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Estimation of Quercetin in *Acacia catechu* Ethanolic Bark Extract by HPLC method

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Abstract: High-performance liquid chromatography (HPLC) is a powerful analytical technique because of its reliability, simplicity, reproducibility, and speed. The aim of this work was to develop an accurate, specific, repeatable and robust method for the estimation of Quercetin content in *Acacia catechu* ethanolic bark extract. The method was validated in compliance with International Conference on Harmonization guidelines. The data obtained from our study conclude that the amount of Quercetin present in *Acacia catechu* ethanolic bark extract is 0.070 % w/w.

Keywords: Acacia catechu Bark, Quercetin, HPLC, ICH guidelines.

INTRODUCTION

Quercetin is a plant pigment found in many foods such as onions, apples, berries, tea, grapes and red wine. It's not a nutrient, but is classified as a flavonoid. Like many other plant chemicals, it is sold as a supplement. Oral quercetin is relatively well absorbed, and it is metabolized mainly to isorhamnetin, tamarixetin and kaempferol.^[1] It often occurs in plants as glycosides, such as rutin (quercetin rutinoside) in tea. Quercetin and rutin are used in many countries for blood vessel health and are ingredients of numerous multivitamin preparations and herbal remedies .Quercetin posses antioxidant, anti inflammatory, anti cancer, anti microbial property. The dry skin of wild onion is used as a yellow dye, it contains Quercetin, which is an anti allergic agent and is helpful in treating IBD.^[2]

Acacia catechu (AC) (Family: Fabaceae, subfamily: Mimosoideae) known as Black cutch.AC is medium sized thorny deciduous tree mainly found in India and also found in deciduous forests around the world. It grows up to 13 meters in height. The foliage is softly textured; light green and oval-shaped. The branches are thin and spike like due to tiny thorns grown around the exterior. The sap wood of AC is large and yellowish white and heart wood is small and red in colour. The leaves, bark and heartwood have many nutritional and medicinal uses.^[3,4]

The chief constituents of the plant are catechin and catechutannic acid.^[5] The wood contains epicatechin.^[5], Atzelchin, catechin tetramer, dicatechin, gallochin, gossypetin, phlobatannin, kaempferol, quercetin.^[6] Catechin is biologically highly active. It is used as a haemostatic. Taxifolin an other important constituent has antibacterial8, anti-fungal , antiviral, anti-inflammatory, and antioxidant activity.^[6].

The extract of *Acacia catechu* extract have been reported to have various pharmacological effects like immuno modulatory¹³, anti pyretic ^[7], hypoglycaemic, hepatoprotective activity. ^[7, 11] Cutch is astringent, cooling, and digestive. It is useful in cold and cough ^[9, 10] ulcers, boils and eruptions of the skin, bleeding piles, uterine haemorrhages, atonic dyspepsia, chronic bronchitis etc. In stomatitis, halitosis, dental caries and cavities. ^[16]The bark is anthelmintic, antipyretic, antiinflammatory and antileprotic. Bark and cutch are antidiarrhoeal, astringent and stomachic. *Catechu* or

Cutch tree bark is useful in melancholia, conjunctivitis and haemoptysis.^[17]

Our literature survey revealed that there are no scientific studies carried out regarding the phytochemical activity of Acacia catechu ethanolic Bark extract compared to standard Quercetin by HPLC method. Hence in our present study the Ethanolic Bark extract of Acacia catechu were examined for its presence of Quercetin content in it which is responsible for its pharmacological actions.

MATERIALS AND METHODS

Extract and Chemicals

The Ethanolic Bark extract of Acacia catechu willd and Quercetin standard was obtained from Green Chem, Herbal Extracts & Formulations, Bangalore. Acetonitrile and methanol HPLC grade solvents; all analytical grade solvents obtained from E-Merck Ltd, Mumbai, India. Potassium dihydrogen orthophosphate Analytical Reagent grade was procured from Qualigens Fine Chemical, Mumbai, India.

Instrumentation

The output signal was monitored and processed using LC Solutions Version 1.21 SP1 software on a Pentium computer (Hewlett Packard).

Preparation of Standard Solution

The standard was prepared by accurately weighing about 10 mg Quercetin in 10 ml of methanol in a volumetric flask. It was then sonicated for 10-15 minutes. The contents of flask were filtered through Whatman no.41 paper (Merck, Mumbai, India) to attain the stock solution of 1 mg/ml.

Preparation of Sample Solution

500 mg Sample (Acacia catechu Bark extract) is accurately weighed and dissolved in 50 ml of methanol in a volumetric flask. It was then sonicated for 10-15 minutes then the contents of the flask were filtered through Whatman No. 41 paper (Merck, Mumbai, India) to attain the stock solution of 10 mg/ml.

Methodology

High-performance liquid chromatography method development ^[18]

The separation was performed by using column C18 ODS thermo 250×4.6 mm, 5 µm on a Shimadzu class LC(California ,USA), which consisted of a FCV-10 ACVP pump, DGU-14A degasser, a thermo

stated CTO-10AVP column oven compartment, an auto sampler, and a SPD-M10AVP diode array detector and Rheodyne 7725i injector with 20 µl loop volume and a flow rate of 1.0 ml/min. The output signal was monitored and processed using SPI Software LC Solutions, Version 1.21 on a Pentium computer (Hewlett Packard). All analyses were performed at a ambient temperature of 37°C, with a mobile phase of orthophosporic acid /Acetonitrile,

The analysis was performed using a highperformance liquid chromatographic system an injection volume of 20 µl, and a flow rate of 1.0 ml/min. The UV absorbance of the eluent was measured at 350 nm.

The developed method was validated according to conference International on Harmonization guidelines (ICH).^[19]

Chromatography

Column C18 ODS thermo
(250 x 4.6 mm) 5µm
350 nm
20 µl
1.0ml/min.
Solvent A-0.1 %
orthophosphoric acid
Solvent B-Acetonitrile
80:20v/v

HPLC ANALYSIS

Equation 1

Calculation of Assay of Quercetin

(Std Wt / Sample Wt) x (Sample Area / STD Area) x Assay of Standard = Assay of Sample.

Calculation of assay of Quercetin in Acacia catechu Bark (Ethanolic) Extract

Sample Weight taken	: 10.02mg
Area of sample	: 410112
Standard Weight taken	: 1 mg
Area of Standard	: 5754965
Assay of Quercetin	: 100%

Assay of Quercetin

(1.0 /10.02) x (410112 /5754965) x 100 =0.070% w/w.

CHROMATOGRAM ANALYSIS

GRAPH 1

ESTIMATION OF STANDARD (QUERCETIN)

Sample Name	: QUERCETIN/STD/100%
Vial	: 1
Injection Volume	: 20ul



PeakTable D:\hpic03-data-ddrive\D\Total flavanoids 1\Flavanoids 147.1cd							
Detector A Ch1 350nm							
Peak#	Name	Ret. Time	Area	Theoretical Plate#	Tailing Factor	Area %	
1	Quercetin	22.839	5754965	197405.868	1.601	100.000	
Total			5754965			100.000	

TABLE 1 – CHROMATOGRAM ANALYSIS OF QUERCETIN

GRAPH 2

ESTIMATION OF QUERCETIN CONTENT IN ACACIA CATECHU ETHANOLIC BARK EXTRACT

Sample Name	: Acacia catechu ethanolic Bark extract
Vial	: 2
Injection Volume	: 20ul



Detector A C	Detector A Ch1 350nm						
Peak#	Name	Ret. Time	Area	Theoretical Plate#	Tailing Factor	Area %	
1	Quercetin	22.761	410112	160391.324	1.630	100.000	
Total			410112			100.000	
Iotal			410112			10	

TABLE 2 - CHROMATOGRAM ANALYSIS OF ACACIA CATECHU ETHANOLIC BARK EXTRACT

RESULT AND DISCUSSION

Flavonoids like Quercetin are a group of polyphenolic compounds, which possess many biochemical effects like inhibition of enzymes, regulatory role on different hormones and pharmacological activities like antimicrobial, antioxidant, and anticancer, antihepatotoxic, protection of cardio vascular system.

Acacia catechu willd contains many active constituents in it like Catechin, Epicatechin, Quercetin, Taxifolin etc., the HPLC studies revealed that the plant extract were also rich in Quercetin (0.070%w/w), the assay of Quercetin was calculated according to the

Equation 1 .The retention times observed for the authentic sample of Quercetin and those of *Acacia catechu* ethanolic Bark extract are given in Table 1 and 2.

The present study reveals that the antioxidant, antimicrobial, antiinflammatory, anticancer activity is not only due to presence of phytochemical constituent like Catechin and Epicatechin, it is also due to the presence of Quercetin which possess lots of medicinal value, since antiquity.

CONCLUSION

Herbal medicines have been used for many years. Phytomedicine with medicinal properties are a useful and effective source of treatment for various diseases. Many of the common drugs used by us today are derived from herbs. Screening of medicinal plants for phytochemical analysis are important for finding potential new compounds for therapeutic use .Hence our present study concludes that the Quercetin content

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present is 0.070%w/w in *Acacia catechu* ethanolic Bark extract determined by HPLC method.

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