Simultaneous quantification of quercetin and gallic acid in methanolic extract of *Heliotropium indicum* (Linn.) through HPTLC analysis

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Abstract

Phytomedicines are increasingly a crucial component of improving healthcare and have promising futures in many nations. The herbaceous medicinal weed *Heliotropium indicum* Linn. also referred to as "Indian heliotrope" (Hathishur), is a member of the "Boraginaceae" family and is very common in India. It has a long history of traditional medicinal uses in many different nations around the world. Along with India, Bangladesh, and a few other African nations, these therapeutic herbs can be found worldwide in both tropical and temperate regions.

Therefore, in the current work, the hydroalcoholic (Polar) and n-hexane (Non-Polar) extracts of *Heliotropium indicum* Linn were analyzed using high-performance thin-layer chromatography (HPTLC) to determine their phytochemical profiles.

Keywords: *Heliotropium indicum*, phytochemicals, HPTLC, n-hexane, and hydroalcoholic extract, Chromatogram.

Introduction

Due to the bioactive phytochemicals found in herbal plants, they are vital to human existence and the maintenance of good health. Several natural items are employed as phototherapeutics for the treatment of many ailments, and the use of medicinal herbs in the treatment of skin infections and other disorders is an ancient practice (Sisodiya D et al 2018).

Since many pathogenic pathogens are developing resistance to synthetic medicines, finding a newer source of antibiotics is a global concern (Latha SP et al 2006). Numerous medicinal plants and herbs have a long history of use for their ability to treat a wide range of serious illnesses and conditions (Sisodiya D et al 2018). As part of complementary and alternative medicine (CAM), the usage of herbal medications is growing (Cooper LN et al 2004). We might claim that there has been a "Return to Herbal & Nature" in the global trend away from synthetic to herbal treatment. Linn's *Heliotropium indicum*. (Family: Boraginaceae) comes from the Greek words helios, which means sun, and trope, which means to tune, implying that the leaves and blossoms face the sun (Chittenden FJ et al 1951). Linn's *Heliotropium indicum* belongs to the Boraginaceae family. Herbs make up the vast majority of the family's plants. While some heliotropes are common garden plants, others are weeds. About 250 species of *Heliotropium* can be found throughout the world's tropical, subtropical, and warm temperate regions (Gurib-Fakim A et al 2008). However, only a small number of species have been thoroughly studied. The biological effects of various extracts of *Heliotropium indicum* have been investigated in a variety of animal models, and it has been found that they have potent antimicrobial, antifertility, antitumor, antituberculosis, anti-inflammatory, histo-gastroprotective, anti-cataract, analgesic, and wound healing effects. In a rat model, the ability of an alcoholic extract of *Heliotropium indicum* to cure wounds was investigated. The percentage of wounds that healed quickly increased after topical treatment of 10% w/v *Heliotropium indicum.* According to this study, *Heliotropium indicum* extract has wound-healing properties (Reddy JS et al 2002). The World Health Organisation (WHO) has put a lot of effort into establishing, developing, and applying good research as well as the requirement for the scientific validity of herbal medicines (Tilburt JC et al 2008). For the qualitative and quantitative evaluation of phytochemicals found in plants, various approaches are available.

Phenolic chemicals are present in aqueous leaf extracts from seedlings of *Heliotropium indicum*. The pyrrolizidine precursor amines putrescine, spermidine, and spermine, as well as the alkaloids trachelanthamidine and retronecine (in leaves and inflorescence), were isolated and identified in the leaves (Birecka H et al 1984).

From the seeds, it has been determined that cynoglossine, europine-N-oxide, heliotridine-N-oxide, heliotrope N-oxide (Willaman J. et al 1961), and heliotrine (Pandey VB et al 1982) are present. The leaves of the plant have also been shown to contain other alkaloids, including putrescine, spermidine, homo spermidine, and spermine (Birecka H et al 1984).

Apart from alkaloids, the entire plant has been shown to contain a number of triterpenes and steroids, such as -amyrin, lupeol (Pandey DP et al 1996), chalinasterol (Andhiwal CK et al.

1985), -sitosterol, stigmasterol, and campesterol (Andhiwal CK et al. 1985). Rapanone (Mehta R et al 1981) and hexacosan-1-ol (Andhiwal CK et al 1985). are two more substances that have been found in the entire plant Estradiol (Mannan A et al 1978) presence has been also reported. These active molecules can now be recognized, screened for, and isolated thanks to improved technology (Sisodiya D et al 2017). High-performance thin-layer chromatography, or HPTLC, is a more advanced kind of TLC because it produces data with greater accuracy and precision. It is acknowledged as one of the most effective analytical techniques for biomedical analysis on a global scale. Since it is an easy and quick approach for estimating the chemical components contained in test samples, the pharmaceutical industry uses it the most frequently while looking for novel drugs. The purpose of the current work was to use HPTLC to profile the phytochemical composition of a methyl extract of *H. indicum*.

Material and Methods

Collection and Plant Authentication

The fresh bulk leaves of *Heliotropim indicum* (Family: Boraginaceae) were taken from the Arjunganj neighborhood Sultanpur Road, Lucknow in the month of May to June.

By sandwiching the freshly cleaned and dried leaves between a book's nonstick paper, a herbarium file (sample) was created. The CSIR-National Institute of Science Communication and Policy Research, located in New Delhi, India, 110012, received this herbarium file for identification and confirmation. NIScPR/RHMD/Consult/2022/4174-75-3 is the authentication number.

Preparation of extract

After being cleaned, the plant material was dried in the shade for a month at room temperature. A grinder or mixer was used to grind the dried leaves of the plant sample into a coarse powder. *Heliotropium indicum* leaves that have been air-dried and coarsely ground amount to 15 grams. This coarse powder was extracted using n-hexane and hydro-alcoholic solvent (50:50 v/v) using the soxhlet apparatus for sequential solvent extraction by hot extraction with n-hexane (highly nonpolar, maintained at 69 ^{0}C and hydro-alcohol (polar, maintained at 60 ^{0}C) for 24 hours separately. Excess solvent was then evaporated in a steam water bath at 50 ^{0}C to 100 ^{0}C after the extraction procedure was finished to produce a concentrated product that was then either stored or used.

Instrumentation:

The system used has a CAMAG HPTLC system, a LINOMAT 5 applicator with a 100 µl syringe, a CAMAG TLC scanner, and Win CATS software.

Chemicals and solvents

All of the chemicals employed were of the analytical reagent grade, and all of the solvents were of the chromatography grade.

Preparation of samples

After being dissolved in 100 ml of HPTLC-grade methanol and filtered, a dried extract of *H. indium* (10 g) was used. For the HPTLC investigation, this solution served as a test solution.

Chromatographic conditions

On an E. MERCK KGaA 20 x10 cm pre-coated silica gel 60 F 254 HPTLC plate, the HPTLC was carried out. The plate was not altered or cleaned beforehand. A 100-µl syringe-equipped CAMAG Linomat applicator was used to apply the sample solution on the plate as bands (Table 1). 150 nl/s was the steady application rate. The mobile phase, Toluene: Ethyl acetate: Formic acid (6:4:0.3 v/v/v), was added to the sample-loaded plate and kept in a manual development chamber. The CAMAG TLC scanner-III with winCATS software was used for scanning. The photos were taken in white light, 254 nm (short UV), and 366 nm (long UV) wavelengths, and the bands were visualized using the CAMAG visualizer (Figs. 1 and 2). UV-active substances will experience fluorescence quenching and show up as black patches on a bright backdrop when exposed to short-wave UV light of 254 nm. Contrarily, substances that absorb UV light with a wavelength of 366 nm will manifest as brilliant spots on a dark backdrop (https://www.merckmillipore.com).

Parameters	Values
Linomat 5 application parameters	
Spray gas	Nitrogen gas
Sample solvent type	Methanol
Dosage speed	150 nl/s
Predosage volume	6 µl
Syringe size	100 µl
Application position	5.0 mm
Band length	6.0 mm
Solvent front position	98.0 mm
Calibration parameters	
Calibration mode	Multiple level
Statistics mode	CV
Evaluation mode	Peak height & Peak Area
Detection-CAMAG TLC scanner	
Number of tracks	04
Position of track X	5.0 mm
Distance between tracks	09.4 mm
Scan start position Y	5.0 mm
Scan end position Y	98.0 mm
Slit dimensions	6.00x 0.30 mm, micro
Optimize optical system	Light
Scanning speed	20 mm/s
Data resolution	100 µm/step
Integration: Properties	
Baseline correction	Lowest slope
Peak threshold min.slope	5
Peak threshold min.height	27.0 AU
Peak threshold min.area	276.0 AU
Peak threshold max.height	727.7 AU
Track start position	5.0 mm
Track end position	98.0 mm
Display scaling	Automatic
Measurement	
Wavelength	254 nm and 366 nm
Lamp	D2/Hg
Measurement type	Remission
Measurement mode	Absorption/fluorescence
Optical filter	Second order/K400
Detector mode	Automatic
PM high voltage	181V

Table 1: Parameters used for HPTLC

Calibration curve of quercetin and gallic acid

With standard values ranging from 2 to 10 µg/spot, the quercetin and gallic acid concentrations were calculated, and the calibration curve was then displayed. A stock solution containing 0.5 mg/ml of methanol was created. In order to obtain standards of 2 µg, 4 µg, 6 µg, 8 µg, and 10 µg/spot, various injection volumes of stock solution, such as 4 µl, 8 µl, 12 µl, 16 µl, and 20 µl, were spotted on the HPTLC plate.

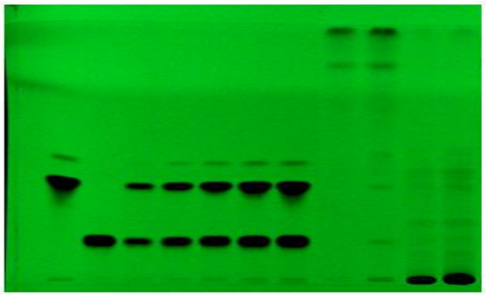
Result

As shown in the chromatograms (Figs. 1, 2, 8, and 9) obtained after scanning at UV 254 nm and 366 nm, phytochemical profiling and quantification using HPTLC analysis of *H. indicum* revealed the presence of many beneficial compounds as well as quantification of quercetin and gallic acid. The tables (Tables 2,3,4, and 5) show the Rf values, peak height, peak area, and percent area of quercetin, gallic acid, as well as other unidentified chemicals.

Discussion

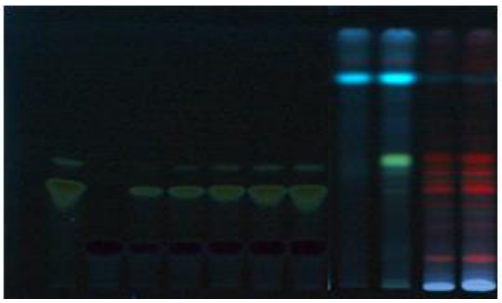
As shown in the figures and tables, *Helitropium indicum* (L.) leaf methanolic extract underwent HPTLC to reveal the presence of several phytoconstituents in a range of concentrations. Sample 1 HPTLC chromatogram indicates the presence of 10 bioactive compounds, while sample 2 chromatogram indicates the presence of 15 bioactive compounds as indicated by a number of peaks. Through the use of a chromatogram, the study additionally confirms the existence of reference quercetin and gallic acid at Rf 0.40 (Table 2, Track 1) and 0.18 (Table 3, Track 2), respectively.

When compared to the typical quercetin Rf (0.40) value (Table 2, Track 1), the HPTLC chromatogram for the samples shows peaks at Rf 0.36 (Sample 1) (Table 4, Track 3), and Rf 0.35 (Sample 2). The HPTLC chromatogram for the samples shows peaks that are almost identical to the normal gallic acid Rf (0.18) value (Table 3, Track 2) at Rf 0.15 (Sample 1) and Rf 0.14 (Sample 2) (Table 5, Track 4). Peak area and peak height measurements of quercetin and gallic acid using HPTLC show that sample 1 had 0.092% of quercetin and 0.089% of gallic acid, whereas sample 2 contained 0.109% of quercetin and 0.075% of gallic acid.



Q GA Q+GA Sample 1 Sample 2

Fig: 1 Image of TLC Plate at 254 nm



Q GA Q+GA Sample 1 Sample 2

Fig: 1 Image of TLC Plate at 366 nm

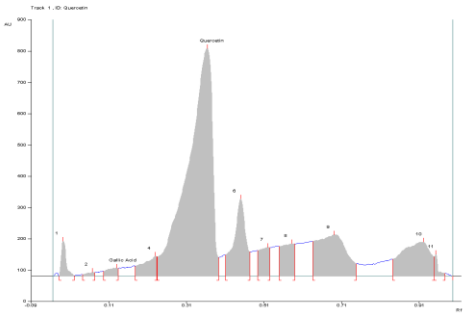


Fig: 3 Standards Peak of Quercetin

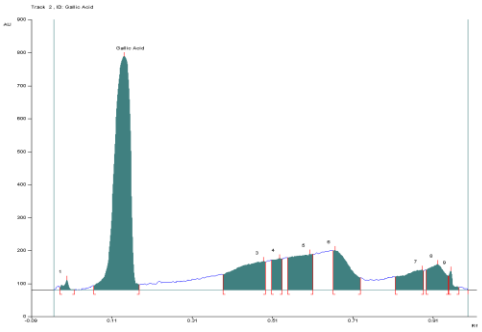


Fig: 4 Standard Peak of Gallic acid

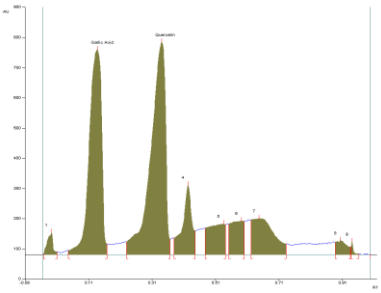


Fig: 5 Standard Peak of Quercetin and Gallic Acid

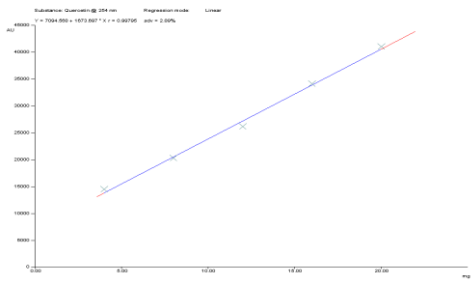


Fig: 6 Calibration curve of Quercetin

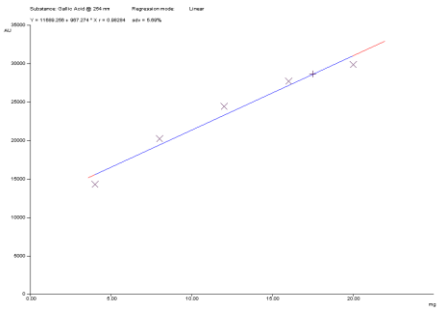


Fig: 7 Calibration curve of Gallic Acid

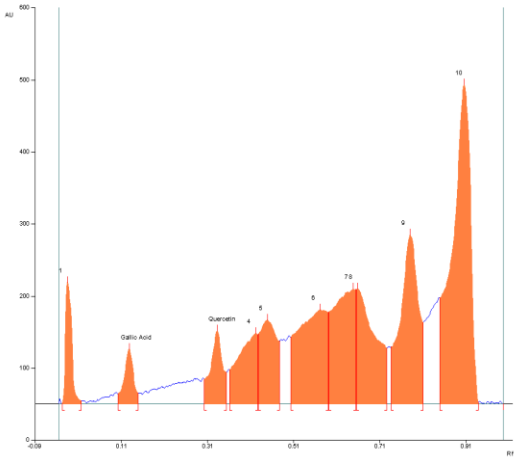


Fig: 8 HPTLC Chromatogram of *H. Indicum* I (Sample 1)

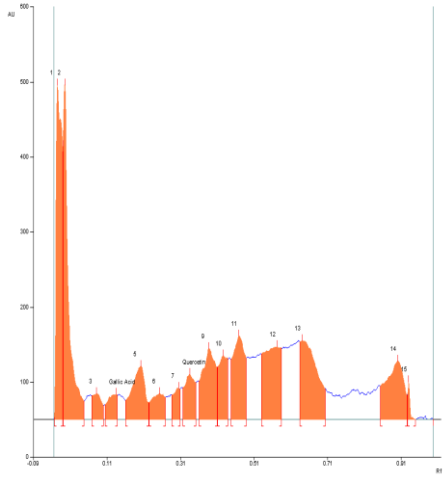


Fig: 9 HPTLC Chromatogram of *H. Indicum* II (Sample 2)

Track 1, ID: Quercetin

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02 Rf	0.6 AU	-0.01 Rf	112.7 AU	6.63 %	0.02 Rf	1.8 AU	1480.1 AU	1.72 %	unknown *
2	0.04 Rf	5.0 AU	0.07 Rf	12.7 AU	0.75 %	0.07 Rf	11.7 AU	276.0 AU	0.32 %	unknown *
3	0.10 Rf	15.3 AU	0.13 Rf	27.0 AU	1.59 %	0.14 Rf	24.6 AU	793.8 AU	0.92 %	Gallic Acid
4	0.18 Rf	32.1 AU	0.23 Rf	64.7 AU	3.80 %	0.24 Rf	31.7 AU	2366.9 AU	2.76 %	unknown *
5	0.24 Rf	62.0 AU	0.37 Rf	727.7 AU	42.76 %	0.40 Rf	30.2 AU	47178.5 AU	54.95 %	Quercetin
6	0.41 Rf	68.1 AU	0.45 Rf	247.7 AU	14.56 %	0.48 Rf	77.5 AU	7806.9 AU	9.09 %	unknown *
7	0.50 Rf	81.8 AU	0.52 Rf	93.0 AU	5.47 %	0.53 Rf	31.0 AU	2548.8 AU	2.97 %	unknown *
8	0.55 Rf	95.4 AU	0.58 Rf	104.0 AU	6.11 %	0.59 Rf	33.4 AU	3697.9 AU	4.31 %	unknown *
9	0.64 Rf	110.7 AU	0.69 Rf	132.3 AU	7.77 %	0.75 Rf	39.9 AU	10610.8 AU	12.36 %	unknown *
10	0.84 Rf	54.7 AU	0.92 Rf	109.3 AU	6.42 %	0.95 Rf	52.3 AU	8413.9 AU	9.80 %	unknown *
11	0.95 Rf	61.8 AU	0.96 Rf	70.5 AU	4.14 %	0.98 Rf	9.1 AU	686.3 AU	0.80 %	unknown *

Table 2: Track 1, ID: QUERCETIN (Standard)

Track 2, ID: Gallic Acid

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02 Rf	6.5 AU	-0.00 Rf	31.1 AU	2.30 %	0.02 Rf	0.6 AU	379.8 AU	0.68 %	unknown *
2	0.07 Rf	12.4 AU	0.14 Rf	708.5 AU	52.48 %	0.18 Rf	15.8 AU	28620.8 AU	51.03 %	Gallic Acid
3	0.39 Rf	46.9 AU	0.49 Rf	87.0 AU	6.45 %	0.49 Rf	35.0 AU	6821.9 AU	12.16 %	unknown *
4	0.51 Rf	90.6 AU	0.53 Rf	95.4 AU	7.07 %	0.54 Rf	33.5 AU	2223.2 AU	3.96 %	unknown *
5	0.55 Rf	97.8 AU	0.61 Rf	109.2 AU	8.09 %	0.61 Rf	38.3 AU	5897.1 AU	10.52 %	unknown *
6	0.66 Rf	118.5 AU	0.67 Rf	120.3 AU	8.91 %	0.73 Rf	36.7 AU	5043.5 AU	8.99 %	unknown *
7	0.82 Rf	40.1 AU	0.88 Rf	61.1 AU	4.52 %	0.89 Rf	57.6 AU	3269.0 AU	5.83 %	unknown *
8	0.90 Rf	60.8 AU	0.92 Rf	78.6 AU	5.82 %	0.95 Rf	42.4 AU	3238.1 AU	5.77 %	unknown *
9	0.95 Rf	42.7 AU	0.96 Rf	58.8 AU	4.35 %	0.98 Rf	8.1 AU	588.3 AU	1.05 %	unknown *

Table 3: Track 2, ID: GALLIC ACID (Standard)

Track 3, ID: Sample 1

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.03 Rf	1.3 AU	-0.01 Rf	168.8 AU	10.01 %	0.02 Rf	4.5 AU	2534.1 AU	3.58 %	unknown *
2	0.10 Rf	14.2 AU	0.13 Rf	76.0 AU	4.51 %	0.15 Rf	15.1 AU	1715.2 AU	2.42 %	Gallic Acid
3	0.30 Rf	34.2 AU	0.33 Rf	101.2 AU	6.00 %	0.36 Rf	44.3 AU	2896.9 AU	4.09 %	Quercetin
4	0.36 Rf	47.8 AU	0.42 Rf	98.1 AU	5.81 %	0.43 Rf	36.3 AU	4533.1 AU	6.40 %	unknown *
5	0.43 Rf	96.7 AU	0.45 Rf	116.2 AU	6.89 %	0.48 Rf	37.2 AU	4694.8 AU	6.62 %	unknown *
6	0.51 Rf	92.3 AU	0.57 Rf	130.8 AU	7.75 %	0.59 Rf	26.7 AU	9145.3 AU	12.90 %	unknown *
7	0.59 Rf	127.0 AU	0.65 Rf	159.4 AU	9.45 %	0.66 Rf	58.9 AU	8462.3 AU	11.94 %	unknown *
8	0.66 Rf	159.0 AU	0.66 Rf	159.5 AU	9.45 %	0.73 Rf	77.5 AU	7273.2 AU	10.26 %	unknown *
9	0.74 Rf	79.2 AU	0.78 Rf	234.7 AU	13.91 %	0.81 Rf	13.4 AU	10203.8 AU	14.40 %	unknown *
10	0.85 Rf	147.5 AU	0.91 Rf	442.5 AU	26.23 %	0.94 Rf	2.6 AU	19413.8 AU	27.39 %	unknown *

Table 4: Track 3, ID: SAMPLE 1

Track 4,ID: Sample 2

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.03 Rf	2.7 AU	-0.02 Rf	445.4 AU	24.96 %	-0.01 Rf	56.7 AU	7223.3 AU	16.05 %	unknown *
2	-0.01 Rf	371.3 AU	-0.00 Rf	445.7 AU	24.97 %	0.05 Rf	24.6 AU	6688.7 AU	14.86 %	unknown *
3	0.07 Rf	31.4 AU	0.08 Rf	34.1 AU	1.91 %	0.10 Rf	18.7 AU	863.2 AU	1.92 %	unknown *
4	0.11 Rf	19.7 AU	0.14 Rf	33.4 AU	1.87 %	0.14 Rf	32.4 AU	862.9 AU	1.92 %	Gallic Acid
5	0.16 Rf	25.7 AU	0.20 Rf	70.3 AU	3.94 %	0.22 Rf	22.1 AU	2648.8 AU	5.88 %	unknown *
6	0.23 Rf	22.2 AU	0.26 Rf	34.1 AU	1.91 %	0.27 Rf	29.5 AU	1239.5 AU	2.75 %	unknown *
7	0.29 Rf	32.7 AU	0.31 Rf	41.5 AU	2.33 %	0.31 Rf	40.0 AU	785.2 AU	1.74 %	unknown *
8	0.32 Rf	42.0 AU	0.34 Rf	59.8 AU	3.35 %	0.35 Rf	49.4 AU	1716.4 AU	3.81 %	Quercetin
9	0.36 Rf	51.3 AU	0.39 Rf	94.4 AU	5.29 %	0.41 Rf	39.3 AU	3386.3 AU	7.52 %	unknown *
10	0.41 Rf	69.6 AU	0.43 Rf	84.8 AU	4.75 %	0.44 Rf	31.2 AU	2057.7 AU	4.57 %	unknown *
11	0.45 Rf	80.3 AU	0.47 Rf	111.3 AU	6.24 %	0.49 Rf	31.5 AU	3713.9 AU	8.25 %	unknown *
12	0.53 Rf	87.0 AU	0.57 Rf	96.7 AU	5.42 %	0.59 Rf	35.1 AU	4652.2 AU	10.34 %	unknown *
13	0.64 Rf	103.2 AU	0.64 Rf	104.8 AU	5.87 %	0.71 Rf	40.7 AU	4997.0 AU	11.10 %	unknown *
14	0.86 Rf	44.4 AU	0.90 Rf	78.0 AU	4.37 %	0.93 Rf	31.7 AU	3878.0 AU	8.62 %	unknown *
15	0.93 Rf	33.1 AU	0.93 Rf	50.3 AU	2.82 %	0.95 Rf	0.0 AU	299.7 AU	0.67 %	unknown *

Table 5: Track 4, ID: SAMPLE 2

Conclusion

By comparing the Rf values of the compounds with the reference standards, the HPTLC study for *H.indicum* phytochemical profiling will be beneficial in identifying bioactive chemicals and markers. The current study found that *H.indicum* contains a number of phytochemicals, which may account for some of its medicinal benefits and hence support its use as a treatment for a variety of diseases.

Data availability statement

This article includes all of the data that were analyzed during the investigation.

Funding

For the submission this study, does not include any research funding.

Competing interests

Since no animals were used in this study, ethical approval was not needed.

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