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Phytochemical analysis of *Clerodendron infortunatum* Linn. leaves in various seasons by different solvent and extraction techniques

Research Article

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Abstract

Clerodendron infortunatum Linn is widely used for the treatment of malaria and vermifuge, scorpion and snake bites, tumors, inflammation, bronchitis and in wound healing. The present study was carried out to estimate the variation in the amounts of Gallic acid, Rutin and Quercetin in *Clerodendron infortunatum* Linn.leaves extracts collected in different seasons using high-performance thin layer chromatography (HPTLC).In this study, leaves of *C. infortunatum* were subjected to methanol extractions using Soxhlet method and cold maceration. The leaves were collected in different seasons in the months of December (Sample A -Shishir rutu-Winter season) and July (Sample B -Varsha Rutu -Rainy season). The methanolic extracts were subjected to HPTLC study & using CAmag HPTLC system, employing a mixture of toluene:ethyl acetate:formic acid:methanol (3:6:1.6:0.4) as the mobile phase with densitometric scanning at 254nm. Quantity of Quercetin is found in both samples, extracted by Soxhlet method. Its quantity is higher in the winter season. Quantity of Gallic acidis found in both samples. Its quantity is the same in Sample B by Soxhlet extraction method & maceration method respectively. Quantity of Rutin is found in both the samples extracted by Soxhlet & maceration methods. It is higher in the sample-A. The present HPTLC method is used for the detection and quantization of Gallic acid, Quercetin and Rutin of *C. infortunatum* leaves. It needs to study the season wise and region wise variation of phytochemicals in useful parts of all other plants by different extraction methods.

Keywords: *Clerodendron infortunatum Linn., HPTLC, Rutin, Quercetin, Gallic acid, Seasonal variations, Ayurveda, Traditional medicine.*

Introduction

In *Ayurveda*, methods of collection of plant parts are described in a systematic manner according to seasons. To get superior results of plant for its pharmacological actions seasonal collection of plant must be taken care of. In *Charak Samhita*, tender leaves of plant should be collected in *varsha Ritu* (Rainy Season) and *Vasant Ritu* (Spring season) (1). According to *Sushrut Samhita* it should be collected in *Varsha ritu*. ((Rainy Season) (2) & *Raj Nighantu* quoted that leaves should be collected in *Shishira ritu* (winter season) and tender leaves in *Grishma Ritu* (Summer season) (3). *Clerodendron infortunatum* Linn.is widely used traditionally. *Clerodendrum infortunatum* belongs to *Verbenaceae* family, an under shrub found in central India and Ceylon. It is a terrestrial shrub having square, blackish stem and simple, opposite, decussate, petiolate, exstipulate, coriacious, hairy leaves with a disagreeable odour .The shrub is of 2-4 feet in height. Flowers are bluish-purple often white in pyramid shaped terminal panicles It is antiperiodic in n Malaria and vermifuge. It is used in infusion as a bitter tonic. Expressed juice of the leaves is laxative and anthelminthic. Decoction of leaves is used as anthelminthic in round worms. The drug is used in scorpion sting and snake bite (4). Leaves of *C. infortunatum* L. shows the sterols, carbohydrates, tannins, terpenoids, flavonoids and saponin (5). Rutin pharmacological activities include antioxidant (6), cytoprotective (7), vasoprotective, anticarcinogenic (8), neuroprotective (9). Quercetin is one of the important bioflavonoids which is known for its anti-inflammatory, antihypertensive, vasodilator effects, antiobesity, antihypercholesterolemic and antiatherosclerotic activities (10, 11). Gallic acidis having antioxidant, astringent, antibacterial, antifungal, antiviral potential and it counteracts the mutagenicity of certain carcinogens (12). Therapeutic potential of drug depends

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upon its active constituents. The interest area of this study is to discover whether the chemical compositions vary according to seasons and extraction methods.

Figure No.1: Leaves & Flowers of *Clerodendron infortunatum* Linn.

Materials & Methods

Chemicals and reagents

Quercetin(99%), Gallic acid(99%) and Rutin (98%) were purchased from Hi Media Laboratories, Mumbai, India. All chemicals and reagents used in the study were of AR grade. The Thin Layer chromatographic analysis was carried out on aluminum-backed TLC plates percolated silica gel 60 F254 (10×10 cm) plates (0.2 mm layer) purchased from E. Merck (Germany).

Collection and authentication of leaves of *Clerodendron infortunatum*-Leaves of *Clerodendron infortunatum* Linn were collected from the *Vidarbha region* (Amravati,Maharshtra,India) in the month of December (Sample A -Shishir rutu-Winter season) and July(-Sample B -Varsha Rutu -Rainy season). It was authenticated at Agharkar Research Institute, Pune (Govt.of India) with Report reference no. AUTH 20-138. The plant material collected was first washed with running tap water, the washed leaves were dried in shade. The air-dried specimen (leaves) were pulverized and sieved through 80# mesh size and stored in air-tight container at room temperature.

Extraction methods

Soxhlet method: 50 gm of shade dried leaves powder of samples A & B were extracted in methanol using Soxhlet apparatus for 17 hours.

Cold Maceration: 50 gm of shade dried leaves powder of samples A & B were extracted in methanol by cold maceration method. In this method, Samples A & B were soaked in a Stoppered container with solvent and allowed to stand at room temperature for a period of 72hrs. with frequent agitation. The ratio of sample: solvent was maintained at 1:10 in the study. The methanolic extracts were filtered through a Whatman filter paper.

The methanolic extracts obtained by both the processes were then concentrated in a rotary evaporator. The residual solvents were further removed by drying using a vacuum oven (Labline) under reduced pressure at 40°C to obtain the dry extracts. The dried extracts were stored in air tight containers in the refrigerator.

High-performance thin layer chromatography

Preparation of Gallic acid, Quercetin and Rutin standard solution

A stock solution of concentration 100 µg/mL each of standard Gallic acid, Quercetin and Rutin were prepared by transferring 10 mg each of Gallic acid, Quercetin and Rutin in separate 100 mL volumetric flask, 75 ml of methanol was added followed by sonication for 10 min, and the volume was made up to the mark with methanol. The resulting standard solutions were suitably filtered through Whatman filter paper for further analysis.

Preparation of sample solutions

100 mg of the concentrated methanolic extracts (A and B) obtained both by Soxhlet extraction and cold maceration was re-dissolved in10ml methanol to obtain sample solutions of concentration 1000 µg/mL.

HPTLC instrumentation and chromatographic conditions

In this study, an attempt was made to study the effect of the period of collection on the amount of active phytoconstituents; mainly Quercetin, Gallic acid and Rutin present in the leaves of Clerodendron infortunatum Linn. The analysis was carried out employing a Camag HPTLC system equipped with a TLC densitometric scanner 3 and win CATS 1.2.2 software (Camag, Muttens, Switzerland). The system also included a UV chamber (Camag, Muttens, Switzerland), a twin trough chamber (10×20 cm or 20×20 cm; Camag, Muttens, Switzerland), and saturation pads (Camag, Muttens, Switzerland). The standard and sample solutions were spotted as bands of 6 mm width using a Camag Linomat V 5 sample applicator (Hamilton, Broadus, Switzerland) equipped with a micro liter syringe (100 µl). The stationary phase consisted of aluminum plates precoated with silica gel 60 F254 (10×10 cm with 0.2 mm thickness, E. Merck, Germany). During the chromatographic analysis, the slit dimension was kept constant (5 mm×0.45 mm) with scanning speed maintained at 20 mm/s. The chromatographic development was carried out using linear ascending development in the twin trough glass chambers. The separation was achieved using a mobile phase consisting of toluene: ethyl acetate: formic acid: methanol (3:6:1.6:04). The chromatograms were allowed to develop up to a length of 80 mm and then dried with air dryer. The densitograms were recorded and the amount of Gallic acid, Quercetin and Rutin in the methanolic extracts were quantified against the standard calibration curves.

Results and Discussion

Table no. 1: Organoleptic examination of leaves of C. infortunatum

Sr.No.	Organoleptic parameters	Sample A	Sample B
1	Appearance	Dark blackish Green, more hairy	Light green, Less hairy
2	Odour	Foul odour	Foul odour
3	Texture	Rough	Rough
4	Sound on fracture	Typical cracking sound	Typical cracking sound
5	Taste	Bitter	Bitter

High-performance thin layer chromatography

The presence of the phytoconstituents; Gallic acid, Quercetin and Rutin in the sample extracts was confirmed by comparing the retention factors of the bands obtained in the sample densitograms with that of the standard solutions of Gallic acid, Quercetin and Rutin. In the standard densitograms, peaks were observed at R_f 0.74, R_f 0.85 and R_f 0.18 for Quercetin, Gallic acid and Rutin respectively. In the sample densitograms, peaks were obtained at Rf values close (Rf +_ 0.2) to that of the respective standards under the selected chromatographic conditions.

The densitograms of standard Gallic acid, Quercetin, Rutin is show in Figure 2.Figure 3 represents the densitograms of extracts of leaves of *C. infortunatum* of sample A.In fig.4,Sample B - respectively by Soxhlet method. The Figure 3a & Figure 4a are showing densitogram by maceration method of sample B & A respectively. The three-dimension UV spectra of standard Gallic acid,Quercetin and Rutin scanned at 254 nm are shown in Figure 5.The calibration curve for Gallic acid, Rutin and Quercetin were liners in the concentration range of range of 100-2000ng/band respectively. From the regression equations the concentration of Gallic acid, Rutin and Quercetin extracts collected at different time intervals was determined.

Figure 2: The chromatograms of standard Gallic acid, Quercetin and Rutin.

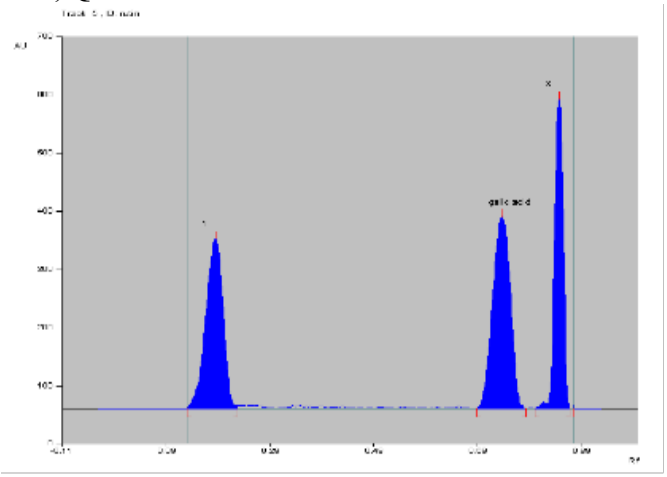


Table No. 2: Showing standards of Gallic acid, Quercetin and Rutin

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.13 Rf	1.2 AU	0.18 Rf	292.9 AU	25.33%	0.23 Rf	5.0 AU	8161.3 AU	32.69%	Rutin
2	0.69 Rf	0.2 AU	0.74 Rf	330.1 AU	28.55%	0.78 Rf	0.2 AU	9439.2 AU	37.81%	Gallic acid
3	0.80 Rf	0.1 AU	0.85 Rf	533.4 AU	46.13%	0.87 Rf	5.9 AU	7367.5 AU	29.51%	Quercetin

Fig. No. 3-Densitogram of methanolic extract of leaves of Cl. Infortunatum by Soxhlet in sample A, showing presence of Rutin, Gallic acid and Quercetin.

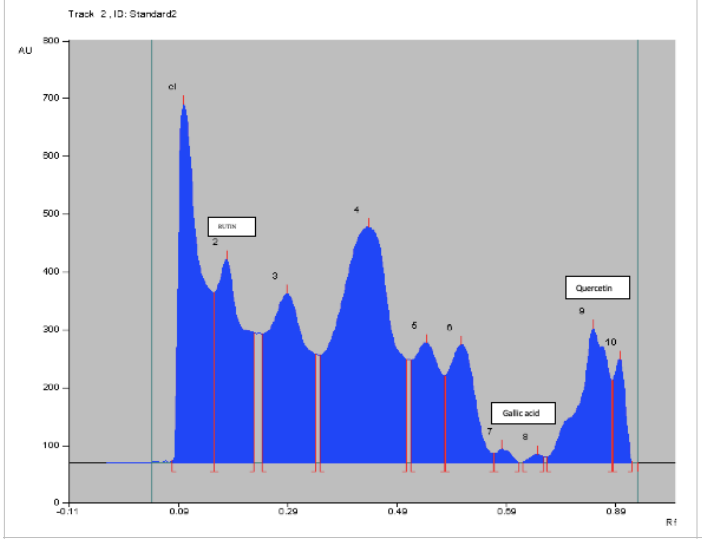


Fig. no.3a- Densitogram of methanolic extract of leaves of *Cl. Infortunatum* showing presence of Rutin by maceration method of sample A.

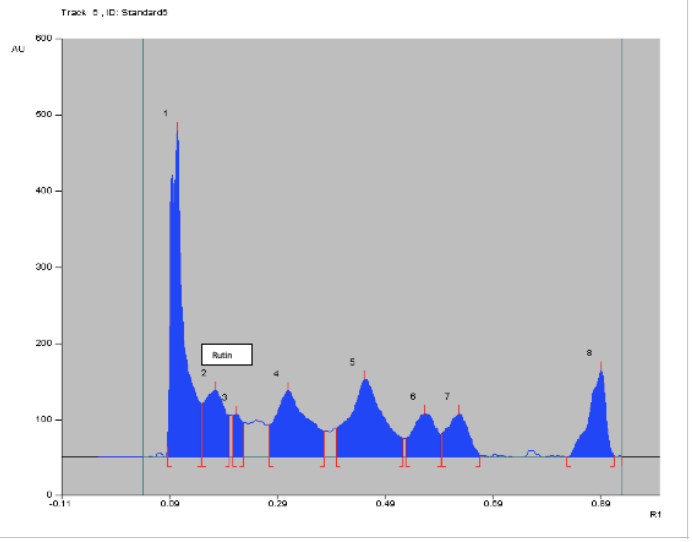


Fig. no.4- Densitogram of methanolic extract of leaves of *Cl.infortunatum* showing presence of Rutin and Quercetin by Soxhlet method of sample B.

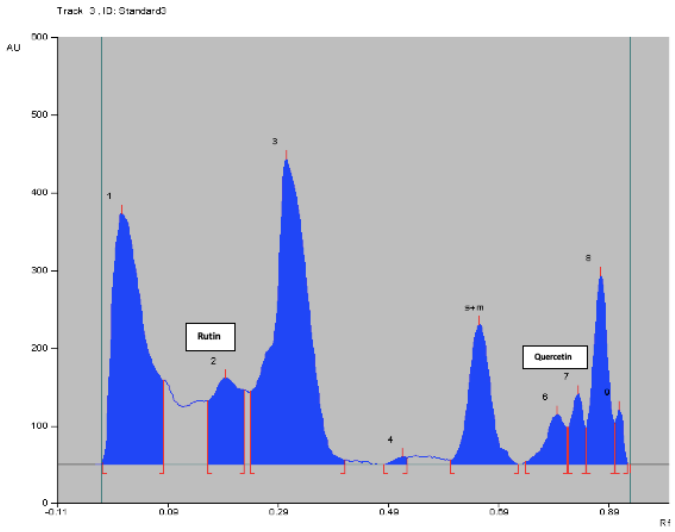


Fig.4a-Densitogram of methanolic extract of leaves of *Cl. Infortunatum* by maceration method showing presence of Rutin in the sample B.

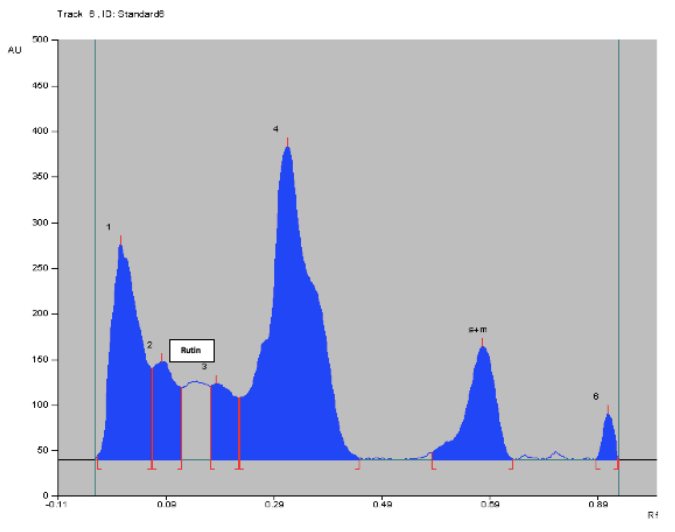


Table No.3: Showing presence of Rutin, Gallic acidand Quercetin at 2, 8 and 9 peaks respectively.

Pea	Start	Start	Max	Max	Max%	End	End	Area	Area
1	0.08Rf	3.1AU	0.10Rf	619.6AU	24.50	0.15Rf	34.0AU	20730.9AU.	17.90%
2	0.16Rf	294.4A	**0.18Rf**	351.8AU	13.91	0.23Rf	24.9AU	14478.1AU	12.50%
3	0.24Rf	2228AU	0.29Rf	291.9AU	11.54	0.34Rf	37.2AU	17014.7AU	14.69%
4	0.35Rf	186.oA	0.44Rf	406.6AU	16.08	0.51Rf	78.3AU	33428.1AU	28.86%
5	0.52Rf	176.6A	0.54Rf	207.3AU	8.20%	0.58Rf	50.2AU	8277.5AU	7.15%
6	0.58Rf	150.3A	0.61Rf	203.5AU	8.05%	0.67Rf	15.1AU	8037.5AU	6.94%
7	0.67Rf	15.3AU	0.68Rf	23.5AU	0.93%	0.71Rf	0.1AU	490.4AU	0.42%
8	0.72Rf	0.6AU	**0.75Rf**	14.5AU	0.57%	0.76Rf	8.9AU	257.5AU	0.22%
9	0.76Rf	9.1AU	**0.85Rf**	231.9AU	9.17%	0.88Rf	40.8AU	10141.8AU	8.76%
10	0.88Rf	142.3A	0.90Rf	172.8AU	7.05%	0.92Rf	1.3AU	2969.3AU	2.56%

Table No 4: showing presence of Rutin at the peak no.2

Peak	Start	Start	Max	Max	Max%	End	End	Area	Area %
2	015 Rf	**70.8 AU**	0.17 Rf	87.9	8.86%	0.20 Rf	55.0 AU	2778.6 AU	9.81%

Table No 5: showing presence of Rutin & Quercetin at the peak no.2 & 7 respectively.

Peak	Start	Start	Max	Max	Max%	End	End	Area	Area %
2	0.16 Rf	**81.9 AU**	0.19 Rf	111.4	7.49%	0.23 Rf	95.6 AU	4866.5 AU	8.61%
7	0.82 Rf	**47.9 AU**	0.83 Rf	90.9 AU	6.11%	0.85 Rf	46.3 AU	1655.5 AU	2.93%

Table No 6: showing presence of Rutin at the peak no.3

Peak	Start	Start	Max	Max	Max%	End	End	Area	Area %
3	0.17 Rf	**80.8 AU**	0.18 Rf	83.9 AU	8.86%	0.22 Rf	67.5 AU	2940.2 AU	6.29%

Figure 5: 3 D Spectra@254nm of Sample B

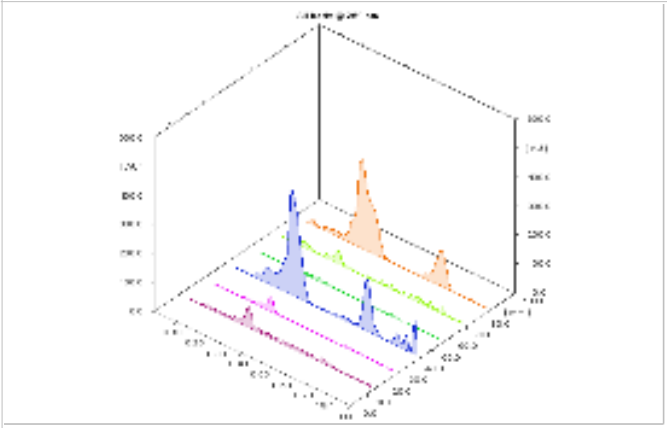
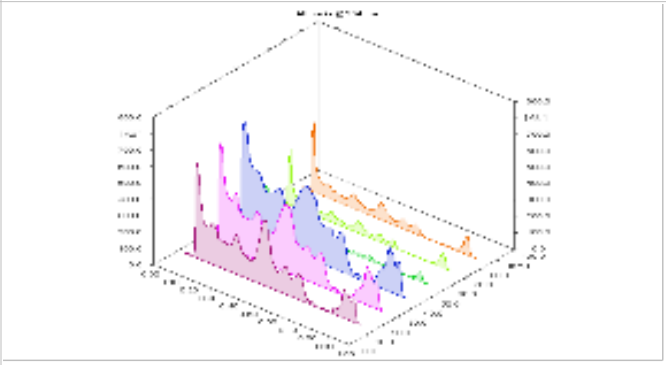


Figure 6: 3 D Spectra@254nm of Sample A.



Discussion

Biodiversity of plants, different seasons and methods of extractions, type of solvents affects the quantity of constituents in the plant. It can be differ accordingly.

Table no.7 -Percentage of chemical constituents according to different seasonal plant material

Sr.No	Chemical constituent	Name of extraction method	Plant material collected in December (sample A)	Plant material collected in July (Sample B)
1	Gallic acid	Soxhlet method	0.0027	absent
		Maceration	Absent	0.0027
2	Rutin	Soxhlet method	0.166	0.056
		Maceration	0.031	0.033
3	Quercetin	Soxhlet method	0.137	0.071
		Maceration	Absent	Absent

The sample A, leaves of *Clerodendron infortunatum* Linn, extracted by Soxhlet extraction method showed the presence of Gallic acid in the winter season & in sample B in rainy season by Soxhlet & maceration method respectively. Quantity of Gallic acidin both the samples is same.. In case of Quercetin, a higher proportion of Quercetin was estimated in the sample A by Soxhlet method. Rutin was however present in both the samples A&B by both the methods. Quantity of Rutin was found higher in sample A by Soxhlet method. In comparison the leaves extracted using maceration showed lower amounts of the phytoconstituents when compared to the Soxhlet extraction method. Sample A exhibit higher content of both Rutin and Quercetin. The variations in the proportion of the phytoconstituents collected in both the seasons indicate that be concerned while collection of the leaves of this plant. Collection of the sample during winter season may be more advantageous due to the secondary metabolites being produced in slightly higher proportions in this period. Rutin($C_{27}H_{30}O_{16}$), bioflavonoid, is responsible for its anti-inflammatory anti-carcinogenic, anti-bacterial, anti-fungal, anti-hypertensive, antiulcer, anticonvulsant, antialzheimer effect. Gallic acid ($C_7H_6O_5$), a phenolic acid is antioxidant, antitumor, antidiabtic, anti-myocardial ischemic in nature. Quercetin($C_{15}H_{10}O_7$), flavonol effective for its anti carcinogenic, anti-diabetic, antihypertensive, anti Alzheimer, anti arthritic activities.

Conclusion

The present study concludes the significance of collection of plant material in specific seasons mentioned in *Ayurveda*.There are variations found in the phytoconstituents according to drug seasonal collection and extraction methods. Rutin is found in the higher percentage than the Gallic acidand Quercetin in winter season. The present study shows higher percentage of Gallic acid, Quercetin and Rutin present in winter season by Soxhlet extraction methods.

Conflict of interest

There is no conflict of interest.

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