Section: Research Paper

Qualitative Analysis of Phytoconstituents of *Buchanania lanzan* Leaf Extracts

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Abstract Tropical dry deciduous forests are famous for their many uses. They are rich storehouses of medicinal plants used as raw material in industry or manufacture of drugs. Medicinal plants are valuable sources of new drugs globally. Medicinal plants are an important part of nature's resources, and they are now accepted worldwide. *Buchanania lanzan* plant, apart from its natural therapeutic value against various diseases, also provides high quality food for livelihood. It is a wild tropical deciduous forest plant that has been used as food around the world. The aim of the present work was to analyze the constituents of various phytochemicals on ethyl acetate, dichloromethane and methanol extracts of *B. lanzan* leaf. The leaves of this plant were collected from Ratapani forest area of Sehore district (Madhya Pradesh), India. Qualitative phytochemical analysis by TLC and HPTLC was attempted in this study. The present study suggested that TLC studies constituted different colored phytochemicals with various Rf values and the leaf contains classes of compounds tested namely alkaloids, flavonoids, phenols and tannins. And by the HPTLC analysis ethyl acetate extract of leaf shows the diversity of phenolic group at the 254 nm and 366 nm wavelengths using Toluene: ethyl acetate (7:3 v/v) mobile phase. These findings suggested that selected extracts of *B. lanzan* leaves could be a potential source of drugs that could be important for the production of synthetically improved therapeutic agents in the future. Medicinal plants can be a substantial source of revenue for rural people in developing nations, mainly through selling wild-harvested material.

Keywords *Buchanania lanzan*, leaf extracts, phytochemical analysis, TLC, HPTLC.

1. Introduction

In developing countries, people are heavily dependent on plants for their livelihood, food, fitness and profits. Tribal's and villagers in India depend on local medicinal plants to cure their ailments. India has the richest ethno-botanical traditions in the world, through the presence of 15,000-20,000 medicinal plant species. Many medicinal plant species are being broadly used to care for acute and chronic diseases all over the world and are regarded as "Chemical Goldmines" due to the presence of natural chemicals [1]. *Buchanania lanzan* Spreng. (Achar Chironji) is known as a forest plant of the Anacardiaceae family and has its origins in the Indian subcontinent [2]. It is found as a wild tree in the tropical deciduous region of northern, western and central India. It is an evergreen moderate-sized deciduous tree [3]. Chironji oil is used as a substitute for almond oil to enhance the flavor of sweets, confectionery and betel nut powder. The mesocarp of this fruit is edible and excellent quality fruit juice can be prepared from ripe pulp of the fruits [4]. The seed oil of this plant is non-repellent and non-toxic and it is good for human use [5]. The kernel is very nutritious and rich in protein and yields sweet oil. The kernels are used as a brain tonic by the tribes of central India, especially in Gujarat. Ointment made from the kernel is used to reduce itching of the skin and blemishes on face and is also used in diarrhea and intercostals pain. Cell mediated immunity (CMI) and humoral immunity is significantly stimulated by kernel. Bark produces a varnish and is used as a tanning agent in Kerala, India [6]. Plant also posses important chemicals like linoleic, myristic, olenie, palmistic, stearic acids, triterpenoids, gallotanins, saponins and vitamins. Chironji plant, similar to various other forest plants, is a storehouse of significant unknown plant drugs. Sporadic reports have been published so far which suggest that especially the leaf, bark, stem and seed are major sources of diverse vital metabolites of enormous medicinal importance. Usually, leaves have been used for the control of wounds as well as a digestive, expectorant and laxative. Chironji leaves are essential for their tonic and cardiotonic properties, and their powder is a general remedy for wounds. Methanolic extract of leaves have antidiabetic, antihyperlipidemic and antioxidant activity. The leaves contain triterpenoid, tannins, saponins, flavonoids, kaempferol-7 o'glucosides, quercetin-3-rahmnoglucoside, gallic acid, quercetin, kaemferol, and reducing sugars, together with a new glycoside, and myricetin-3-rhmnoside 3-galactoside [7,8].

2. Material and Methods

2.1. Plant Identification

For this purpose mature plant was selected. Plant

identification and certification was done from Vedanta Testing and Research Laboratory, Bhopal India and Minor Forest Produce Processing and Research Centre, Bhopal India.

2.2. Sample Collection

Mature leaves of *B. lanzan* were collected from Ratapani forest region, Sehore (MP) India. The different considerations occupied in the accurate collection of the leaf for investigation of novel constituents, the leaf was selected on the basis of its excellent activity by traditional medicine for treatment of diseases.

2.3. Preparation of Leaf Material

Collected leaves were washed in the laboratory with detergent and distilled water and the leaves were left to dry. After each leaf was dried it was finely grind in a mixture machine. The powder was kept in a bottle for future analysis.

2.4. Successive Extraction

Extraction was carried out via the soxhlet extraction method using various solvents. Dried leaf powder 23 gm was suspended in 220 ml of Ethyl acetate, Dichloromethane and methanol solvents, run sequentially for 4-5 cycles at a specific temperature for solvent but not exceeding the boiling point. Then, the crude materials were evaporated at 80^{o}C on a water bath for 4-5 days to obtain the extracts. After evaporation of organic solvents all extracts were stored at 10°C until analysis [9].

2.5. Preparation of Extract

0.5 gm of each extract sample was prepared in 2 ml NaOH (1 M) and 2 ml distilled water.

2.6. Thin Layer Chromatography (TLC) of Leaf Extract

Silica plates were used for the initial detection and estimation of four major phyto chemicals from crude methanolic extracts of the leaf. The silica gel G slurry was spread on a glass plate to make a silica gel plate. Glass plates were activated for 30 min in an oven at 60 °C. Plate was marked with a pencil on both the bottom and top side. Capillaries tubes were used to spot the sample on the TLC plate on the pencil mark bottom line. After that, about 20 ml of different solvents were taken into the chamber (Table 1). After run, the plates were dried to room temperature and then spots were detected. All plates were dried and spots were detected through the help of UV light at 254 nm. After this, the number of spot was noted and Rf values were calculated using the following formula [10, 11].

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

2.7. Column Chromatography

The ethyl acetate sample or extract of the leaves were selected on the basis of good Rf value for the phenolic compound and also its colour spots and number of spots of TLC and separated from the crude extract by column chromatography. The column was placed in an upright position in the stand, and cotton was pushed into the bottom of the column. After this, powder of silica gel G (60-120 mesh) was added to the column and cotton was applied upper side of the column. Once the column was prepared, mobile solvent was allowed to run down the entire column and 1 ml of sample was loaded inside the top of the column. Chloroform: ethyl acetate: formic acid (12.5: 10: 1) mobile phase was used and the column was run with this solvent system. Fraction was collected in vials and continuously numbered for further analysis on HPTLC [12].

Table 1. Mobile phases of the leaf extracts of *B. lanzan* plant

Test for	Extracts	Mobile Phase
Alkaloids	Ethyl acetate	Chloroform: Methanol:
	Methanol	Glacial acetic acid (4.75: 4.75:
		0.5)
Flavonoids	Ethyl acetate	Ethyl acetate: Formic acid:
	Dichloromethane	Glacial acetic acid: Water (10:
	Methanol	1.1: 1.1: 2.6)
Phenols	Ethyl acetate	Chloroform: Ethyl acetate:
	Dichloromethane	Formic acid (12.5: 10: 1)
	Methanol	
Tannins	Ethyl acetate	Water: Methanol: Chloroform
	Dichloromethane	(2.5: 8.9: 16.3)
	Methanol	

2.8. High Performance Thin Layer Chromatography (HPTLC)

The column purified fractions were assayed on HPTLC to detect the active compounds present in them. Toluene: ethyl acetate (7:3 v/v) was used as the mobile phase. 4.0 µl (track 1) and 6.0 µl (track 2) samples were applied in the form of bands on HPTLC plate silica gel 60 F 254 (10.0 x 10.0 cm) which was programmed by winCATS software version 1.4.6. After development, plate was dried at 60 °C through a plate drier for 5 min, after which the developed plate was visualized under the wavelengths of 254 nm and 366 nm. Photographs were taken by placing the plates in a photo documentation chamber and Rf values were recorded by WinCATS software.

3. Results

3.1. Extract Yield (%)

The yield of leaves extracts of various solvents was

calculated using following formula: percentage or extraction yield (%) = W1/W2×100; Where W1 is the mass of crude extract and W2 is the mass of the sample [13]. The amounts of ethyl acetate, dichloromethane and methanol extracts obtained were 4.00%, 1.56% and 18.51%, respectively. The highest mass (18.51%) was obtained from the methanolic extract.

3.2. Thin Layer Chromatography

Conducted thin layer chromatography (TLC) was used to determine the components of secondary metabolites of plant extracts. A wide range of solvent systems were tried for good resolution. Rf values obtained from TLC analysis are listed in the Table 2. All spots were colored under UV light (Figure 1). There were 3 spots for phenols in ethyl acetate and dichloromethane extract. While in methanolic extracts had 2 spots for flavonoids and phenols. Dichloromethane extract had the highest Rf value (0.71) for flavonoids.

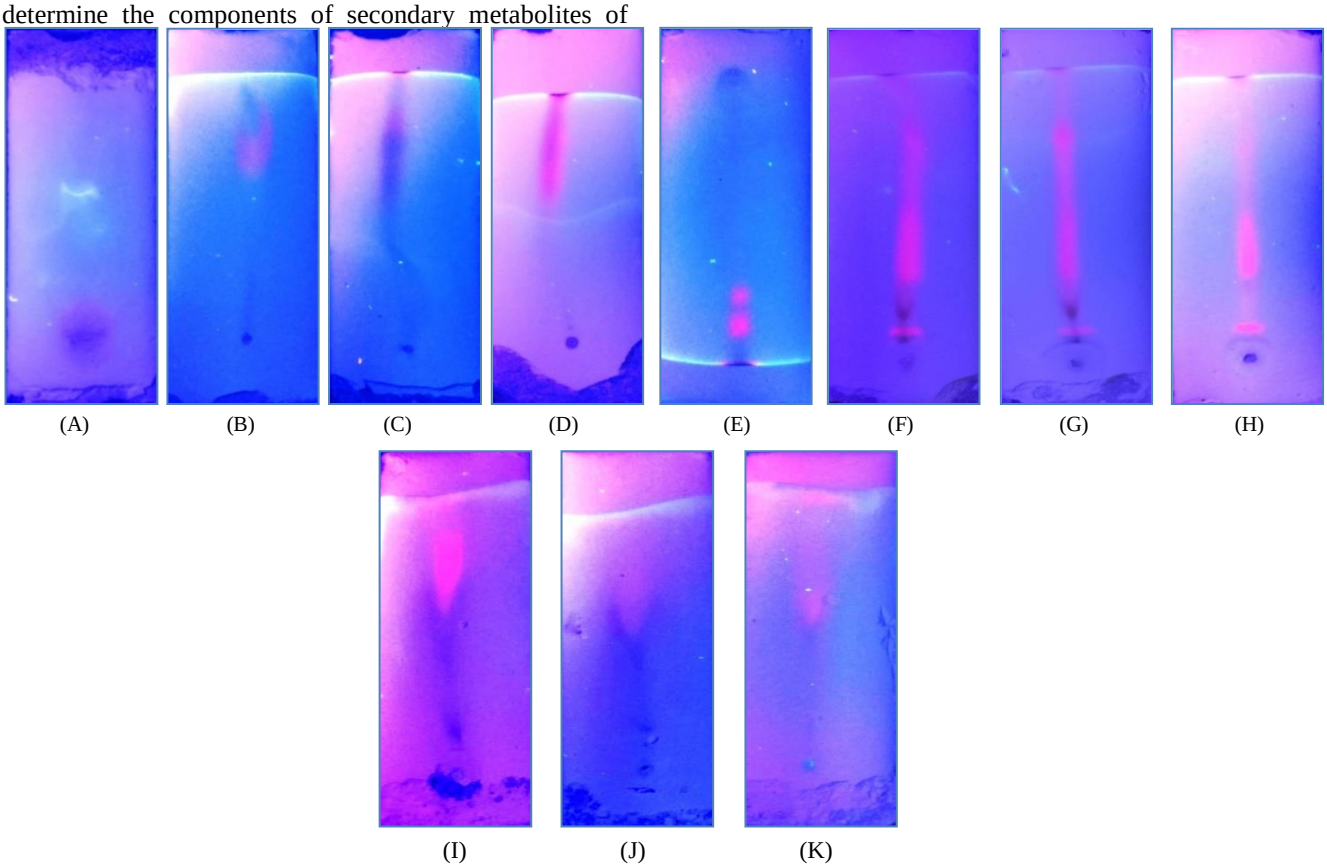


Figure 1. Chromatogram of leaf extract (A) Ethyl acetate (B) Methanol (C) Ethyl Acetate, (D) Dichloromethane (E) Methanol (F) Ethyl acetate (G) Dichloromethane (H) Methanol (I) Ethyl acetate (J) Dichloromethane (K) Methanol.

Table 2. Rf value of leaf extracts by TLC

S. No.	Extracts	Phytochemicals	No. of spots	Rf Value
A	Ethyl acetate	Alkaloids	1	0.46
B	Methanol		1	0.65
C	Ethyl acetate	Flavonoids	1	0.63
D	Dichloromethane		1	0.71
E	Methanol		2	0.10, 0.17
F	Ethyl acetate	Phenols	3	0.09, 0.16, 0.70
G	Dichloromethane		3	0.10, 0.15, 0.42
H	Methanol		2	0.09, 0.36
I	Ethyl acetate	Tannins	1	0.64
J	Dichloromethane		2	0.15, 0.56
K	Methanol		1	0.61

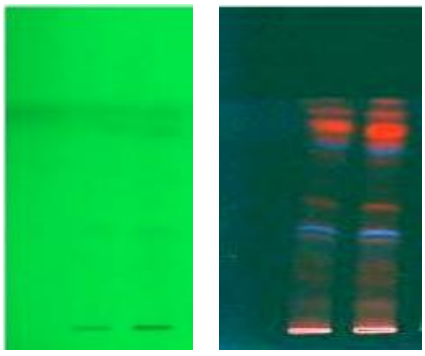
3.3. Column Chromatography

Selected extract was separated by column chromatography using Chloroform: Ethyl acetate: Formic acid (12.5: 10: 1) solvent system. Fraction was obtained from plant extract and collected in separate vials (Figure 2).

Figure 2. Fraction of leaf extract obtained by column chromatography

3.4. Qualitative Analysis by HPTLC:

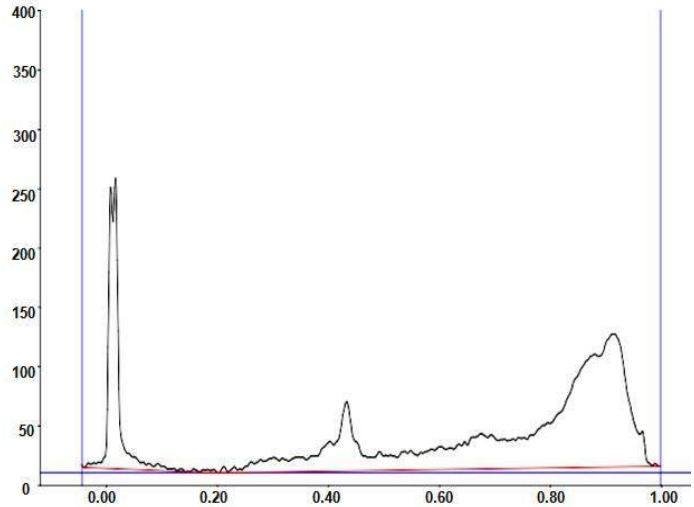
HPTLC analysis of the fractions revealed several bands, indicating the presence of diverse groups of phytochemicals. Overall maximum Rf (0.97) was recorded in track 2, ID: S1 at 254 nm, start height was 4.2 to 20.7 and end height was 12.3 to 2.0 where 446.6 was the maximum height. A total of seven peaks were seen in this track. In track 1, ID: S1 at 366 nm presence of confirmed values was maximum Rf 0.02 to 0.95, start height was 0.2 to 30.2 and End height 30.5 to 0.1 where 617.9 was Maximum height. Total of 11 peaks were observed in this track, whose area ranges from 8581.2 to 693.2 and the area of 6 peaks is 730.2 (2.86%). The Rf value range of 0.54 confirmed the presence of a phenolic group at peak number six [14]. HPTLC fingerprint results scanned at different wavelengths for phenolic group of selected extracts of *B. lanzan* leaf confirmed the presence of this phytochemicals. Different range band Rf values were obtained for hot ethyl acetate extracts at different wavelengths as shown in Table 3 (A, B, C, D) and Figure 3 and Graph 1 (A, B, C, D).



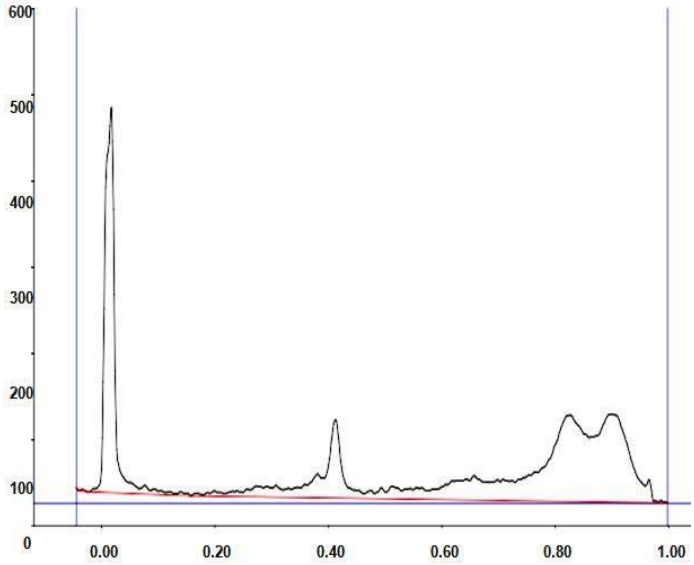
(A) (B)

Figure 3. HPTLC plates, visualized under: (A) UV 254 nm (B)

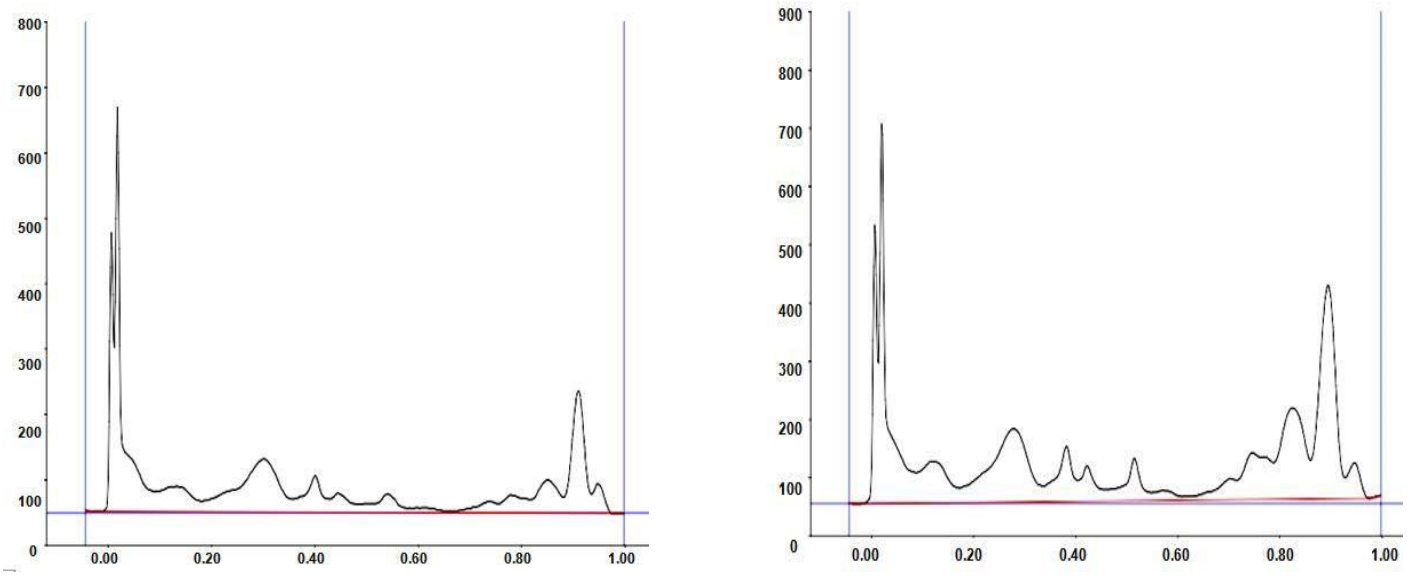
UV 366 nm



(A)



(B)



(C) (D)

Graph 1. HPTLC densitogram of leaf fractions (ethyl acetate extract) scanned at 254 nm and 366 nm (A. B. C. D)

Table 3. Rf value of fractions (ethyl acetate extract) by HPTLC at 254 and 366 nm (A. B. C. D)

A. Track 1, ID: S1 at 254 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	3.9	0.02	245.0	43.35	0.06	5.8	3196.2	23.15
2	0.38	13.0	0.40	25.1	4.44	0.41	22.0	428.5	3.10
3	0.41	24.6	0.43	58.1	10.27	0.46	12.2	1129.7	8.18
4	0.64	19.7	0.68	29.5	5.22	0.69	26.3	840.2	6.09
5	0.80	37.6	0.88	95.3	16.86	0.89	93.7	4138.7	29.98
6	0.89	93.7	0.91	112.2	19.85	0.96	27.2	4071.2	29.49

B. Track 2, ID: S1 at 254 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.01	4.2	0.02	446.6	54.18	0.05	12.3	5578.3	31.04
2	0.35	11.2	0.38	27.7	3.36	0.39	22.6	541.2	3.01
3	0.39	22.8	0.41	91.2	11.06	0.44	9.8	1451.9	8.08
4	0.61	17.7	0.66	28.8	3.49	0.67	21.6	973.9	5.42
5	0.77	33.3	0.83	100.6	12.21	0.86	74.8	4498.5	25.03
6	0.86	74.9	0.90	102.3	12.42	0.96	19.9	4755.7	26.47
7	0.96	20.7	0.97	27.0	3.27	0.98	2.0	169.7	0.94

C. Track 1, ID: S1 at 366 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.01	0.2	0.02	617.9	52.38	0.09	30.5	8581.2	33.57
2	0.10	30.8	0.13	38.8	3.29	0.18	16.1	1783.6	6.98
3	0.18	16.3	0.30	81.1	6.87	0.36	19.6	4982.3	19.49
4	0.36	19.8	0.40	55.7	4.72	0.42	20.8	1414.2	5.53
5	0.43	20.0	0.44	29.2	2.47	0.48	12.3	773.7	3.03

6	0.52	13.4	0.54	28.5	2.42	0.58	7.0	730.2	2.86
7	0.70	5.9	0.74	17.6	1.49	0.76	13.9	479.7	1.88
8	0.76	14.0	0.78	27.6	2.34	0.82	20.4	932.6	3.65
9	0.82	20.7	0.85	50.8	4.30	0.88	24.1	1583.5	6.20
10	0.88	24.3	0.91	187.4	15.89	0.94	29.6	3604.6	14.10
11	0.94	30.2	0.95	45.2	3.83	0.98	0.1	693.2	2.71

D. Track 2, ID: S1 at 366 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.01	1.2	0.02	652.1	36.34	0.09	52.5	10500.2	22.38
2	0.09	52.8	0.12	71.4	3.98	0.17	25.4	3009.4	6.42
3	0.17	25.4	0.28	126.3	7.04	0.34	26.6	7415.5	15.81
4	0.34	26.9	0.38	94.7	5.28	0.41	36.3	2346.0	5.00
5	0.41	36.8	0.42	61.4	3.42	0.46	19.6	1301.8	2.77
6	0.46	20.1	0.52	72.7	4.05	0.55	13.8	1803.7	3.84
7	0.56	14.1	0.57	17.0	0.95	0.61	6.1	485.0	1.03
8	0.63	6.1	0.70	36.0	2.01	0.72	33.4	1188.8	2.53
9	0.72	33.5	0.75	80.0	4.46	0.79	63.0	3182.2	6.78
10	0.79	63.2	0.83	156.2	8.70	0.86	66.4	5652.8	12.05
11	0.86	68.0	0.90	366.0	20.40	0.93	35.2	8932.2	19.04
12	0.93	35.6	0.95	60.6	3.37	0.98	0.1	1094.3	2.33

4. Discussion

B. lanzan plant like many other forest plants is a storehouse of important unknown phyto-medicines. Phytochemicals have the potential to act as a source of useful drugs and cure many infections as a result of the presence of various bioactive compounds that clearly show immense activity against diseases [15]. Three major chemical constituents were isolated from the methanolic extract of leaves such as epinitol, vomicine, and celidoniol, these constituents were characterized based on chemical tests and spectral analysis such as infrared, H nuclear magnetic resonance, mass spectroscopy [16]. Preliminary phytochemical based analysis of leaf samples of *B. lanzan* revealed the presence of glycosides, flavonoids and phenolic compounds in various leaves extracts [8]. TLC based analysis of various leaf extracts for the detection of mobile phases is relatively new and HPTLC based analysis of ethyl acetate extracts as well, since the leaf of this plant has been reported to be therapeutically potent [17]. The leaf extract was reported to contain several Medicinal properties by many researchers. The present study provides a qualitative analysis of phytochemicals based on TLC and HPTLC analysis comparing different solvents and mobile phases. The TLC Rf values showed significant diversity of the compounds determined from the different extracts. The chromatograms have validated the presence of a variety of alkaloids, flavonoids, phenols and tannins in the leaves extracts. HPTLC is an advanced type of planar chromatography which has been extensively used in recent years for fingerprinting of medicinal plants, products and screening of plant substances [18]. The chromatogram scanned at 254 nm (Table. 4 A, B) represents 06 and 07 peaks for track 1 and track 2, respectively, whereas the chromatogram scanned at 366 nm (Table. 4 C, D) indicates 11 and 12 peaks for track 1 and track 2, respectively. The number of peaks indicates the presence of different phytoconstituents present in the sample. And HPTLC analysis confirmed the presence of phenolic group at 254 nm and 366 nm wavelengths in the leaf ethyl acetate extract. These Rf values obtained from the active phytoconstituents provide the important knowledge about their polarity and important clues for the separation of these phytochemical in the separation process. Bands of the separated compounds can be observed on HPTLC plates under UV of wavelength 254 nm and 366 nm (Figure 3). Diverse Rf values of the sample also reflect an idea regarding their polarity by the use of the various solvent systems for TLC and HPTLC studies, this could be important for the selection of the appropriate mobile phase and also useful for drug development for different pathogenic diseases from extracts of these plants.

5. Conclusion

In conclusion, the study of the selected plant leaf contains many useful phytochemical compounds with important properties. The presence and absence of compounds in plants depend on the solvent medium used for extraction and the physical property of the individual taxa. Since methanol is polar among the solvents used, they contain a higher yield of phytoconstituents than other solvents. Results of this research provide some important factors that are responsible for the qualitative evaluation of *B. lanzan* leaf. Toluene: ethyl acetate (7:3 v/v) is the best mobile phase for HPTLC analysis. The present study proved that the leaf extracts contains alkaloids, flavonoids, phenols and tannins. The leaves of these plant studied here could be used as a potential source of useful medicines.

6. Abbreviations

TLC: Thin layer chromatography; HPTLC: High Performance Thin Layer Chromatography; Rf: Retention factor; Max: Maximum; nm: Nanometer.

REFERENCES

[1] Sharma RS., Ramakrishnan RS., Sharma S., Kumar A., Singh M., Dwivedi N., Singh SV., Purohit A, "Present status and future outlook for conservation of chironji (*Buchanania lanzan* Spreng.)," Octa Journal of Biosciences, 9, 1, pp. 12-20, 2021.

[2] Zeven AC., de Wet JM, "Dictionary of cultivated plants and their regions of diversity: Excluding most ornamentals, forest trees and lower plants," Pudoc Wageningen, 2. pp. 227, 1982.

[3] Rai PK., Sharma DR., Sharma A, "*Buchanania lanzan* is a pharmacognostic miracle herb," Research Journal of Pharmacognosy and Phytochemistry, 7, 3, pp. 182-188, 2015.

[4] Munde., VM., Shinde GS., Sajindranath AK., Prabu T., Machewad PM, "Correlation and path analysis studies in charoli (*Buchanania lanzan* Spreng)," South Indian Horticulture, 50, pp. 517-521, 2003.

[5] Banerjee A., Jain M, "Investigations on *Buchanania lanzan* Spreng seed oil," Fitoterapia, 59, 05, pp. 406. 1988.

[6] Kumar J., Vengaiah PC., Srivastav PP., Bhwmick PK, "Chironji nut (*Buchanania lanzan*) processing, present practices and scope," Indian Journal of Traditional Knowledge, 11,1, pp. 202-204, 2012.

[7] Nasim KT., Arya R., Babu V., Ilyas M, "Myricetin 3-rhamnoside-3-galactoside from *Buchanania lanzan* (Anacardiaceae). Phytochemistry," 31, 7, pp. 2569-2570. 1992.

[8] Mehta SK., Mukherjee S., Jaiprakash B, "Preliminary phytochemical investigation on leaves of *Buchanania lanzan*," International Journal of Pharmaceutical Sciences Review and Research, 3, 2, pp. 55-59. 2010.

[9] Handa SS., Khanuja SPS., Longo G., Rakesh DD, "Extraction technologies for medicinal and aromatic plants," United Nations Industrial Development Organization and the International Centre for Science and High Technology, Italy, 2008.

[10] Harborne JB, "Phytochemical methods: A guide to modern techniques of plant analysis," 3rd ed, Hall Chapman and Co, 1998, New York.

[11] Wagner H, Bladt S, "Plant drug analysis: A thin layer chromatography atlas," 2nd ed, Springer, 2001, New York.

[12] Coskun O, "Separation techniques: chromatography," North Clinics of Istanbul, 3, 2, pp. 156-160, 2016.

[13] Ngamkhae N., Monthakantirat O., Chulikhit Y., Boonyarat C., Maneenet J., Khamphukdee C., Kwankhao P., Pitiporn S., Daodee S, "Optimization of extraction method for Kleeb Bua Daeng formula and comparison between ultrasound-assisted and microwave-assisted extraction," Journal of Applied Research on Medicinal and Aromatic Plants, 28, pp. 1-11, 2022.

[14] Dinakaran SK., Sujiya B., Avasarala H, "Profiling and determination of phenolic compounds in Indian marketed hepatoprotective polyherbal formulations and their comparative evaluation," Journal of Ayurveda and Integrative Medicine, 9, pp. 3-12, 2018.

[15] Mahalle D., Gupta A, "Comparative qualitative phytochemical analysis of the different parts of *Tinospora crispsa:* A contribution to sustainable use of the plant species," Journal of Drug Delivery and Therapeutics, 9, 4, pp. 795-798, 2019.

[16] Mehta SK., Jaiprakash B., Nayeem N, "Isolation and phytochemical investigation on leaves of *Buchanania lanzan* (Chironji)," Annals of Biological Research, 02, 03, pp. 469-73, 2011.

[17] Banerjee S., Bandyopadhyaym A, "*Buchanania lanzan* Spreng: A veritable storehouse of phytomedicines," Asian Journal of Pharmaceutical and Clinical Research, 8, 5, pp. 18-22, 2015.

[18] Ravisankar P., Lokapavani C., Devadasu C., Rao GD, "HPTLC: A versatile method for rapid analysis of pharmaceutical formulations and comparison with other chromatographic techniques and its applications," Indian Journal of Research in Pharmacy and Biotechnology, 2, 3, pp. 1209-1214, 2014.