



Research Article

NEW APPROACH FOR ESTIMATION OF BIOMARKER QUERCETIN IN ETHANOLIC LEAF EXTRACT OF *ACACIA CAESIA* (L.) WILLD., BY HPLC METHOD

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ABSTRACT

Acacia caesia is a medicinal plant rich in phytochemical compounds which are used for the treatment of skin diseases, sexual problems and wound healing, stomach and tooth problems. The present study deals with the preliminary phytochemical screening of *A. caesia* for various bioactive compounds such as alkaloids, anthraquinones, flavonoids, glycosides, phenols, tannins, saponins, steroids and terpenoids. And also quantify the bioactive compound flavonoid in the ethanol extract of *A. caesia* from by High Performance liquid chromatography (HPLC) analysis. The main objective of our study is to estimate the quercetin content present in *A. caesia* ethanolic leaf extract by HPLC method. The estimation of quercetin content in *A. caesia* ethanolic leaf extract was performed using shim-pack CLC ODS (4.6×15 mm) column and a 350 nm detector. The mobile phase consisted of 0.1 % phosphoric acid in water and acetonitrile at 80:20 v/v. The method was validated in compliance with International Conference on Harmonization [ICH] guidelines. In conclusion, the quercetin content present in the ethanolic leaf extract of *A. caesia* was found to be 0.76% w/w.

Keywords: *Acacia caesia*, phytochemical, ethanol, HPLC, quercetin

INTRODUCTION

India has a rich part of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for ayurvedic, unani and siddha traditional medicines but only very few medicinal plants have been studied chemically and pharmacologically for their potential medicinal value^{1,2}. The medicinal importance of a plant is due to the presence of some special substances like alkaloids, glycosides, resins, volatile oils, gums and tannins etc. which can be later designed into drugs. This compound may enhance the possibility of designing novel drugs to life-threatening diseases³.

Acacia caesia (L.) Willd., is a species of plant in the Mimosaceae family. It is an armed woody straggling shrub growing throughout India especially in the foot hills of Western Ghats of Coimbatore and Erode District, Tamil Nadu. In the present study *Acacia caesia* was selected for further HPLC studies. The local name of the species is 'Kari indu or Indamul'. It is used for the treatment of skin, sexual problems, wound, stomach and tooth problems. Still many herbal products derived from *Acacia* species are sold in markets in pure or mixed forms like babool tooth paste, ayur shampoo, nyle shampoo, etc. All *Acacias* are suitable materials for fuel, forage, soil fertility and soil conservation. In Indian systems of medicine *Acacia caesia* is a strong antioxidant rich medicinal plant. Hence, this plant was selected for evaluating new bioactive compounds.

In the current era, we evaluated the preliminary phytochemical constituents and also estimate the quercetin content from ethanolic extract of *Acacia caesia* leaves by High Performance Liquid Chromatography (HPLC). This technique is used to determine species in mixture of organic, inorganic, biological, ionic and polymeric materials⁴. Our literature survey revealed that there are no scientific reports carried out regarding the screening

of phytochemical flavonoid from *Acacia caesia* compared to standard quercetin by HPLC method. Hence in our present study the ethanolic leaf extract of *Acacia caesia* were examined for its presence of quercetin content in it which is responsible for its pharmacological actions.

MATERIALS AND METHODS

Chemicals and instruments

All the chemicals used for present study were of laboratory grade. Quercetin standard was obtained from Green Chem, Herbal Extracts and Formulations, Bangalore. All analytical grade solvents were obtained from E-Merck Ltd, Mumbai, India.

Collection and authentication of plant material

Fresh and healthy leaves were collected from the Maruthamalai Hill (arid; 540 m above msl; dry deciduous forest), Coimbatore District (a part of the Western Ghats of Western Tamil Nadu). The plants were collected in their flowering and fruiting seasons from the natural habitat. While collecting the study plant, a thorough observation was made regarding the location, natural habitat, distribution pattern, habit, floral and fruit characteristics etc. The collected study plant was identified with the help of the existing Floras⁵⁻⁷ and compared with type specimens available in the herbarium of Botanical Survey of India, Southern Circle, Tamil Nadu Agricultural University Campus, Coimbatore, Tamil Nadu (Voucher specimen No. BSI/SRC/5/23/2015/TECH/343).

Preparation of extract

Five hundred grams of coarse powder of shade dried leaves of *Acacia caesia* was extracted successively with petroleum ether and ethanol in Soxhlet extractor for 8 hours. The extract was

filtered, concentrated in a vacuum evaporator and dried in a desiccator to obtain constant weight. The percentage of ethanol soluble extractive was calculated with reference to the air dried sample. The obtained extracts were stored in desiccators for further phytochemical and HPLC analysis to find the bioactive components.

Phytochemical studies

Qualitative phytochemical analysis

Phytochemical screening of different successive solvent extracts was carried out following the methods⁸⁻¹⁰. Carbohydrates, proteins and amino acids, alkaloids, anthraquinones, flavonoids, glycosides, phenols and tannins, saponins, steroids and terpenoids were qualitatively analyzed.

Quantitative phytochemical analysis

Determination of total flavonoid contents

0.5 ml aliquot of properly diluted sample solution (10 mg / 2 ml) was mixed with 2 ml of distilled water and 0.15 ml of 5 % NaNO₂ solution. After 6 minutes, 0.15 ml of 10 % AlCl₃ solution was added and allowed to stand for 6 minutes. In this mixture, 2 ml of 4 % NaOH solution was added and water make up to a final volume of 5 ml. Finally, the mixture was thoroughly mixed and allowed for another 15 minutes; determination of mixture Absorbance at 510 nm versus water blank. The results were expressed as rutin equivalent¹¹.

HPLC analysis

The HPLC analysis of ethanolic leaf extract of *Acacia caesia* was carried out with Chromatographic system (Shimadzu, Japan), using shim-pack CLC ODS (4.6 × 15 mm) column and a 350 nm detector.

Calculation of Assay

$$\text{Quercetin percentage} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Concentration of standard}}{\text{Concentration of sample}} \times \text{Purity of standard (70\%)}$$

The developed method was validated according to International conference on Harmonization guidelines (ICH)¹²

RESULTS AND DISCUSSION

Qualitative phytochemical evaluation

Phytochemical constituents such as alkaloids, flavonoids, glycosides and several other aromatic compounds are secondary metabolites in plants that have alleviated the pathogenic and environmental stress¹³. To investigate the chemical constituents of plant powder of *Acacia caesia*, the successive solvent extracts were subjected to qualitative phytochemical screening. The preliminary phytochemical screening of all three extracts revealed the presence of carbohydrates, proteins, amino acids, alkaloids, glycosides and phenols. From the twelve identified compounds petroleum ether and ethanol extraction showed best results. The petroleum ether and ethanol extracts were more efficient than water extract (Table 1). These activities may be related to their activity¹⁴

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 filter paper (Merck, Mumbai, India) to attain the stock solution of 1 mg/ml. The injected volume of standard was 2 µl (2 µg).

Preparation of sample solution

50 g sample (*Acacia caesia* leaf extract) is accurately weighed and dissolved in 250 ml of ethanol 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. The injected volume of standard was 20 µl (400 µg).

Methodology

High-performance liquid chromatography method development:

HPLC instrument: Chromatographic system (Shimadzu, Japan), using shim-pack CLC ODS (4.6 × 15 mm) column and a 350 nm detector was used with data acquisition by SPI software. All chromatographic data were recorded and processed using autochro-3000 software.

HPLC conditions

The mobile phase consists of two pumping systems (Pump A – 0.1 % phosphoric acid in water; Pump B – Acetonitrile at 80:20 v/v) and the separations were performed by using isocratic mode, elution performed at a flow rate of 1 ml/minutes. The samples were run for 30 minutes and detection was done at 350 nm by UV detector.

Calculate the content of the Quercetin in the test sample.

Quantitative phytochemical evaluation

Total flavonoid content

The flavonoids have been found to possess anti-inflammatory properties in various studies¹⁵. Total flavonoid content of different solvent extracts of *Acacia caesia* leaf powder was studied and expressed as rutin equivalent and shown in Table 2. The total flavonoid content was maximum in water extract (5.52 mg/g) followed by ethanol (2.23 mg/g) and water (2.13 mg/g).

HPLC analysis

Standardization and characterization of herbal drugs is a focus of permanent systematic awareness in the herbal drug production. The modern chromatographic systems are an ever growing plan to create and extend simple, speedy, suitable and cost effective methods for standardization. Behind this, for standardization of ethanolic leaf extract of *Acacia caesia*, HPLC is a sensitive and accurate tool that widely used for the quality assessment of plant extract and its derived product/formulation.

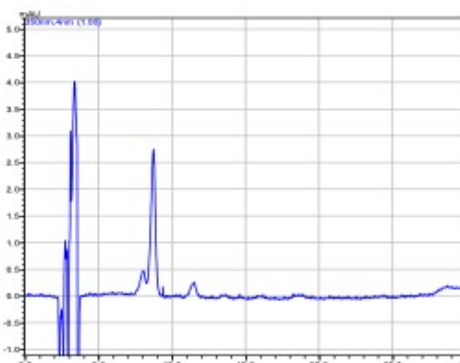
Table 1: Qualitative phytochemical screening of different extracts of *Acacia caesia*

S. No.	Constituents	Petroleum ether	Ethanol	Water
1.	Carbohydrates	+	+	+
2.	Proteins	+	+	+
3.	Amino acids	+	+	+
4.	Alkaloids	+	+	+
5.	Anthraquinones	+	+	-
6.	Flavonoids	+	+	-
7.	Glycosides	+	+	+
8.	Phenols	+	+	+
9.	Tannins	+	+	-
10.	Saponins	+	+	-
11.	Steroids	+	+	-
12.	Terpenoids	+	+	-

Table 2: Estimation of total flavonoid content of different solvent extracts of *Acacia caesia* plant powder

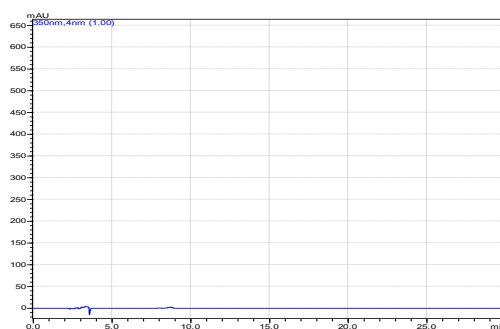
S. No.	Extraction medium	Total flavonoid (mg RE/g extract) [#]
1.	Petroleum ether	2.13
2.	Ethanol	2.23
3.	Water	5.52

[#] Values are means of three independent analysis $\pm$ Standard Deviation RE - Rutin equivalent



Peak number	Retention time (min)	Peak area	Peak height	Name of the compound
1	8.707	53153.8	2610.3	Quercetin

Figure 1: HPLC Profile of standard Quercetin at 350 nm



Peak number	Retention time (min)	Peak area	Peak height	Name of the compound
1	9.201	115918.2	4492.7	Quercetin

Figure 2: HPLC profile of ethanolic leaf extract of *Acacia caesia* at 350 nm

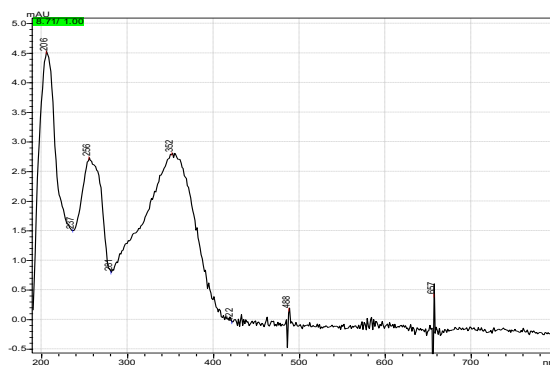
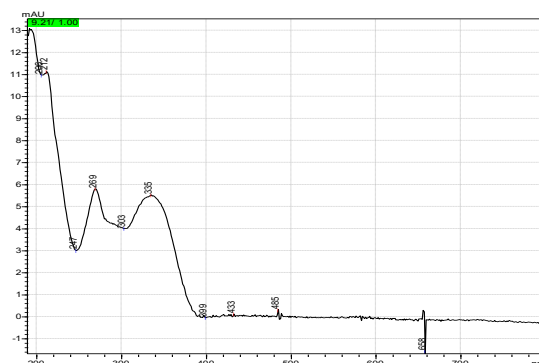


Figure 3: UV Absorbance spectrum of standard Quercetin at 8.71 minutes

Figure 4: UV absorbance spectrum of Quercetin in ethanolic leaf extract of *Acacia caesia* at 9.21 minutes

Quercetin is a polyphenolic flavonoid that is found in buckwheat, apples, onions and certain teas. As a flavonoid, quercetin is a substance that gives most fruits and vegetables in their pigmentation. It is almost present in plants as a food source primarily relates to the prevention of cancer, heart disease and other diseases. Quercetin possesses antioxidant, anti-inflammatory, anti cancer and anti microbial property. Many *Acacia* species contains different active constituents in it like catechin, epicatechin, quercetin, taxifolin etc., The present HPLC studies of *Acacia caesia* also revealed that the ethanolic leaf extract contain more quantity of quercetin (0.76 % w/w), the quercetin assay was calculated to the standard method. The retention times observed for the reliable sample of quercetin was 8.707 minutes and those of *Acacia caesia* was in 9.201 minutes (Figure 1 and 2). The results were noted at 350 nm. UV absorbance spectrum of standard quercetin at 8.71 minutes and absorbance spectrum of quercetin in ethanolic leaf extract of *Acacia caesia* at 9.21 minutes were shown in Figure 3 and 4. The present study investigated the antimicrobial, anti inflammatory, antioxidant and anticancer activity due to the presence of phytochemical component like catechin epicatechin and also the presence of quercetin which possesses loads of medicinal significance. This is in agreement with our present finding¹⁶

CONCLUSION

Screening of photochemical from plants is a very important scientific and laboratory process. This process is used for identifying the crucial components of any plant part such as leaves, stem, root and bark. Difficult to know exactly loads of different medicinal plants are used in the world today, but we know that medicinal plants are extremely important in both traditional and western medicine. Therefore it is important to evaluate the phytochemicals present in the plant parts through a potential sophisticated method. HPLC analytical technique is a

convenient method to recognize the occurrence of various constituents present in the ethanolic extract of plant leaves. The present findings supported that, HPLC is a sensitive and accurate tool that widely used for the quality assessment of plant extract and its derived new product (Quercetin). In conclusion, our present study provides new scientific information about ethanolic leaf extract of *Acacia caesia*, based on its antibacterial potential and chemical profiling that has never been reported.

REFERENCES

1. Gupta MP, Solis PN, Calderon AJ, Guineau – Sinclair F. and Correa M. Medical ethnobotany of the tribes of Bocas del Toro Panama. *Journal of Ethnopharmacology*. 2005; 96: 389-401.
2. Sandhu DS. and Heinrich M. The use of health foods, spices and other botanicals in the Sikh community in London. *Phytotherapy Research* 2005; 19: 633-642.
3. Yadav M, Chatterji S, Gupta SK. and Watal G. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2014; 6(5): 539-542.
4. Kaushal R, Kaur N, Upadhyay A, Suri OP and Thakkar A. High Performance Liquid Chromatography Detectors- A review. *International Research Journal of Pharmacy* 2011; 2(5): 1-7.
5. Gamble GS. and Fischer CEF. The flora of presidency of madras Vol. III. Adlard and son Ltd. London; 1957.
6. Fyson P F: The Flora of the Nilgiri and Pulney hill tops Vol III. Superintendent, Government Press, Madras 1915-20.
7. Mathew KM. Illustrations on the flora of the Tamilnadu Carnatic Vol 2. English, Book, Illustrated edition; 1983.
8. Harborne JB. *Phytochemical methods: A guide to modern techniques of plant analysis*. London, New York: Chapman and Hall, 1984; 12(288): 320.

9. Kokate CK, Purohit AP. and Gokhale SB. Pharmacognosy, 3rd edition, Nirali; 1995.
10. Prabhakaran P. Chemical investigation of finding medicinal plants and related synthetic studies. Ph.D. Thesis, M.K.U. Madurai, Tamil Nadu, India; 1996.
11. Zhishen J, Mengcheng T. and Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999; 64: 555-559.
12. Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP. and Yacobi A. Analytical methods validation: Bio availability, bioequivalence, and pharmacokinetic studies. International Journal of Pharmacy 1992; 82: 1-7.
13. Edreva A, Velikova V, Tsonev T, Dagnon S. and Gurel A. Stress-protective role of secondary metabolites: diversity of functions and mechanisms, General and Applied Plant Physiology. 2008; 34: 67-78.
14. Lambert JD. and Yang CS. Cancer chemopreventive activity and bioavailability of tea polyphenols: a review. Mutation Research. 2003; 523: 208-210.
15. Amaral S, Mira L, Nogueira JM, Da Silva AP. and Helena Florencio M. Plant extracts with anti-inflammatory properties-a new approach for characterization of their bioactive compounds and establishment of structure-antioxidant activity relationships, Bioorganic and Medical Chemistry 2009; 17(5): 1876-1883.
16. Neelam Verma. and Nitu Trehan. HPLC Analysis of Methanolic extract of herbs for quercetin content, Journal of Pharmacognosy and Phytochemistry 2013; 2(1): 159-162.

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