
4.	Acid insoluble ash (% w/w)	0.18	0.12
5.	Alcohol soluble extractive value (% w/w)	1.71	5.55
6.	Water soluble extractive value (% w/w)	4.90	8.00
7.	pH of 10% aqueous solution	5.55	5.66

Phytochemical analysis of different extracts of heartwood and small branches are shown in Table 2. Results reveal the presence of similar phytochemicals in heartwood and small branches in *n*- hexane, ethyl acetate and ethanol extracts.

Table 2: Phytochemical analysis of extracts of heartwood and small branches of *A. catechu.*

Phytochemicals	Heartwood			Small branches		
	n-Hexane	Ethyl acetate	Ethanol	*n*-Hexane	Ethyl acetate	Ethanol
Phenols	-ve	-ve	+ve	-ve	-ve	+ve
Tannins	-ve	-ve	+ve	-ve	-ve	+ve
Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve
Carbohydrates	-ve	+ve	+ve	-ve	+ve	+ve
Saponins	-ve	-ve	+ve	-ve	-ve	+ve
Steroids	-ve	-ve	+ve	-ve	-ve	+ve
Flavonoids	-ve	-ve	+ve	-ve	-ve	+ve
Coumarins	-ve	-ve	+ve	-ve	-ve	+ve
Quinone	-ve	-ve	+ve	-ve	-ve	+ve
Furanoids	-ve	-ve	+ve	-ve	-ve	+ve
Terpenoids	-ve	-ve	+ve	-ve	-ve	+ve

(+ve) = Present, (-ve) = Absent

Total amount of phenolics and flavonoids content of ethanolic extract of heartwood and small branches of *A. catechu* are summarized in Table 3. Results indicate that in comparison to heartwood, small branches had high total phenolic and flavonoid contents.

Table 3: Total phenolic and total flavonoid content of ethanolic extracts of heartwood and small branches of *A. catechu.*

S. No.	Plant parts	Total phenolics mg of GAE/ g dry weight*	Total flavonoids mg of QUE/ g dry weight*
1.	Heartwood	17.40±0.82	41.02±1.28
2.	Small branches	31.06±3.36	50.48±1.94

*Values are expressed as Mean ± SD

Comparative HPTLC profile of *n*-hexane, ethyl acetate and ethanol extracts of heartwood and small branches of *A. catechu* were recorded to reveal the chemical pattern of each extract.

HPTLC profile of *n*-hexane extract of heartwood and small branches (Figure 3 and Table 4) showed no bands when visualized under UV at 254 nm. At UV 366 nm, both heartwood and small branches showed five bands respectively, out of which one band at R_f 0.73 (red) was found similar. Bands at R_f 0.61, 0.69, 0.82, 0.95 were also found common to both parts but with different colour. Visualization under white lights after derivatization with anisaldehyde sulphuric acid reagent both heartwood and small branches showed four bands and all were found similar. It is interesting that all the bands of heartwood were found present in the small branches. This denotes that in *n*-hexane extract all the compounds which are present in heartwood are also present in small branches.

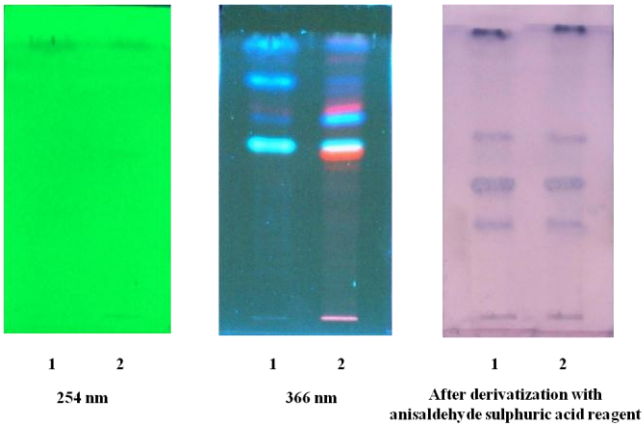

Fig 3: HPTLC profile of *n*-hexane extracts of heartwood and small branches of *A. catechu.* (Track 1: heartwood, track 2: small branches)

Table 4: R_f value of *n*-hexane extract of *A. catechu*.

S. No.	Wavelength	R_f value	
		Heartwood	Small branches
1.	254 nm	No band	No band
2.	366 nm	0.61, 0.69, 0.73, 0.82, 0.95.	0.61, 0.69, 0.73, 0.82, 0.95.
3.	Visible light after derivatization	0.38, 0.54, 0.68, 0.94.	0.38, 0.54, 0.68, 0.94.

HPTLC profile of ethyl acetate extract of heartwood and small branches (Figure 4 and Table 5) showed no bands when visualized under UV at 254 nm. At UV 366 nm heartwood and small branches showed two and five bands, respectively and both the two bands at R_f 0.66 (green) and 0.74 (blue) of HPTLC profile of heartwood were also found present in small branches. Visualization under white light after derivatization with anisaldehyde sulphuric acid reagent, both heartwood and small branches showed four bands and all were found similar. This indicates the presence of almost similar compounds also in ethyl acetate extract of heartwood and small branches.

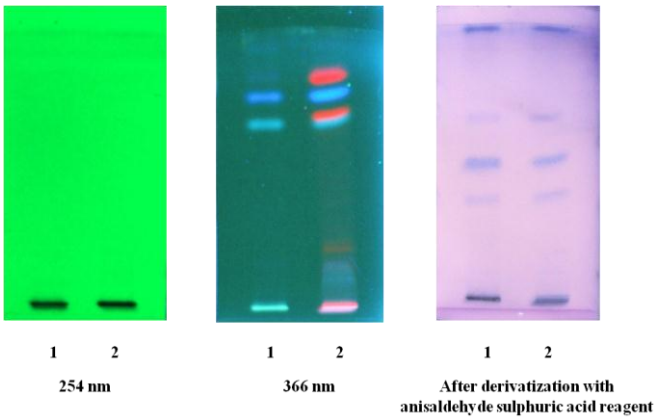

Fig 4: HPTLC profile of ethyl acetate extracts of heartwood and small branches of *A. catechu*. (Track 1: heartwood, track 2: small branches)

Table 5: R_f value of ethyl acetate extract of *A. catechu*.

S. No.	Wavelength	R_f value	
		Heartwood	Small branches
1.	254 nm	No band	No band
2.	366 nm	0.66, 0.74.	0.23, 0.66, 0.70, 0.74, 0.82.
3.	Visible light after derivatization	0.40, 0.52, 0.67, 0.98.	0.40, 0.52, 0.67, 0.98.

HPTLC profile of ethanol extract of both heartwood and small branches (Figure 5 and Table 6) showed no bands when visualized under UV at 254 nm. At UV 366 nm heartwood and small branches showed three and six bands, respectively out of which two bands at R_f 0.74 (blue), 0.98 (blue) were found similar. Band at R_f 0.66 was also found common to both parts but with different colour. Visualization under white lights after derivatization with anisaldehyde sulphuric acid reagent both heartwood and small branches showed three bands and all were found similar. This indicates the presence of many similar compounds in ethanol extract of heartwood and small branches also.

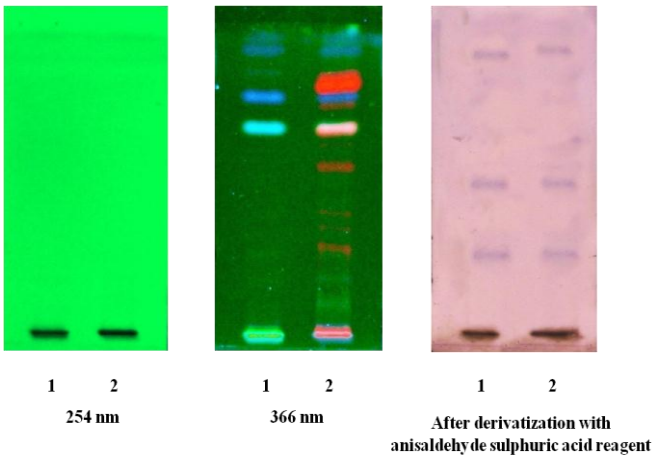

Fig 5: HPTLC profile of ethanol extracts of heartwood and small branches of *A. catechu*. (Track 1: heartwood, track 2: small branches)

Table 6: R_f value of ethanol extract of *A. catechu*.

S. No.	Wavelength	R_f value	
		Heartwood	Small branches
1.	254 nm	No band	No band
2.	366 nm	0.66, 0.74, 0.98.	0.23, 0.66, 0.70, 0.74, 0.82, 0.98.
3.	Visible light after derivatization	0.38, 0.52, 0.97.	0.38, 0.52, 0.97.

4. Conclusion

Many similarities in HPTLC profiles and phytochemical analysis of *n*-hexane, ethyl acetate and ethanol extracts of *A. catechu* heartwood and small branches suggests that small branches may have almost similar active potency like heartwood and may be used as a substitute of heartwood after comparison and confirmation of same for pharmacological activities. The study provides the base for further study to recommend small branches in place of heartwood which will help sustainable utilization. The study will also be useful in identification and quality control of drug and can provide standard HPTLC profiles with selected solvent system for proper identification/ authentication of drug.

5. Acknowledgement

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