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Phytochemical standardization of the leaves of a medicinal plant *Cipadessa baccifera* Roth Miq.

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ABSTRACT

Background: The present study was undertaken for qualitative, quantitative estimation of phytochemicals and standardization of *Cipadessa baccifera* leaf and to develop HPTLC finger print profile for its alcoholic extract. **Methodology:** The shade dried leaves were subjected to analyse physico-chemical parameters as per standard procedures. The qualitative analysis and quantitative estimation of flavonoids, tannins and phenols were carried out by subjecting the specific extraction procedures using UV –Visible Spectrophotometer. TLC/HPTLC finger print profile was developed by using suitable solvent system. **Results and discussion:** Physicochemical analysis of the leaves showed moisture content (9.39 %); ash content (4.58%) acid insoluble ash (0.61%); water soluble extractive value (18.63 %) and alcohol soluble extractive value (13.56%). The qualitative analysis of alcohol extract showed positive test for triterpenoid, flavonoid, alkaloid, coumarin, saponin, tannin and phenol. Quantitative estimation of the plant observed 1.90 % of tannin, 4.79 % of flavonoid and 2.48% of phenol. **Conclusion:** HPTLC finger print profile was shown the presence of various phytochemical constituents. The present study on physicochemical analysis, qualitative and quantitative analysis revealed the presences of various biologically active constituents in this plant. This information can be utilised for authentication of this plant for the use of drug preparation.

KEYWORDS: UV, TLC, HPTLC, Tannin, Phenol, Flavonoids.

1. INTRODUCTION:

Cipadessa baccifera (syn*: Cipadessa fruticosa* Blume; *Cipadessa cinerascens* (Pellegr) Hand.-Mazz **)** family: Meliaceae. *Cipadessa baccifera* is a bushy shrub widely cultivated in the southwest of China and hilly areas of India. The plant is being employed for treating malaria, skin itch, dysentery and it is internally administered for cobra, scorpion and insect bites. In addition, it is also used against piles, diabetes, diarrhoea and headache. This plant has been reported to contain *ent*- clerodane, labdane, limonoids, sterols, sesquiterpenoids, heneicosene derivates and one coumarin.[1-3] The seed has been reported to contain cipadesin, 17α,20R-dihydroxypregnan-3,16-dione, 1,4-epoxy-16-hydroxyheneicos-1,3,12,14,18-pentaene, and 1,4-epoxy-16-hydroxyheneicos-1,3,12,14-tetraene.[4] The aerial parts have been already reported for diterpenoids 15, 16-dihydroxy-*ent*-cleroda-3,14-diene($C_{20}H_{34}O_2$), 12,16-di-hydroxy-*ent*-cleroda-3,14-diene ($C_{20}H_{34}O_2$), and 8, 15-dihydroxy-13-*E*-labdane($C_{20}H_{36}O_2$).[5] Cipadessalide, rubralin D, 3*β*,4*β*-dihydroxy-2*β*-acetoxypregnan-16-one, bacciferins A and B have been reported from the stem of *cipadessa baccifera*.[6] The present study was carried out to investigate qualitative analysis for the presence of phytochemicals, and the physicochemical analysis of the leaves. Quantitative estimation of total flavonoid, phenol and tannin from the leaves of the plant was carried out by using Perkin Elmer lambda EZ 201 Ultra Visible-Spectroscopy. The plant was subjected to TLC and HPTLC finger print profile studies.

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2. MATERIAL AND METHOD:

2.1. Collection of plant material:

The plant *Cipadessa baccifera* Roth Miq**.** was collected from Tirupati in the month of June 2014. The plant was authenticated by Prof. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy and Research Centre, Chennai, based on the organoleptic, and macroscopic examination of fresh sample. The specimen voucher is PARC/2015/3196 deposited for future reference.

2.2. Physico-chemical parameters

The collected leaves were dried and made in to coarse powder. The plant powder was studied for various physico-chemcial standards like loss on drying at 105°C, alcohol soluble extractive, water-soluble extractive, total ash, acid-insoluble ash using standard methods.[7]

2.3. Qualitative estimation of Phytochemicals

The plant material was extracted with absolute alcohol. This extract was screened for preliminary phytochemical tests for the presence of steroid, triterpenoid, flavonoid, alkaloid, coumarin, saponin, tannin and phenol were carried out as per the standard methods.[8]

2.4. Quantitative Estimation of Phytochemicals:

2.4.1. Estimation of total flavonoid content

This estimation was carried out based on the following procedure.[9] Quercetin was used as standard. 10 mg of quercetin was dissolved in methanol and made up to 100 ml in a standard flask. 5 gm of plant material was extracted with ethanol using a soxhlet apparatus. Then the extract obtained is filtered and concentrated. 10 mg of the plant extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol. 0.5 (TS 1) and 1.5 (TS 2) ml of extract stock solution, were taken in two different 10 ml volumetric flask. To each flask 3 ml of methanol 0.2 ml of 10% aluminum chloride, 0.2 ml of 1M potassium acetate and distilled water were added and made up to mark. The reaction mixture was allowed to stand at room tempt for 30 min. Sample blank was prepared in similar way without sample and standard solution.

2.4.2. Preparation of calibration Curve for flavonoid:

Aliquots of 0.5, 1.0, 1.5, 2.0 and 2.5 ml from the above standard quercetin solution were taken in six different 10 ml volumetric flask. To each flask 3ml of methanol 0.2 ml of 10% Aluminum chloride, 0.2 ml of 1M potassium acetate and distilled water were added. The reaction mixture was allowed to stand at room tempt for 30 min. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λ_{max} of quercetin) with a Perkin Elmer lambda EZ 201 Ultra Visible-Spectroscopy. The calibration curve was prepared by plotting absorbance *vs* concentration.

2.4.3. Estimation of total phenolic content

This estimation was carried out based on the following procedure.[10] 10 mg of phenol was dissolved in 100 ml of water to give a concentration of 100µg/ml. 1 gm of the plant material was boiled with water for 30 min. The extract was filtered and made up to 100 ml with distilled water in a standard flask. 0.1 (TS 1) and 0.3 (TS 2) ml of sample solution, were taken in two different 10 ml volumetric flask. To each flask 2.5 ml of 1N Folin- Ciocalteu reagent and 2ml of 20% sodium carbonate were added and made up to mark with distilled water. The mixture was allowed to stand for 15 mins.

2.4.4. Preparation of calibration curve for phenolic

Aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0 ml from the above standard solution were taken in 5 different 10 ml volumetric flask. To each flask 2.5 ml of 1N Folin- Ciocalteu reagent and 2 ml of 20% sodium carbonate were added. The mixture was allowed to stand for 15 minutss and the volume was made up to mark with distilled water. Blank sample was prepared in similar way without the sample.The absorbance of the resulting solutions was measured at 765 nm against reagent blank. A standard calibration curve was prepared by plotting absorbance *vs* concentration and it was found to be linear over this concentration range.

2.4.5. Estimation of Tannin

This estimation was carried out based on the following procedure. [11] 10 mg of the tannic acid dissolved and made up to the 100 ml standard flask using distilled water. 1gm of the plant material was boiled with water for 30 min. The extract filtered and made up to 100 ml with distilled water. 0.2 (TS 1) and 0.3(TS 2) ml of sample solution, were taken in two different 10 ml volumetric flask. To each flask 0.5ml of 1N Folin- phenol reagent and 1 ml of saturated sodium carbonate were added. The mixture was allowed to stand for 30 minutes and the volume was made up to the mark with distilled water.

2.5. Preparation of calibration curve for Tannin

Aliquots of 0.2, 0.6 and 0.8 ml from the above standard solution were taken in 3 different 10 ml volumetric flask. To each flask 0.5ml of 1N Folin- phenol reagent and 1 ml of saturated sodium carbonate were added. The mixture was allowed to stand for 30 mins and the volume was made up to the mark with distilled water. Sample blank was prepared in similar way without the sample. Absorbance was measured at 760 nm against reagent blank. A standard calibration curve was prepared by plotting absorbance *vs* concentration and it was found to be linear over this concentration range.

2.6. TLC and HPTLC Studies

These studies were carried out based on the following procedures.[12-14] 2 gm of the plant material was soaked in 20 ml of alcohol and kept overnight. Next day the solution was boiled for 10 minutes and filtered. The filtrate was concentrated and made up to 10 ml with alcohol in a standard flask. Precoated silica gel on aluminium plates were used as a stationary phase. 5 and 10 µl of the extract was applied on Merck aluminum plate pre-coated with silica gel 60 F_{254} of 0.2 mm thickness using Linomat IV applicator. The plate was developed in *Chloroform: methanol* (9:1) and observed under UV 254 and UV 366 nm. The plate was then dipped in Vanillin-sulphuric acid reagent and heated in a hot air oven at 105°C until the colour of the spots appeared and the photos were taken.

The TLC plate developed above, before dipping in vanillin-sulphuric acid was scanned at 254 nm and 366 nm in scanner 3, Camag HPTLC instrument using Deuterium and Mercury lamp respectively. The TLC plate was derivatizated with vanillin-sulphuric acid reagent and scanned at 520 nm using tungsten lamp.

3. RESULT:

Physicochemical analysis of this plant leaves revealed the following results. Water soluble extractive value was found to be 18.63%, alcohol soluble extractive was found to be 13.56%, loss on drying was found to be 9.39%, ash value was found to be 4.58% and acid insoluble ash was found to be 0.61%. These parameters play an important role in the standardization of medicinal plants.

The estimation of phytochemical was performed both qualitatively and quantitatively. Qualitative estimation of the alcohol extract revealed the presence of steroid, triterpenoid, flavonoid, alkaloid, coumarin, saponin, tannin and phenol. Quantitative estimation was performed for total tannin, phenol and flavonoid. Quantitative estimation of the plant observed 1.90 % of tannin, 4.79 % of flavonoid and 2.48% of phenol. The result and standard calibration graph for flavonoids give in Table 1 and Fig 1. The total flavonoids present in the plant was 4.79 %. The result and standard calibration graph for phenol give in Table 2 and Fig 2. The amount of phenol was 2.48 %. The result and standard calibration graph for tannic acid give in Table 3 and Fig 3. The total tannin present in the plant was 1.90%.

Table 1. Results of calibration curve of Quercetin

Sample No	ABS	Concentration (µg/ml)
STD 1	0.249	50
STD 2	0.594	100
STD 3	0.94	150
STD 4	1.295	200
STD 5	1.693	250
Test sample1 (TS1)	0.141	27.18
Test sample2 (TS2)	0.377	62.13

Fig 1. Standard calibration curve of Quercetin

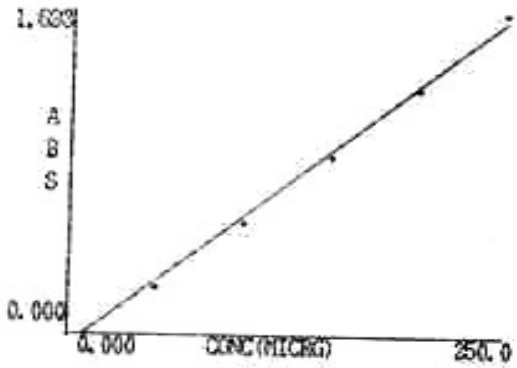


Table 2. Results of calibration curve phenol

Sample No	ABS	Concentration (µg/ml)
STD 1	0.325	20
STD 2	0.603	40
STD 3	0.815	60
STD 4	1.125	80
STD 5	1.351	100
Test sample1(TS 1)	0.355	24.08
Test sample2(TS 2)	1.059	76.51

Fig 2. Standard calibration curve of phenol

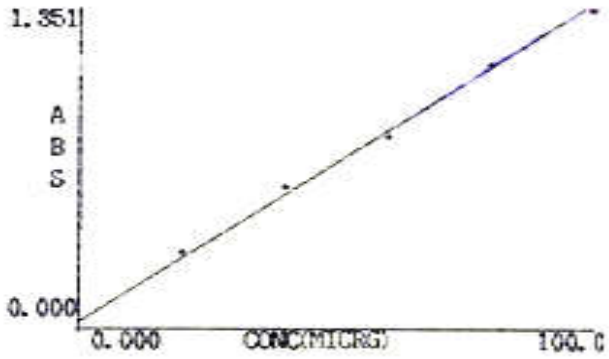


Table 3. Results of calibration curve

Sample No	ABS	Concentration (µg/ml)
STD 1	0.234	20
STD 2	0.754	60
STD 3	0.990	80
Test sample 1 (TS1)	0.472	38.18
Test sample 2(TS 2)	0.701	56.50
STD 1	0.234	20
STD 2	0.754	60

Fig 3. Standard calibration curve of tannic acid

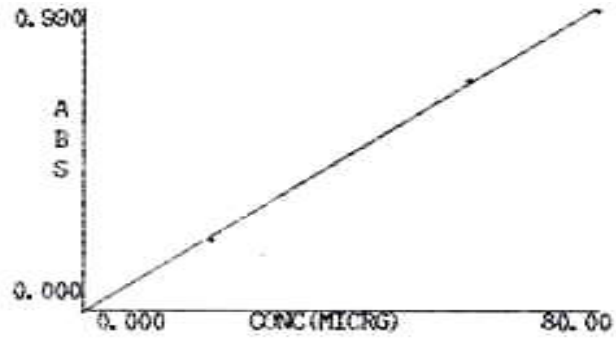


Fig 4. TLC Photo documentation of *Cipadessa baccifera* (Alcohol Extract)

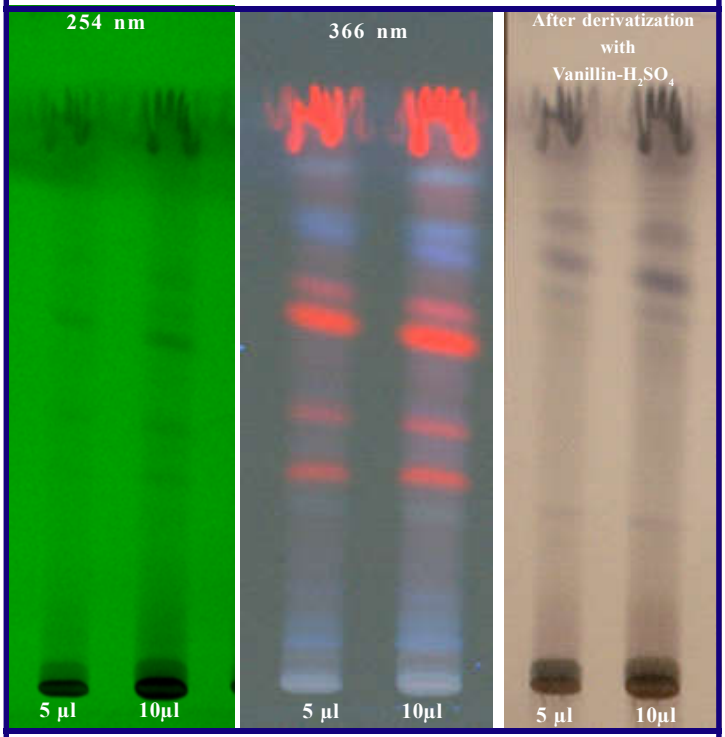


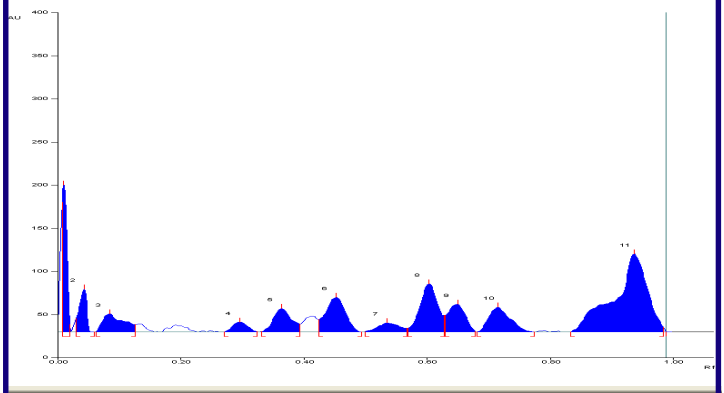
Table 4. TLC Rf values of alcohol extract of *Cipadessa baccifera*

254 nm		366 nm		After derivatization with Vanillin- sulphuric acid	
R_f	colour	R_f	color	R_f	colour
0.1	green	0.1	blue	0.1	grey
o.44	green	0.3	red	0.29	grey
0.55	green	0.45	red	0.62	grey
0.6	green	0.55	red	0.7	grey
0.7	green	0.61	red	0.75	grey
0.75	green	0.7	blue	0.83	grey
		0.75	blue		
		0.84	blue		

TLC analysis of alcoholic extract of leaves showed many spots when observed under 254 nm, 366 nm and after derivatization with vanillin-sulfuric acid (Fig 4). The spot R_f and its color were given in Table 4. This extract showed 6 spots under 254 nm, 8 spots under 366 nm and 5 spots when viewed after derivatization. Of these three spots (R_f 0.10, 0.70, 0.75) were common in all three detections systems.

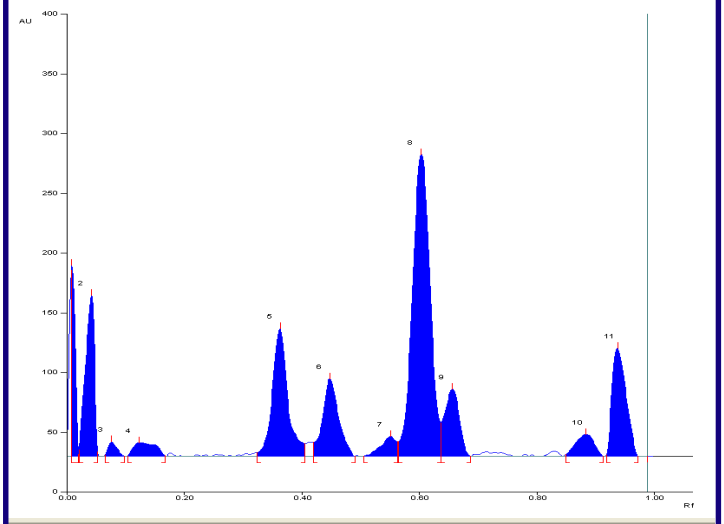
HPTLC analysis of this extract revealed 11 spots at various R_f when viewed under UV 254 nm and 366 nm (Fig 5 & Fig 6). The derivatization with vanillin-sulpuric acid and heating at 105^{0}C for 5 mins, 14 spots were observed at various R_f (Fig 7). The major peak was at R_f 0.83, 0.56 when viewed under UV 254 and 366 nm (area 35.50, 37.02% respectively). The major peak was at R_f 0.67 when viewed after derivatization (area 29.31%).

Fig 5. HPTLC finger print profile of *Cipadessa baccifera* (Alcohol Extract) 10 µl -Scanning for 254 nm



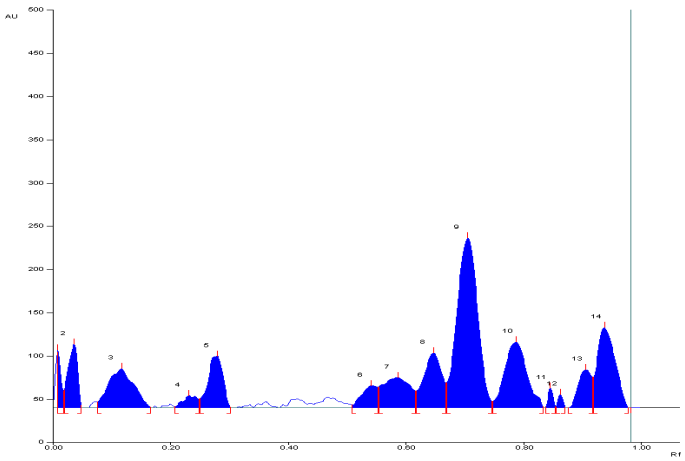
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	150.4 AU	0.01 Rf	170.1 AU	31.69 %	0.02 Rf	8.3 AU	1150.7 AU	9.73 %
2	0.03 Rf	15.3 AU	0.04 Rf	49.8 AU	9.29 %	0.06 Rf	0.0 AU	511.0 AU	4.32 %
3	0.06 Rf	0.1 AU	0.08 Rf	21.1 AU	3.92 %	0.13 Rf	8.3 AU	620.0 AU	5.24 %
4	0.27 Rf	0.2 AU	0.30 Rf	11.4 AU	2.12 %	0.32 Rf	0.3 AU	239.2 AU	2.02 %
5	0.33 Rf	0.5 AU	0.36 Rf	27.2 AU	5.07 %	0.39 Rf	9.2 AU	689.3 AU	5.83 %
6	0.42 Rf	14.4 AU	0.45 Rf	40.0 AU	7.45 %	0.49 Rf	0.1 AU	1103.4 AU	9.33 %
7	0.50 Rf	0.2 AU	0.53 Rf	10.5 AU	1.95 %	0.57 Rf	4.2 AU	314.9 AU	2.66 %
8	0.57 Rf	4.2 AU	0.60 Rf	55.6 AU	10.36 %	0.63 Rf	19.1 AU	1380.7 AU	11.68 %
9	0.63 Rf	19.2 AU	0.65 Rf	32.0 AU	5.96 %	0.68 Rf	0.3 AU	768.6 AU	6.50 %
10	0.68 Rf	0.0 AU	0.71 Rf	28.7 AU	5.34 %	0.77 Rf	0.1 AU	847.3 AU	7.17 %
11	0.83 Rf	0.1 AU	0.94 Rf	90.4 AU	16.84 %	0.98 Rf	5.5 AU	4196.8 AU	35.50 %

Fig 6. HPTLC finger print profile of *Cipadessa baccifera* (Alcohol Extract) 10 µl- Scanning for 366 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	155.8 AU	0.01 Rf	159.7 AU	17.29 %	0.02 Rf	1.8 AU	993.5 AU	5.63 %
2	0.02 Rf	5.6 AU	0.04 Rf	134.5 AU	14.57 %	0.05 Rf	3.7 AU	1750.2 AU	9.91 %
3	0.07 Rf	0.8 AU	0.08 Rf	12.1 AU	1.31 %	0.10 Rf	0.0 AU	153.8 AU	0.87 %
4	0.10 Rf	0.1 AU	0.12 Rf	11.4 AU	1.23 %	0.17 Rf	0.3 AU	382.7 AU	2.17 %
5	0.32 Rf	3.3 AU	0.36 Rf	107.1 AU	11.59 %	0.41 Rf	10.5 AU	2410.0 AU	13.65 %
6	0.42 Rf	11.9 AU	0.45 Rf	64.9 AU	7.03 %	0.49 Rf	0.3 AU	1506.7 AU	8.53 %
7	0.51 Rf	0.2 AU	0.55 Rf	16.7 AU	1.81 %	0.56 Rf	12.1 AU	374.3 AU	2.12 %
8	0.56 Rf	12.3 AU	0.60 Rf	252.2 AU	27.30 %	0.64 Rf	27.7 AU	6537.0 AU	37.02 %
9	0.64 Rf	27.7 AU	0.66 Rf	56.3 AU	6.10 %	0.69 Rf	0.1 AU	1189.0 AU	6.73 %
10	0.85 Rf	1.8 AU	0.88 Rf	18.3 AU	1.98 %	0.91 Rf	0.1 AU	500.2 AU	2.83 %
11	0.92 Rf	0.9 AU	0.94 Rf	90.4 AU	9.79 %	0.97 Rf	0.2 AU	1859.8 AU	10.53 %

Fig 7. HPTLC finger print profile of *Cipadessa baccifera* (Alcohol Extract) 10 µl- Scanning for 520 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	64.4 AU	0.01 Rf	66.8 AU	8.06 %	0.02 Rf	19.4 AU	391.1 AU	1.93 %
2	0.02 Rf	21.0 AU	0.04 Rf	73.4 AU	8.84 %	0.05 Rf	2.6 AU	1008.8 AU	4.99 %
3	0.07 Rf	6.4 AU	0.12 Rf	45.2 AU	5.44 %	0.17 Rf	0.6 AU	1679.4 AU	8.31 %
4	0.21 Rf	1.1 AU	0.23 Rf	14.2 AU	1.72 %	0.25 Rf	10.1 AU	291.4 AU	1.44 %
5	0.25 Rf	10.6 AU	0.28 Rf	59.5 AU	7.17 %	0.30 Rf	0.5 AU	1306.2 AU	6.46 %
6	0.51 Rf	3.3 AU	0.54 Rf	25.6 AU	3.08 %	0.55 Rf	23.7 AU	580.7 AU	2.87 %
7	0.55 Rf	23.9 AU	0.59 Rf	34.9 AU	4.21 %	0.62 Rf	19.5 AU	1396.5 AU	6.91 %
8	0.62 Rf	19.5 AU	0.65 Rf	63.3 AU	7.63 %	0.67 Rf	28.7 AU	1659.5 AU	8.21 %
9	0.67 Rf	29.0 AU	0.71 Rf	196.2 AU	23.65 %	0.75 Rf	7.2 AU	5924.3 AU	29.31 %
10	0.75 Rf	7.2 AU	0.79 Rf	76.2 AU	9.18 %	0.83 Rf	1.1 AU	2463.8 AU	12.19 %
11	0.84 Rf	0.4 AU	0.85 Rf	22.5 AU	2.71 %	0.85 Rf	1.4 AU	151.7 AU	0.75 %
12	0.86 Rf	0.9 AU	0.86 Rf	15.4 AU	1.86 %	0.87 Rf	0.5 AU	95.9 AU	0.47 %
13	0.88 Rf	0.0 AU	0.91 Rf	43.8 AU	5.28 %	0.92 Rf	35.2 AU	849.4 AU	4.20 %
14	0.92 Rf	35.3 AU	0.94 Rf	92.7 AU	11.18 %	0.98 Rf	0.3 AU	2416.9 AU	11.96 %

4. DISCUSSION:

Physicochemical analysis of the leaves showed moisture content (9.39%); ash content (4.58%) indicating presence of inorganic content, acid insoluble ash (0.61%) indicating presence of silicates, water soluble extractive value (18.63 %) and alcohol soluble extractive value (13.56%) indicating polar constituents. The qualitative analysis of alcohol extract showed positive test for triterpenoid, flavonoid, alkaloid, coumarin, saponin, tannin and phenol. Flavonoid are polyphenolic compounds, these are extremely common and wide spread in the plant kingdom as their glycosides. The flavonoids and phenol have been reported to possess antioxidant properties [15]. Total flavonol is determined by colorimetric method using aluminium chloride. TLC and HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. It provides chromatographic finger print of phytochemical which is suitable for confirming the identity and purity of medicinal plant as raw materials.

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CONFLICT OF INTEREST:

We declare that we have no conflict of interest.

REFERENCES:

1. Rojatkar SR, Nagasampagi BA. Diterpenes from *Cipadessa fruticosa.* Phytochemistry 1994; 37: 505 –07.
2. Rojatkar SR, Chiplunkar YG, Nagasampagi BA. A diterpene from *Cipadessa fruticosa* and *Grangea maderaspatana*. Phytochemistry 1994; 37:1213 –14.
3. Luo XD, Wu SH, Ma YB, Wu DG. Components of *Cipadessa baccifera.* Phytochemistry 2000; 55: 867 –72.
4. Luo XD, Wu SH, Ma Y B, Wu DG. 2001. Studies on chemical constituents of *Cipadessa baccifera.* Zhongcaoyao 2001; 32: 778 –80.
5. Anonymous. The Wealth of India (Raw Materials) Publication and Information Directorate, CSIR, New Delhi, 262.
6. Lin LG, Tang CP, Ke CQ, Zhang Y, Yang Y. Terpenoids from the stems of *Cipadessa baccifera.* J Nat Prod 2008; 71: 628–32.
7. Anonymous. Quality Control Methods for Medicinal Plant Materials. World Health Organisation, Geneva, 1998.
8. Harborne JB. Phytochemical Methods. 2nd ed. London: Chapman & Hall; 1973.
9. Chang CC, yang MH, Wen HM, Chern JC. Estimation of Total Flavonoid Content in propolis by two complementary colorimetric methods. Journal of food and drug analysis 2002; 10 (3): 178-82.
10. The Ayurvedic pharmacopoeia of India, 1st ed, part –I, Government of India ministry of health & family welfare department of Ayush, New Delhi, 2008, 336.
11. Anonymous. Official Methods of Analysis of AOAC International, Horwitz W, Latimer GW, editor. 18th ed, AOAC International; Maryland, Chapter 10, 2005,17-23.
12. Stahl E. Thin layer Chromatography 2nd ed. New york: 1969, 30-160.
13. Wagner H, Baldt S, Zgainski EM. Plant Drug Analysis. Berlin: Springer; 1996.
14. Chatwal GR, Anand SK. Instrumental methods of chemical analysis. Himalaya publishing house, Mumbai, 2007.
15. Rajanandh MG, Kavitha J. Quantitative estimation of β-sitosterol, total phenolic and flavonoid compounds in the leaves of *Moringa oleifera*. International Journal of Pharm tech research 2010; 2 (2): 1409- 14.

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