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Chromatographic fingerprint analysis of Burmese grape (*Baccaurea ramiflora* Lour.) by HPTLC technique

Antara Choudhury, Nikunjana A Patel and Nagaraj Gowda

Abstract
The objective of the present study is to investigate the phytochemistry along with TLC and establish the High Performance Thin Layer Chromatography (HPTLC) fingerprint profile for the first time of the three different extracts viz. acetone, methanol and aqueous extracts of the bark of *Baccaurea ramiflora*, the wild edible plant used by the tribal people of Meghalaya, India. Camag HPTLC system equipped with TLC Linomat V applicator, Camag TLC scanner III and win CATS software were used. Acetone, Methanol and Aqueous extracts of the bark of *Baccaurea ramiflora* were developed in suitable mobile system using standard procedures and scanned under UV at 254 nm and 366 nm. Phytochemical screening showed the presence of phytosterols, gums and mucilage. The HPTLC fingerprint scanned at 254 nm revealed 11 peaks with R_f value in the range of 0.07 to 0.92 for acetone extract, 7 peaks with R_f value 0.08 to 0.77 for methanol extract and 6 peaks with R_f value 0.07 to 0.62 for aqueous extract with mobile system I, similarly 11 peaks with R_f value in the range of 0.07 to 0.87 for acetone extract, 10 peaks with R_f value 0.09 to 0.88 for methanol extract and 8 peaks with R_f value 0.07 to 0.58 for aqueous extract with mobile system II. Phytochemical screening, TLC and HPTLC analysis of *Baccaurea ramiflora* can provide standard reference for the proper identification/authentication and quality control of the drug and will be helpful in differentiating the species.

Keywords: *Baccaurea ramiflora*, Bark, HPTLC fingerprinting, Standardization.

1. Introduction
North Eastern states of India are one of the richest repositories of medicinal and aromatic plants in the World due to its diverse culture and home of large number of tribal people [1]. Tribal knowledge on wild edible plants of Meghalaya, India brought to light a number of wild plant species used as edibles either in raw or in cooked forms [2]. This knowledge is passed on from generation to generation which is based on their needs, instinct, observation, trial and error and long experience [1]. Modern medicine has evolved from traditional system only after thorough chemical and pharmaceutical screening of plants. The WHO has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards [3]. Standardization of plant materials is the need of the day. Several pharmacopoeias containing monographs of the medicinal plant materials describe only the physicochemical parameters, hence the modern methods of identification and quantification of active constituents in the plant material may be useful for proper standardization of medicinal plants and its formulations [4, 5]. HPTLC fingerprinting has found to be an effective tool for quantification of active ingredients, authentication and quality control of medicinal plants. It is a modern, rapid, accurate and simple tool for detecting the marker compounds in the plant sample [6]. *Baccaurea ramiflora* Lour. belongs to the family Euphorbiaceae, an evergreen dioecious tree, 12-15 m in height and 0.6-1.5 m in girth, found wild in sub-Himalayan tract in Eastern India from Bihar to Arunachal Pradesh and in lower hills and valleys of Assam, Meghalaya, Nagaland, Manipur, Mizoram and Tripura at an altitude of 900 m, chiefly in moist tropical forests[7]. The juice of the bark is used in constipation [8]. Leaves elliptical, lanceolate or obovate and flowers are unisexual. Leaves and flowers are reported to be eaten. Leaves and barks yields green dye and are used in dying [7]. Literature survey of the selected plant contains friedelin and epi-friedelanol, β-sitosterol, methyl-betulinate and an unknown ester ($C_{31}H_{48}O_2$). The literature survey revealed that *Baccaurea ramiflora* bark has no scientific claims for HPTLC fingerprint profile.

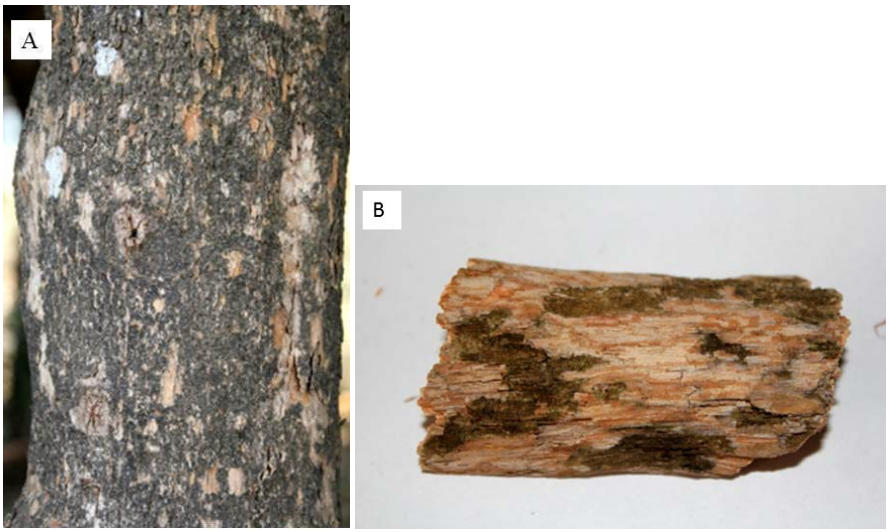


Fig 1: (A) *Baccaurea ramiflora* – Stem, (B) *Baccaurea ramiflora* – Bark

2. Materials and methods

2.1 Plant Materials

The fresh barks of *Baccaurea ramiflora* Lour (Euphorbiaceae) were collected in bulk from the Experimental Botanic Garden, Barapani under Botanical Survey of India, Shillong in the month of June-July 2014, and was authenticated by the Botanist Dr. A. A. Mao, Scientist-E, Eastern Regional Centre, Shillong, India and the herbarium was deposited in Shree S.K Patel College of Pharmaceutical Education and Research, Department of Pharmacognosy, Ganpat University, India.

2.2 Preliminary phytochemical screening

The powdered drug was subjected to systematic phytochemical screening by successively extracting them in different solvents and testing for the presence of various chemical constituents such as phytosterols, gums and mucilage in different extracts by using standard procedures [9, 10].

2.3 Thin Layer Chromatography (TLC)

Thin layer chromatography studies of the different extracts were carried out in various solvents using Precoated silica gel 60 F_{254} as an adsorbent which were procured from (E. Merck Ltd, Germany) [11, 12]. The plates were developed and observed under UV at both 254nm and 366nm and showed prominent band separation with chloroform: toluene: methanol (4:4:2) and later sprayed with 5% sulphuric acid. The R_f values were calculated for different bands.

2.4 Preparation of sample

The barks were shade dried at room temperature for 10 days and coarsely powdered. The powdered crude drug was macerated with acetone, methanol and water respectively. The extracts were filtered, and the filtrate were evaporated and dried under reduced pressure to yield the acetone, methanol and aqueous extracts. All the extracts were dissolved in respective solvents and these were centrifuged at 3000 rpm for 5 minutes and then filtered. The filtrate was used as the sample solution. The samples were inoculated on the precoated silica gel 60 F_{254} aluminum sheets.

2.5 Chromatographic condition

Chromatogram was developed on 10 x 20cm aluminum Thin Layer Chromatography (TLC) plate precoated with 0.2mm layer of silica gel 60 F_{254} (E. Merck Ltd, Germany) stored in a desiccator. The application was done by Camag Linomat syringe (100µL sec^{-1}), mounted on a Linomat V applicator. Application of bands of each extract with different concentration were carried out at a distance of 8mm with the help of Linomat V applicators attached to Camag HPTLC system, which was programmed through winCATS software (Version 1.3.0) at λ_{max} 254 and 366nm using Deuterium light source, the slit dimensions were 8 x 0.4mm and at λ_{max} 410nm using Tungsten light source. The chromatograms were recorded [11-13].

2.6 Developing solvent system

The spotting was done on the TLC plate, ascending development of the plate, migration distance 8mm (distance to the lower edge was 10mm) was performed with dichloromethane: methanol: ammonia (90:9:1) as mobile system I and chloroform: toluene: methanol (4:4:2) as mobile system II in a Camag chamber previously saturated with solvent vapor for 20 minutes. The concentration of the sample 8, 16, 24, 36µl for acetone and methanol and 5, 10, 15, 20µl for aqueous extract were applied on the track at a distance of 8 mm. After development, the plate was dried at $60^{O}C$ in an oven for 5 minutes. Densitometric scanning was then performed with a Camag TLC Scanner III equipped with the win CATS Software.

2.7 Detection of spots

The chromatograms were scanned by the densitometer at 254 and 366nm. The R_f values and fingerprint data were recorded and the plate was kept in photo documentation chamber and captured the images.

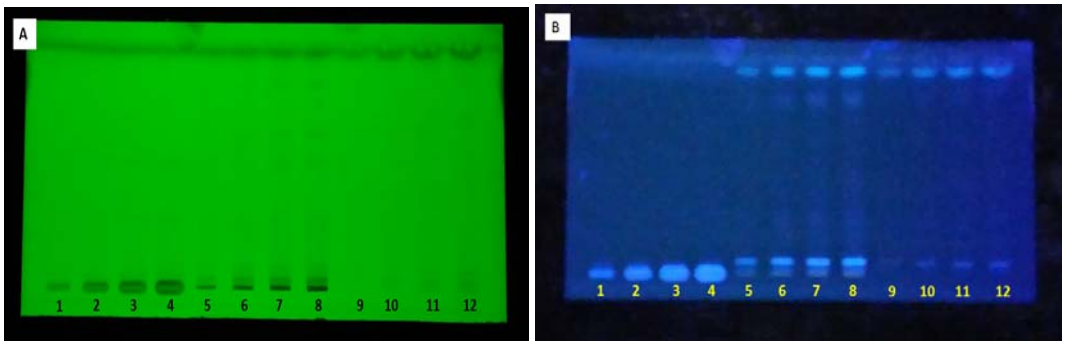


Fig 2: (A) TLC profile at 254 nm, (B) TLC profile at 366 nm with mobile system I - Dichloromethane: methanol: ammonia (90:9:1).
Track 1, 2, 3, 4 - 5, 10, 15, 20µl respectively (Aqueous extract)
Track 5, 6, 7, 8 – 8, 16, 24, 32µl respectively (Methanol extract)
Track 9, 10, 11, 12 – 8, 16, 24, 32µl respectively (Acetone extract)

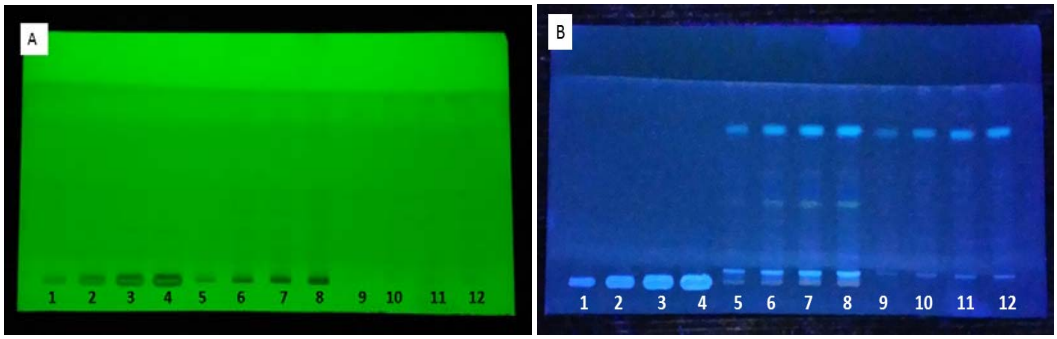


Fig 3: (A) TLC profile at 254 nm, (B) TLC profile at 366 nm with mobile system II - Chloroform: Toluene: Methanol (4:4:2).
Track 1, 2, 3, 4 - 5, 10, 15, 20µl respectively (Aqueous extract)
Track 5, 6, 7, 8 – 8, 16, 24, 32µl respectively (Methanol extract)
Track 9, 10, 11, 12 – 8, 16, 24, 32µl respectively (Acetone extract)

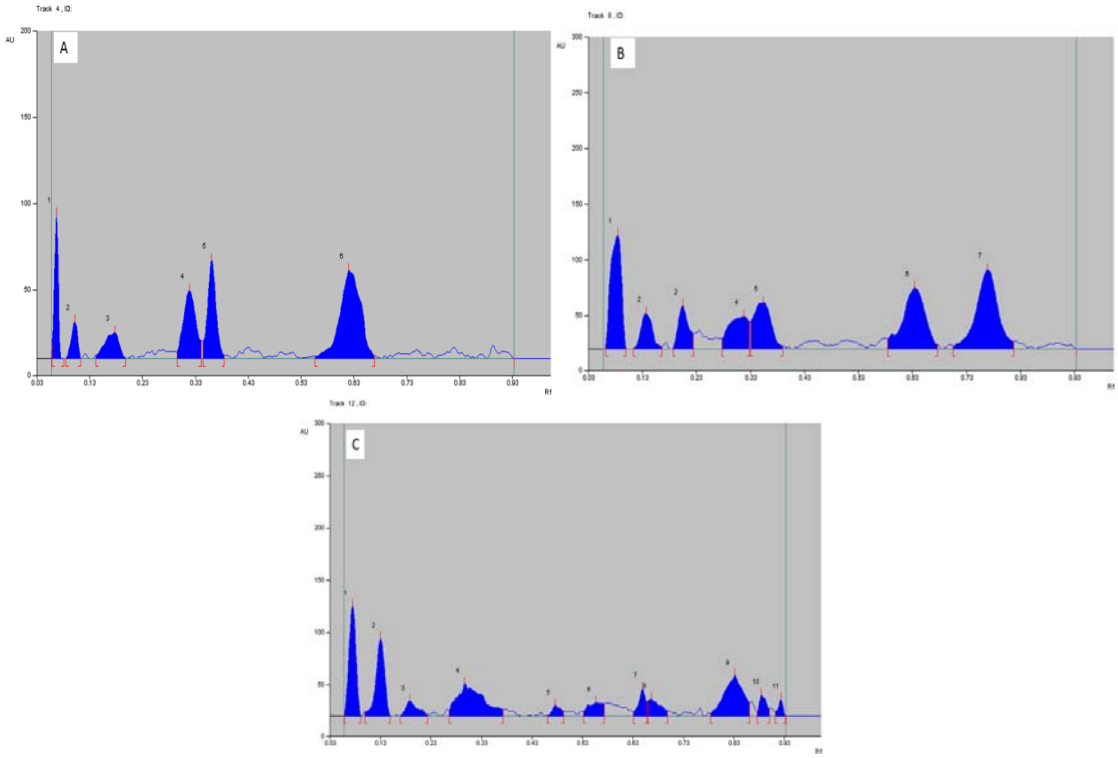


Fig 4: A- HPTLC Chromatogram of track 4(Aqueous extract) at 254nm, B- HPTLC Chromatogram of track 8(Methanol extract) at 254nm, C- HPTLC Chromatogram of track 12(Acetone extract) at 254nm with mobile system Dichloromethane: methanol: ammonia (90:9:1).

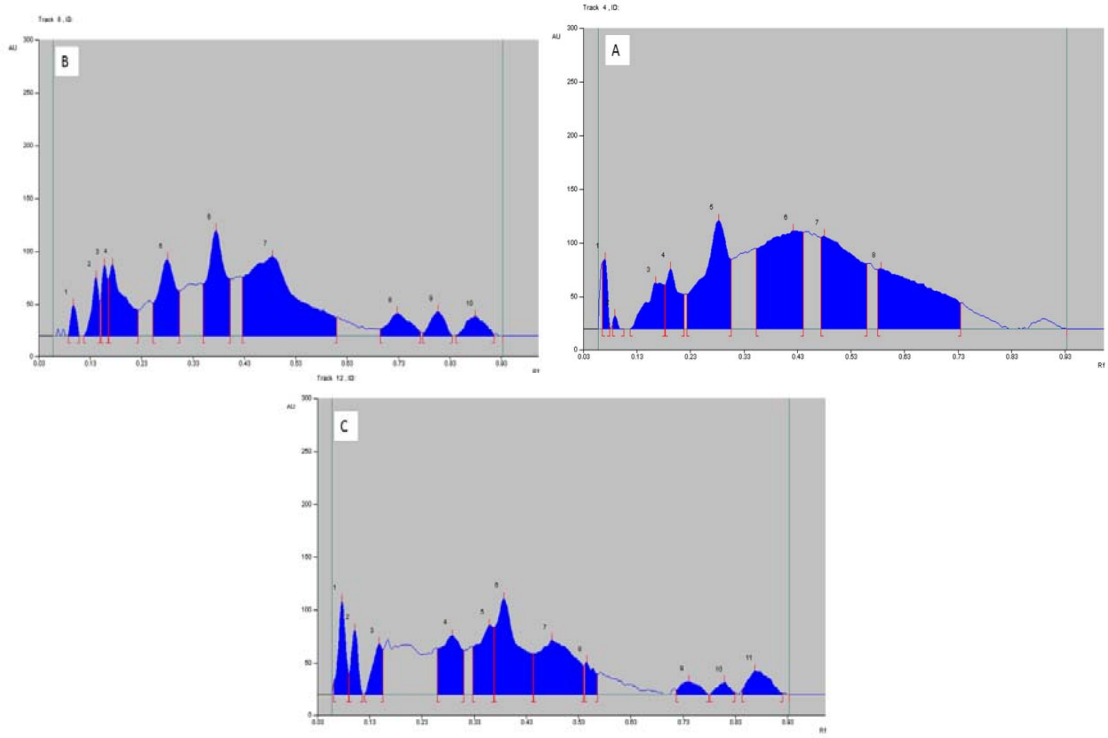


Fig 5: A- HPTLC Chromatogram of track 4(Aqueous extract) at 254 nm, B- HPTLC Chromatogram of track 8(Methanol extract) at 254 nm, C- HPTLC Chromatogram of track 12(Acetone extract) at 254 nm with mobile system Chloroform: Toluene: Methanol (4:4:2).

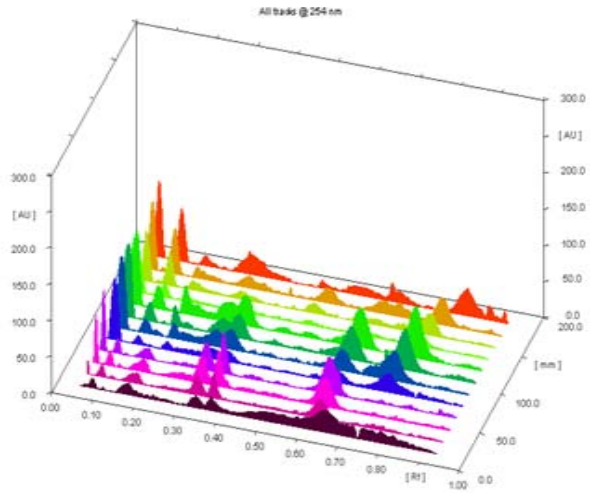


Fig 6: HPTLC fingerprint profile of all the tracks at 254nm of the bark of *Baccaurea ramiflora* with mobile system Dichloromethane: methanol: ammonia (90:9:1).

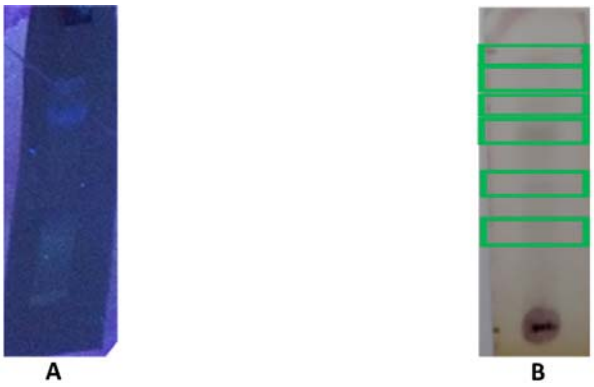
3. Results

The preliminary phytochemical screening of petroleum ether, chloroform, methanol, acetone and aqueous extracts of the bark of *Baccaurea ramiflora* showed the presence of phytosterols, carbohydrate, gums and mucilage (Table 1).

Table 1: Phytochemical constituents of the bark of *Baccaurea ramiflora*.

Phytochemical constituents	Bark extract
Alkaloids	-
Glycosides	-
Saponins	-
Phytosterols	+
Phenolics and tannins	-
Carbohydrates	+
Gums and mucilage	+

The different solvent systems with different polarities were prepared for developing the TLC system for identification of constituents in aqueous extracts and the one showing better resolution was selected as the solvent system for the study. The plates were developed and observed under UV at both 254 nm and 366nm (Figure 7). The R_f values were calculated for different bands (Table 2).



(A)-Aqueous extract at 366nm; (B)-Aqueous extract with 5% sulphuric acid.

Fig 7: (A, B) TLC Photograph: Solvent system - Chloroform: Toluene: Methanol (4:4:2) - 6 bands.

Table 2: Qualitative chemical examination of the aqueous extract of bark.

BANDS	R_f VALUES
1	0.25
2	0.44
3	0.57
4	0.69
5	0.85
6	1.02

The HPTLC fingerprint scanned at 254nm revealed 6 peaks with R_f value 0.07, 0.10, 0.17, 0.32, 0.36 and 0.62 for aqueous extract (Table 3), 7 peaks with R_f value 0.08, 0.13, 0.20, 0.32, 0.35, 0.63 and 0.77 for methanol extract (Table 4) and 11 peaks with R_f value 0.07, 0.13, 0.19, 0.29, 0.47, 0.55, 0.65, 0.66, 0.83, 0.88 and 0.92 for acetone extract (Table 5) when developed in mobile system dichloromethane: methanol: ammonia (90:9:1).

Similarly, HPTLC fingerprint when developed in chloroform: toluene: methanol (4:4:2), when scanned at 254nm revealed 8 peaks with R_f value 0.07, 0.09, 0.16, 0.19, 0.28, 0.42, 0.48 and 0.58 for aqueous extract (Table 6), 10 peaks with R_f value 0.09, 0.14, 0.16, 0.17, 0.28, 0.37, 0.48, 0.72, 0.80 and 0.88 for methanol extract (Table 7) and 11 peaks with R_f value 0.07, 0.10, 0.15, 0.29, 0.36, 0.38, 0.48, 0.54, 0.74, 0.81 and 0.87 for acetone extract (Table 8).

The photo documentation of aqueous, methanol and acetone extracts observed at 254 and 366nm is given (Figure 2A, B) when developed in the mobile system I and (Figure 3A, B) when developed in the mobile system II.

Photo documentation of HPTLC Chromatogram of track 4, 8 and 12 are given (Figure 4A, B, C) with mobile system I and similarly (Figure 5A, B, C) with mobile system II. Photo documentation of HPTLC fingerprint profile of all the tracks at 254nm are also given (Figure 6).

These separated spots had different R_f values and the percentage areas of these are given in the Table 3, 4, 5 for solvent system I and Table 6, 7, 8 for solvent system II respectively. The HPTLC images shown in Figure 2A, B and Figure 3A, B indicated that all sample constituents were clearly separated without any tailing and diffuseness. The difference in number of peaks and R_f values evidences qualitative variations of the components in the extracts. The appearance of the peaks, R_f values and their areas provide corresponding fingerprint profiles for the bark of *Baccaurea ramiflora*. The chromatographic fingerprints obtained can be stored as an electronic image without any errors and change for further investigation.

Table 3: R_f value of the chromatogram of track 4 Aqueous extract of the bark of *Baccaurea ramiflora* when developed in the solvent system Dichloromethane: methanol: ammonia (90:9:1).

Track	Peak	Max R_f	Area %
4	1	0.07	11.95
4	2	0.10	4.93
4	3	0.17	8.02
4	4	0.32	18.05
4	5	0.36	17.82
4	6	0.62	39.23

Table 4: R_f value of the chromatogram of track 8 Methanol extract of the bark of *Baccaurea ramiflora* when developed in the solvent system Dichloromethane: methanol: ammonia (90:9:1).

Track	Peak	Max R_f	Area %
8	1	0.08	18.62
8	2	0.13	6.06
8	3	0.20	6.68
8	4	0.32	9.79
8	5	0.35	12.38
8	6	0.63	20.57
8	7	0.77	25.91

Table 5: R_f value of the chromatogram of track 12 Acetone extract of the bark of *Baccaurea ramiflora* when developed in the solvent system Dichloromethane: methanol: ammonia (90:9:1).

Track	Peak	Max R_f	Area %
12	1	0.07	18.50
12	2	0.13	16.27
12	3	0.19	4.29
12	4	0.29	20.10
12	5	0.47	2.18
12	6	0.55	4.97
12	7	0.65	4.61
12	8	0.66	5.03
12	9	0.83	18.45
12	10	0.88	3.65
12	11	0.92	1.96

Table 6: R_f value of the chromatogram of track 4 Aqueous extract of the bark of *Baccaurea ramiflora* when developed in the solvent system Chloroform: Toluene: Methanol (4:4:2).

Track	Peak	Max R_f	Area %
4	1	0.07	2.42
4	2	0.09	0.37
4	3	0.16	5.56
4	4	0.19	5.00
4	5	0.28	17.89
4	6	0.42	25.58
4	7	0.48	21.63
4	8	0.58	21.55

Table 7: R_f value of the chromatogram of track 8 Methanol extract of the bark of *Baccaurea ramiflora* when developed in the solvent system Chloroform: Toluene: Methanol (4:4:2).

Track	Peak	Max R_f	Area %
8	1	0.09	1.65
8	2	0.14	4.01
8	3	0.16	3.88
8	4	0.17	10.74
8	5	0.28	12.52
8	6	0.37	17.31
8	7	0.48	38.26
8	8	0.72	4.74
8	9	0.80	3.33
8	10	0.88	3.56

Table 8: R_f value of the chromatogram of track 12 Acetone extract of the bark of *Baccaurea ramiflora* when developed in the solvent system Chloroform: Toluene: Methanol (4:4:2).

Track	Peak	Max R_f	Area %
12	1	0.07	7.23
12	2	0.10	4.69
12	3	0.15	5.37
12	4	0.29	13.34
12	5	0.36	11.95
12	6	0.38	22.99
12	7	0.48	21.11
12	8	0.54	3.43
12	9	0.74	2.65
12	10	0.81	1.63
12	11	0.87	5.60

4. Discussion

Phytochemical screening of the bark showed the presence of phytosterols, gums and mucilage. An important observation from phytochemistry point is the absence of alkaloids. The TLC profile of aqueous extract indicated presence of six compounds with the solvent system chloroform: toluene: methanol (4:4:2).

HPTLC fingerprinting has found to be an effective tool for quantification of active ingredients, authentication and quality control of medicinal plants. It is a modern, rapid, accurate and simple tool for detecting the marker compounds in the plant sample. The separation and resolution are much better, and the results are much more reliable and reproducible as compared to the TLC technique. Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment of the traditional system of medicine throughout the world [6]. HPTLC was performed in various solvents, dichloromethane: methanol: ammonia (90:9:1) and chloroform: toluene: methanol (4:4:2) were found to be the most suitable solvents for proper elution of compounds with good separations.

The present study gives information regarding various phytoconstituents present in acetone, methanol and aqueous extracts when scanned at 254 and 366 nm. The separated spots had different R_f values and the percentage areas. The HPTLC images indicate that all sample constituents were clearly separated without any tailing and diffuseness. The difference in number of peaks and R_f values indicates qualitative variations of the components in the extracts. The appearance of the peaks, R_f values and their areas provide corresponding fingerprint profiles for the bark of *Baccaurea ramiflora*. The chromatographic fingerprints obtained can be stored as an electronic image without any errors and change for further investigation.

5. Conclusion

Phytochemical screening of the bark showed the presence of phytosterols, gums and mucilage. The TLC profile of aqueous extract indicated presence of six compounds. Thus the results obtained from qualitative evaluation of HPTLC fingerprint images will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy. HPTLC analysis of *Baccaurea ramiflora* can provide standard fingerprint with selected solvent system and can be used as a reference for the proper identification/authentication and quality control of the drug and will be helpful in differentiating the species. Further, separation and characterization of the bioactive compounds from the plant is to be evaluated and reported in near future.

6. Acknowledgement

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