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Preliminary phytochemical screening and HPTLC finger printing profile of leaf extract of *Lippia alba* (Mill.) N.E. ex Britton and P.Wilson

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ABSTRACT

*T*o establish the preliminary phytochemical screening and fingerprint profile of leaf extract of *Lippia alba*(Mill.) N.E. ex Britton and P.Wilson using thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) technique. different Rf value of various phytochemicals provide valuable clue regarding their polarity and selection of solvents for separation of phytochemicals. The study will help in future for identifying this plant for further research work.

INTRODUCTION

Awareness of the herbal plant's chemical constituents is helpful in the discovery of effective therapeutic agents. Extraction and characterization of several active phytocompounds from the medicinal plants is the foundation for the formation of some high activity profile drugs[1]. High Performance Thin Layer Chromatography (HPTLC) finger print analysis developed into an effective and powerful tool for linking the chemical constituents profile of the plants with botanical identify. *Lippia alba* (Mill.) N.E. Br. ex Britton & P. Wilson (Verbenaceae) is an aromatic plant widely used all over South and Central America for different purposes [2].

Therefore, the main aim of the present investigation was to study the preliminary phytochemical screening along with high performance thin layer chromatographic studies on leaves of *Lippia alba* N.E. ex Britton & P. Wilson which could be of use in preparing monograph of plant.

Material and Methods

1. Collection, Identification and Preparation of plant extract

The plant specimen Lippia alba N.E. ex Britton & P. Wilson leaves were collected from Boreya Village, Kanke, Ranchi, Jharkhand and authenticated by Dr.S. Ranjan, Botanical Survey of India, Howrah and deposited at the college herbarium. The leaves were washed, shade dried and is made powder mechanically and the fine powder was used for extraction procedure.

15g of the shade dried and powdered leaves material was taken for extraction. The crude powder was subjected for extraction by Soxhlet apparatus in round bottom flask with solvent ethanol (72℃) for 24 hours. The extract were filtered over Whatman No. 1 filter paper and the filtrates were concentrated under reduced pressure to pasty mass for further studies.

2. Preliminary Phytochemical Studies

The preliminary phytochemical screening done by the following methods[3].

(i) **Tests for Alkaloids**

The 2 ml of leaves or stems extract is dissolved in dilute hydrochloric acid and filter. A pinch of picric acid added with filtrate. Presence of alkaloids was confirmed by the yellow colored precipitate.

(ii) **Test for Flavonoids**

2 ml of extract mix with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colour less on further addition of dilute acid, indicate the presence of flavonoids.

(iii) **Test for Steroids**

2 ml of extract dissolved in 2 ml. of chloroform. On adding 2-3 drops of conc. Sulphuric acid. Allow the solution to stand. Formation of brown ring indicated the presence of steroids.

(iv) **Test for Tannins**

2 ml of plant extract taken in the test tube.

Add 3-4 drops of 0.1% ferric chloride. Presence of brownish green or a blue black colouration indicated the presence of tannins.

(v) **Test for Saponins**

2 ml of extract was diluted with distilled water to 5 ml and shaken in a graduated test tube for 15 minutes. Layers of foam indicated the presence of saponins.

(vi) **Test for Amino Acid**

2 ml of plant extract taken in the test tube. Add Ninhydin reagent to the test solution and boiled for few minutes. Development of blue colour indicated the presence of amino acid.

(vii) **Test for Reducing Sugars**

2 ml of extract is dissolved in dilute hydrochloric acid and filter. Filtrate was treated with Benedict's reagent & boil for 5 minutes. Orange red precipitate was obtained shows the presence of reducing sugar.

(viii) **Test for Phenols**

Adding 2 ml of leaves or stems extract with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicted the presence of phenols.

3. Thin Layer Chromatography

Thin Layer Chromatography (TLC) studies were carried out following the methodology[4]

Silica gel plate having 5 x 15 cm was taken and it was marked with the help of pencil. The distance between the spots was 1.5 cm while the distance from the spot of the margin of plant was 1.0 cm. These plates were allowed to dry for 15 minutes. The spotted plates were put in a chromatographic solvent chamber which contain solvent system Hexane: Ethyl acetate (8:2where they placed in such a way that the loaded spot should not touch the solvent. The chamber was covered with proper lid and then it allowed to stand for sufficient time to reach the solvent phase at maximum out and dried at room temperature.

The term retention factor (Rf is commonly used to describe the chromatographic behaviour of sample solute. Rf value was calculated for well using the following formula.

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

4. High Performance Thin Layer Chromatography (HPTLC)

(i) Chromatographic conditions

Chromatogram was performed on 10 × 10 cm aluminum packed TLC plate coated with a 0.2 mm layer of silica gel 60F254 (E. Merck Ltd, Darmstadt, Germany) stored in a desiccator, application was done by Hamilton micro syringe (Switzerland), mounted on a Linomat V applicator. Application of bands of each extract was carried out using spray technique. Sample were applied in duplicate on precoated silica gel 60F254 aluminum sheets (5 × 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software (Version 1.3.0) at λmax = 254 nm and 366 nm using Deuterium light source, the slit dimensions were 6.00 × 0.45 mm and at λmax = 620 nm using Tungsten light source. The chromatograms were recorded.

(ii) Developing Solvent System

Spotting was done on the TLC plate, ascending development of the plate, migration distance 80 mm (distance to the lower edge was 10 mm) was performed at 20ºC solvent system as a mobile phase in a CAMAG chamber previously saturated for 30 mins. The concentration of the sample 5 µl, 10 µl and 15 µl were applied in the track as 8 mm bands at a spraying rate of 15s/L and Rf value were calculated. After development the plate was dried at 60ºC in an oven for 5 mins. Densitometric scanning was then performed with a Camag TLC Scanner 3 equipped with the win CATS Software[5]

(iii) Development of Chromatogram

After the application of the sample, the chromatogram was developed in Twin through glass chamber 10 × 10 cm saturated with different solvent system for 15 mins.

(iv) Detection of Spots

The air-dried plates were viewed in ultraviolet radiation to mid-day light. The chromatograms were scanned at 200 nm to 440 nm staining with Anisaldehyde-sulphuric acid stains and Iodine vapour. The Rf values and finger print data were recorded by WIN CATS software.

Results and Discussion

Lippia alba(Mill.) *N.E.Br. ex Britton &P.Wilson* preliminary photochemical screening shows that the

plant contains Alkaloid, Flavonoid, Tannin, Saponin, Steroid, Reducing sugar, Amino acid and Phenols.
TLC studies of the ethanol extract of *Lippia alba* solvent system Hexane: Ethyl acetate (8:2) was used and 2 spots were visible and the Rf values were 0.67 and 0.81 respectively.

The occurrence of 16 different components in the ethanolic extract. With the Rf value 0.02, 0.43, 0.50, 0.67, 0.81, 0.92, 0.98, 0.02, 0.54, 0.81, 0.92, 0.97, 0.03, 0.83, 0.93 and 0.99 respectively. As the percentage area were 6.32%, 6.82%, 8.93%, 7.87%, 23.46%, 34.52%, 12.09%, 11.85%, 7.50%, 29.25%, 34.07%, 17.34%, 10.91%, 30.91%, 46.08% and 12.11%. These compounds were found to be more prominent at 230nm. The developed chromatogram will be specific with selected solvent system (Table 1).

Rf value of *Lippia alba* visualized at 260nm shows that there are 17 peaks 0.02, 0.50, 0.54, 0.67, 0.81, 0.92, 0.98, 0.02, 0.64, 0.81, 0.92, 0.97, 0.05, 0.58, 0.83, 0.93 and 0.98 Rf respectively with the percentage area showed in all peaks 8.71%, 8.22%, 2.89%, 6.24%, 23.62%, 38.25%, 12.08%, 14.66%, 7.16%, 28.40%, 34.75%, 15.03%, 12.14%, 4.17%, 28.10%, 46.02% and 9.57% (Table 2).

There are 14 spots were visualized from the developed chroma-togram of *Lippia alba* scanned at 290nm with the Rf value of 0.02, 0.50, 0.67, 0.81, 0.94, 0.98, 0.02, 0.64, 0.81, 0.56, 0.04, 0.58, 0.83 and 0.93 sequentially as the percentage area were 11.81%, 9.23%, 8.00%, 21.70%, 43.09%, 6.17%, 28.06%, 14.47%, 39.67%, 17.80%, 30.34%, 10.9%, 43.53% and 16.04% respectively (Table 3).

Rf value of *Lippia alba* visualized at 320nm shows that there are 4 peaks 0.02, 0.98, 0.03 and 0.04 Rf respectively with the percentage area 42.50%, 57.50%, 100.00% and 100.00% (Table 4).

There are 3 spots were visualized from the developed chromatogram of *Lippia alba* scanned at 350nm with the Rf value is 0.02, 0.02 and 0.04 respectively as the percentage area were 100% at 350nm (Table 5)

Rf value of *Lippia alba* visualized at 380nm shows that there are 3 peaks 0.02, 0.03 and 0.04 Rf respectively with the percentage area all peaks was 100.00% (Table6).

The occurrence of 12 different components in the ethanolic extract. With the Rf value 0.02, 0.36, 0.53, 0.68, 0.03, 0.58, 0.64, 0.81, 0.98, 0.04, 0.59 and 0.82 Rf respectively. As the percentage area were 48.81%, 14.42%, 23.13%, 14.26%, 70.32%, 7.05%, 12.33%, 9.09%, 1.20%, 71.90%, 14.91% and 13.19%. These compounds were found to be more prominent at 410nm (Table 7).

There are 12 spots were visualized from the developed chroma-togram of *Lippia alba* scanned at 440nm with the Rf value of 0.02, 0.36, 0.53, 0.68, 0.04, 0.58, 0.63, 0.81, 0.98, 0.05, 0.58 and 0.81 sequentially as the percentage area were 43.44%, 14.55%, 28.30%, 13.70%, 74.79%, 5.85%, 8.79%, 9.17%, 1.39%, 72.84%, 13.00% and 14.08% respectively (Table 8)

Conclusion

Further works have to be carried out for the characterization of other chemical constituents and the quantitative estimation with marker compounds. Even though data can be considered along with the other values for fixing standard to the plants.

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Table 1.Peak list and Rf value of chromatogram of the ethanolic leaves extract of Lippia alba at 230nm

1	1	0.01 Rf	69.5 AU	0.01 Rf	74.8 AU	28.09%	0.02 Rf	2.1 AU	519.7 AU	6.32%
1	2	0.37 Rf	15.4 AU	0.38 Rf	17.2 AU	6.45%	0.43 Rf	5.7 AU	560.8 AU	6.82%
1	3	0.44 Rf	12.3 AU	0.48 Rf	19.7 AU	7.41%	0.50 Rf	15.6 AU	734.3 AU	8.93%
1	4	0.62 Rf	11.4 AU	0.65 Rf	21.9 AU	8.22%	0.67 Rf	17.6 AU	647.2 AU	7.87%
1	5	0.72 Rf	13.8 AU	0.78 Rf	43.9 AU	16.49%	0.81 Rf	30.8 AU	1930.1 AU	23.46%
1	6	0.83 Rf	33.9 AU	0.88 Rf	47.3 AU	17.78%	0.92 Rf	36.4 AU	2839.4 AU	34.52%
1	7	0.92 Rf	36.4 AU	0.94 Rf	41.4 AU	15.56%	0.98 Rf	1.3 AU	994.5 AU	12.09%
2	1	0.01 Rf	77.2 AU	0.01 Rf	90.2 AU	38.13%	0.02 Rf	0.8 AU	763.8 AU	11.85%
2	2	0.59 Rf	7.2 AU	0.62 Rf	17.0 AU	7.19%	0.54 Rf	13.5 AU	483.7 AU	7.50%
2	3	0.72 Rf	9.1 AU	0.78 Rf	42.2 AU	17.85%	0.81 Rf	28.4 AU	1885.4 AU	29.25%
2	4	0.85 Rf	35.0 AU	0.88 Rf	44.9 AU	18.96%	0.92 Rf	36.8 AU	2196.1 AU	34.07%
2	5	0.92 Rf	36.9 AU	0.94 Rf	42.3 AU	17.87%	0.97 Rf	0.5 AU	1117.8 AU	17.34%
3	1	0.01 Rf	47.9 AU	0.01 Rf	65.9 AU	35.20%	0.03 Rf	0.0 AU	654.1 AU	10.91%
3	2	0.74 Rf	8.9 AU	0.78 Rf	41.8 AU	22.32%	0.83 Rf	26.3 AU	1853.7 AU	30.91%
3	3	0.83 Rf	26.6 AU	0.88 Rf	43.2 AU	23.4%	0.93 Rf	34.3 AU	2763.9 AU	46.08%
3	4	0.94 Rf	34.1 AU	0.95 Rf	36.4 AU	19.44%	0.99 Rf	0.6 AU	726.2 AU	12.11%

Table2.Peak list and Rf value of chromatogram of the ethanolic leaves extract of Lippiaalba at 260nm

1	1	0.01 Rf	95.1 AU	0.01 Rf	103.3 AU	35.28%	0.02 Rf	0.9 AU	695.0 AU	8.71%
1	2	0.43 Rf	0.2 AU	0.48 Rf	17.5 AU	5.98%	0.50 Rf	12.7 AU	656.2 AU	8.22%
1	3	0.50 Rf	12.7 AU	0.51Rf	14.9 AU	5.08%	0.54 Rf	1.0 AU	230.3 AU	2.89%
1	4	0.62 Rf	4.1 AU	0.65 Rf	19.4 AU	6.63%	0.67 Rf	14.4 AU	497.8 AU	6.24%
1	5	0.72 Rf	11.3 AU	0.78 Rf	44.1 AU	15.05%	0.81 Rf	29.4 AU	1885.2 AU	23.62%
1	6	0.83 Rf	33.2 AU	0.88 Rf	50.5 AU	17.25%	0.92 Rf	40.7 AU	3052.9 AU	38.25%
1	7	0.92 Rf	40.9 AU	0.94 Rf	43.1 AU	14.73%	0.98 Rf	1.0 AU	963.8 AU	12.08%
2	1	0.01 Rf	108.8 AU	0.01 Rf	125.5 AU	44.42%	0.02 Rf	1.2 AU	1015.6 AU	14.66%

2	2	0.58 Rf	3.6 AU	0.63 Rf	18.1 AU	6.42%	0.64 Rf	14.7 AU	495.9 AU	7.16%
2	3	0.72 Rf	8.2AU	0.78 Rf	45.6 AU	16.15%	0.81 Rf	29.5 AU	1966.8 AU	28.40%
2	4	0.85 Rf	36.9 AU	0.88 Rf	49.5 AU	17.51%	0.92 Rf	40.5 AU	2407.1 AU	34.75%
2	5	0.93 Rf	41.0 AU	0.94 Rf	43.8 AU	15.50%	0.97 Rf	0.7 AU	1041.1 AU	15.03%
3	1	0.01 Rf	65.4 AU	0.01 Rf	89.9 AU	37.50%	0.05 Rf	0.0 AU	854.3 AU	12.14%
3	2	0.54 Rf	3.2 AU	0.58 Rf	16.1 AU	6.71%	0.58 Rf	14.4 AU	293.6 AU	4.17%
3	3	0.74 Rf	7.3 AU	0.78 Rf	45.5 AU	18.97%	0.83 Rf	28.7 AU	1976.9 AU	28.10%
3	4	0.83 Rf	28.9 AU	0.88 Rf	48.6 AU	20.29%	0.93 Rf	37.9 AU	3237.6 AU	46.02%
3	5	0.94Rf	38.0AU	0.95 Rf	39.6 AU	16.53%	0.98 Rf	1.7 AU	673.3 AU	9.57%

Table 3.Peak list and Rf value of chromatogram of the ethanolic leaves extract of Lippia alba at 290nm

1	1	0.01 Rf	76.1 AU	0.01 Rf	83.3 AU	46.48%	0.02 Rf	1.1 AU	571.1 AU	11.81%
1	2	0.42 Rf	0.0 AU	0.48 Rf	10.9 AU	6.95%	0.50 Rf	8.8 AU	656.2 AU	9.23%
1	3	0.59 Rf	1.1 AU	0.65 Rf	12.8 AU	7.08%	0.67 Rf	10.6 AU	386.9 AU	8.00%
1	4	0.72 Rf	8.8 AU	0.78 Rf	21.8 AU	12.10%	0.81 Rf	16.2 AU	1049.9 AU	21.70%
1	5	0.81 Rf	16.2 AU	0.88 Rf	26.9 AU	14.91%	0.94 Rf	23.2 AU	2084.6 AU	43.09%
1	6	0.94 Rf	23.3 AU	0.94 Rf	24.1 AU	13.40%	0.98 Rf	0.6 AU	298.3 AU	6.17%
2	1	0.01 Rf	92.5 AU	0.01 Rf	105.1 AU	62.25%	0.02 Rf	0.0 AU	848.2 AU	28.06%
2	2	0.58 Rf	3.9 AU	0.62 Rf	14.2 AU	8.38%	0.64 Rf	12.5 AU	437.3 AU	14.47%
2	3	0.72 Rf	8.2 AU	0.78 Rf	24.9 AU	14.74%	0.81 Rf	16.7 AU	1199.3 AU	39.67%
2	4	0.93 Rf	23.5 AU	0.95 Rf	24.7 AU	14.63%	0.56 Rf	3.2 AU	538.2 AU	17.80%
3	1	0.7 Rf	56.8 AU	0.01 Rf	77.7 AU	56.97%	0.04 Rf	0.1 AU	765.7 AU	30.34%
3	2	0.53 Rf	1.6 AU	0.58 Rf	12.1 AU	8.94%	0.58 Rf	11.7 AU	254.7 AU	10.9%
3	3	0.73 Rf	6.9 AU	0.78 Rf	24.4 AU	17.88%	0.83 Rf	17.4 AU	1098.8 AU	43.53%
3	4	0.94 Rf	20.7 AU	0.95 Rf	21.9 AU	16.21%	0.93 Rf	0.9 AU	404.9 AU	16.04%

Table 4.Peak list and Rf value of chromatogram of the ethanolic leaves extract of Lippiaalba at 320nm

1	1	0.01 Rf	73.4 AU	0.01 Rf	80.4 AU	87.66%	0.02 Rf	1.0 AU	543.8 AU	42.50%
1	2	0.85 Rf	6.3 AU	0.93 Rf	11.3 AU	12.34%	0.98 Rf	0.2 AU	735.8 AU	57.50%
2	1	0.01 Rf	88.3 AU	0.01 Rf	100.0 AU	100.0%	0.03 Rf	0.2 AU	801.5 AU	100.00%
3	1	0.01 Rf	53.0 AU	0.01 Rf	71.8 AU	100.0%	0.04 Rf	0.1 AU	709.0 AU	100.00%

Table5. Peak list and Rf value of chromatogram of the ethanolic leaves extract of Lippia alba at 350nm

1	1	0.01 Rf	76.7 AU	0.01 Rf	82.7 AU	100.0%	0.02 Rf	0.5 AU	544.1 AU	100.00%
1	2	0.01 Rf	91.3 AU	0.01 Rf	102.7 AU	100.0%	0.02 Rf	1.1 AU	613.4 AU	100.00%
2	1	0.01 Rf	53.3 AU	0.01 Rf	72.3 AU	100.0%	0.04 Rf	0.1 AU	717.4 AU	100.00%

Table6Peak list and Rf Value of chromatogram of the ethanolic leaves extract of Lippia alba at 380nm

1	1	0.01 Rf	79.9 AU	0.01 Rf	83.3 AU	100.0%	0.02 Rf	0.6 AU	531.7 AU	100.00%
1	2	0.01 Rf	95.1 AU	0.01 Rf	107.1 AU	100.0%	0.03 Rf	0.0 AU	813.4 AU	100.00%
2	1	0.01 Rf	52.6 AU	0.01 Rf	71.9 AU	100.0%	0.04 Rf	0.0 AU	723.2 AU	100.00%

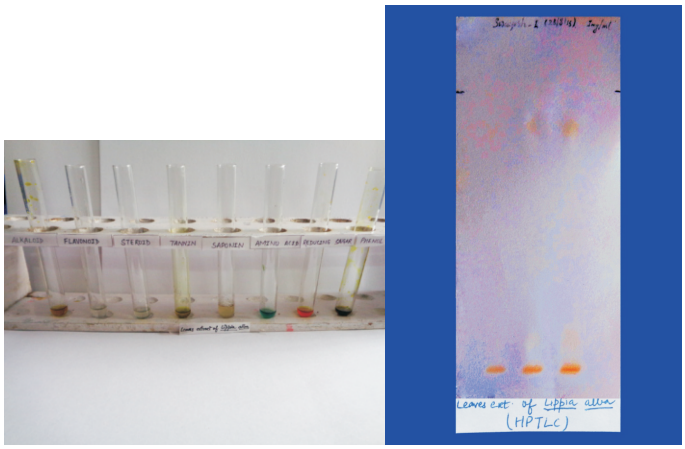
Table7.Peak list and Rf value of chromatogram of the ethanolic leaves extract of Lippia alba at 410nm

1	1	0.01 Rf	392.5 AU	0.01 Rf	407.3 AU	66.70%	0.02 Rf	6.2 AU	2631.4 AU	48.81%
1	2	0.29 Rf	4.0 AU	0.33 Rf	22.6 AU	4.82%	0.36 Rf	9.1 AU	787.7 AU	14.42%
1	3	0.43 Rf	10.9 AU	0.48 Rf	20.5 AU	4.36%	0.53 Rf	10.9 AU	1263.3 AU	23.13%
1	4	0.62 Rf	10.1 AU	0.65 Rf	19.4 AU	4.13%	0.68 Rf	15.9 AU	778.8 AU	14.26%
2	1	0.01 Rf	479.6 AU	0.01 Rf	535.1 AU	87.79%	0.03 Rf	0.9 AU	4461.8 AU	70.32%
2	2	0.51 Rf	1.8 AU	0.56 Rf	17.2 AU	2.83%	0.58 Rf	9.0 AU	447.4 AU	7.05%
2	3	0.58 Rf	9.1 AU	0.62 Rf	21.3 AU	3.49%	0.64 Rf	17.5 AU	782.6 AU	12.33%
2	4	0.76 Rf	20.2 AU	0.78 Rf	23.5 AU	3.86%	0.81 Rf	8.6 AU	576.7 AU	9.09%

2	5	0.96 Rf	4.1 AU	0.97 Rf	12.3 AU	2.02%	0.98 Rf	0.1 AU	76.3 AU	1.20%
3	1	0.01 Rf	249.4 AU	0.01 Rf	346.5 AU	89.88%	0.04 Rf	0.4 AU	3612.7 AU	71.90%
3	2	0.53 Rf	4.2 AU	0.56 Rf	21.5 AU	5.58%	0.59 Rf	19.4 AU	749.1 AU	14.91%
3	3	0.75 Rf	14.1 AU	0.77 Rf	17.5 AU	4.53%	0.82 Rf	5.2 AU	662.5 AU	13.19%

Table 8 :Peak list and Rf value of chromatogram of the ethanolic leaves extract of *Lippia alba* at 440nm

1	1	0.01 Rf	358.8 AU	0.01 Rf	371.0 AU	85.31%	0.02 Rf	5.0 AU	2340.1 AU	43.44%
1	2	0.29 Rf	4.7 AU	0.33 Rf	22.3 AU	5.12%	0.36 Rf	9.3 AU	784.0 AU	14.55%
1	3	0.43 Rf	7.3 AU	0.48 Rf	22.4 AU	5.14%	0.53 Rf	12.0 AU	1524.7 AU	28.30%
1	4	0.62 Rf	9.7 AU	0.65 Rf	19.2 AU	4.42%	0.68 Rf	16.1 AU	738.0 AU	13.70%
2	1	0.01 Rf	450.8 AU	0.01 Rf	502.4 AU	88.36%	0.04 Rf	0.9 AU	4216.6 AU	74.79%
2	2	0.53 Rf	3.8 AU	0.56 Rf	13.4 AU	2.36%	0.58 Rf	7.2 AU	329.9 AU	5.85%
2	3	0.58 Rf	7.4 AU	0.62 Rf	19.5 AU	3.42%	0.63 Rf	18.5 AU	495.6 AU	8.79%
2	4	0.76 Rf	18.4 AU	0.78 Rf	21.5 AU	3.79%	0.81 Rf	7.1 AU	517.2 AU	9.17%
2	5	0.96 Rf	4.2 AU	0.97 Rf	11.8 AU	2.07%	0.98 Rf	1.6 AU	78.4 AU	1.39%
3	1	0.01 Rf	227.7 AU	0.01 Rf	318.1 AU	89.91%	0.05 Rf	0.1 AU	3342.4 AU	72.84%
3	2	0.53 Rf	6.1 AU	0.57 Rf	21.5 AU	6.01%	0.58 Rf	20.9 AU	600.1 AU	13.00%
3	3	0.75 Rf	15.1 AU	0.778 Rf	18.2 AU	5.08%	0.81 Rf	8.0 AU	646.3 AU	14.08%



Preliminary phytochemical screeningLeaves sample loaded in Silica gel plate

Graph Rf value of chromatogram ofthe ethanolic leaves extract of *Lippiaalba*