ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal
www.orientjchem.org
ISSN: 0970-020 X
CODEN: OJCHEG
2025, Vol. 41, No.(5):
Pg. 1518-1527

A Novel Densitometric HPTLC Method Integrated with FTIR for The Detection of Rutin in *Senna auriculata* Flower Extract

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http://dx.doi.org/10.13005/ojc/410508

(Received: May 23, 2025; Accepted: September 17, 2025)

ABSTRACT

The objective of this research was to create a reliable way to analyze flavonoids, mainly Rutin, in extract from the *Senna auriculata* flower with densitometric HPTLC and FTIR. Fresh flowers were extracted using methanol and analyzed on silica gel plates using toluene\:ethyl acetate\:formic acid as the mobile phase, looking for compounds with toluene (254nm) and ethyl acetate (366nm). Rutin was confirmed in the Standard Rutin and the extract, as the Rf values for both were 0.014, 0.017 for Standard Rutin and 0.032, 0.026 for the extract. There was 1.12% Rutin present in the raw material. The presence of phenolic, aromatic and ether functional groups in FTIR confirmed that the compounds are flavonoids. Using this approach, the process was reliable, effective, pollution-free and needed very little solvent. The method enables the generation of a radiation-proof, non-rinse herbal sheet mask for space missions, specifically those on the International Space Station (ISS).

Keywords: *Senna auriculata*, Rutin, Densitometric HPTLC with FTIR, Radiation protection non rinse herbal formulation sheet mask and Aerospace medication.

INTRODUCTION

Senna auriculata (L.) Roxb.belonging to the family Fabaceae (Previously known as Caesalpiniaceae), is recognized as one of the medicinal flora utilized traditionally in Ayurveda practices since the 15th century (Fig. 1.)[1]. *Senna auriculata* commonly referred to as Tanner's Cassia in English and Avaram poo in Tamil represents a notable traditional plant this evergreen species is native to India[2]. The shrub is a small, perennial plant that can grow as tall as 2 meters[3]. The flowers are large, asymmetrical and hermaphroditic vibrant yellow, measuring almost 5 cm in diameter. The pedicels are sleek and have a length of 2.5 cm. The racemes are brief, sparsely flowered and upright, clustered in the axils of the upper leaves to form a distinct terminal inflorescence. The stamens are non-functional and the ovary is superior, single-chambered, with ovules situated along the edges[4]. The word 'Flavonoid' originates from the Latin term 'flavus', which translates to yellow. The examination of flavonoids has a longstanding history that extends over several decades[5]. Flavonoids are secondary metabolites found in plants that constitute a category of naturally occurring polyphenols found widely in the

plant kingdom. These extraordinary substances are chemically characterized by a structure comprising fifteen carbon atoms. These molecules possess a carbon backbone organized as C6-C3-C6, featuring a benzo-γ-pyrone structure features a phenyl ring. In this configuration the benzene and phenyl rings are referred to as the A ring and B ring respectively while the oxygen containing γ-pyrone ring is classified as the C ring[6].

The main subcategories consist of flavones, flavonols, flavanones, flavanonols, flavan-3-ols, anthocyanins, isoflavones and chalcones. Each category of bioflavonoids fulfills a unique biochemical role and demonstrates a specific distribution among plant species[7]. The word Quercetin comes from the Latin term "Quercetum", it falls under the category of flavonols, which are not synthesized by the human organism[8].Quercetin is a yellow compound that exhibits low solubility it is soluble in alcohol and lipids but remains insoluble in cold water. It is recognized as one of the most commonly utilized bioflavonoids for addressing metabolic and inflammatory conditions 9. Rutin is a type of flavonols often referred to by several names including rutoside, sophorin, vitamin P, or quercetin-3-O-rutinoside (Fig. 2.)[10]. Rutin being a lipophilic compound is soluble in organic solvents additionally it exhibits poor stability and bioavailability primarily attributable to its low solubility in water[11]. Phytochemical screening analysis of *Senna auriculata* (L.) Roxb. shade dried flowers extract identified the existence of different categories of secondary plant metabolites including flavonoids (Shinoda test), alkaloids (Dragendroff's test), phenolic compounds (Gelatin test), proteins and amino acids (Millon's test), tannins (Braymer's test), saponins (Foam test), glycosides (Legal's test)[12]. HPTLC is a widely utilized technology for the identification and analysis of the stability of herbal raw materials and formulations. Its minimal solvent usage, cost-effectiveness and shorter analysis time are making the HPTLC method a promising analytical tool[13]. As per the reviewed literature the identification, quantification and estimation of flavonoids in HPTLC analytical technique by using different crude extract. This study develops a High Performance Thin Layer Chromatography profile for flavonoids, identifying quercetin in *Senna auriculata* flowers revealing its antioxidant potential and suggesting further exploration of its chemical composition and medicinal use[14]. *Cassia auriculata* shows significant antioxidant activity in leaves and twigs with quercetin presence confirmed through HPTLC analysis[15]. This study analyzed flavonoids, phenolic acids and xanthones in six herbal materials using HPTLC. Quercetin was found in varying concentrations except *Cassia lanceolate* establishing a reliable methodology for routine phytochemical analysis[16]. HPTLC method validates emodin and quercetin quantification in *Cassia fistula* and *Cassia tora* identifying optimal solvent systems with high linearity and low detection limits[17]. Study presents a HPTLC method to quantify quercetin in *Michelia champaca* flowers revealing its antioxidant potential and benefits for herbal pharmaceutical quality control and standardization[18]. Research developed an HPTLC method to quantify flavonoids in *Cassia occidentalis* demonstrating significant larvicidal activity against malaria vectors[19]. Study developed a phenolic fingerprint for differentiating genus *Cassia L*. sensu lato using HPTLC identifying compound concentrations notably higher Quercetin and Curcumin aiding taxonomic classification and chemotaxonomic insights[20]. Study identifies 19 flavonoids in *Aerva lanata* using HPTLC emphasizing its medicinal potential especially against cancer and heart issues while supporting quality assurance in herbal products[21]. Study presents a validated HPTLC method for quantifying rutin, quercetin and gallic acid in *Pterospermum acerifolium* extracts showing excellent linearity and high reliability for quality control[22]. Study analyzes the phenolic content, rutin levels and bioactive properties of methanolic extracts from *Verbesina sphaerocephala* leaves and flowers. HPTLC confirmed rutin presence with total phenolic content varying and significant antioxidant and antibacterial activities observed[23]. Study investigates *Dodonaea angustifolia* using HPTLC and ultrasound assisted methanol extraction to quantify flavonoids and phenolic acids in leaves and flowers. Strong antibacterial activity was observed[24]. Research examines HPTLC to analyze flavonoid content in safflower varieties identifying APRR3 as the safest and most bioactive emphasizing the need for analytical assessment in herbal applications[25]. Assessment considers analyzes flavonoids in *Cyperus rotundus* using TLC and HPTLC identifying quercetin, establishing essential metrics for assessing its medicinal quality[26]. Inquiry delves into uses HPTLC to quantify quercetin and rutin in Melia azedarach extracts validating HPTLC for phytochemical assessment[27]. This study develops a validated HPTLC method for rutin quantification in *Morus alba*, *Morus nigra* and *Morus indica* leaves achieving for quality assurance[28]. This study establishes HPTLC technique for assessing rutin, quercetin and liquiritin in *Cocculus hirsutus* leaves

achieving high accuracy and precision for quality assurance in herbal products[29]. Study developed HPTLC method for quantifying rutin and quercetin in *Ocimum basilicum* seeds achieving effective separation and good precision with low detection limits[30]. Research developed HPTLC method to quantify gallic acid rutin and quercetin in *Terminalia chebula* revealing significant phytochemical properties[31]. A validated HPTLC technique measures flavonoids rutin and quercetin in *Anogeissus latifolia* bark achieving ideal separation with high linear regression coefficients and specific concentrations[32]. Quercetin and rutin content in *Benincasa hispida* seeds and *Carissa congesta* roots using HPTLC and HPLC, revealing significant levels and highlighting their ethnopharmacological importance[33]. Analyzes six Senna species using HPTLC revealing variations in chrysophanol levels. Morphological and anatomical assessments aid species identification ensuring accuracy in herbal medicine[34]. HPTLC to analyze flavonoids in *Syzygium cumini* leaves finding quercetin, rutin and others. Ethanol extract showed high Quercetin content and significant antimicrobial effects against various pathogens[35]. HPTLC and HPLC to analyze flavonoid content in *Capparis moonii* extracts revealing notable immune modulating effects but limited anticancer activity[36]. Introduces a validated HPTLC method for quantifying berberine and rutin in *Tinospora cordifolia* extracts and formulations confirming specificity, linearity and accuracy with notable antioxidant activity[37]. HPTLC method for rutin quantification in Hibiscus micranthus, achieving accurate results rutin content with excellent precision and reliability[38]. An advanced HPTLC method was developed for simultaneous quantification of quercetin and rutin in *Punica granatum*, *Tamarindus indica* and *Prunus domestica*. Technique is efficient, accurate, cost effective and meets ICH validation standards ensuring reliable assessment of bioactive flavonoids[39]. HPTLC to quantify flavonoid rutin in *Verbesina sphaerocephala* revealing high phenolic content, strong antioxidant potential and antibacterial activity, supporting its ethnopharmacological value[40]. Compares HPTLC and spectrophotometry for rutin measurement in *Amaryllis belladonna L.* confirming HPTLC's superior accuracy, efficiency and lower solvent use for bioactive compound analysis[41]. Developed a cost effective HPTLC method for analyzing flavonoids in eight Indocalamus species revealing six key markers and specific totals[42]. Flavonoids and phenolic acids in seven *Croatian stachys* taxa using HPTLC. Notable include chlorogenic acid in six taxa and the highest flavonoid content in *S. recta subsp. recta*[43]. Flavonoids in bamboo leaf extract offer health benefits. A precise HPTLC method measures vitexin and related compounds in *Phyllostachys* and *Pleioblastus* species differing significantly in flavonoid concentrations[44]. Examines HPTLC techniques to evaluate flavonoids in *Cyclanthera pedata* extracts revealing significant antioxidant activity particularly in leaf extracts compared to fruits[45]. HPTLC method for flavonoid analysis, detecting apigenin, quercetin, rutin and luteolin in medicinal plants like *Bauhinia variegate* and *Ginkgo biloba* with high accuracy and efficiency[46]. Quantitatively evaluated flavonoids from *Satureja hortensis* L. using HPTLC with Reflux extraction with ethanol yielded highest levels of rosmarinic acid and luteolin[47]. Research combines HPTLC with MALDI-TOF MS to effectively identify flavonoids like rutin and luteolin, enhancing resolution and mass identification for complex phytochemical analysis[48]. Developed a novel method using HPTLC and multivariate image analysis to differentiate Apiaceae species analyzing samples and enhancing quality control in herbal drugs through flavonoid measurement[49]. Enhances methods for separating phenolic compounds notably flavonoids, using RP-HPLC and NP-HPTLC, improving identification in herbal extracts like Polygonum species[50]. Employs HPTLC and ATR-FTIR to analyze flavonoids and polyphenolics in Olive leaf extracts revealing enhanced extraction through fermentation, improving antioxidant activity[51]. Clinacanthus nutans highlights antiviral and anti-inflammatory properties. An analytical technique using HPTLC and HPLC-UV/DAD identified key flavones. Prominent indicator for quality control in herbal products[52]. A rapid UPLC method analyzes five indole alkaloids and four flavonoids from Passiflora species in eight minutes providing high sensitivity and improved authentication for supplements with HPTLC supporting quality assurance[53]. Research employed HPTLC and HPLC-DAD-MS to identify flavonoids and phenolic compounds in extracts, caffeoyl glucaric acids and consistent results for *Galinsoga parviflora* and Galinsoga[54]. Study employs HPTLC, HPLC and GC-MS to analyze flavonoids in *Morinda tinctoria* leaves identifying rutin and scopoletin. Results confirm significant flavonoid content supporting its pharmacological potential[55]. HPTLC, HPLC-MS and NMR to analyze flavonoids in Cymbopogon giganteus identifying epicatechin, luteolin 8-C-glucosideand luteolin 6-C-glucoside. Results confirm high flavonoid content[56]. Develops a method

combining HPLC, ESI-MS and HPTLC to distinguish Dendrobium officinale from *D.devonianum* identifying flavonoids for accurate differentiation essential for consumer safety[57]. Study uses HPTLC-DPPH to isolate a novel flavonoid from Abrus precatorius leaves showing strong antioxidant activity[58]. Antioxidant properties of *Asparagus racemosus* aerial segments through HPTLC and ATR-FTIR high phenolic and flavonoid content in methanolic extracts, significant DPPH scavenging activity and identification of beneficial compounds[59]. This analytical study investigates the Bioactive compound profile of *Senna auriculata* methanolic flower extract, with focus on quantification of the flavonoids especially rutin and quercetin using densitometry analysis employing High Performance Thin Layer Chromatography (HPTLC)[60]. The concentrations of these flavonoids in the extract will be measured and compared with standard references to confirm their presence and assess their potential for future non rinse radiation-induced skin protection herbal formulation sheet mask in the International Space Station (ISS). Additionally, the study aims to identify and characterize the chemical constituents of the extract through Fourier Transform Infrared Spectroscopy (FTIR), enabling detailed elucidation of its phytochemical profile[61].

MATERIALS AND METHODS

Plant Collection and Authentication

Fresh flowers of Senna auriculata were systematically collected from Chengalpattu district in December month. The botanical identity of the flower was confirmed through Identification of crude drug –Macroscopic study of the coded Drug examination authenticated and officially certified by the Captain Srinivasa murthy Central Ayurveda Research Institute, Anna Nagar, Chennai, Tamil Nadu, India. A voucher specimen (No.2502005) has been archived for future reference.

Chemicals and Solvents

Rutin reference standard (90-101%) was obtained from HIMedia Laboratories Pvt. Ltd., Maharashtra, India. Quercetin reference standard (95%) was obtained from LOBA CHEMIE Pvt. Ltd., Mumbai, Maharashtra. Solvent for mobile phase used in the developed were toluene, methanol, ethyl acetate, formic acid all of HPLC grade quality. HPTLC plate used; E.Merck KGaA silica gel 60 F254.

Instruments

HPTLC instruments of Aetron AE260321 include an automatic syringe sample applicator with a 10ml capacity in Glass spraylin HPTLC - Thin Layer Chromatography software. Photo documentation was made using Aetron IDS, quantification using just TLC software.

Preparation of Senna auriculata Flower Extract

Senna auriculata flowers are thoroughly rinsed with tap water and then exposed to drying in a shaded environment at ambient temperature for a duration 15 days. The dried flowers are grind using an electrical blender equipped with a 40-mesh sieve to obtain a fine powder. From this powder, 10 g are taken and dissolved in 100 mL of HPLC-grade methanol. The maceration extractive technique is employed in this process. On the third day the sample undergoes purification using Whatman Grade 1 filter paper. The filtered extract is then transferred into a Petri dish to undergo open evaporation for one day. After this period, a solid residue is obtained for further use.

Preliminary Phytochemical Test for *Senna auriculata* Flower Extract

Initial phytochemical analysis performed according to Indian pharmacopoeia procedure the liquid extract from the flowers of *Senna auriculata* demonstrated the presence of flavonoids, alkaloids, phenolic compounds, proteins and amino acids, saponins, tannins and glycosides (Table 4).

Preparation of Standard Stock Solutions

Standard stock solutions were constituted by dissolving 10.2 mg of Rutin and 10.8 mg of Quercetin in 50mL of methanol. The final concentrations obtained were 0.204 mg/mL for Rutin and 0.216 mg/mL for Quercetin.

Preparation of Sample Stock Solution

1.003 g of *Senna auriculata* flower solid extract was dissolved in 10 mL HPLC grade methanol. Then 1.5 mL of this was diluted to 10 mL, yielding a working concentration of 15.046 mg/mL for HPTLC Densitometry analysis.

Method Development

The employed HPTLC plates were HPTLC silica gel 60 F254 (E. Merck KGaA) having 0.2mm layer thickness and 10x10cm in plates size; The standard and sample solutions of Rutin and Quercetin were applied to the HPTLC plates; Each at 10 µL. The composiure of the overall mobile phase that was applied in gas chromatography

was toluene, ethyl acetate and formic acid, 25% each for the formic and 20% respectively for the two and 1% in volumes for both detection types. The analysis was done in a twin trough glass chamber 10×10 cm pre saturated with the mobile phase and maintained for 20 min with the moving front up to 85 mm. They were then left in the air to dry for a period of forty–five minutes after going through the development stage. At 254 nm detection, eight bands were distinguished with the following values: 3-4=82px, 5-6=84px and for the flower extract of the Senna auriculata 5-6=80px, across the total length of 190mm (Fig. 3, Fig. 4, Table 1). The standard Rf values recorded for Rutin at 254 nm wavelength was 0.014 and for Quercetin 0.385 and the sample Rf value for the *Senna auriculata* extract was 0.032. Under 366 nm detection, six bands were recorded, with band widths of 81 px for Rutin, 75 px for Quercetin, and 65 px for the *Senna auriculata* extract, maintaining the same band length of 190 mm. The corresponding standard Rf values at 366 nm were 0.017 for Rutin and 0.388 for Quercetin, whereas the sample Rf value was 0.026. These optimized chromatographic conditions enabled efficient separation and detection of the bioactive constituents present in the *Senna auriculata* flower extract at both detection wavelengths.

Fig. 1. *Senna auriculata* (L.) Roxb.syn. flower

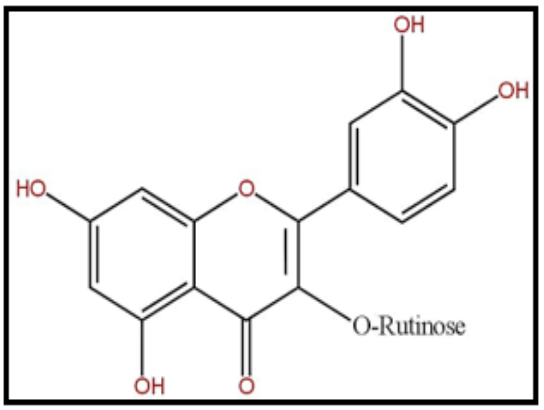


Fig. 2. Chemical structure of Rutin

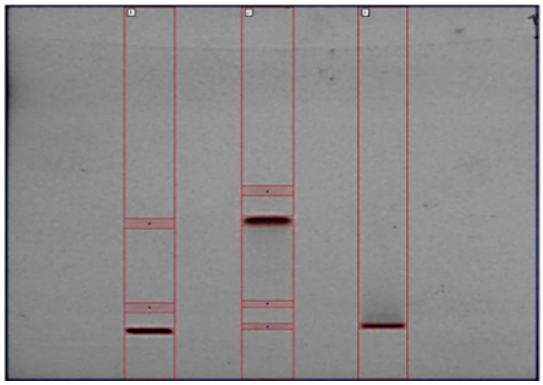


Fig. 3. HPTLC plate at 254nm showing visible bands for Rutin (Lane 1), Quercetin (Lane 2) and the *Senna auriculata* flower extract (Lane 3), Lane 3 *Senna auriculata* flower extract indicating the presence of Rutin through band alignment with the respective standards

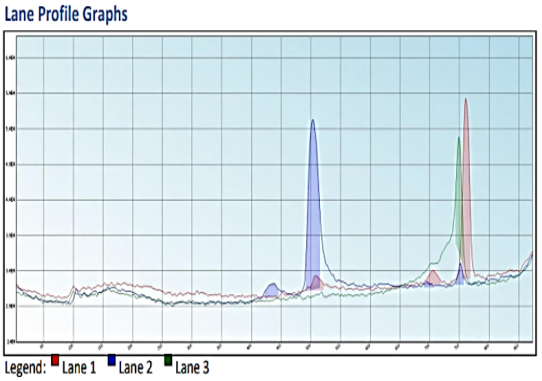


Fig. 4. The HPTLC Densitometric Chromatogram recorded at 254nm showing the lane profiles of Standard Rutin (Lane 1), Standard Quercetin (Lane 2), and the *Senna auriculata* flower extract (Lane3). The observed chromatographic bands and their respective Rf values the detection and quantification of Rutin in the *Senna auriculata* flower extract through co-elution with the standard compounds, confirming their identity under UV 254 detection

Lanes

ID	Width	Bands	Volume	Displayed Volume	Notes
1	82	3	28734440	287.35	Rutin
2	84	4	41726580	417.26	Quercetin
3	80	1	12736240	127.36	*Senna auriculata* flower extract

Bands

Table 1: Densitometric analysis at 254nm revealed bands corresponding to Rutin in the herbal extract, confirmed by matching Rf values and peak profiles with those of standard compounds

Lane ID	Band ID	Rf	Area	Volume	Displayed Volume	Notes
1	1	0.376	1886	2786032	27.86	
1	2	0.094	1886	2801612	28.02	
1	3	0.014	1148	23146796	231.47	Rutin
2	1	0.484	1932	2995944	29.96	
2	2	0.385	2016	35849100	358.49	Quercetin
2	3	0.107	1344	778176	7.78	
2	4	0.029	1176	2103360	21.03	
3	1	0.032	1040	12736240	127.36	*Senna auriculata* flower extract (Rutin)

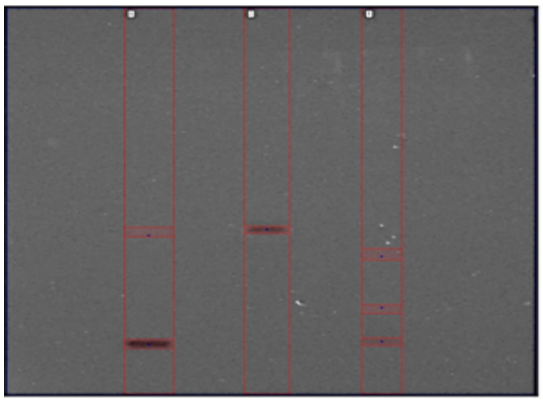


Fig. 5. HPTLC plate at 366nm showing fluorescent bands for Rutin (Lane 1), Quercetin (Lane 2), and the *Senna auriculata* flower extract (Lane 3) confirming the presence of Rutin in the flower extract through band alignment with the standard

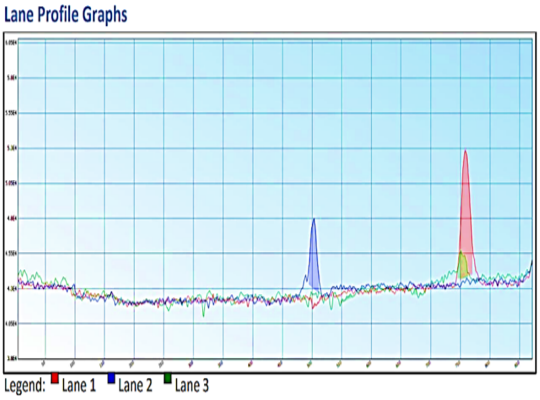


Fig. 6. HPTLC Densitometric Chromatogram captured at 366nm illustrating the lane profiles of standard Rutin (Lane 1), Standard Quercetin (Lane 2) and the *Senna auriculata* flower extract (Lane 3). The fluorescence characteristics and matching Rf values substantiate the detection and quantification of Rutin in the flower extract as evidenced by the alignment of bands with those of the respective standards

Lanes

ID	Width	Bands	Volume Volume	Displayed	Notes
1	81	2	11306304	113.06	Rutin
2	75	1	4352175	43.52	Quercetin
3	65	3	1875250	18.76	*Senna auriculata* flower extract (Rutin)

Bands

Table 2: Densitometric analysis at 366nm the flower extract demonstrated fluorescent bands corresponding to those of the Rutin standard, suggesting its presence based on closely aligned Rf values and fluorescence intensities

Lane ID	Band ID	Rf	Area	Volume	Displayed Volume	Notes
1	1	0.369	1701	216837	2.17	
1	2	0.017	1701	11089467	110.89	Rutin
2	1	0.388	1275	4352175	43.52	Quercetin
3	1	0.302	1690	178555	1.79	
3	2	0.136	1300	119535	1.2	
3	3	0.026	1105	1577160	15.77	*Senna auriculata* flower extract (Rutin)

FTIR Spectrum for Identification and Characterization of Phytoconstituents in Senna auriculata

Preparation of Senna auriculata Flower Extract

Senna auriculata flowers are thoroughly rinsed with tap water and then exposed to drying in a shaded environment at ambient temperature for a duration 15 days. The dried flowers are grind using an electrical blender equipped with a 40-mesh sieve to obtain a fine powder. From this powder, 10 g are taken and dissolved in 100 mL of HPLC-grade methanol. The maceration extractive technique is employed in this process. On the third day the sample undergoes purification using Whatman Grade 1 filter paper. The filtered extract is then transferred into a Petri dish to undergo open evaporation for one day. After this period, a solid residue is obtained for further use.

Instrument

Spectrum 2 Type: ATR-FTIR Spectrometer Service Spectrometer Make: PerkinElmer Location: USA.

FTIR Analysis working Procedure

The solid extract of *Senna auriculata* flowers was analyzed using an ATR-FTIR

spectrometer. The powder sample was placed and pressed on the ATR crystal and spectra was recorded from 4000 to 450 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans. Absorption bands appeared at 3270.78 cm^{-1} (O-H), 2924.00 and 2853.37 cm^{-1} (C-H), 1611.62 and 1505.51 cm^{-1} (C=C) ,1448.44 cm^{-1} (CH_2),1226.57-1068.10 cm^{-1} (C-O/C-O-C), 967.30 cm^{-1} (=C-H), 834.53 cm^{-1} (C-H bending), and 499.00 cm^{-1} (C-Br). These peaks indicate the presence of phenolic, aromatic, ether these functional groups (Fig. 7. and Table 3).

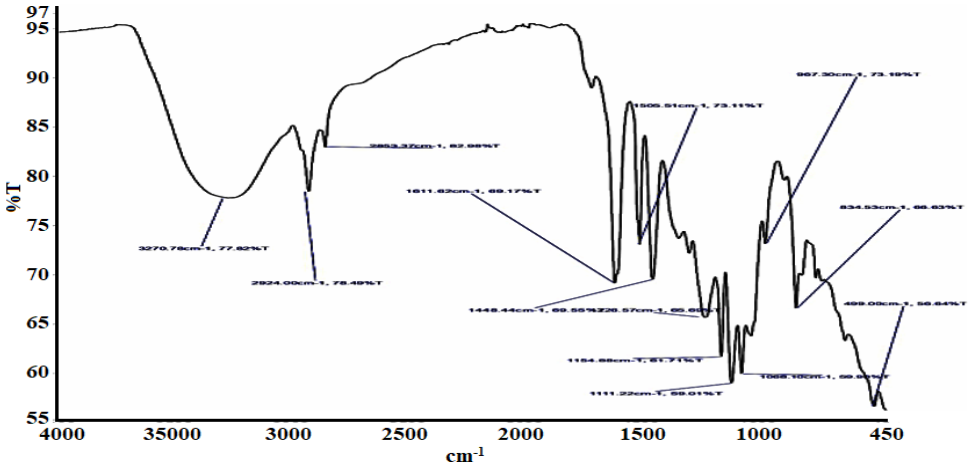


Fig. 7. FTIR spectrum of Senna auriculata shows prominent peaks confirmed by Phenols or Alcohols, Alkanes, Aromatic compounds, and Ethers functional group vibrations

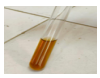
Table 3: FTIR peak table of *Senna auriculata* identifies functional groups indicating the presence of phenols, alcohols, alkanes, aromatic compounds, ethers, alkenes and halides reflecting a rich phytochemical composition

Sr. No	Wavenumber (cm^{-1})	%Transmittance	Phytochemical class	Functional Group Assignment
1	3270.78	77.82	Phenols/Alcohols	O-H stretch
2	2924.00	78.49	Alkanes	C-H stretch
3	2853.37	82.98	Alkanes	C-H stretch (symmetric)
4	1611.62	69.17	Aromatic compounds	C=C stretch (Aromatic ring)
5	1505.51	73.11	Aromatic compounds	C=C stretch (Aromatic)
6	1448.44	69.55	Alkanes	CH2 bending
7	1226.57	65.69	Phenols / Ethers	C-O stretch
8	1154.88	61.71	Ethers	C-O-C stretch
9	1111.22	59.01	Alcohols /Ethers	C-O stretch
10	1068.10	59.98	Carbohydrates/ Alcohols	C-O stretch
11	967.30	73.19	Alkenes	=C-H bending
12	834.53	66.63	Aromatic compounds	C-H bending (out-of-plane)
13	499.00	56.64	Alkyl halides	C-Br stretch

Qualitative Phytochemical Profiling of Senna auriculata Flower Extract

Qualitative Phytochemical analysis was conducted following the established standard protocols of Trease and Evans (1989) (Table 4).

Table 4: Preliminary Phytochemical Analysis of Senna auriculata Flower extract

Sr. No	Phytochemical Tested	Test Method	Observation	Senna auriculata flower extract Inference	Photographic Evidence
1	Flavonoids	Alkaline reagent test	An intense yellow colour	+	
		Alkaline reagent test	A yellow fluorescence	+	

		Ferric chloride test	A green Precipitate	+	
		Conc.H_2SO_4 test	An orange colour	+	
2	Alkaloids	Dragendroff's test	A reddish brown precipitate	+	
		Mayer's test	A creamy white/ yellow precipitate	+	
		Wagner's test	A brown/reddish precipitate	+	
3	Phenolic compounds	Iodine test	A transient red colour	+	
		Gelatin test	A white precipitate	+	
		Ellagic Acid test	Solutions turns muddy/ Niger brown precipitate	+	
4	Proteins and amino acids	Millon's test	A white precipitate	+	
		Biuret test	A pink coloured solution in ethanolic layer	+	
5	Saponins	Froth test	Persistent forth	+	
		Foam test	Stable foam	+	
6	Tannins	Gelatin test	A white precipitate	+	
7	Glycosides	10% NaOH	A brick red precipitate	+	

RESULTS AND DISCUSSION

This work describes the optimized HPTLC-Densitometry method for the qualitative analysis of the extract of *Senna auriculata* flower and consists Toluene: Ethyl acetate: Formic acid (25: 20: 1) is used on the plates silica gel 60 F 254 and the detection was done at 254 nm and also at 366 nm. The Rf values for the standard Rutin and Quercetin were determined as 14 and 385 at 254nm and 17 and 388 at 366nm respectively. Therefore, the *Senna auriculata* flower extract has provided the Rf values of 32 at 254nm and 26 at 366nm almost close to the standard Rutin is indicating the presence of Rutin in the extract. The selected mobile phase toluene, ethyl acetate and formic acid were found to be effective as far as resolution and separation of phytoconstituents is concerned. Total Rutin concentration of the extract was analysed using densitometry quantification based on the ration of the sample 'peak area' with the standard 'peak area' (Formulas 3 & 4) The functional groups of hydroxyl -OH, aromatic C=C, and ether C-O-C were detected in the extract based on the characteristic IR peaks of Rutin.

CONCLUSION

The study successfully developed a dual- analytical approach combining HPTLC densitometry with FTIR spectroscopy for the quantification and characterization of Rutin in *Senna auriculata* flower extract. The HPTLC method exhibited high specificity, precision and

reproducibility under dual wavelength detection enabling accurate quantification of flavonoid. FTIR analysis confirmed the presence of functional groups associated with flavonoid structures, supporting compound integrity and identity. This integrated cost effective and time efficient method provides a reliable phytochemical standardization and quality control indicates strong potential for future non rinse radiation-induced skin protection herbal formulation sheet mask during space missions particularly on the International space station (ISS).

ACKNOWLEDGEMENT

The authors would like to deeply thank writers VISTAS-Vels Institute of Science, Technology & Advanced Studies (VISTAS) old pallavaram Chennai-600117, Tamilnadu, India for their unending support for all the facilities and ideas provided, which have had a direct effect in developing the current work.

Conflict of Interest

The author declares that there are no conflicts of interest.

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