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Chromatographic fingerprint analysis of *Acacia Catechu Ethanolic* leaf extract by HPTLC Technique

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Abstract

Acacia catechu have been in use in Indian traditional medicine, 'Ayurveda' since antiquity. However, there has been no attempt to standardize the herbal extract as the main ingredient in terms of its active principle or marker compound. Chromatographic fingerprint symbolize the active chemical constituents of herbal medicines for desired therapeutic action. This study presents a simple, rapid and selective HPTLC method for the quantitative estimation of rutin and quercetin from ethanolic leaf extract of *Acacia catechu*, precoated HPTLC silica gel 60 F254 as stationary phase and mobile phase for Formic Acid: water: Ethyl acetate [10:10:80 v/v]. detection and quantification were performed densitometrically at 366nm for rutin and quercetin.

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INTRODUCTION

Acacia catechu Willd. (*Mimosaceae*),also known as Khair, is a medium sized deciduous tree with crooked and forked trunk. It is found growing in both natural and plantation forms in most of the parts of country[1] .In India, there are three varities of *A. catechu* namely, *Catechu, Catechuoides* and *Sundra. Catechu* is commercially used to obtain *Katha* (a concentrated filtered extract) in North India. It is found widely distributed in Jammu, Punjab, Himachal Pradesh, Uttar Pradesh, Madhya Pradesh, Bihar, Andra

Pradesh and Orrissa. *Catechuoides* is found in terrai region of Sikkim, Assam and West Bengal, whereas *Sundra*, generally known as Lal Khair (red catechu) is found in Deccan, Gujrat, Rajasthan and southern Maharashtra.[2]

Acacia catechu is used in traditional medicinal system for its wider range of therapeutic properties. various parts of the tree ex. ,leaf, bark, heartwood possess medicinal value.[3] The decoction of bark mixed with milk is taken to cure cold and cough. The bark decoction is either alone or used in combination with opium to cure severe diarrhea.[4] Heartwood of Khair is boiled with other ingredients to prepare the decoction. It is taken as tea by the pregnant ladies to keep warm their body. It is also given to cure fever due to cold during the pregnancy.

Acacia catechu is highly valuable for its powerful astringent and antioxidant activities. It is useful in dental, oral, throat infections and as an astringent for reducing oozing from chronic ulcers and wounds. The extracts of *Acacia catechu* exhibits various pharmacological effects like antipyretic, anti-inflammatory, antidiarrhoeal, hypoglycemic, hepatoprotective, antioxidant and antimicrobial activities .[5- 9]

Main chemical constituents of *Acacia catechu Willd* are catechin, (-) epicatechin,epigallocatechin, epicatechin gallate, epigallocatechin, phloroglucin, protocatechuic acid, quercetin, poriferasterol glucosides, poriferasterol acyglucosides, lupenone, kaempferol, dihydrokaempferol, taxifolin, (+)-afzelchin gum and minerals .[10-15]The chief phytoconstituent of the heartwood are catechin and epicatechin. Catechins have siginificant antioxidant and antimicrobial effects. [16,17]

Both rutin and quercetin possess antioxidant activity and reduce low density lipoproteins (LDL) oxidation .[18] quercetin in combination with other flavonoids, are inhibiting a number of enzymes like bradykinin [19], tyrosine kinase [20], and 5'-nucleotidase activity[21]. Rutin and quercetin have shown regulatory activity of hormones like affect the transport, metabolism and action of thyroid hormones. High Performance Thin Layer Chromatography (HPTLC) method is the suitable method for estimation of chemical constituents present in plant materials. Hence an attempt was made to estimate the presence of rutin and quercetin present in *Acacia catechu Ethanolic leaf extract* by HPTLC finger printing method.

MATERIALS AND METHODS

Chemicals and Reagents

Rutin, quercetin [Green chem. herbal extracts and formulations, Bangalore], ethyl acetate, formic acid, glacial acetic acid and methanol [all Reagents of analytical grade, E-Merck] and silica plate with Linomat applicator precoated TLC aluminium plates [E-Merck].

Apparatus

Spotting device: Linomat IV automatic sample spotter;CAMAG (Muttenz, Swizerland)

Syringe: 100µL Hamilton syringe (Bonadug, Swizerland)

TLC chamber: Glass twin trough chamber (20× 10× 4).

Densitometer: TLC scanner 3 with SPI software 1.21 version; CAMAG

HPTLC Plate: 10×10cm, 0.2mm precoted with silica gel 60F254; Merck

pH meter: Elico Ltd., Hyderabad, India.

Flame Photometer: Digital Biomed Flame Photometer, Hyderabad.

Muffle furnace: Dolphin Industries Ltd., Mumbai.

Preparation of standard Solution

Quercetin RS solution: 10 mg of Quercetin RS were accurately weighed into a 10 mL volumetric flask, dissolved in 25mL methanol, it was sonicated for 30 minutes and the solution was made up to 10 mL.

Rutin RS solution: 10 mg of Rutin RS were accurately weighed into a 10 mL volumetric flask, dissolved in 25mL methanol, it was sonicated for 30 minutes and the solution was made up to 10 mL.

Preparation of sample solution

200 mg of *Acacia catechu* leaf extract was accurately weighed into a 10 mL volumetric flask, was extracted by heating 40°C for 10 minutes, dissolved in methanol and then solution was filtered through Whatman filter paper No. 42 and the filtrate was made up to the mark with methanol and proceeded for spotting.

Development of HPTLC Technique

The samples were spotted in the form of bands with hamilton syringe on a precoated silica gel plates 60F 254 [10 cm X 10 cm with 0.2 mm thickness, E.Merck] using Camag linomat IV applicator. Automatic sample spotter of band width 7 mm. The plates were developed in a solvent system in CAMAG glass twin through chamber previously saturated with the solvent for 30 min. the distance was 8 cm. subsequent to the scanning, TLC plates were air dried and scanning was performed on a Camag TLC Scanner 3 in absorbance at 366 nm and operated by LC Solutions Version 1.21 SP1 software on a Pentium computer (Hewlett Packard).

Rutin and Quercetin estimation in Herbal extract

Stationary Phase: Silica gel 60 F 254 plates, Mobile phase ethyl acetate: glacial acetic acid: formicacid:water [10:10:80v/v],Standard:Rutin1mg/ml[5µL],Standard : Quercetin 1 mg/ml [5 µL],Sample : Herbal extract 10 mg/ml [10 µL],Migration distance : 60 mm, Detection wavelength : 366nm, Mode of scanning :Absorption

CHROMATOGRAPHY [22,23]

A. 10 ml of mixture of Formic Acid: Water: Ethyl acetate (mobile phase-10: 10: 80] is transferred to the chromatographic tank. What man Filter paper disc is placed and kept closed with the lid (for faster saturation of the tank with the solvent system). now the tank is allowed to saturate for 30 minutes.

B. 10 µl of sample(s) and 10 µl Standard (as 10 mm bands separated by a distance of 15 mm; at 10mm from the base) is applied on a HPTLC silica plate using a Linomat HPTLC applicator.

C. the plate is kept in Fume hood to let the solvent to evaporate and the plate is placed in the tank as near vertical as possible ensuring that the line of application is well above the solvent level. the lid is tightly replaced and the solvent are allowed to ascent to 1.5cm below the top of the plate.

D. the plate is removed and let to air dry in fume hood

Spraying reagent

The plate is kept heated at 105 ° C for 5 min. the warm plate is sprayed with 10g/L solution of Diphenyl boric acid amino ethyl ester reagent and 50g/L solution of Macrogol 400 in methanol. the plate is allowed to dry and examined in UV 366nm.

Detection

After Spraying UV 366nm

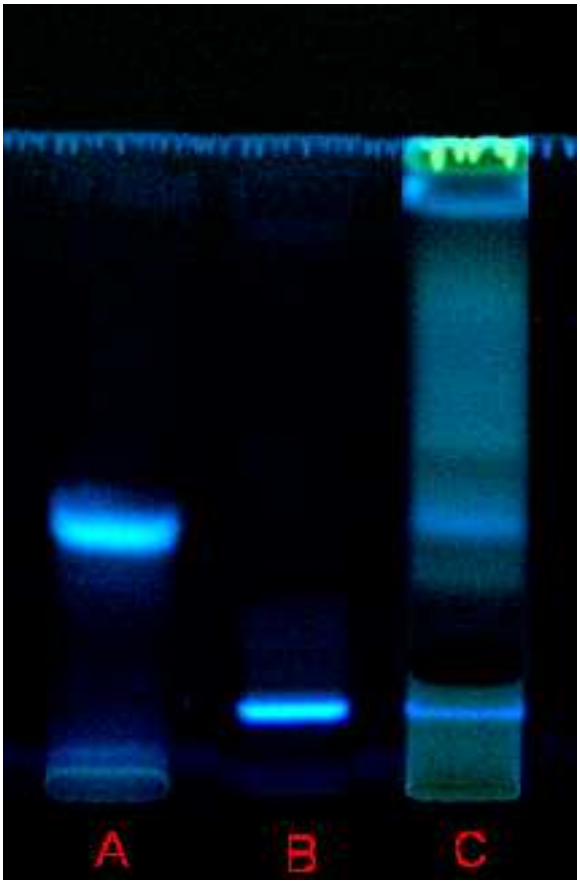

FIG 1 – TLC FINGERPRINT PROFILE OF *ACACIA CATECHU*

A – Quercetin/Std/99.0%
B – Rutin/Std/98.0%
C – *Acacia catechu* ethanolic leaf extract

RESULT & DISCUSSION

The chromatogram obtained with the reference solutions shows in the middle part a Light greenish blue fluorescent zone (Quercetin), in the lower part a bluish fluorescent zone (Rutin). The chromatogram obtained with the test solution shows a light greenish blue fluorescent zone corresponding in position to the zone due to Quercetin in the chromatogram obtained with the reference solution and it shows a bluish fluorescent zone corresponding to the zone due to Rutin in the chromatogram obtained with the reference solution. Further zones are present.

CONCLUSION

From the superimposition study the HPTLC fingerprinting is ideal which involves comparison between a standard and a sample. The use of markers like quercetin and rutin ensures that the concentration and ratio of components in the herbs are present in reproducible levels in raw materials and in the final dosage form. Therefore HPTLC fingerprinting is proved to be a linear, precise, accurate method for herbal formulation and can be used further in quality control of not established herbals.

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