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HPTLC QUANTIFICATION & PHYTOCHEMICAL INVESTIGATION OF ROOTS OF *ACACIA CATECHU* WILLD

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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 [1]. *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.

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It is also distributed in Ganjam, Burma, throughout the Konkam, S.M. country & Deccan [2, 3].

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[4]. The leaves contain the trace elements: Cu, 8.90; Fe, 126.08; Mn, 25.31; and Zn, 24.26 ppm on dry matter basis[5]. The chief constituents of the heartwood are catechin and catechutannic acid, acacatechin, epicatechin, catechin tetramer, dicatechin, gallocatechin, gossypin, kaempferol and dihydro derivative, taxifolin, procyanidine, isorharnnetin, (+) afzelchin and flavonoids like quercetin.[6, 7] 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[8]. Catechu

resin contains catechin, catechu-tannic acid & tannins [9]. Acid hydrolysis of the gum afforded L-arabinose, D-galactose, Dvrhamnose, aldobiuronic 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 [6, 7, 10]. *A. Catechu* Willd. plant possess various biological activities like antipyretic, hepatoprotective hypoglycaemic & antidiarrhoeal activity [11]; antimicrobial activity [12] and found effective in the treatment of lepromatous leprosy [13]. The leaves, roots & bark of *Acacia catechu* Willd. showed potent anti-mycotic activity [14]. The plant is used for hypotensive activity [15], antifertility activity [16,] and immunomodulatory activity [17].

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, Punchkula. 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[18]. (**Fig. 1-4**)

Fluoroscence 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[19-22]. (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 [18]. Ethanol-soluble, and water-soluble extractive values were determined [23]. Loss on drying and swelling index was also determined[18]. Preliminary phytochemical screening was carried out (**Table 4**), by using standard methods, to identify the presence of various phytoconstituents [24]. (**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

No.	Appl. Position	Appl. Volume	Vial#	Sample ID	Active
1	15.0mm	5.0 µl	1	Sample A	Yes
2	29.0mm	10.0 µl	1	Sample A	Yes
3	43.0mm	5.0 µl	2	Std-Quercetin	Yes
4	57.0mm	10.0 µl	2	Std-Quercetin	Yes

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 **c**olour, 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

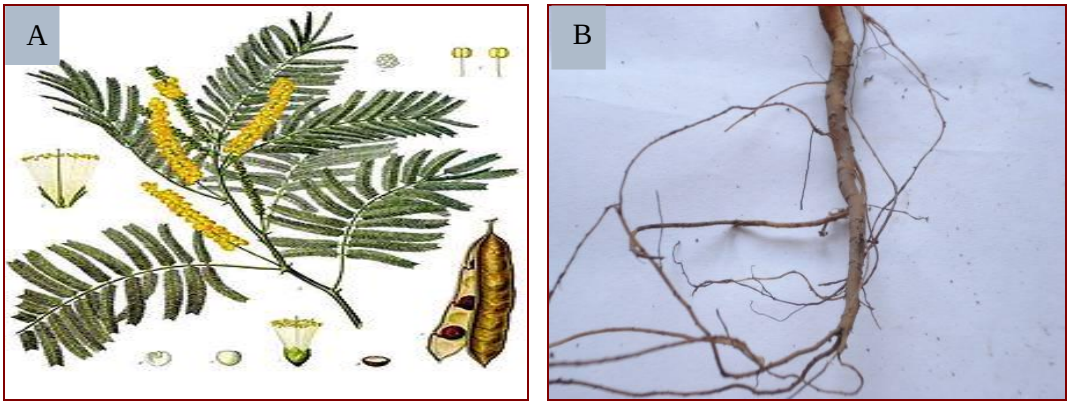


FIG. 1: A: *ACACIA CATECHU* WILLD. STEM, LEAVES AND FRUIT; B: *A. CATECHU* WILLD. ROOT

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.

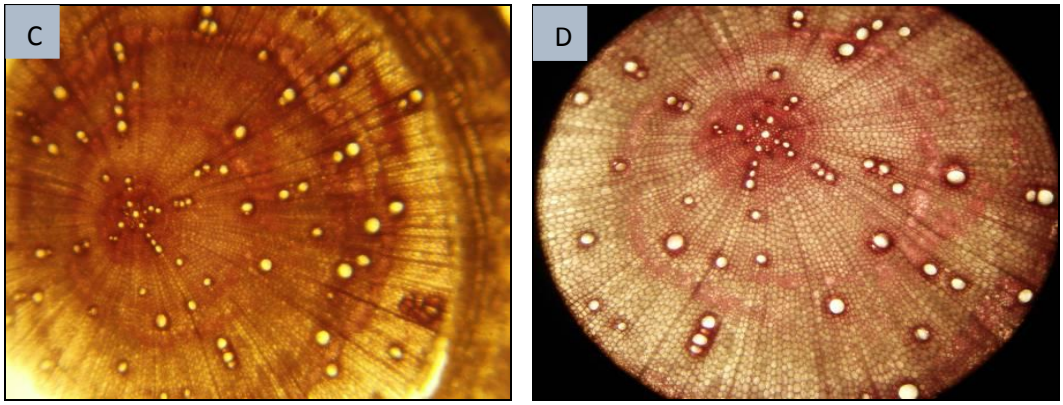


FIG.2: ROOT MICROSCOPY AT 10 X - C: SHOWS EPIDERMIS LAYER, XYLEM CELLS, VESSELS, MEDULLARY RAYS AND PITH; D: PRIMARY AND SECONDARY XYLEM CELLS, MEDULLAY RAYS, AND PITH

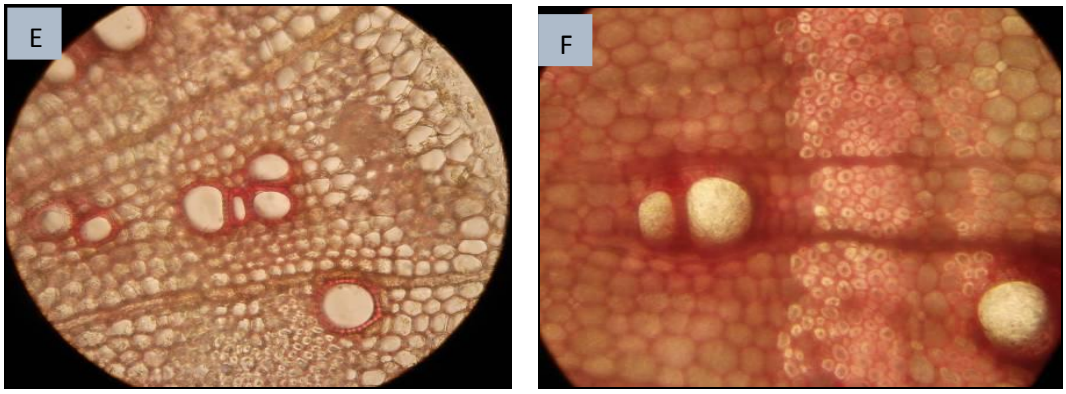


FIG. 3: ROOT MICROSCOPY AT 45X- E: MEDULLARY RAYS, PRIMARY AND SECONDARY XYLEM CELLS, VESSELS GRANULAR CELLS; F: MULTILAYERED GRANULER CELLS WITH CONSISTENT MEDULLARY RAYS, CORK CELLS

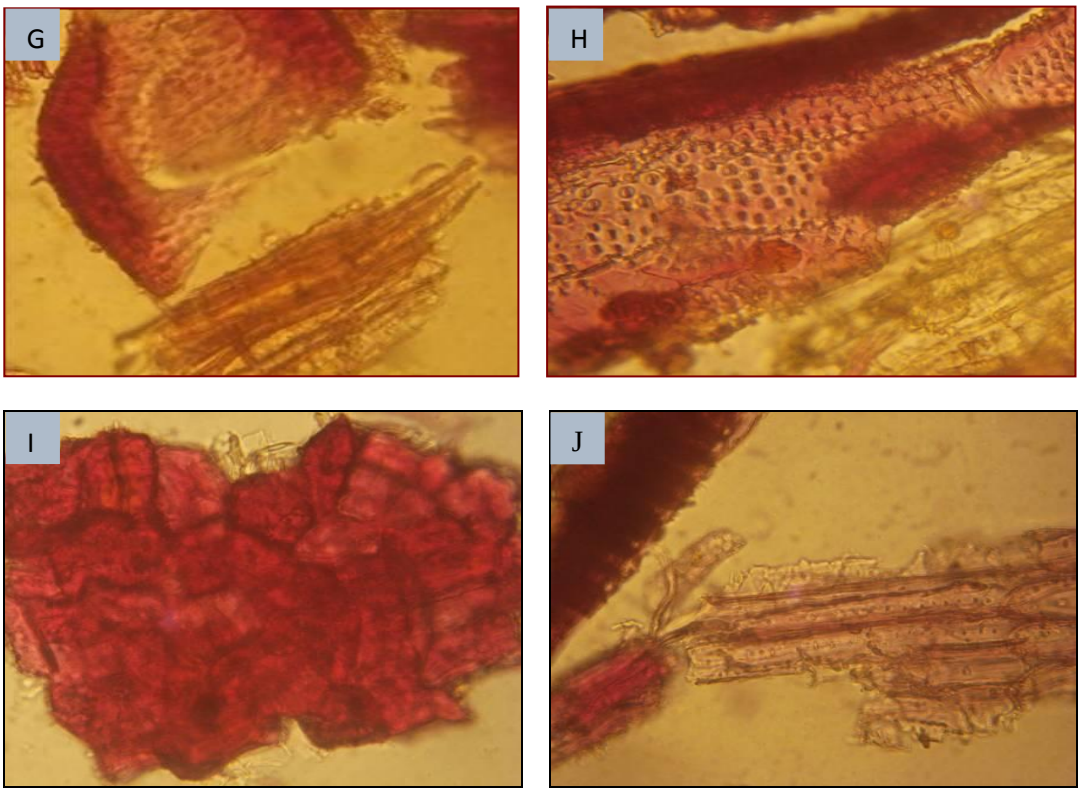


FIG.4: POWDER MICROSCOPY- G: FIBRES & PITTED CELLS; H: CORK CELLS, PITTED CELLS AND PHLOEM FIBRE; I: CORK CELLS; J: SCLEROID FIBRE WITH ATTACHED TRACHEIDS, CORK CELLS

Fluoroscence 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% H_2SO_4, 50% HNO_3, 50% HCl and change in colour was observed under UV light.

TABLE 2: FLUORESCENCE BEHAVIOR OF ROOT POWDER OF *A. CATECHU* WILLD. WITH DIFFERENT REAGENTS.

Sr. no.	Treatment	Visible (400-800nm)	U.V. short (254 nm)	U.V. Long (366 nm)
1.	Powder as such	Light Brown	Light Green	Brown
2.	1N NaOH in Methanol	Brown	Dark Green	Brown
3.	1N NaOH in Water	Brown	Yellowish Brown	Brown
4.	1N HCl	Brown	Yellowish Brown	Dark Brown
5.	50% HNO_3	Dark Brown	Dark Green	Black
6.	50% HCl	Brown	Dark Green	Dark Brown
7.	50% H_2SO_4	Brown	Light Green	Blackish brown

TABLE 3: FLUORESCENCE OF *A. CATECHU* WILLD. ROOT EXTRACTS WITH DIFFERENT SOLVENTS AND RESPECTIVE EXTRACTIVE VALUES

Sr. no.	Treatment	Visible (400-800nm)	U.V. short (254 nm)	U.V. Long (366 nm)	Extractive values
1.	Petrolium ether	Yellow	Leafy Green	Lime Yellow	0.24%
2.	Chloroform	Dark Yellow	Leafy Green	Lime Yellow	0.18%
3.	Ethyl acetate	Yellowish Brown	Dark Green	Brown	0.89%
4.	Successive ethanol	Dark Brown	Dark Green	Brownish Black	3.64%
5.	Aqueous	Dark Brown	Dark Green	Black	5.07%
6.	Total ethanolic	Dark Brown	Dark Green	Dark Green	5.69%

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.

TABLE 4: PHYTOCHEMICAL SCREENING OF *A. CATECHU* WILLD. ROOTS

Sr. No.	Compounds	Petrolium Ether	Chloroform	Ethyl Acetate	Ethanolic	Total Ethanolic	Aqueous
1	Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve
2	Carbohydrates	-ve	-ve	-ve	-ve	-ve	-ve
3	Steroids	-ve	-ve	-ve	-ve	-ve	-ve
4	Saponins	-ve	-ve	-ve	+ve	+ve	+ve
5	Proteins	-ve	-ve	-ve	-ve	-ve	-ve
6	Fixed Oils/Fats	+ve	-ve	-ve	-ve	-ve	-ve
7	Flavanoids	-ve	-ve	+ve	+ve	+ve	+ve
8	Tannins & Phenols	-ve	-ve	-ve	+ve	+ve	+ve
9	Gums& Mucilages	-ve	-ve	-ve	-ve	-ve	-ve
10	Glycosides	-ve	-ve	-ve	-ve	-ve	-ve
11	Reducing Sugars	-ve	-ve	+ve	+ve	+ve	-ve
12	Amino Acids	-ve	-ve	-ve	-ve	-ve	-ve

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.

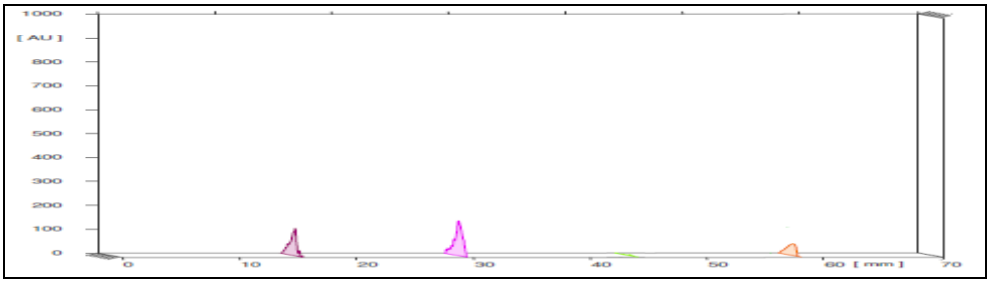
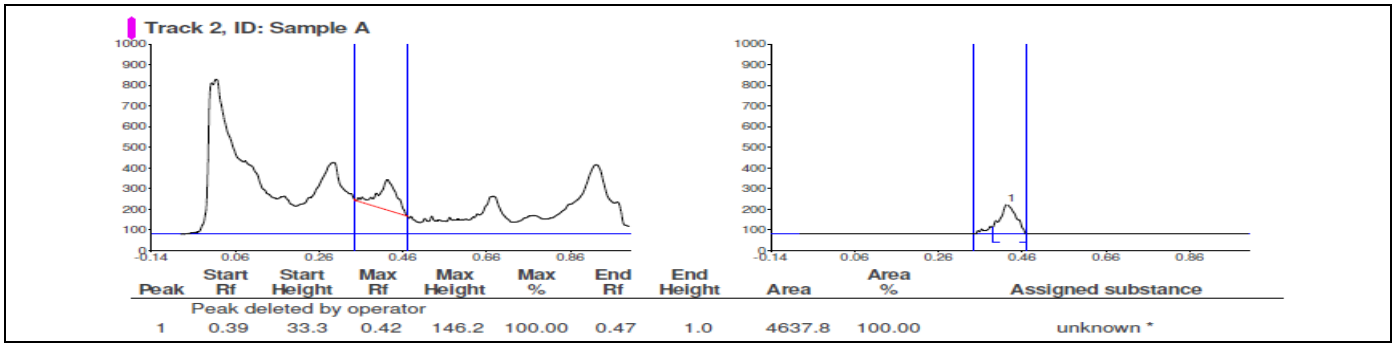
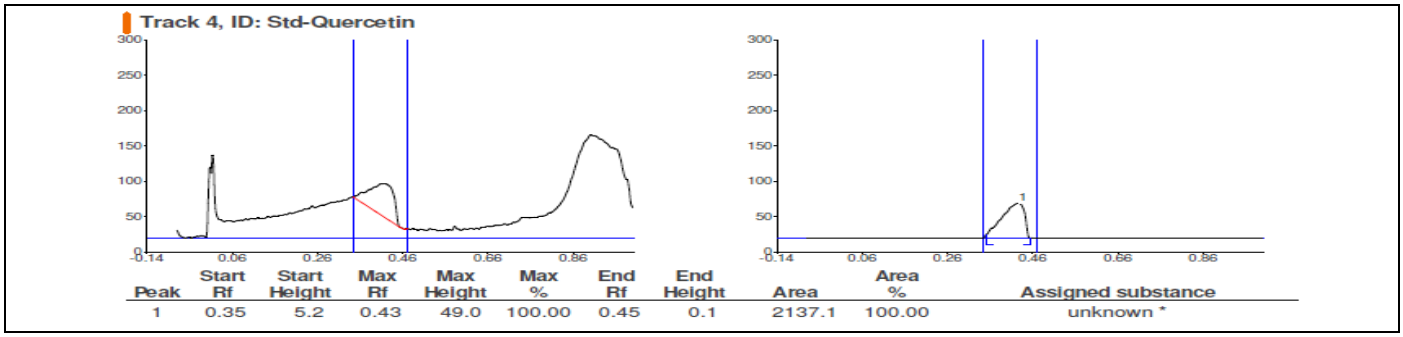


FIG. 5: HPTLC PEAKS OF *A. CATECHU* WILLD. EXTRACT WITH QUERCETIN (ALL TRACKS AT WAVELENGTH)





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.35	5.2	0.43	49.0	100.00	0.45	0.1	2137.1	100.00	unknown *

FIG. 6: PEAKS OF QUERCETIN DETECTED BY HPTLC IN TRACKS OF SAMPLE AND STANDARD.

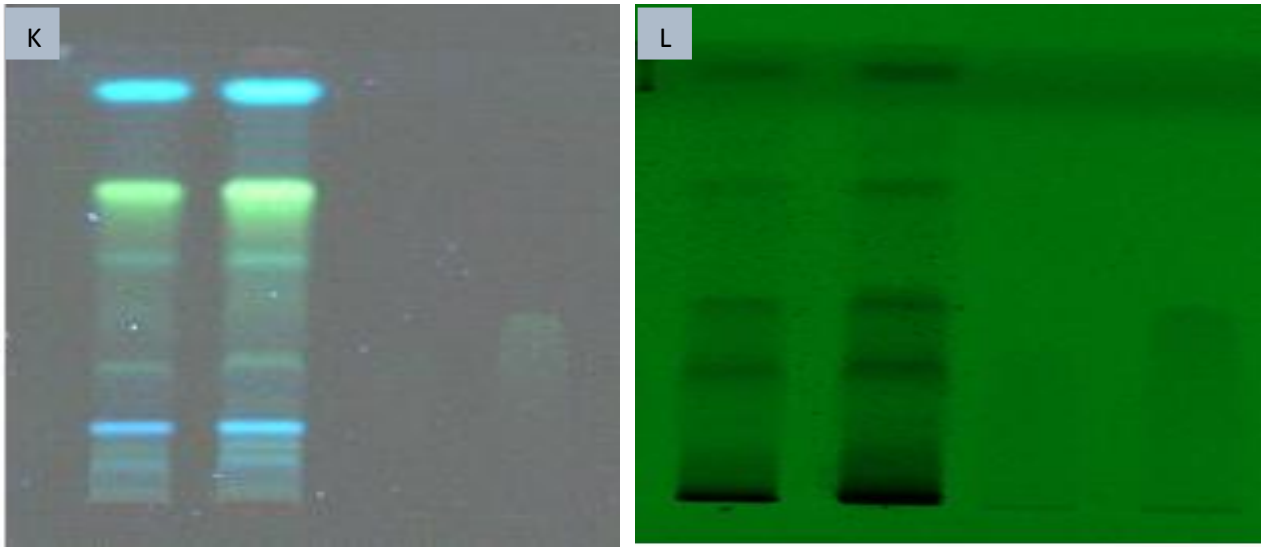


FIG.7: HPTLC OF EXTRACT WITH STANDARD- K: AT 366 nm; L: AT 254nm

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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