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ISOLATION AND IDENTIFICATION OF THE FLAVONOID "QUERCETIN" FROM *TRIDAX PROCUMBENS* LINN.

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ABSTRACT: *Tridax procumbens* (Linn.) is an important medicinal plant belonging to the family Asteraceae. It is known for a number of pharmacological activities like antidiabetic, anticancer, anti-inflammatory, wound healing, hepatoprotective and antioxidant activity. Flavonoid "Quercetin" was isolated from the flowers of this plant. Dried flowers were Soxhlet with petroleum ether, chloroform, and methanol successively. The methanolic fraction so obtained was successively extracted with petroleum ether, diethyl ether and ethyl acetate. The ethyl acetate fraction was hydrolyzed with 7% H_2SO_4 and extracted with ethyl acetate to obtain crude Quercetin. The crude product was recrystallized with dilute ethanol to get pure Quercetin. The purified material was subjected to various chromatographic and spectral techniques such as UV, IR, HPTLC, HPLC, NMR etc. and was identified as "Quercetin". This study is also of practical importance because quercetin is an important constituent of *Tridax procumbens* and it has many uses such as in cancer, diabetes, inflammation, & in viral infections etc.

INTRODUCTION: Ayurveda, the ancient healing system of India, has been steadily gaining importance and acceptance as the dangers and shortcomings of modern medicines are getting more apparent [1].

Man is able to obtain from them a marvelous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc [2]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant [3].

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Tridax procumbens L. (family – Asteraceae) is commonly known as *Jayanthi* in Ayurveda/Sanskrit and Coat buttons/Tridax daisy in English. It is native of America and widely distributed throughout the tropics and sub-tropical countries such as India, Brazil, Mexico, Australia, Indonesia, Sri Lanka, Bangladesh, Africa etc [4]. The plant has number of chemical constituents like alkaloids, tannins, flavonoids like luteolin, quercetin, keampherol, saponins, carotenoids, β-Sitosterol, n-hexane, and various acids like fumaric, lauric, myristic, palmitic, stearic, arachidic, benenic, palmitoloic, linoleic acid etc [5].

It is known for a number of pharmacological activities like antidiabetic, anti-inflammatory, wound healing, hepatoprotective and antioxidant activity. It has antimicrobial activity against both gram-positive and gram-negative bacteria. It is also known to have hypotensive effect and an immunomodulating property.

It is use in bronchial catarrh, dysentery and diarrhoea. Studies also suggest that it is used to stimulate hair growth and prevent hair fall. It shows anticancer activity and is being studied for prostate cancer and skin cancer [6, 7].

The present study deals with the isolation and identification of the flavonoid Quercetin from *Tridax procumbens* L. Flavonoids are a group of about 4000 naturally occurring polyphenolic compounds, found universally in foods of plant origin [8]. They are usually subdivided according to their substituents into flavanols (kaempferol, quercetin), anthocyanins, flavones, flavonones and chalcones. These flavonoids display a remarkable array of biochemical and pharmacological actions *viz*., anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticancer activities [9, 10].

Quercetin belongs to this group of plant pigments called flavonoids which is known to have antioxidant, anticancer, anti-inflammatory and antiviral activity. It is also useful for a variety of cardiovascular diseases [11, 12].

MATERIALS AND METHODS:

Chemicals: Quercetin standard was procured from Yucca Enterprises (Mumbai). All other chemicals, solvents and reagents were obtained from SD Fine chemicals (India).

Plant Material: Flowers of *Tridax procumbens* were handpicked from a private farm in the eastern region of Nashik and were authenticated at the Botany department of KTHM College, Nashik.

EXPERIMENTAL: The flowers of *Tridax procumbens* L. were shade dried and grinded to obtain a coarse powder. 100 gm. of the coarse powder was successively soxhlet extracted with 750 ml. each of petroleum ether, chloroform and methanol respectively. Each of the solvent was used for extraction for a period of 24 hrs. The methanolic fraction was concentrated to obtain a semisolid consistency (13 gm). 2 gm of this fraction was successively extracted with 50 ml of petroleum ether (fraction I), 50 ml of diethyl ether (fraction II) and 50 ml of ethyl acetate (fraction III) with the help of a separating funnel. Each extraction was repeated three times to ensure complete extraction in each case. This was done for the entire methanolic extract. Fraction I and II were rejected because of the presence of fatty acids and free flavonoids respectively. Fraction III was used for further processing as it contained Quercetin in its glycoside form. Fraction III was concentrated and hydrolyzed using 7% H_2SO_4 (10 ml/ gm extract) for 5 hrs. The hydrolyzed fraction was filtered and extracted with ethyl acetate (1:1/ thrice) by using a separating funnel. It was then concentrated to get the crude Quercetin which was later crystallized with 10% ethanol to get pure Quercetin. Quercetin was subjected to various spectral and chromatographic techniques.

Characterization of Quercetin:

UV Spectroscopy:

Sample preparation: Sample solution of the strength 1000.0 ppm was prepared by dissolving accurately weighed powder of isolated Quercetin (10.0 mg) with ethanol (10.0 ml) in a standard volumetric flask. From this, a stock solution of 10.0 ppm was prepared.

Preparation of standard solution: Solution of standard Quercetin of the strength 1000.0 ppm was prepared by dissolving the accurately weighed standard (10.0 mg) with ethanol (10.0 ml) in a standard volumetric flask. From this, a stock solution of 10.0 ppm was prepared.

Infrared Spectroscopy (IR): The sample was prepared by mixing the isolated fraction of Quercetin with KBr (1:100) and subjected to Fourier Transform Infrared Spectroscopy (FTIR) using Diffused Reflectance Spectroscopy (DRS) assembly.

Thin Layer Chromatography (TLC): The precoated TLC plate (3.0×8.0 cm) were activated in hot air oven at 105^0 C for 30 min and cooled to room temperature. Quercetin was dissolved in ethanol and was applied 1 cm. above the edge of the plate along with the standard Quercetin. This plate was developed in an air tight chromatography chamber containing about 8.5 ml of solvent mixture of ethyl acetate, toluene, formic acid (4:3.5:0.5).

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 alc. Aluminum chloride.

High Performance Thin Layer Chromatography (HPTLC):

Sample preparation: Sample solution of the strength 1000.0 ppm was prepared by dissolving accurately weighed powder (10.0 mg) with methanol (10.0 ml) in a standard volumetric flask. From this, a stock solution of 10.0 ppm was prepared.

Preparation of standard solution: Solution of standard Quercetin of the strength 1000.0 ppm was prepared by dissolving the accurately weighed standard (10.0 mg) with methanol (10.0 m) in a standard volumetric flask. From this, a stock solution of 10.0 ppm was prepared. Optimized HPTLC conditions are given in **Table 1**.

TABLE 1: OPTIMIZED HPTLC CONDITIONS

Parameters	Description
Stationary Phase	Merck Silica gel 60 F_{254} HPTLC pre-coated plates
Plate size	4.0 cm x 10.0 cm
Mode of separation	Normal phase
Mobile phase	Ethyl acetate: Toluene: Formic acid (4:3.5:0.5, v/v/v)
Development chamber	Camag twin trough chamber
Chamber saturation	30 min
Sample applicator	Camag Linomat V
Syringe	Hamilton, 100.0 µL
Band width	7.0 mm
Space between the bands	7.0 mm
Distance from the edges of the plate	13.0 mm
Rate of sample application	150 nL/sec
Development distance	85.0 mm
Densitometric scanner	Camag Scanner IV equipped with winCATS Planar Chromatography manager software version 1.4.7
Lamp and wavelength	Deuterium, 254 nm

High Performance Liquid Chromatography (HPLC): The purified material was also subjected to HPLC studies. Optimized HPLC conditions are given in **Table 2**.

TABLE 2: OPTIMIZED HPLC CONDITIONS

Parameters	Description
Column	HiQ Sil C18HS
Column size	4.6 mm × 250 mm × 5µ
Mobile phase	Methanol: 0.1% ortho phosphoric acid (65:35%)
Flow rate	1 ml/min
Detector and wavelength	UV, 369 nm

RESULTS: The λ_{max} of isolated sample and the standard Quercetin are same (i.e. 255 and 372 nm) (**Figure 1, 2**). The characteristic IR peaks were found to be matching with those of their respective standard reference compound of Quercetin (**Figure 3, 4**). The TLC plate developed under UV light shows spots with the standard quercetin. R_f value (0.43) of Quercetin isolated from sample coincided with the R_f value of standard Quercetin (**Figure 5**). HPTLC overlay at 254 nm and spectrum scan of isolated and standard Quercetin are comparable (**Figure 6, 7**). When isolated Quercetin was subjected to HPLC, it showed retention time of 8.4 min which coincided with that of standard Quercetin (**Figure 8, 9**). H1 NMR spectra of both reference and isolated compounds are matching (**Figure 10, 11**). Results of tests carried on standard and isolated Quercetin are summarized in **Table 3**.

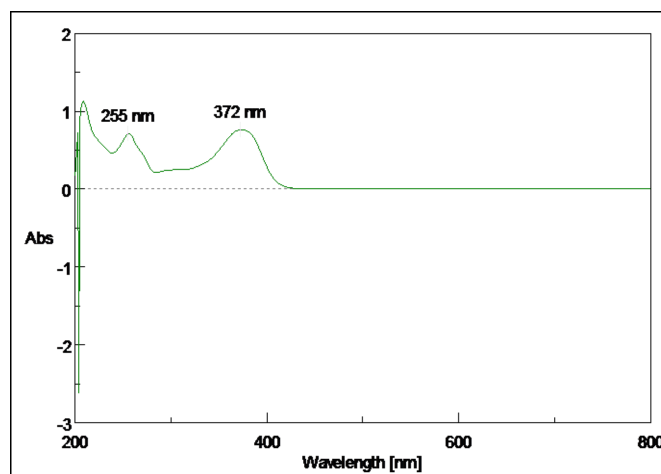

FIGURE 1: UV SPECTRA OF STANDARD QUERCETIN

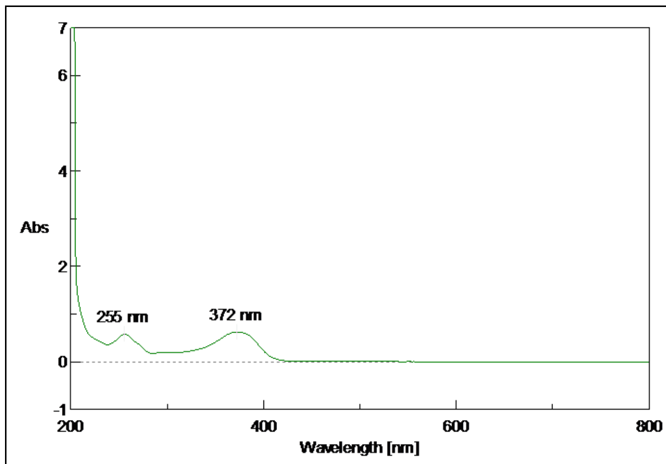

FIGURE 2: UV SPECTRA OF ISOLATED QUERCETIN

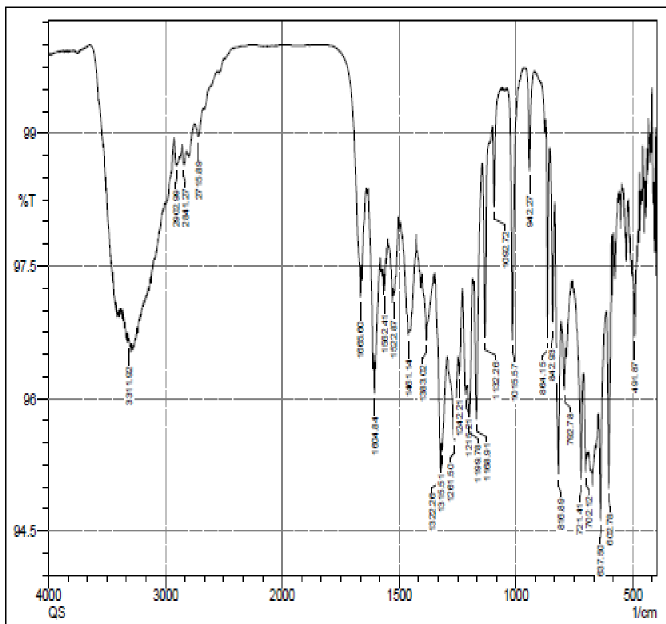

FIGURE 3: IR SPECTRA OF STANDARD QUERCETIN

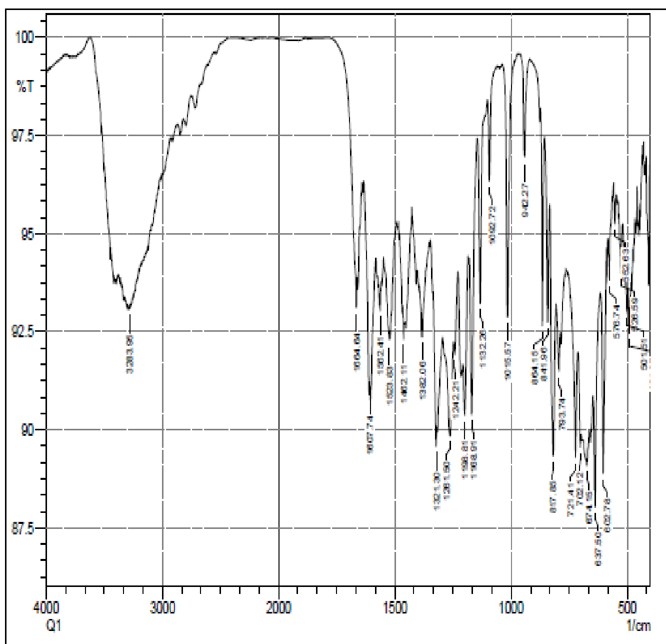

FIGURE 4: IR SPECTRA OF ISOLATED QUERCETIN

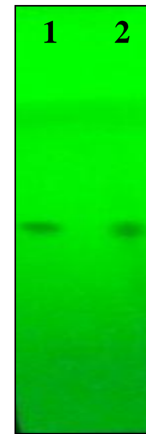

FIGURE 5: TLC PLATE SHOWING PRESENCE OF QUERCETIN 1) STD., 2) ISOLATED

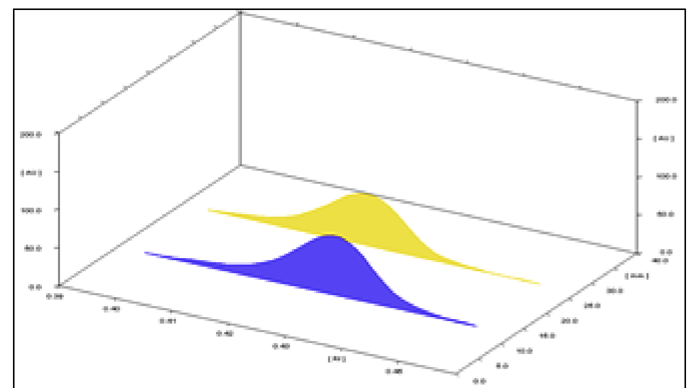

FIGURE 6: OVERLAY AT 254 NM OF STANDARD AND ISOLATED QUERCETIN IN HPTLC

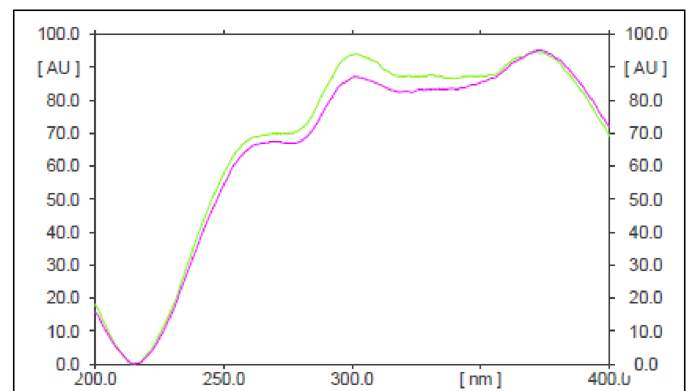

FIGURE 7: SPECTRUM SCAN OF STANDARD AND ISOLATED QUERCETIN IN HPTLC

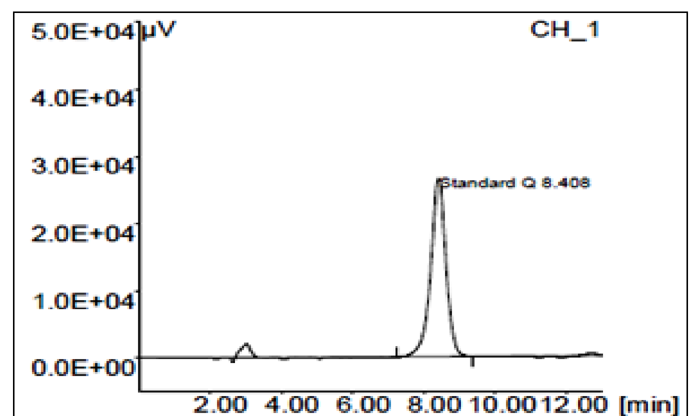

FIGURE 8: HPLC OF STANDARD QUERCETIN

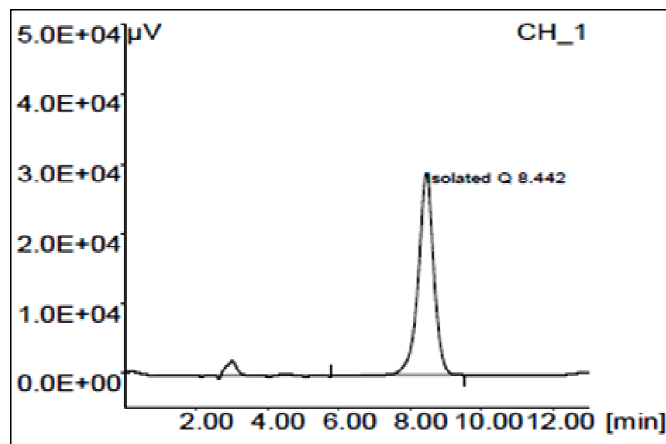

FIGURE 9: HPLC OF ISOLATED QUERCETIN

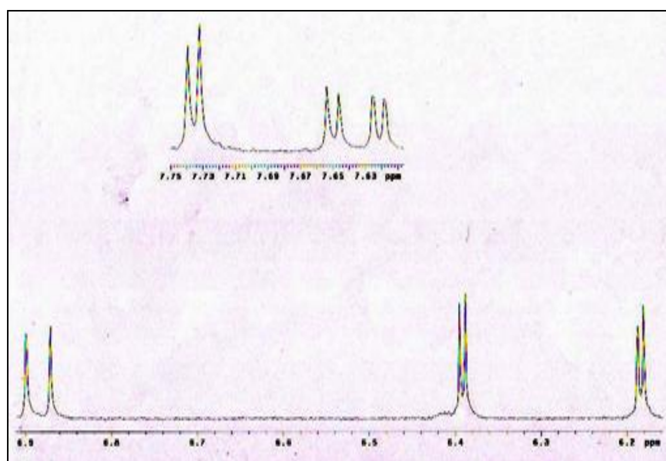

FIGURE 10: H^1-NMR SPECTRA OF STANDARD QUERCETIN

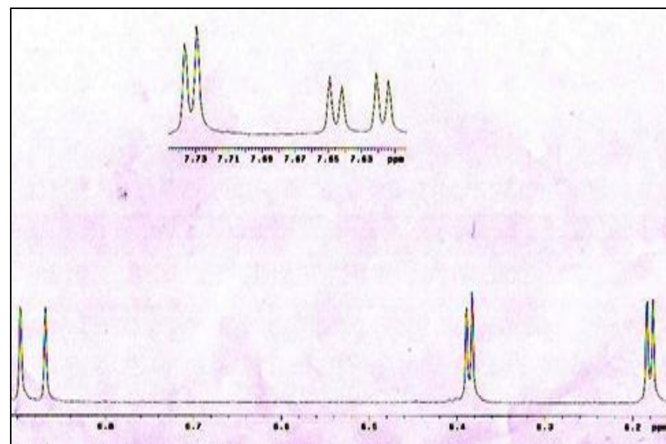

FIGURE 11: H^1-NMR SPECTRA OF ISOLATED QUERCETIN

DISCUSSION: Quercetin is an important constituent of *Tridax procumbens* which has number of uses such as anticancer, antidiabetic, hepatoprotective, antioxidant, etc. was isolated from flowers of *Tridax procumbens* using simple, rapid and convenient isolation procedure. The yield was found to be 0.072%.

The isolated fraction was characterized using sophisticated method of analysis from physicochemical, spectral and chromatographic studies, the structure of standard and isolated Quercetin was confirmed.

TABLE 3: RESULTS OF TESTS CARRIED ON STANDARD AND ISOLATED QUERCETIN

Parameters	Isolated Quercetin	Standard Quercetin
Elemental analysis	C, H, O present	C, H, O present
UV λ_{max}	255 nm, 372 nm	255 nm, 372 nm
IR	3411 cm^{-1}, 1663 cm^{-1}, 1608cm^{-1} 1523 cm^{-1}, 1496cm^{-1}, 1383 cm^{-1}, 1318 cm^{-1}, 1203 cm^{-1}	3411 cm^{-1}, 1663 cm^{-1}, 1608cm^{-1} 1523 cm^{-1}, 1496cm^{-1}, 1383 cm^{-1}, 1318 cm^{-1}, 1203 cm^{-1}
R_f value	0.43	0.43
Retention time	8.4 min	8.4 min
Spray reagents		
• **Ferric Chloride**	Dark brown spot	Dark brown spot
• **Alc. Aluminum Chloride**	Fluorescent yellow spot	Fluorescent yellow spot

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