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Research Article

APPLICATION AND EVALUATION OF MULTIPLE EXTRACTION TECHNIQUES (BIOPHARMACEUTICAL RECYCLING) FOR OPTIMIZATION OF KHADIRA KWATHA; AN AYURVEDIC FORMULATION

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ABSTRACT

In Ayurvedic pharmaceutics and therapeutics, heartwood of several precious plants is used. Increasing global demands of the Ayurvedic formulations containing heartwood may lead to damage or destroy the plants. With aim to develop some alternative pharmaceutical methods, repeated extraction process was applied on *Acacia catechu* heartwood. Coarse powder of *Acacia catechu* heartwood (group A), dry residue after its first decoction (group B) and dry residue after second decoction (group C) were analysed by pharmacognostical and physicochemical tests including pH, loss on drying, ash value, acid insoluble ash, water soluble extractive, methanol soluble extractive and tannin content, qualitative, colorimetric and HPTLC analysis. Pharmacognostic characteristic like oil globules, crystalline fibres, fibres passing through medullary rays, simple fibres, calcium oxalate crystals, Group of pitted scleroids, border pitted vessels, starch grains, prismatic crystals, yellowish or brown contents, lignified fibres etc were found in all the three samples; but the intensity of the characters was found decreasing respectively. In physicochemical tests except Tannin and Loss on drying, more than 75% and 65% extraction values were unchanged in group B and C respectively with compare to group A. Tannin and Protein were absent in group C, while alkaloids, flavonoids, saponins, steroids, carbohydrates were present even after extraction for third time. Residue of drug after preparation of decoction for two times may be considered for reutilization and should be studied for pharmacological activities.

KEY WORDS: Ayurveda, extraction, HPTLC, Khadira, Repeated decoctions

INTRODUCTION

There are at least 18664 different species of vascular plants in India, among them 26.8% are endemic.[1] The estimate of gross deforestation was 0.43% in India and 0.6% in global for 2009–2011.[2] Traditional system of medicine (Ayurveda) is depending upon the wild or cultivated medicinal plants. Globalization and scientific validation of the traditional system has been received commercial attention that will ultimately lead to overharvesting and risk of extinction of many medicinal spices.[3] In Ayurveda, average 30% of medicinal preparations are prepared from roots, 14% from bark, 16% from whole plants and 3% from heartwood or sapwood.[4] Collection and use of these plants may lead to damage or destroy them. Hence, it is the need of hour to think in a way to cultivate these valuable plants, to use them properly and to find some alternative pharmaceutical methods. The concept of recycling of residual part in drug preparation may be an alternative measure to optimize its utilization in therapeutics. In Ayurvedic literature, some references are found regarding repeated immersion with prolong duration of immersion to facilitate better extraction of the heartwood of *Khadira* (*Acacia catechu* willd., Family Leguminosae).[5] In Ayurveda, heartwood of *Acacia catechu* is used in various medicinal formulations internally as well as externally.[6] It shows proven pharmacological actions such as anti-mycotic[7], antibacterial[8], antimicrobial[9], immunomodulatory[10], antioxidant[11], insecticidal[12], antipyretic, hypoglycaemic, anti-diarrhoeal and hepatoprotective activities.[13] It is also reported as endangered species in Nepal.[14] Pharmacognostical characteristics of *Acacia catechu* willd. have been already reported, but the changes in its characteristics due to repeated decoction are not reported till date. So, it was planned to find out the difference in macroscopic, microscopic and analytical parameters between *Acacia catechu* heartwood and its dry residues after repeated decoctions and in view of consideration of the usage of repeated decoction for medicinal purpose.

MATERIAL AND METHODS

Procurement of raw plant material

The heartwood of *Acacia catechu* was procured from the village named Motipanchasara situated in Saurashtra region of Gujarat, India in the month of December, 2013. It was identified with the help of their taxonomy from various floras and research articles and was authenticated by the Department of Pharmacognosy, I.P.G.T. and R.A., Gujarat Ayurved University, Jamnagar.

Sample preparation

Branches of *Acacia catechu* were shade dried for 4 weeks and then heartwood was separated and cut into small pieces with the help of saw mill. Coarse powder (Sieved through 10 no. mesh) was prepared and taken as a sample A. Sample A was soaked in 16 time's of potable water for 12 hrs and then its decoction was

prepared by reducing 1/8th on mild heating. The residual coarse powder of the decoction was shade dried and named as a Sample B. Same procedure was repeated on Sample B and its residual coarse powder was taken as sample C. Three batches of each group were prepared and were taken for pharmacognostical, analytical and HPTLC evaluations.

Pharmacognostical study

It includes macroscopic, powder microscopic, preliminary phytochemical and physicochemical studies. For macroscopic study, organoleptic features like colour, odour, taste, and texture of the untreated powdered drugs were noted. Examination of the colour was done under diffuse daylight. In all samples, texture, Surface and fracture characteristic were examined. Softness or hardness was decided by touch and rubbing. Odour strength (none, weak distinct, strong) and odour sensation (aromatic, musty, mouldy, etc.) were assessed. Taste was perceived carefully by taking fixed quantity of the powdered material. For powder microscopy, fine powder of the samples was done in 60 no. mesh size and stored well separately in air tight glass bottles. All the samples were individually spread on glass slides and observed under microscope at different magnifications. For the detection of lignified tissues (stone cell, scleroids, xylem vessel, etc.) the powder was stained with phloroglucinol and hydrochloric acid and to observe the starch grains the powder was stained with iodine solution. [15]

Physicochemical investigation

Different physicochemical investigations of Khadira heartwood powder and its reused forms were carried out using standard pharmacopoeial methods, including pH, loss on drying, ash value, acid insoluble ash, water soluble extractive, methanol soluble extractive and tannin contents were carried out as per standard methods.[16] Qualitative analysis of various functional groups was carried out on methanol soluble extractives of the samples.[17] Infusion and decoction of all three samples were analysed by colorimetric analysis.

HPTLC analysis

All the samples of *Acacia catechu* were individually dissolved in methanol to get standard solutions having concentration of 1 mg/ml. silica gel GF 254(E.Merk) coated TLC plates were taken as a Stationary phase and the combination Toluene: Ethyl acetate: Formic acid (7:2:0.5 v/v/v) was taken as Mobile phase. 5µl Sample volume was taken**.** Vaniline sulfuric acid and Dragendorff's reagent were used as Spray reagents.

Table 1: Comparative organoleptic characters of three groups

Parameters	**Group** A	**Group** B	**Group** C
Colour	Brick Red	Cream	Light brown
Taste	Taste-less	Taste-less	Taste-less
Odour	Characteristic- Woody	Characteristic- Woody	Characteristic- Woody
Consistency	Fine smooth	Smooth with fibres	Smooth with more fibres

Table 2: Comparative Physicochemical characterization of three groups (Mean ± S.D., n=3)

Characteristic	**Group A (%)**	**Group B (%)**	**Group C (%)**
Loss on drying at 110º C	8.84 ±0.151	12.37 ±0.460	11.27 ±0.4
Total ash	8.83 ±0.862	7.73 ±0.378	7.33 ±0.23
Acid insoluble ash	0.68 ±0.02	0.60 ±0.005	0.60 ±0.015
Water soluble extractive	14.78 ±0.588	11.67 ±0.416	10.27 ±0.50
Methanol soluble extractive	13.11 ±0.136	10.64 ±0.348	9.28 ±0.249
pH of Water extract	5.24 ±0.0814	5.99 ±0.070	6.65 ±0.090
Quantitative estimation of tannin	7.3 ±0.529	3.05 ±0.181	0.46 ±0.178

Table 3: Determination of presence of various functional groups in three groups

Sr. No.	**Parameters**	**Group A**	**Group B**	**Group C**
1	Alkaloid	+	+	+
2	Flavonoids	+	+	+
3	Starch	-	-	-
4	Tannin	+	+	-
5	Saponin	+	+	+
6	Steroid	-	-	-
7	Carbohydrate	+	+	+
8	Protein	+	+	-

Table 4: HPTLC profile (254 nm and 366 nm) of different groups of *Acacia catechu*

Groups	**Visualizing Condition (254 nm)**		**Visualizing Condition (366 nm)**	
	No. of spots	**Rf Value**	**No. of spots**	**Rf Value**
Group 1	10	0.02, 0.14, 0.23, 0.32, 0.36, 0.52, 0.65, 0.71, 0.79, 0.88	07	0.01, 0.08, 0.20, 0.32, 0.37, 0.43, 0.66
Group 2	05	0.02, 0.08, 0.43, 0.78, 0.89	01	0.06
Group 3	07	0.01, 0.13, 0.30, 0.37, 0.67, 0.84, 0.88	02	0.00, 0.67

Instrumental conditions

Sample was developed in Camag twin trough chamber under Camag linomat V mode. Chamber saturation time and development time was 30 min for each while development distance was 7 cm. Sample was scanned in Camag scanner III under linear mode at 254 nm and 366 nm. Camag reprostar was used for photo documentation, while deuterium and tungsten lamp were used for detection. Oven U.V. Spectrum 200 nm to 700 nm was used as drying device.

Statistical Analysis

Measures of central tendency (Mean) and standard deviation (SD) were applied for the measurement of physicochemical parameters. Win cats software was used to generate and analysed the HPTLC data.

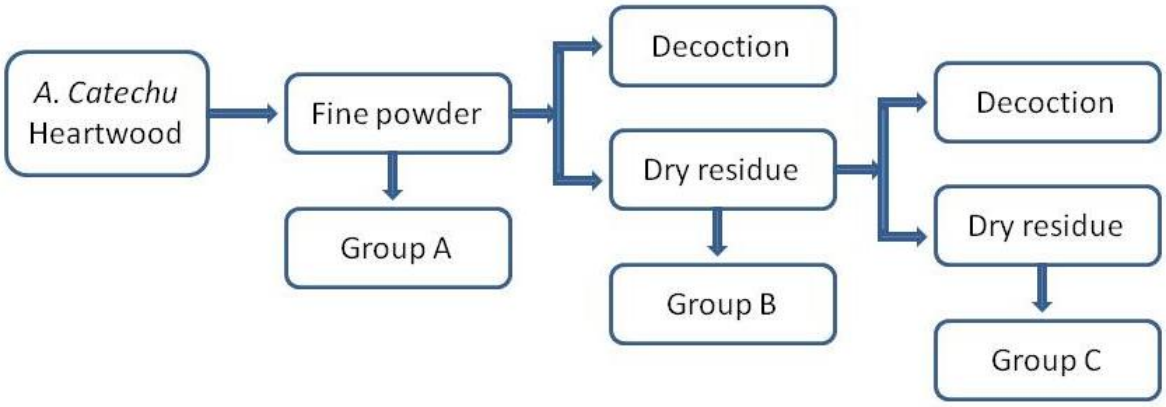

Figure. 1 Manufacturing scheme of the sample

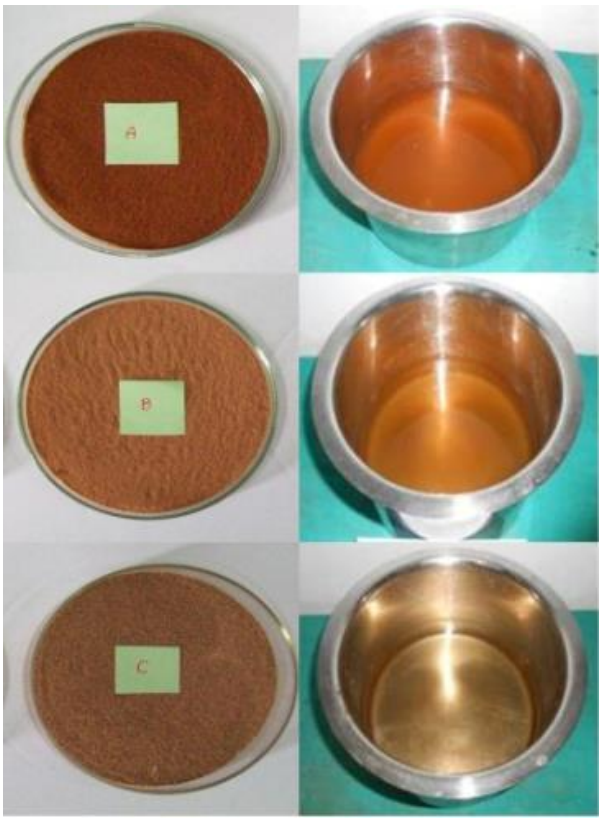

Figure. 2 Photographs of powder and decoctions (A) Powder of Khadira heartwood (Group A), (B) Powder of dry residue (Group B), (C) Powder of dry residue (Group C), (D) Decoction of Group A, (E) Decoction of Group B, (F) Decoction of Group C.

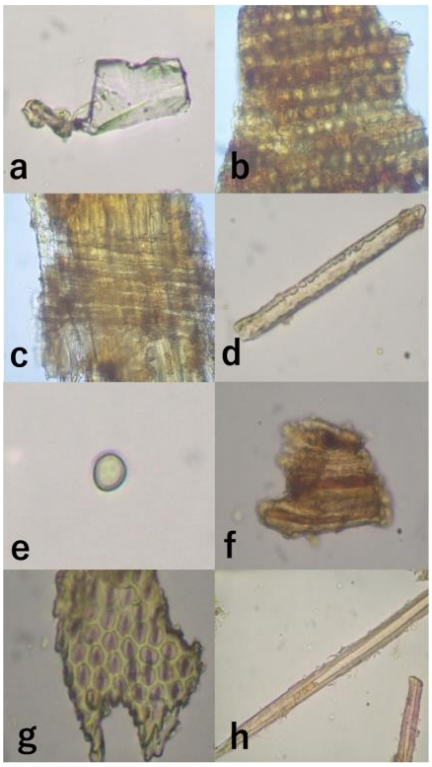

Figure. 3 Powder microscopy of Group A : (a) Calcium oxalate crystals, (b) Group of crystaline fibres, (c) Fibres passes through medullary rays, (d) Border pitted vessels, (e) Oil globules, (f) Brown content, (g) Group of pitted scleride, (h) Simple fibres

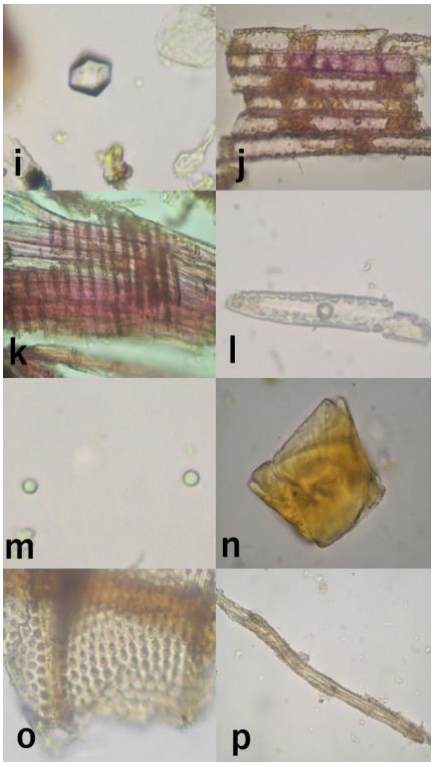

Figure. 4 Powder microscopy of Group B : (i) Calcium oxalate crystals, (j) Group of crystaline fibres, (k) Fibres passes through medullary rays, (l) Border pitted vessels, (m) Oil globules, (n) Brown content, (o) Group of pitted scleride, (p) Simple fibres

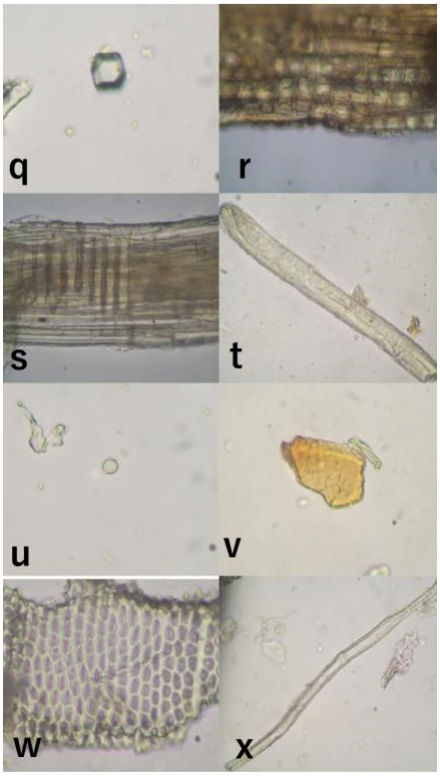

Figure. 5 Powder microscopy of Group C : (q) Calcium oxalate crystals, (r) Group of crystaline fibres, (s) Fibres passes through medullary rays, (t) Border pitted vessels, (u) Oil globules, (v) Brown content, (w) Group of pitted scleride, (x) Simple fibres

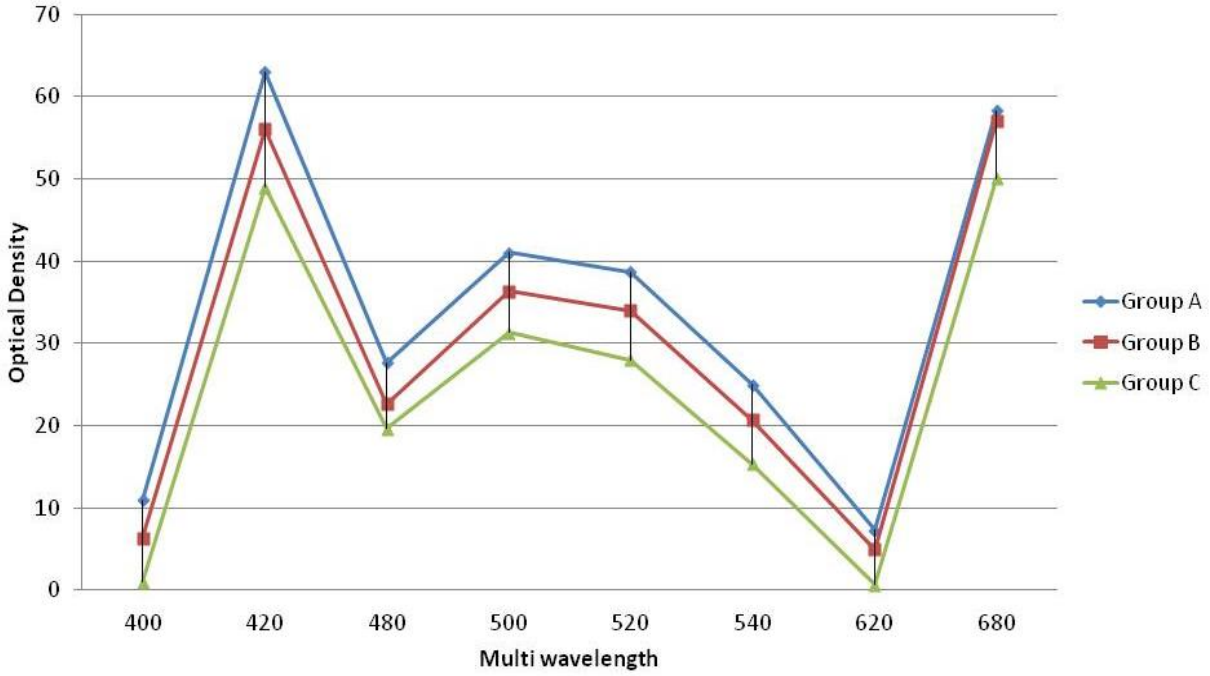

Figure. 6 Colorimetri chart for the infusions of all the groups

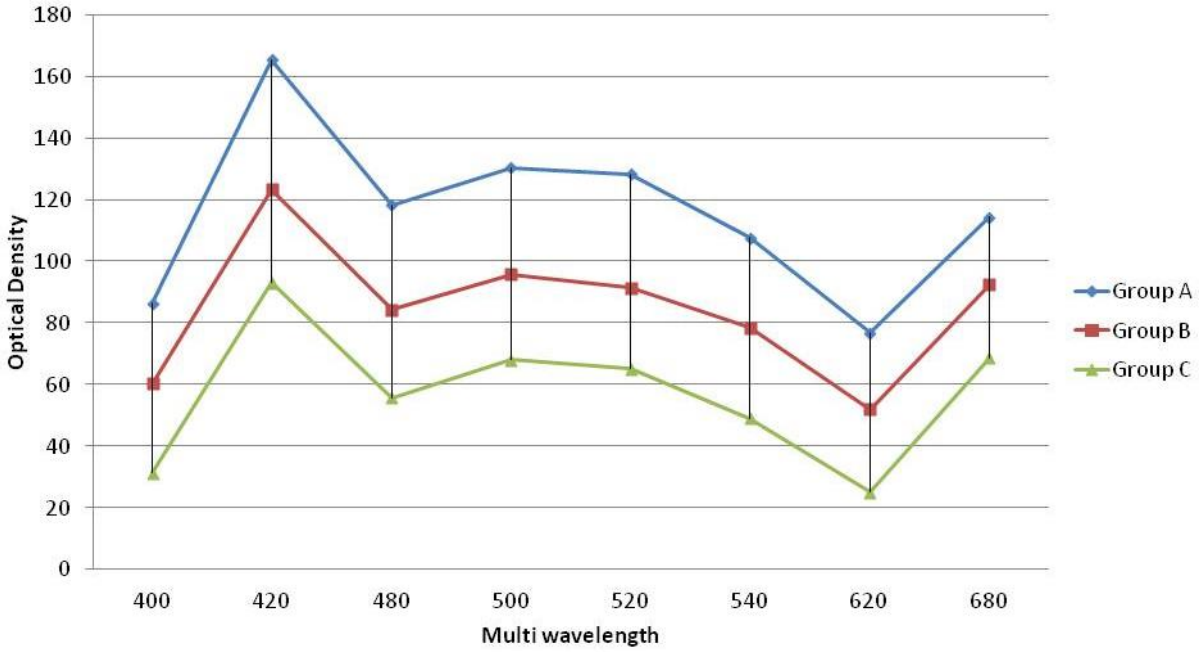

Figure. 7 Colorimetri chart for the decoction of all the groups

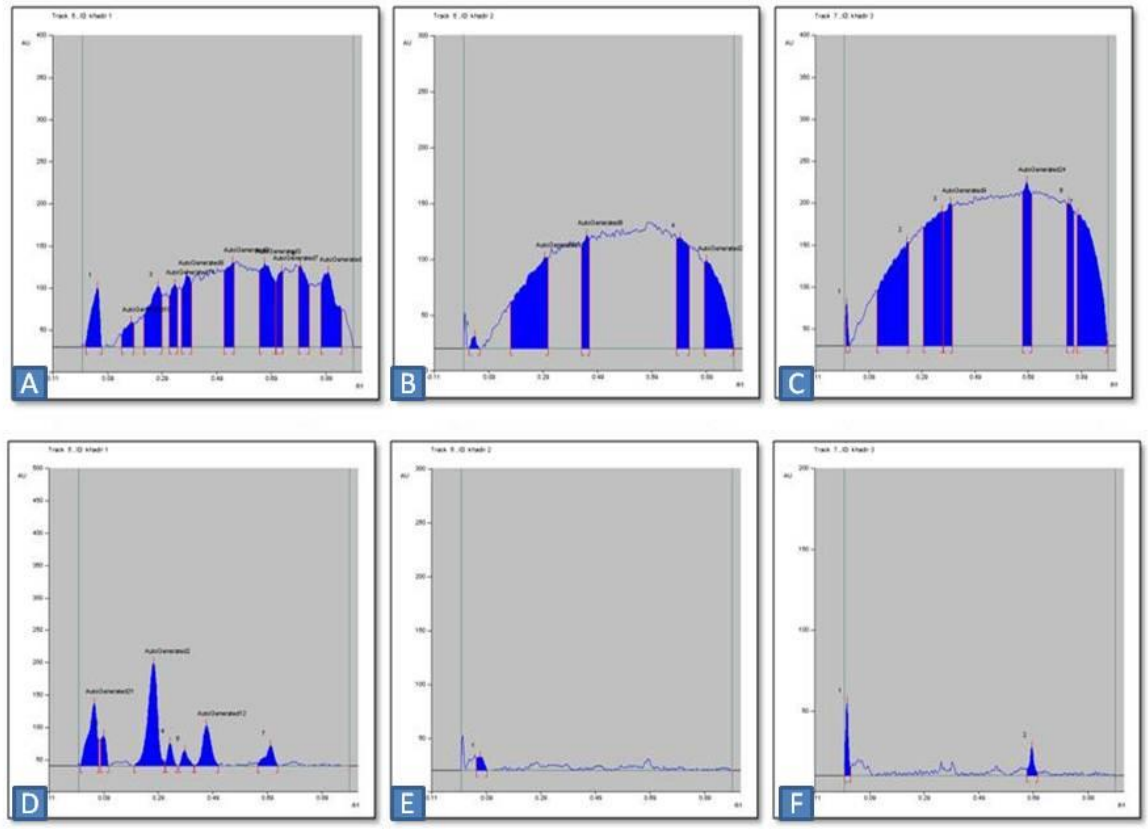

Figure. 8 HPTLC finger prints of Acacia catechu and its reused forms at 254 nm after derivatization (A) Group A, (B) Group B, (C) Group C; at 254 nm after derivatization (D) Group A, (E) Group B, (F) Group C.

RESULTS AND DISCUSSION

Macroscopic features

While comparing the organoleptic characteristic of the powders, some changes were observed in colour and consistency. Colour changed from brick red to creamish and creamish to light brown in the samples respectively. Decoctions of the samples were also became lighten in colour respectively. Consistency changed from fine to fibrous powder respectively. (Table 1) (Figure 1)

Microscopic features

Diagnostic powder microscopic characters of heartwood of *Acacia catechu* are oil globules, crystalline fibres, fibres passing through medullary rays, simple fibres, calcium oxalate crystals, Group of pitted scleroids, border pitted vessels, starch grains, prismatic crystals, yellowish or brown contents, lignified fibres etc.[18] All above characters were found in all the three samples; but the intensity of the characters was found decreasing respectively. (Figure 2-5) Colour of dark yellowish brown contents turned to light yellowish brown. Amount of oil globules was also found decreased in sample B and C. Group of pitted scleroids and border pitted vessels were filled with brownish matter in group A, which were found with decreased in group B, while in group C, the brownish matter was found very less. In group C, broken parts of some characteristics were found in comparison to group A and B, which may be due to the process of repeated heating.

Physicochemical investigation

Loss on drying was more in group B and C in comparison to group A because in group B and C, the residue was shade dried after squeezing the decoction. Ash value, water soluble extractives and alcohol soluble extractives were decreasing in respective groups. Group A showed acidic pH, Group B showed mild acidic pH, while Group C was found almost neutral in pH value. Quantitatively tannin was found decreased in group B which is more than half to the value of group A. In group C value of tannin was negligible. (Table 2)

In qualitative estimation of various functional groups, starch and steroids were found absent in all the groups. (Table 3) Alkaloids, Flavonoids, carbohydrates and saponin were present in all three groups. Protein and tannin were present in group A and B, while they were found absent in group C. Similar heat stress may results in the denaturation of existing proteins.[19]

Spot tests for organic functional groups reveals presence of alkaloids, flavonoids, tannins, saponins, steroids, carbohydrates, protein after processing for second time and alkaloids, flavonoids, saponins, steroids, carbohydrates were present even after extraction for third time. (Figure 6-7)

HPTLC analysis

In the results of HPTLC, 10, 5 and 7 spots were found in the groups respectively under 256 nm visualisation. That reveals that number of spots were half in group B with compare to

group A and group C shows more spots than group B. under 366 nm visualized condition, 7, 1 and 2 spots were found in respective groups. (Table 4) (Figure 8)

Comparatively more number of spots in chromatographic study reveals presence of newer peaks which raise the possibility formation of new chemical moieties due to processing. Similar processes like further heating of Kwatha (Khadira sara) and prolonged immersion of Khadira Kwatha (Arishta, Bhavana etc) are done in Ayurvedic pharmaceutical processes.

Data of area under curve of observed peaks reveals that it has been significantly reduced in both the samples B and C as that of A, which is suggestive of extraction of maximum extractives in first processing which is further supported by comparatively more and significant reduction in Total Tannin content of Samples B and C respectively as that of sample A. Tannins in Acacia catechu being non hydrolysable (condensed) tannins, are less likely to undergo hydrolysis as cause of newer peaks and less percentage in subsequently treated samples B and C. These tannins have high temperature of degradation (ca. 190 °C) and a high glass transition temperature (ca. 140 °C), further nullifying the possibility of thermal degradation.

CONCLUSION

In view of probable scarcity of drug source, presence of significant quantity of water soluble and methanol soluble extractives, generation of new peaks in samples B and C, it can be concluded that, residue of drug after preparation of decoction for two times may be considered for reutilization and should be studied for pharmacological activities.

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