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Research Article

Extraction, Isolation and Identification of Quercetin from *Acacia Arabica L.* Pod Extract

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ABSTRACT

Acacia Arabica L. is an important medicinal plant in Ayurveda and Unani system of medicine. Nowadays it is renamed as *Vachellia arabica* which belonging to family Fabaceae and subfamily Mimosaceae. The phytochemical screening of *Acacia Arabica L.* shows the presence of alkaloids, volatile oil, polyphenols, flavonoids, tannins, resins, steroids, and terpenes. Since all the parts of this plant are useful; it has been recognized worldwide as a multipurpose tree. The bark, root, fruit, flower, and leaf has been used for many therapeutic activities like skin disease, diarrhoea, eczema, diabetes and wound healing and it also acts as astringent, demulcent and anti-asthmatic. The plants have medicinal value due to the presence of various phytochemical constituents like carbohydrate, tannins, saponins, glycosides, and flavonoids. Since the massive study of plant *Acacia arabica L* has been carried out. But still, no extensive reports are available dealing with the isolation of quercetin from *Acacia arabica L*. pods. Thus present study deals with isolation of quercetin from pod extract of *Acacia arabica L*. and that was confirmed by different chromatographic and techniques like column chromatography, thin layer chromatography (TLC), high-performance liquid chromatography (HPTLC) and spectral technique like UV spectrometry, IR spectrometry, etc.

INTRODUCTION

According to the World Health Organization (WHO), about 80% of the population still depends upon medicinal plants for their primary healthcare needs. The plant *Acacia arabica L*. having a medicinal value from the Ayurveda system of medicine [1]. *Acacia arabica L*. commonly called *Mimosa arabica* or *Mimosa nilotica*, belonging to the subfamily Mimosoideae of the family Fabaceae [2]. Nowadays it is renamed as *Vachellia Arabica* [3]. It is known for its number of pharmacological activities like anti-diabetic, anti-inflammatory, wound healing, antiulcer and antioxidant [4].

Traditionally plant it is effective in the treatment of cholera, hair fall, syphilis, gonorrhoea, leucorrhea, diarrhea, dysentery, diabetes, and is also acts as a styptic, astringent, antiprotozoal, hypotensive, spasmolytic, hypoglycaemic, CNS depressant, antifungal [5].

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The therapeutic activity has shown due to the presence of phytoconstituents like tannins, m-digallic acid, and chlorogenic acid, galloylated flavan-3,4-diol and 7,3',4',5'-tetrahydroxyflavan-3,4-diol, Kaempferol-3-glucoside, Isoquercetin, leucocyanidin; hentriacontane, n-hentriacontanol, and paulownia betulin and sitosterol [6]. Modern pharmacological studies have proven that quercetin has resourceful biological functions on human health, including anticancer, anti-inflammatory, and, antioxidant, cardiovascular protection, Asthma, antiulcer, neuroprotective [7].

Quercetin possesses two major forms of glycosides and non-glycosides, which has been studied in cell-based assays and experimental animal models in recent years and is used as a natural therapy for Diabetes Mellitus and anti-obesity [8].

Since, the massive study has been performed for separation of quercetin from various part of the plant like bark, seed, root, fruit, etc. Still, no extensive reports are available deals with the

separation of quercetin from pods of *Acacia arabica L*. Hence in this attempt we planned to isolate the quercetin from pod extracts of *Acacia arabica L.*

MATERIALS AND METHODS

Material

Chemicals: Quercetin standard was procured from Loba Chemie, Mumbai. All other chemicals, solvents, such as n-hexane, chloroform, ethyl acetate, ethanol, glacial acetic acid, ferric chloride lead acetate, and reagents like Mayer's reagent, Dragendorff's reagent, Molischs solution, Barfoed's reagent were procured from Loba Chemie, Mumbai.

Plant Material: Pods of *Acacia arabica L* were handpicked from a local area in the south region of Maharashtra (India) and were authenticated from Department of Botany, Shivaji University, Kolhapur. Plant authentication voucher specimen number (SPA-01)

Methods

Extraction of *Acacia arabica L* pods

Shade-dried pods of *Acacia arabica* were powdered (250g), soxhlet extracted (Fig. 1) with ethanol 70% (v/v). After drying in hot air oven at 45°C, it was stored in an airtight container in the refrigerator at 5°C. The residue was designed as an ethanolic extract of *Acacia Arabica L*. pods. Dried pods of *Acacia arabica* (250 g) were also extracted successively with n-Hexane and Chloroform to get respective extracts. Later on, the obtained residue was further concentrated, dried and stored in desiccators to perform further experiment and its analysis.

Figure 1: Soxhlet extraction of *Acacia arabica* L. Pods

Isolation, identification, and quantification of quercetin was done by column, Thin layer chromatography (TLC), High- Performance thin layer chromatography (HPTLC), Ultraviolet spectrometry (UV), Fourier transform infrared spectroscopy (FTIR) [9, 10].

Phytochemical Screening of Isolated Compound

Preliminary phytochemical screening was carried out, by using standard methods, to identify the presence of various phyto constituents like alkaloids, carbohydrates, steroids, saponins, proteins, fixed oils or fats, flavonoids, tannins and phenols, and glycosides [11-13].

Column Chromatography

5 gm of ethanolic extract was exposed for the isolation of active phytochemical constituent to a prepaid column of silica gel (60-120) mesh for column chromatography. The extract was gradiently eluted with the solvent ratio (Ethyl acetate: Formic acid: Glacial acetic acid: Water (100:11:11:26). The eluted obtain each of 5 ml was collected at the uniform interval and efficiency of separation were analyzed. (Fig. 2) [14-17].

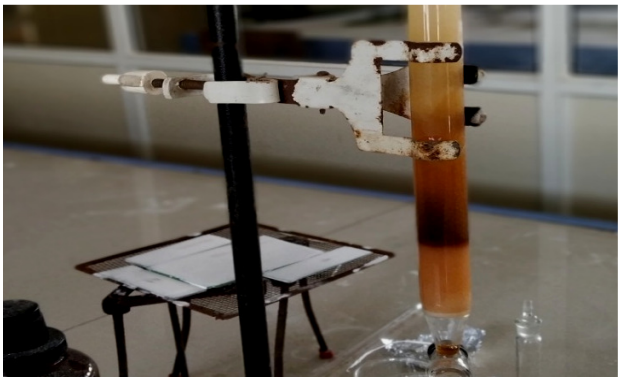


Figure 2: Column chromatography

Thin Layer Chromatography (TLC)

The precoated TLC plate (3.0×8.0 cm) were activated in a hot air oven at 45°C for 30 min and cooled to room temperature. Isolated quercetin was dissolved in ethanol and was placed 1 cm above the edge of the plate along with the standard quercetin. This plate was developed in an airtight chromatography chamber containing solvent system Ethyl acetate: Formic acid: Glacial acetic acid: Water in the ratio of (100.11.11:26). The developed plates were air dried and visualized under UV. These TLC plates were also subjected to spraying reagents for flavonoids such as ferric chloride and aluminium [18-19].

Characterization of Quercetin

UV Spectroscopy

Preparation of Standard Solution of Quercetin and Isolated Quercetin

1 mg sample of standard quercetin was taken and diluted to 10 ml of ethanol to get (100µg/ml) solution. 1ml of above solution further diluted with 10 ml of ethanol to get (10µg/ml) solution. Same procedure was adopted to prepare solution of isolated quercetin. The prepared solution was analyzed for the presence of quercetin using UV spectrometer in the range of 200-400 nm [20].

Attenuated Total Reflectance - Fourier Transform Infrared Spectrophotometer (ATR-FTIR)

ATR-FTIR spectra of isolated quercetin and standard quercetin were recorded by using Infrared spectrophotometer (Jasco-V-530 model). About 2 mg of sample was kept in sample holder and spectra were recorded over the wave number 400-4000cm^{-1} on the spectrophotometer and analyzed [21].

High Performance Thin Layer Chromatography (HPTLC)

Sample Preparation

1 mg sample of isolated quercetin was taken and diluted to 10 ml of ethanol to get (100µg/ml) solution. 1ml of above solution further diluted with 10 ml of ethanol to get (10µg/ml) solution.

Preparation of Standard Solution

1 mg sample of standard quercetin was taken and diluted to 10 ml of ethanol to get (100µg/ml) solution. 1ml of above solution further diluted with 10ml of ethanol to get (10µg/ml) solution.

HPTLC

HPTLC was performed by application of the standards and sample dissolved in ethanol on HPTLC plate silica gel 60F254 (5 cm × 10 cm) with Ethyl acetate: Formic acid: Glacial acetic acid: Water (10:1.1:1.1:2.6) as mobile phase in a Camag glass twin-trough chamber previously saturated with mobile phase vapour for 20 minutes [20]. The details of the instrument are as CAMAG Linomat 5 with 5 application parameters. N_2 was used spray gas; sample solvent type was ethanol with dosage speed 150 ml/s and dosage volume 0.2 µl. The syringe size was 10 µl; application position Y was 8.0 mm and band length was 5 mm. Calibration parameters used were multilevel calibration mode, CV statistics mode and peak area as the evaluation mode. Spots of isolated quercetin (0.3 µg/ml), and standard quercetin (0.3µg/ml) were applied on the plates [22-25].

Table 1: Sequence of application of isolated extract and standard on TLC Plate

Sr. No.	Application Position	Application. Volume	Vial	Active
1	0.15 mm	5 µl	1	Yes
2	0.29 mm	5µl	1	Yes

RESULTS AND DISCUSSION

Extraction was done by using different solvents. The % yield of different solvent extracts from the pods of *Acacia arabica* Linn are shown in Table 2.

Table 2: % Yield of different solvent extracts from the pods of *Acacia arabica* Linn

Sr. No.	Solvent Extract	% Yield
1.	n-Hexane	11.01
2.	Chloroform	16.01
3.	Ethanol	22.14

Table 3: Organoleptic characters of solvent extract

Parameter	Characteristics		
	n-Hexane	Chloroform	Ethanol
Colour	Brownish yellow	Light Brown	Light Brown
Odour	Odourless	Odourless	Odourless
Taste	Tasteless	Bland	Mucilaginous
Nature	Oily	Amorphous	Amorphous

Table 4: Phytochemical Screening of Isolated Compound from different extracts of *Acacia arabica* Linn. pods

Compounds	n-Hexane	Chloroform	Ethanol
Alkaloids	- ve	- ve	- ve
Carbohydrates	- ve	+ ve	- ve
Steroids	- ve	- ve	+ ve
Saponins	- ve	- ve	+ ve
Proteins	- ve	- ve	- ve
Fixed Oils/Fats	- ve	+ve	- ve
Flavonoids	- ve	- ve	+ve
Tannins and phenols	- ve	- ve	+ ve
Glycosides	- ve	- ve	- ve
Reducing sugars	- ve	- ve	+ ve
Amino Acids	- ve	- ve	- ve

Phytochemical Studies

Preliminary phytochemical screening of the different extract showed the presence of secondary metabolites like alkaloids, flavonoids, tannins, sugars, saponins, etc. The maximum constituents, as well as yield, were found in the ethanolic extract which includes tannins, steroid, flavonoids, and saponin glycosides (Fig. 3).

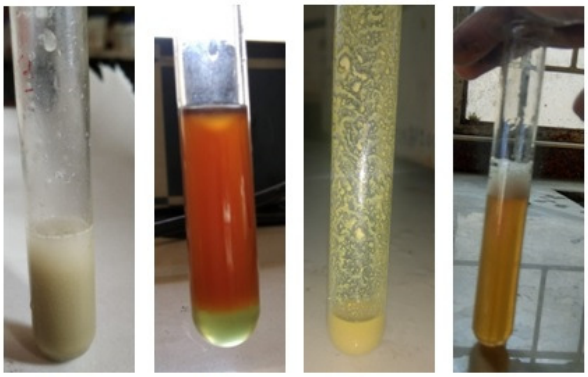


Figure 3: Phytochemical test which shows the positive test of (a) Tannins and phenolic compounds, (b) Terpenes and steroids, (c) Flavanoids (d) Saponins glycoside.

UV Spectra

The wavelength of maximum absorbance for standard quercetin and that of isolated quercetin was found to be equivalent i.e. at 254 nm. Thus it reveals that extracts significantly contain quercetin as a main phytochemical constituent.

IR Spectra

The spectra of isolated quercetin shows the peaks value for characteristic vibration bands of OH Stretching, CH Stretching, -C-O-C Stretching, -C=C Bending , C-H ending at wave number 3322.06 , 2945.28, 1652.65 , 1449.06 respectively. The spectra of standard quercetin showed characteristic peaks at 3320.04, 2938.95, 1418.41 and 1028.54. It confirms that, quercetin is present in isolated compound.

TLC:

The flavonoids present in the extract were found to be standard quercetin as retention factor (R_f) of standard quercetin (0.72) matches with the R_f value of isolated Quercetin (0.71).

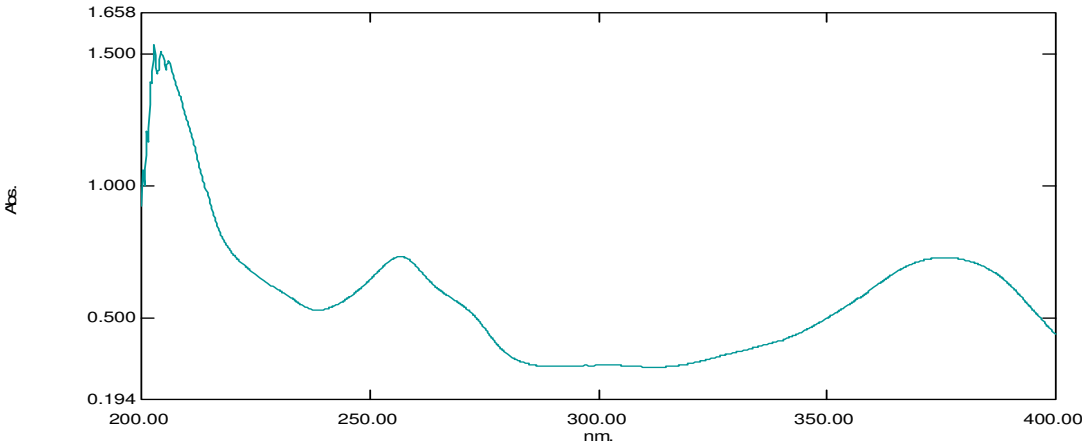


Figure 4: Absorbance maxima of standard quercetin (λ_{max}: 254 nm)

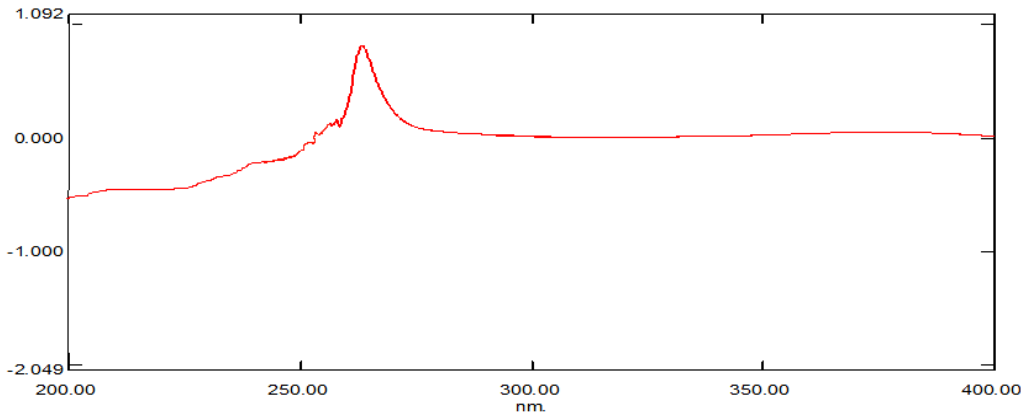


Figure 5: Absorbance maxima of isolated quercetin (λ_{max}: 254 nm)

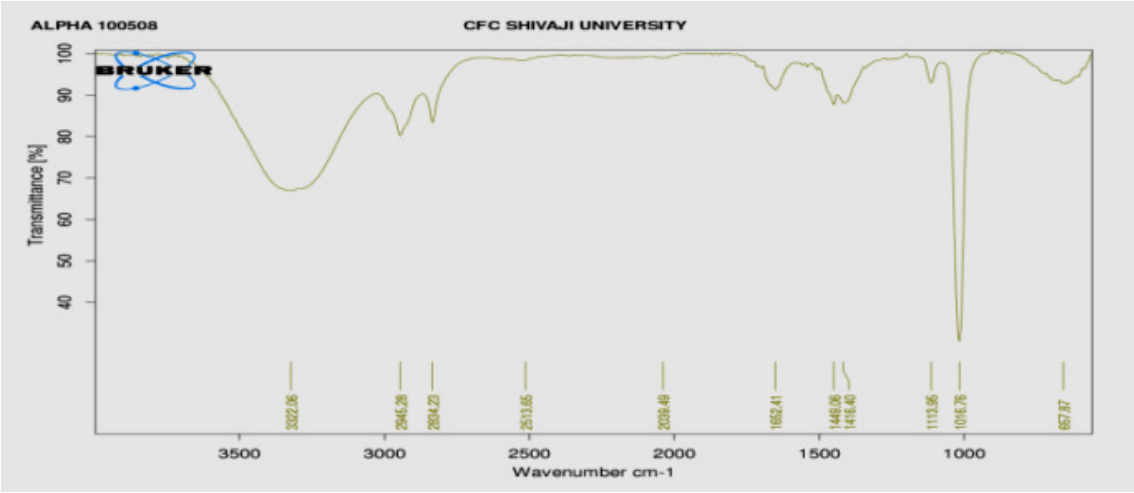


Figure 6: FT-IR spectra of isolated quercetin

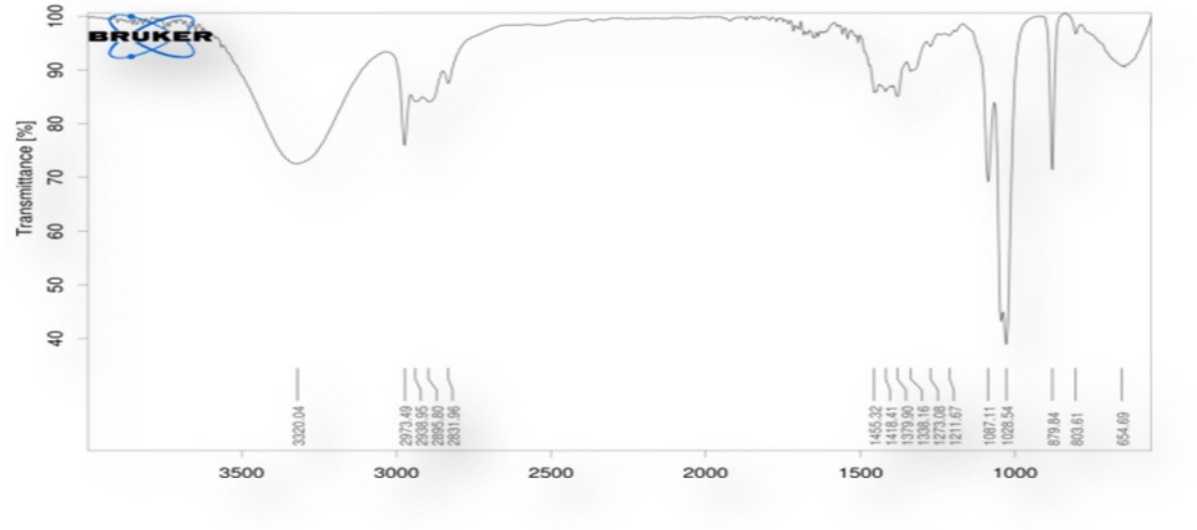


Figure 7: FT-IR spectra of standard quercetin

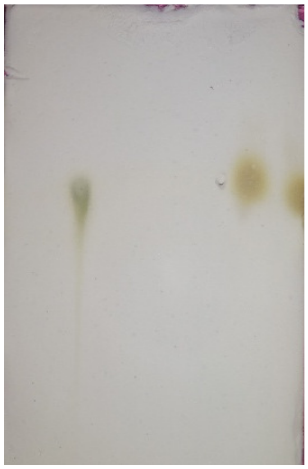
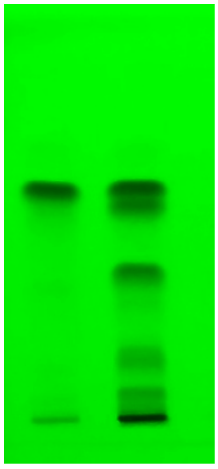


Figure 8: TLC plate showing the presence of 1) Standard quercetin 2) isolated quercetin.



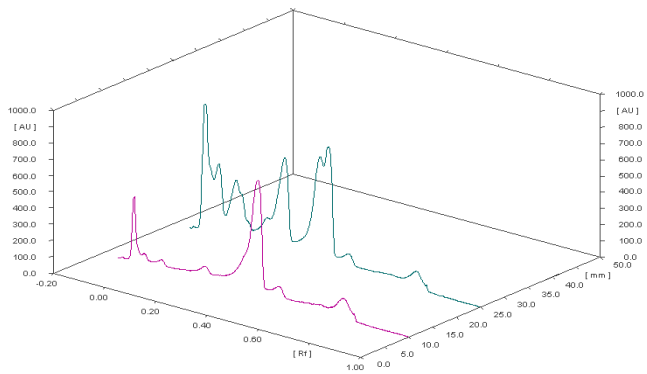


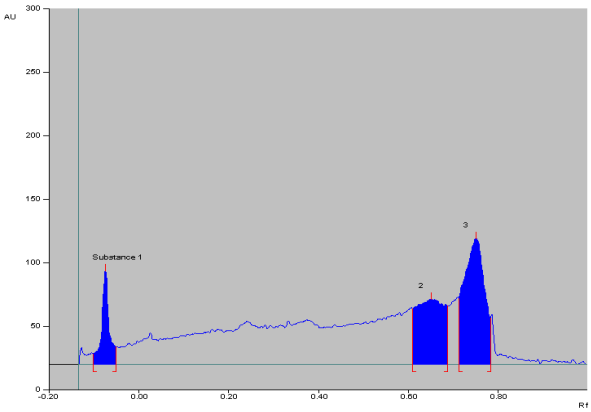
Figure 9: Overlay of standard and isolated quercetin in HPTLC (at 254 nm)

HPTLC

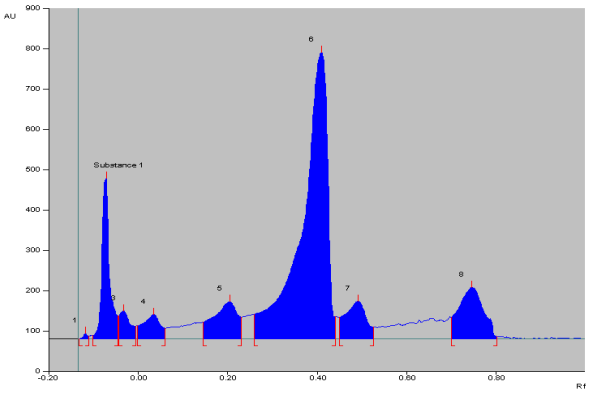
The extract showed the presence of the quercetin at tracks 2 in comparison to standard tracks 1 and isolated extract in the presence quercetin and standard quercetin showed R_f values at 0.75, 0.75 respectively.

Table 5: Chromatographic data for HPTLC

Sample	Rf	Maximum height	Area	Peak
Standard	0.75	12.2 AU	5845.2 AU	3
Isolated quercetin	0.75	99.0 AU	3917.6 AU	8



(a)



(b)

Figure 10: HPTLC chromatogram of (a) Standard quercetin (b) Isolated quercetin

CONCLUSION

From the present study, it was concluded that the plant contains tannins and flavonoids in its pods as main constituents and can be medicinally used for various purpose. The present study was a successful attempt to isolate the quercetin from pods of *Acacia arabica L.* using simple, rapid and convenient isolation procedure. The maximum yield was found to be 22.14 % in ethanolic extract. IR spectra confirmed the presence of characteristic peaks of quercetin. HPTLC chromatogram confirmed that, isolated compound is quercetin.

ACKNOWLEDGMENT

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