J. Phytol. Res. **20**(1) : 43-45, 2007

ISOLATION AND IDENTIFICATION OF FLAVONOID "QUERCETIN" FROM *ACACIA CATECHU* (L.F.) WILLD – A KATHA YIELDING PLANT

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Acacia catechu (Mimosoidae) is an important source of the industrial product katha obtained from heart wood which is used in paan preparation. Flavonoid "quercetin" was isolated from heart wood of the species. The dried samples were separately soxhlet extracted in 80% methanol and then reextracted with petroleum ether, diethyl ether and ethyl acetate. The fraction was concentrated and subjected to TLC plate. The Rf value of isolated quercetin and standard quercetin was calculated. The purified material was subjected to its IR spectral analysis and identified as "quercetin". This study is also of practical importance because quercetin is an important ingredient of Katha.

Keywords: *Acacia catechu;* Flavonoid; Heart wood; Quercet in.

Introduction

Arid and semi arid plants are good sources for the production of various types of secondary metabolites which make them resistant to various environmental stress e.g. scarcity of water, salinity, pathogens etc. They are also important for the primary metabolism of plants. These compounds include alkaloids, flavonoids, steroids, phenolics, terpenes, volatile oils etc. Man has been exploiting these natural plant products for use in medicines, cosmetics, dyes, flavors and foods.

Flavonoids are one of the major secondary compounds which occur ubiquitously in higher plants. They are synthesized from phenyl propanoid and acetate derived precursors. Flavonoids are important for human beings due to their antioxidative and radical scavenging effects as well as their potential estrogenic and anticancer activities[1].Quercetin belongs to this group of plant pigments called flavonoids that are largely responsible for the colours of many fruits, flowers and vegetables. Quercetin works as antiinflammatory, antioxidant, anticancer agents[2].

Acacia catechu (L.f.) Willd (Mimosoidae) one of the most important tree species is locally known as katha or khair tree. Various products from the plant are extensively used in ayurvedic system of medicine and also manufacture of food and furniture. Three important products are extracted from it viz. katha (catechu), cutch, kheersal. Katha is obtained from red heart wood of 10-20 years old tree used as an ingredient of paan (betal leaf masticatory) and gives red colour to saliva. It is used in treatment of diarrhoea, dysentry, ailments of mouth, gums, tonsil. It also finds use as flavoring agents in condiments, icecream, candy, beverages.

The present study deals with the isolation and identification of flavonoid "quercetin" from heart wood of *Acacia catechu* (L.f.) Willd. Quercetin is one of important constituents of katha.

Material and Methods

Five heart wood samples of *Acacia catechu* were collected from Jhalawar, Lucknow, Sariska, Ghati (Malpura) and Jaipur. Samples were collected from 10-20 years old tree and dried at 100°C for 15 minutes and then at 60°C untill a constant weight was achieved.

Isolation of flavonoid: The dried samples were separately soxhlet extracted in 80% methanol (100 ml/gm dry weight) on a water bath for 24 hrs. Each of the extracts was concentrated and reconcentrated in petroleum ether (40°-60°C) (fraction I), ethyl ether (fraction II) and ethyl acetate (fraction III) in succession. Each of the steps were repeated three times to ensure complete extraction in each case. Fraction I was rejected due to its being rich in fatty substances whereas fraction II was analysed for the free flavonoids in each of the samples.

Fraction III of each of the test samples was hydrolysed by refluxing with 7% H_2SO_4 (10 ml/gm residue) for 2 hours. The mixture was filtered and the filtrate extracted with ethyl acetate in a separating funnel. The ethyl acetate layer was washed with distilled water till neutrality, dried *in vacuo* and was analysed for bound flavonoids.

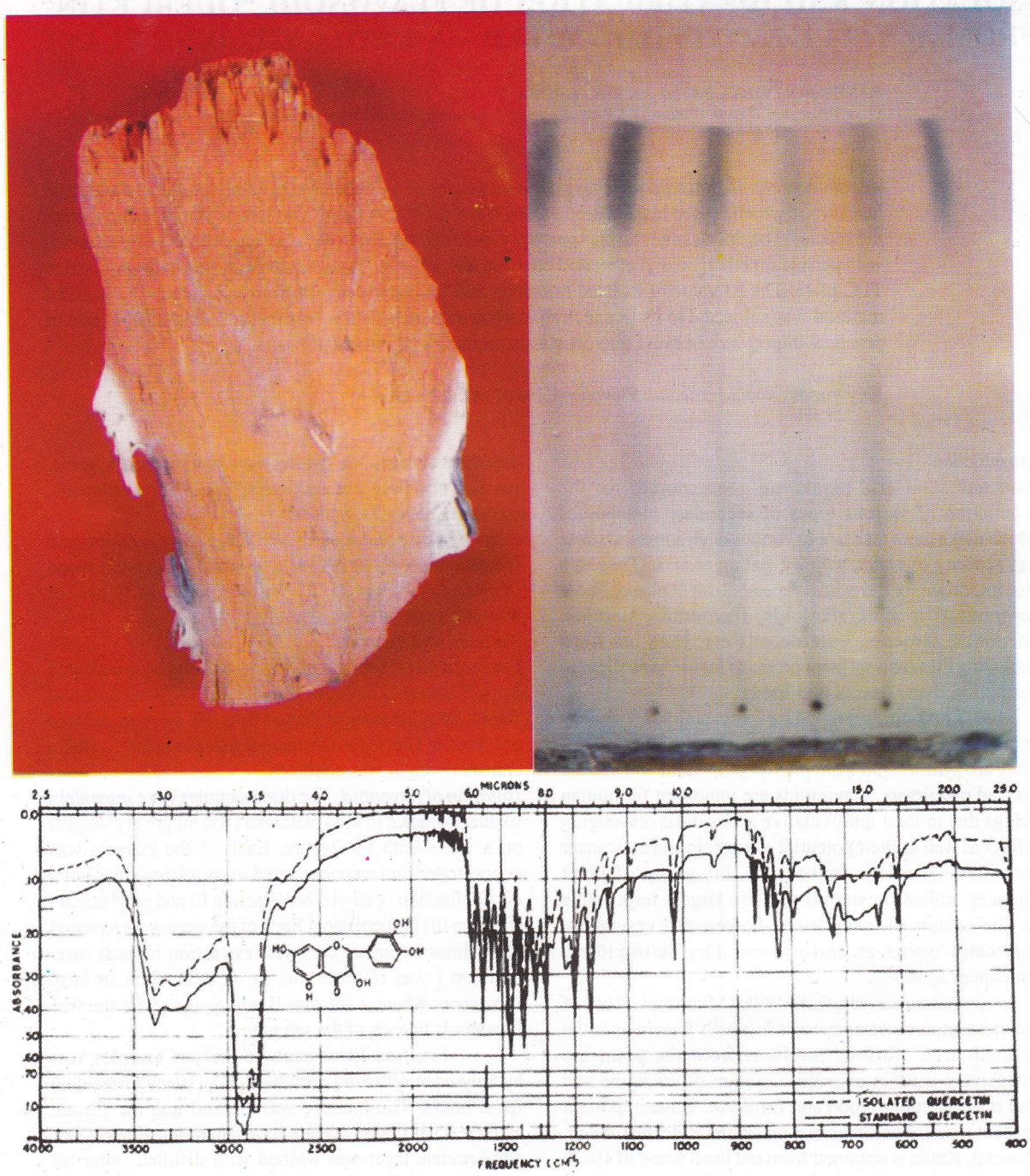

Fig.1.(a) Heart wood sample of *Acacia catechu.*

(b) TLC plate showing presence of quercetin in isolated samples of heart wood.

(c) Superimposed IR spectra of isolated quercetin and standard quercetin.

Identification of flavonoid: The glass plates (20×20 mm) coated with silica gel 'G' (0.2-0.3 mm thick and 30 gm/60 ml) were dried at room temperature. The dried plates were activated at 100°C for 30 minutes in an oven and cooled at room temperature. Ethyl ether and ethyl acetate fractions from each sample were separately applied 2 cm above the edge of the plates along with standard reference compound quercetin. These glass plates were developed in an air tight chromatography chamber containing, about 200 ml of solvent mixture of n-butanol, acetic acid and water (4:1:5).

The developed plates were air dried and sprayed with 5% ethanolic ferric chloride solution to observe the colour of the spots. These plates were also placed in a chamber saturated with ammonia vapours to observe the colour of the spots. Rf value was calculated for isolated sample and coinciding standard.

The developed plates were also visualized under UV light. These fluorescent spots were marked, scraped and eluted with ethanol. Each of the elutes were then crystallized with chloroform. The purified material was subjected to its IR spectral analysis.

Results and Discussion

When the developed plates were sprayed with 5% ethanolic ferric chloride solution, it showed spots which coincided with that of the reference quercetin (bluish grey, Fig. 1b) and when plates were placed in a chamber saturated with ammonia vapours, it showed deep yellow colour of quercetin. Rf value (0.82) of quercetin isolated from the samples coincided with the Rf value of standard quercetin.

The plates developed under UV light showed fluorescent spots in both the fraction II and III coinciding with the standard sample of quercetin (Blue). The characteristic IR spectral peaks were found to be superimposable with those of their respective standard reference compounds of quercetin (Fig. 1c). Quercetin was detected in all the five samples of heart wood of *Acacia catechu.*

More than 2000 flavonoids have been reported among woody and non-woody plants[3]. Biosynthesis, isolation techniques and preparative chromatography[4], TLC, UV and IR spectral studies has provided new dimensions to the chemistry of flavonoids to such an extent that their presence have become important taxonomically[5]. Presence of flavonoids has been reported from many plant species like *Lycium barbarum*[6] , *Arachis hypogea*[7] , *Passiflora plamer*[8], *Heliotropium* species , *Cassia angustifolia*[10], *Jatropa curcas* L.[11]. Quercetin has been reported from many plant species like *Emblica officinalis*[12] and *Cicer arietinum* Linn.[13]

Hence, in the present studies isolation and identification of flavonoid quercetin from the heart wood of *Acacia catechu* has been carried out. This study is also of practical importance because quercetin is an important ingredient of Katha.

Acknowledgement

The first author is highly thankful to University of Rajasthan for Departmental Scholarship.

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