Phytochemical analysis and simultaneous quantification of quercetin and gallic acid in *Ipomoea carnea* Jacq. through HPTLC

Anant Kumar Srivastav[1*], Shikhar Verma[2], Himani Awasthi[1], Santosh Kumar[1], Dharmendra Yadav[1], Rajni Devi[1], Alok Kumar Srivastava[1], Madhaw Dwivedi[1], Anurag Singh[3], Anurag Kumar[4]
[1]Hygia Institute of Pharmaceutical Education and Research, Ghaila Road,Balrampur, Lucknow, Uttar Pradesh 226020, India.
[2] Maharishi School of Pharmaceutical Sciences, IIM Road, Indrapuri Colony, Diguria, Aziz Nagar, Lucknow, Uttar Pradesh 226013.
[3]Goel Institute of Pharmacy and Sciences, Anora Kala, Faizabad Road, Near Indira Canal, Uttar Pradesh, 226028.
[4]Rama University Kanpur.

Abstract:

Ipomoea carnea commonly known as beshram or pink morning glory have several bioactive compounds of therapeutic needs as indicated through several peaks obtained and represented through chromatogram. Aim and objectives: Objective of this study is to investigate the presence of bioactive compounds (Phytochemical profiling) along with quantification of quercetin and gallic acid. Methods: HPTLC analysis of *Ipomoea carnea* test samples extracted from n-hexane (sample 1) and hydro-alcohol (1:1, v/v) (sample 2) extract reveals information for the presence or absence of Quercetin and gallic acid. The phytochemical profile of plants was determined and presented in the figures and tables showed the total number of peaks, peak height, peak area and Rf values. Result: CAMAG HPTLC analysis reveals the absence of quercetin and gallic acid in plant samples. Importance of this research is to set the further research of other flavonoids to set the flavanoidal actions of *I. carnea.*

Keywords: *Ipomoea carnea*, HPTLC, Chromatogram, Quercetin, Gallic acid.

Introduction

Weeds are plants that don't have specific requirements with respect to climatic condition, nutrients and space. They grow at disturb and inhabited places [1]. Biodiversity of a plant community adversely affected by the invasive plant species like *Ipomoea carnea Jacq.* popularly known as beshram, behaya, morning glory, perennial pantropical dicot shrub with milky juice (family: convolvulaceae) mostly at riverbank, canal and water drains and all over the world has become economic and ecological disaster for the water bodies by blocking water flow [2-6].This inhibitory effect of *I. carnea* associated with its allelopathy nature that affects the growth of adjacent species of plant [7-11]. Traditionally leaves of *I. carnea* used in the treatment of wounds healing [12-13]. Anti-inflammatory, antioxidant, anti-bacterial activity, muscular pain, swelling stomach ache, nodules in breast [14-15]. Preliminary qualitative phytochemical screening of *I. carnea* reveals presence of phenolic compounds, terpenoides, flavonoids, and steroids, some of them have antioxidant and antimicrobial activity [16]. The WHO has recommended researchers for scientific validation of herbal drugs for acknowledging sound science and nowadays there are several new technologies made possible for identification, screening and isolation of these active compounds [17-18]. HPTLC is a commonly used method for quantitative and qualitative estimation of several markers in plant

materials. This is very simple, sensitive, and accurate and one of the most approached technique in recent time [19]. The present study was performed for the phytochemical profiling and quantification of quercetin and gallic acid in aerial part of *Ipomoea carnea*.

Materials and Methods

Chemicals and solvents

All the chemical compounds were of chromatographic and analytical grade. Reference markers were gifted by the National botanical research institute, NBRI Lucknow.

Preparation of standard solution

Standard solution initially prepared with strength 1mg/1ml is finally diluted with methanol up to 0.5mg/ml for HPTLC analysis.

Collection, Identification and Authentication

Aerial part of *Ipomoea carnea* Jacq. collected in the month of 20 July, 2022 from ghaila road, faizullaganj lucknow. The identification has been done on the basis of macroscopic studies of the sample followed by detailed scrutiny of literature and matching the sample with authentic samples deposited in the Raw Material Herbarium and Museum, Delhi (RHMD. The plant authentication was done from CSIR-NIS$_C$PR under authentication no. NIScPR-RHMD/Consult/2022/4173-74-2.

Extraction of plant material

Authenticated plant material was properly washed and kept for shade drying for 11 days. The dried plant material was grounded to fine powder by mechanical grinder and then successive extraction by soxhlet apparatus was performed. Initially extraction performed by using non polar solvent n-hexane. After 11 cycles excess solvent evaporated and converted into semi solid mass on water bath at 69 ^{o} C. Parellaly removal and air drying of powder from thimble was done. Same dried powder was packed into thimble and extraction performed by using polar solvent hydro-alcohol (ethyl alcohol and water in 1:1 ratio, v/v) (60 ^{o} C) and same process were performed till semi solid mass was procured. This obtained crude semi-solid form of drug stored in air tight container used for HPTLC analysis.

Preparation of test samples

Test solution for HPTLC study were obtained by dissolving semi-solid mass (10 mg) in 1 ml of HPTLC grade methyl alcohol and then filtered through 0.22 µm filters.

Preparation of calibration curve

Calibration curves were obtained by plotting absorbance unit against concentration of standards. A stock solution prepared with methanol of concentration 0.5mg/ml. Five amounts of standards quercetin and gallic acid injected ranging from 2-10 microgram/spot from injecting volume 4µl, 8µl, 12 µl, 16µl, 20µl of stock solution.

Instrumentation

A CAMAG HPTLC system loaded with LINOMAT 5 applicator with 100 µl syringe, winCATS software and CAMAG TLC scanner was used.

Chromatographic condition

HPTLC plate 60 F 254 (E-Merck KGaA) coated with 0.2mm silica gel of dimension 20 x 10 cm was used. Pre washing was not performed instead manual modification to obtain required size of plate, cutting and then plate activation in an oven at 40 ^{o} C before the HPTLC analysis was done. Sample volumes 10, 20µl and standard solution 5 mm above from the bottom with CAMAG Linomator-5 applicator fitted with 100 µl syringe at a constant application rate of 150 nl/s.

Plate loaded with samples kept in manually developed saturated chamber with mobile phase toluene: ethyl acetate: formic acid in 6:4:0.3(v/v/v) ratios. After development of bands plate was dried in air for 5 minutes and then scanning was performed at 254nm and 366nm using CAMAG scanner III equipped with winCATS software.The bands were seen using CAMAG visualizer. UV active compounds undergo into Fluorescence quenching and seen as dark spot on a white background while compounds absorb 366 nm seen as bright spot on a dark background **[20] (Table. 1).**

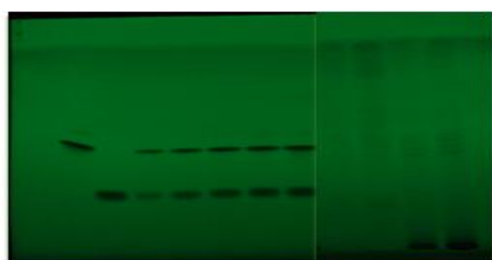
Parameters	**Values**
Calibration Parameter	
Calibration mode	Multiple level
Statistics mode	CV
Evaluation mode	Peak height and peak area
Linomat5 applicationparameters	
Spray gas	Nitrogen
Sample solvent type	Methanol
Dosage Speed	150 nl/s
Predosage volume	6 µl
Syringe size	100 µl
Application position	5.0 mm
Band length	6 mm
Solvent front position	98.0 mm
Detection CAMAG TLC scanner	
Number of track	4
Position of track	5.0 mm
Distance between track	9.4 mm
Scan start position Y	5.0 mm
Scan end position Y	98.0 mm
Slit dimension	6.00 x 0.30 mm, micro
Optimize optical system	Light
Scanning speed	20 mm/sec
Data resolution	100 µm/step
Integration: properties	
Baseline correction	Lowest slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 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 fibre	Second order/K400
Detector mode	Automatic
PM high voltage	181V

Table 1: Parameters used for HPTLC

Results and Discussion

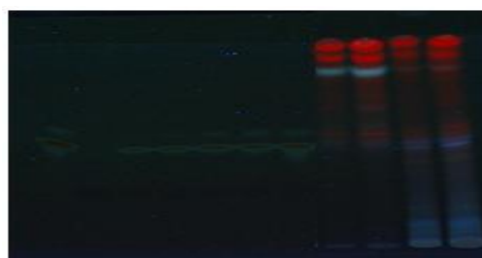
Phytochemical profiling and quantification of quercetin and gallic acid in the methanolic extract of *I. carnea* (Aerial part) was studied and results were obtained in the form chromatograms depicted in figures (1,2,3,4, 5,6,7,8,9) and tables (2, 3, 4, 5). Chromatograms from standards and test samples were obtained

by CAMAG TLC scanner III at short (254 nm) (fig. 1) and long (366 nm) (fig.2) wavelength. The peaks at position second in reference quercetin (fig. 3) and gallic acid (fig.4) chromatogram was found to be maximum in height and area from comparison to other peaks which were found to be at same baseline denotes quercetin and gallic acid at Rf 0.50 (track 1) and Rf 0.29 (track 2). Chromatograms of plants samples (fig. 8 and fig. 9) reveal several peaks representing number of compounds present in the methanolic extract of plant samples. There were ten (fig. 8, track 3) and 11(fig.9, track 4) bioactive compounds in sample 1 and sample 2 respectively. The densitometry chromatogram of HPTLC fingerprint of the methanolic extract is shown in fig. 8 and fig. 9 when compared to chromatograms of reference markers fig. 3 and fig. 5 reveals their absence in plant samples. The peak resolving at Rf 0.50 (Track 1) in the chromatogram of reference quercetin defines its presence when compared to test samples (Track 3 and Track 4) Rf values, peak area and peak height of several bioactive compounds indicates that quercetin was not detected in the both samples. The peak resolving at Rf 0.29 (Track 2) in the chromatogram of reference gallic acid defines its presence when compared to test samples Rf values, peak area and peak height of several bioactive compounds indicates that gallic acid is not detected in both samples.



Q GA Q + GA Sample 1 Sample 2

Fig. 1 Image of TLC plate at 254nm



Q GA Q + GA Sample 1 Sample 2

Fig. 1 Image of TLC plate at 366nm

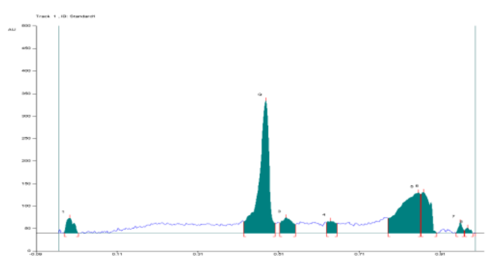


Fig. 3: HPTLC chromatogram of standard quercetin

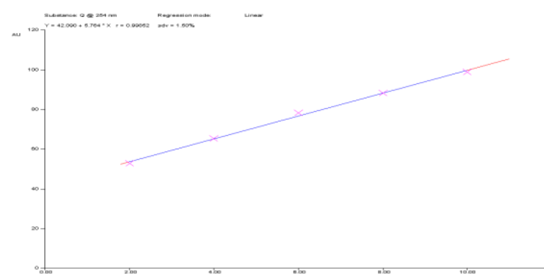


Fig. 4: Calibration curve of standard quercetin

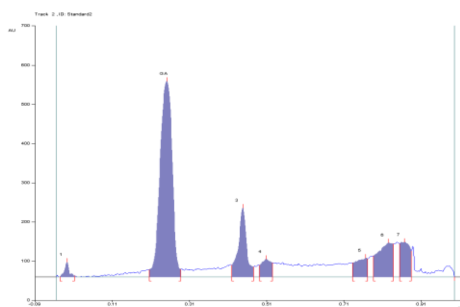


Fig. 5: HPTLC chromatograms of standard gallic acid

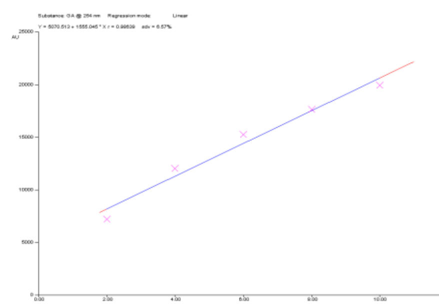


Fig. 6: Calibration curve of standard gallic acid

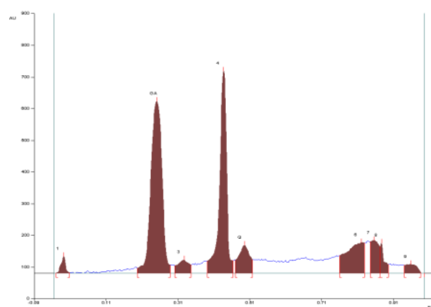


Fig. 7: HPTLC chromatograms of standard Q and GA

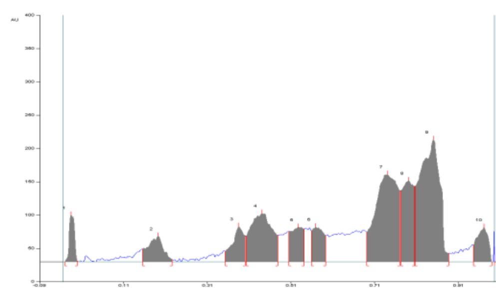


Fig. 8: Chromatogram of sample 1

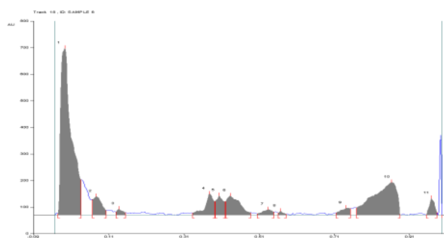


Fig. 9: Chromatogram of sample 2

Track 1, ID: Standard 1										
Peak	**Start Position** Rf	**Start Height** AU	**Max. Position** Rf	**Max. Height** AU	**Max %**	**End Position** Rf	**End Height** AU	**Area** AU	**Area %**	**Assigned substance**
1.	-0.02	0.5	-0.01	33.5	5.52	0.02	0.1	628.8	3.75	Unknown
2.	0.43	25.9	0.48	294.5	48.59	0.50	22.5	7340.2	43.81	Q
3.	0.51	22.9	0.53	34.9	5.76	0.55	19.2	991.6	5.92	Unknown
4.	0.63	23.4	0.64	27.1	4.47	0.66	22.0	618.6	3.69	Unknown
5.	0.78	33.4	0.86	89.8	14.82	0.86	36.8	4713.5	28.13	Unknown
6.	0.86	86.9	0.87	90.9	15.00	0.90	0.7	2086.8	12.46	Unknown
7.	0.95	0.4	0.96	23.6	3.90	0.97	5.8	229.9	1.37	Unknown
8.	0.97	6.2	0.98	11.7	1.94	0.99	0.0	144.9	0.87	Unknown

Table 2: HPTLC peak table of standard quercetin

Track 2, ID: Standard 2										
Peak	**Start Position** Rf	**Start Height** AU	**Max. Position** Rf	**Max. Height** AU	**Max %**	**End Position** Rf	**End Height** AU	**Area** AU	**Area %**	**Assigned substance**
1.	-0.02	0.5	-0.01	38.0	3.86	0.01	3.2	471.0	1.60	Unknown
2.	0.21	18.5	0.25	499.5	50.76	0.29	20.0	16358.1	55.64	GA
3.	0.42	29.7	0.45	176.0	17.88	0.48	26.0	3926.3	13.36	Unknown
4.	0.49	29.9	0.51	45.5	4.62	0.53	35.6	1245.5	4.24	Unknown
5.	0.74	36.1	0.77	48.4	4.92	0.77	46.2	1536.1	5.23	Unknown
6.	0.79	53.7	0.83	87.8	8.92	0.84	34.4	3525.3	11.99	Unknown
7.	0.86	84.9	0.87	88.9	9.03	0.89	35.4	2335.1	7.94	Unknown

Table 3: HPTLC peak table of standard gallic acid

Track 3, Sample 1										
Peak	**Start Position** Rf	**Start Height** AU	**Max. Position** Rf	**Max. Height** AU	**Max %**	**End Position** Rf	**End Height** AU	**Area** AU	**Area %**	**Assigned substance**
1	-0.03	0.2	-0.01	70.2	8.50	0.00	1.0	909.1	2.81	Unknown*
2	0.16	19.3	0.19	38.2	4.63	0.23	3.3	1462.4	4.52	Unknown*
3	0.35	15.7	0.39	52.7	6.38	0.40	39.5	1528.5	4.72	Unknown*
4	0.40	38.2	0.44	73.0	8.84	0.48	39.9	3967.1	12.25	Unknown*
5	0.51	45.2	0.53	52.0	6.29	0.54	49.5	1661.3	5.13	Unknown*
6	0.56	46.7	0.57	52.1	6.30	0.60	39.5	1524.3	4.71	Unknown*
7	0.69	44.9	0.74	130.9	15.85	0.77	xxx	7374.5	22.78	Unknown*
8	0.78	107.2	0.79	121.7	14.73	0.81	13.3	3467.4	10.71	Unknown*
9	0.81	113.4	0.85	183.3	22.19	0.89	12.9	9007.2	27.82	Unknown*
10	0.95	25.5	0.97	52.0	6.29	0.99	0.0	1473.7	4.55	Unknown*

Table 4: HPTLC peak table sample 1

Track 4, Sample 2										
Peak	**Start Position** Rf	**Start Height** AU	**Max. Position** Rf	**Max. Height** AU	**Max %**	**End Position** Rf	**End Height** AU	**Area** AU	**Area %**	**Assigned substance**
1	-0.02	4.9	-0.01	626.8	52.76	0.04	32.8	18722.3	50.3	Unknown*
2	0.07	59.0	0.08	69.5	5.85	0.10	17.4	1688.2	4.54	Unknown*
3	0.13	11.8	0.14	21.9	1.84	0.16	9.4	372.6	1.00	Unknown*
4	0.33	7.8	0.38	79.4	6.68	0.39	50.8	1860.3	5.01	Unknown*
5	0.39	51.0	0.41	72.7	6.12	0.42	50.1	1474.4	3.97	Unknown*
6	0.42	50.3	0.44	71.8	6.05	0.49	8.7	2675.6	7.20	Unknown*
7	0.51	4.1	0.54	20.3	1.71	0.55	11.4	558.2	1.50	Unknown*
8	0.56	8.7	0.57	14.4	1.21	0.58	4.0	176.7	0.48	Unknown*
9	0.72	7.9	0.74	26.0	2.19	0.76	24.2	687.9	1.85	Unknown*
10	0.77	27.2	0.86	122.7	10.33	0.89	2.1	8027.6	21.61	Unknown*
11	0.96	1.1	0.97	62.5	5.26	0.99	10.7	907.5	2.44	Unknown*

Table 5: HPTLC peak table sample 2

Conclusion

As HPTLC phytochemical profiling reveals the presence of several bioactive compounds in the methanolic extract of I. carnea that can be further explored up to their identification and future application in pharmacological treatment. Quantification reveals the absence of quercetin and gallic acid and this concludes that further study must be performed to check total flavanoidal contents and other markers.

Data availability statement

All data included in this article.

Funding

No funding was received for this research.

Competing interest

Authors declare no interest at all.

Ethical approval

Not applicable.

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