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An Experimental Study on Preliminary Phytochemical Screening, FTIR Analysis and HPTLC Fingerprinting of Fruits and Leaves Extract of Luffa Acutangula

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ABSTRACT:

Introduction: Luffa acutangula belongs to family cucurbitaceae, called as ridge gourd, angled gourd or angled luffa with greater health benefits. The present study was aimed to characterize the phytochemical profile for various secondary metabolites using phytochemical screening, FTIR analysis and HPTLC fingerprinting of ethanolic fruits and leaves extract of Luffa acutangula.

Methods: The fresh fruits and leaves were extracted using Soxhlet apparatus, extract was further used for analysis of Phytochemical compounds by chemical reaction. Then identified by Fourier Transform Infrared Spectrophotometer (FTIR) profiling and High-Performance Thin Layer Chromatography (HPTLC) analysis.

Results: Quantitative analysis revealed that ethanolic fruit and leaves extract consists of alkaloids, glycosides, saponins, tannins, flavonoids, terpenoids and phenols. The FT-IR spectrum of ethanolic fruit and leaves extract of Luffa acutangula demonstrated the presence of C-H alkanes, O-H Hydroxyl, C=O carboxylic acid, ether and glycosidic bond. The HPTLC densitometric analysis of the ethanolic fruit and leaves extract of Luffa acutangula was carried out using CAMAG HPTLC system and chromatograms were scanned at 254 nm representing several peaks. HPTLC data of extract shows ten to fifteen different peaks confirming that bioactive compounds are present. HPTLC finger printing of ethanolic fruit extract revealed 11 peaks with Rf values in the range of 0.05 to 0.92; extract of leaves showed 14 peaks with Rf values in the range of 0.05 to 0.99.

Conclusion:

The study concluded that the fruit and leaves extract of Luffa acutangula contain a rich variety of phytochemicals. FTIR and HPTLC fingerprint analysis of extracts of Luffa acutangula can be done as a characteristic tool for the correct identification of the plant.

Keywords: Luffa acutangula, Ethanol extract, phytochemical screening, FTIR, HPTLC fingerprinting.

1. Introduction

Herbal plants have played an important role in the prevention and treatment of diseases since ancient times. The humans began using herbal plant extracts to protect himself from several diseases and to live healthy lifestyle.[1] In many developed countries, traditional herbal plants are making a comeback as alternatives to modern medicine to treat different ailments.[2] The presence of traditional herbs depends on their diversity and the knowledge related to use as herbal medicine for prevention and treatment.[8] Phytochemical investigation is the study of secondary metabolites present in plants.[4,5] Phytochemical analysis is an important technique used for the detection of various bioactive compounds in plants. Plant based natural constituents can be derived from different parts of the plant like leaves, fruits, roots, bark,

seeds and flowers.[6] Plants contain different phytoconstituents like alkaloid, flavonoid, phenol and tannins, carboxylic acids, terpenes, amino acids and inorganic acids. These phytoconstituents shows specific properties to medicinal plants.[3] Hence, the analysis of these chemical constituents would help in detection of biological properties in plants. There are a variety of techniques which can be used for the detection of phytoconstituents present in medicinal herbs. [7,9]

Spectroscopic and chromatography techniques are the most popular methods used for this purpose. The Fourier Transform Infrared Spectrophotometer (FT-IR) was the most recommended tool for the identification of chemical bonds/functional groups present in the phyto chemicals.[8] The wavelength of light absorbed was the salient feature of the chemical bond, as can be seen in the annotated spectrum. Moreover, FTIR spectroscopy is time-saving method for characterisation and identification of functional groups. On the other hand, High-Performance Thin Layer Chromatography (HPTLC) analysis facilitates enables the most complicated separation of the phyto constituents and empowers more accurate quantitative analysis.[10,11]

Luffa acutangula belongs to family cucurbitaceae, called as ridge gourd, angled gourd or angled luffa. It is one of the perennial plant used as vegetable in Asian countries. It is climbing herb grown and cultivated in northwest India, Bihar, West Bengal, Sikkim and Tamil Nadu. [3] The unripe fruit cooked as vegetable, while in its dry form it is used as a cleaning sponge. It is dark green in color and has a tapering end to it.[12] The pulp of this vegetable is white in color and consumed after peeling off the skin. The parts used from luffa are fruit, seeds, leaves, and roots, almost all parts of the plant can be used.[16],[17,44] Luffa acutangula fruits, leaves, seeds and roots have good source of phenolic acids, glycosides, saponins flavonoids, isoflavonoids, flavones etc. with greater health benefits.[18,19,30] Luffa acutangula called as powerhouse of nutrient because it contains high nutrient content.[18,26] The plant has been reported to have various medicinal properties such as treatment of jaundice, strengthening the spleen, laxative, antioxidant and antiinflammatory.[17,19,27,34]

The purpose of the present study was to investigate the various preliminary phytochemical constituents, FTIR Spectrum profile and HPTLC analysis of *Luffa acutangula* fruits and leaves.

2. Material and Methods

Collection of Plant Material

The fresh fruits and leaves of *Luffa acutangula* was collected from vendors of local market Pune, Maharashtra, India. The identification of these crude samples was authenticated by Agharkar Research Institute, Pune. All the analytical grade chemicals used were purchased from Tirummala chemicals, Pune.

Preparation of Extracts of Luffa Acutangula

Preparation of Fruit Extract

The fruits of *L. acutangula* were dried under shade and made powdered, stored in a well closed airtight container. The dried fruit powder of the plant (50gm) were packed in a Soxhlet apparatus and continuously extracted with ethanol till complete the extraction. After completion of extraction cycle the solvent was evaporated and concentrated to dry residue and stored in an air tight container free from any contamination for further evaluation. The percentage yields were calculated of the dried extracts. [20,21]

Preparation of Leaves Extract

The shade dried leaves of *Luffa actangula* were powdered properly. The dried powder of the leaves (50gm) were packed in in a Soxhlet apparatus and continuously extracted with ethanol over a thermo statistically controlled heating mantle (70-75^{0}c) till complete the extraction. After completion of extraction cycle the solvent was evaporated and concentrated to dry residue under reduced pressure at a temperature not exceeding 40°C and then given moderate heating on water bath. The dried extract was stored in an air tight container free from any contamination for further evaluation. The percentage yield was identified.[22,23]

Calculation of Percentage Yield

The percentage yield of extract was calculated by using following formula:

$$\text{Yield\%} = \frac{\text{Wt. of the dry extract}}{\text{Wt. of the dry plant}} \times 100$$

Preliminary Phytochemical Investigations

Phytochemical screening Preliminary phytochemical analysis was carried out to detect the presence of bioactive agents as per standard methods described by Khandelwal and Kokate.[4,5] The extract was screened to identify the presence or absence of various active phytochemicals like alkaloids, glycosides, flavonoids, terpenoids, phenolic compounds, carbohydrates, saponins, tannins, and protein and amino acid.[15] After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.[24,25]

Detection of Alkaloids

Mayer's test: The extracts were treated with Mayer s reagent for identification of alkaloids. Formation of a yellow cream precipitate indicates the presence of alkaloids.[28]

Wagner s test: Wagners reagent was added in the extracts. Formation of brown/reddish brown precipitate indicates the presence of alkaloids.[29]

Detection of Glycosides

Borntragers Test: About 5 ml of the extract was boiled with10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of Chloroform was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. Formation of pink colour indicates the presence of glycosides.[30]

Detection of Flavonoids

Lead acetate test: About few drops of lead acetate solution was added in the extract shows yellow color precipitate which indicates the presence of flavonoids.[32]

H_2SO_4 test: Orange colour was formed after addition of few drops of H_2SO_4 indicates the presence of flavonoids.[33]

Detection of Terpenoids

Salkowski s Test: 5 ml of the extract of the peel, flesh and seeds were mixed with 2 ml of chloroform and then added carefully the 3 ml of concentrated H2SO4 to form a layer. An appearance of reddish brown colour in the inner face indicates the presence of terpenoids.[35]

Detection of Phenols:

Ferric chloride test: Few drops of ferric chloride solution was treated with 10ml of the extracts. Formation of bluish black colour shows the presence of phenol.

Lead acetate test: lead acetate solution was reacted with 10 ml of the extracts shows yellow colour precipitate which indicates the presence of phenol. [36]
Detection of Saponins: About 0.5ml of the extracts was reacted with 5ml of distilled water. Formation of frothing (appearance of creamy of small bubbles) results the presence of saponins. [37]
Detection of Tannins: Extract was mixed with water and heated on a water bath. Filter the mixture and add ferric chloride to the filtrate. A dark green colour indicates the presence of tannins.
Detection of Carbohydrates: 0.5ml extract was dissolved in 5ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.[43]

Molisch test
The filtrate was treated with a few drops of α- napthol (20% in ethyl alcohol). Then 1 ml of concentrated H_2SO_4 was added along the sides of inclined test tube and observed for formation of violet coloured ring at the interface. [38,42]

Detection of Protein & Amino acids.
Biuret test: To 0.5 ml of extract equal volume of 40% NaoH solution and two drops of one percent copper sulphate solution was added. The appearance of violet colour indicates the presence of protein.[39,40]
Ninhydrin test: About 0.5 ml of extract was taken and two drops of freshly prepared 0.2% Ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates that the presence of proteins, peptides or amino acids.[41]

Fourier Transform Infra-red (FTIR)
About 1 mg of the dried ethnolic fruit and leaves extract was mixed with 10 mg of KBr pellet, the sample was scanned to analyse the presence of functional groups using Fourier Transform Infrared Spectrometer (Shimadzu, Japan). The dried potassium bromide was used for baseline correction. Subsequently, the spectrum of mixture of extract and potassium bromide was recorded and the peaks belonging to major functional groups were identified. The scanning range was 4000 cm-1- 400 cm-1 with a resolution of 4 cm-1. This was done to find out the presence of functional groups in the plant extract.[8]

High Performance Thin Layer Chromatography (HPTLC)
High-performance thin layer chromatography fingerprinting of ethanolic fruits and leaves extracts of Luffa acutangula:
HPTLC studies were carried out in the following steps;
• **Sample Preparation:** Take a 2mg Luffa sample in a tube add a 15ml methanol in it, and this tube is placed in a sonication for a 1hr.Then take a supernatant liquid used for a sample spotting and its TLC is checked in 2.5: 2.5 Ethyl Acetate: n hexane. [45, 50]
• **Sample Application:** 2, 4, 6,8,10 µl sample were applied on pre-coated silica gel on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.[51]
• **Development of Chromatogram:** After the application of sample, the chromatogram was developed in twin through glass chamber 10 x 10 cm saturated with Ethyl acetate: n-hexane (2.5: 2.5) for 15 minutes.

• **Detection of Spots**
The air-dried plates were observed in ultraviolet radiation to mid-day light. The chromatograms were scanned by densitometer at UV 254nm. WIN CATS software was used

for recording of the Rf values and finger print data. Rf, colour of the spots and densitometric scan were demontrated.[52,53]

3. Results and discussion

Preparation of Extract: Percentage yield of the extract in both Ethanolic fruit leaves extract of Luffa acutangula shown in Table No. 1.

Table No.1 Percentage yield of the extract:

Sr. no.	Extract	Percentage yield
1.	Ethanolic fruit extract of *Luffa acutangula*	6.8
2.	Ethanolic leaves extract of Luffa acutangula	5.1

Table no. 2 Phytochemical evaluation of ethanolic fruit and leaves extracts of Luffa acutangula:

Sr. No.	Phytochemicals	Test	Result	
			Ethanolic fruit extract	Ethanolic leaves extract
1.	Alkaloids	Mayer's test Wagners test	+ve	-ve
2.	Glycosides	Borntragers Test	+ ve	+ ve
3.	Flavonoids	Lead acetate test H_2SO_4 test	+ ve	+ve
4.	Terpenoids	Salkowski s Test	+ ve	-ve
5.	Phenols	Ferric chloride test Lead acetate test	- ve	+ve
6.	Saponins	Froth test	+ ve	+ve
7.	Tannins	Ferric chloride test	+ ve	+ve
8.	Carbohydrate	Molish test	+ ve	+ve
9.	Protein and amino acids	Biuret test, Ninhydrin test	+ ve	+ve

Positive (+), Negative (-).

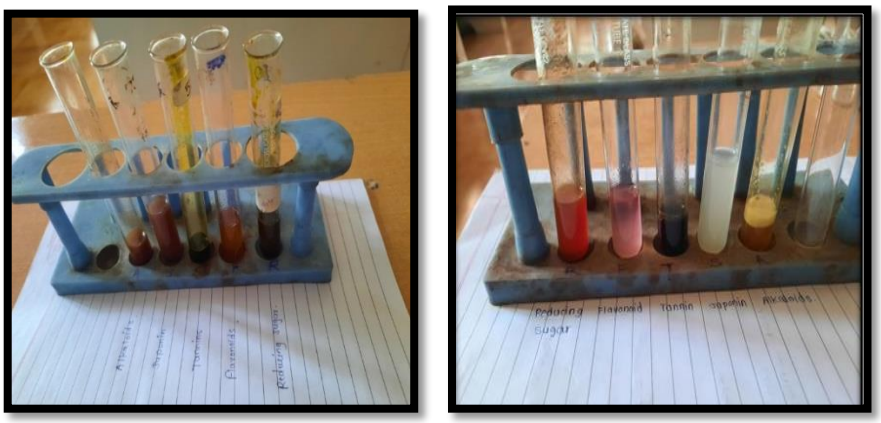


Fig. No. 1 Phytochemical Detection of ethanolic fruit and leaves extracts of *Luffa acutangula*

FT-IR Analysis

FT-IR Analysis of ethnolic fruit and leaves extract of L. acutangula

The analysis of FT-IR peak values and functional groups are represented in Table 4 and 5. The FT-IR spectrum profile is illustrated in Fig. 2 and 3. The FT-IR of ethnolic fruit extract gave a broad peak at 3269 and 2621 cm-1, which indicated the presence of O-H, C-H stretching. The peaks obtained at 1593cm-1 indicated the presence of C=O stretching. Peaks at 1028, 774 and 707cm-1, which indicated the presence of C-H bending. The FT-IR of ethnolic leaves extract gave a broad peak at 3335 and 2919 cm-1, which indicated the presence of O-H, C-H stretching. The peaks obtained at 2851cm-1 indicated the presence of C-H stretching and peak at 1704 cm-1 indicated presence C=O stretching. Peaks at 1375 and 910 cm-1, which indicated the presence of C-H bending. The FT-IR spectrum confirmed the presence of characteristic functional groups carboxylic acid, ethers, alcohols, phenols, alkanes, ketones, in ethnolic fruit and leaves extract of *L. acutangula.*

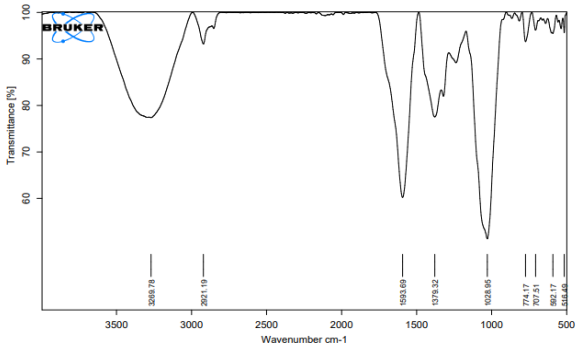


Fig.2 FT-IR spectrum of ethnolic fruit extract of L. acutangula

Table no. 3 Peak frequencies and functional groups in the FTIR spectra of fruit extract

Peak No.	Observed frequency (cm^{-1})	Functional group	Functional group name	Type of molecular vibration
1	3269	O-H	Hydroxyl	Stretching, H-bonding (alcohol and phenols)
2	2921	C-H	Alkane	Stretching
3	1593	C=O	Carboxylic acid	Stretching (Aromatic)
4	1379	C-H	Alkane	Bending
5	1028	C-O-C	Ether	Stretching
6	774	Glycosidic bond	Glycosidic bond	Bending
7	707	Di Substituted Benzene	Di Substituted Benzene	Bending

FT-IR Analysis of ethnolic leaves extract of *L. acutangula*

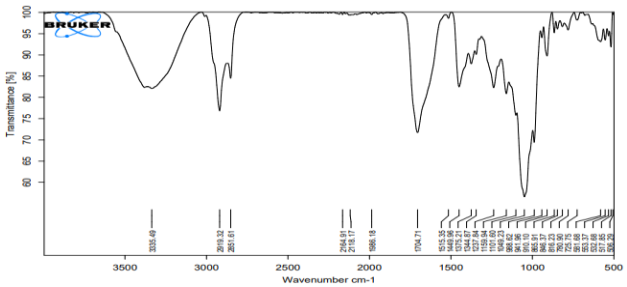


Fig.3 FT-IR spectrum of ethnolic leaves extract of L. acutangula

Table no.4 Peak frequencies and functional groups in the FTIR spectra of leaves extract

Peak No.	Observed frequency (cm^{-1})	Functional group	Functional group name	Type of molecular vibration
1	3335	O-H	Hydroxyl	Stretching, H-bonding (alcohol and phenols)
2	2919	C-H	Alkane	Stretching
3	2851	C-H	Alkane	Stretching
4	1704	C=O	Carboxylic acid	Stretching (Aromatic)
5	1375	C-H	Alkane	Bending
6	910	Substituted Benzene ring	Substituted Benzene ring	Bending

HPTLC Fingerprint Analysis

HPTLC analysis is commonly used for the testing of raw materials and formulated products for their purity, stability, content uniformity or dissolution. The solvent system used for this study was Ethyl Acetate (2.5): n hexane (2.5), and there was a good separation of the compound in this solvent system.

The HPTLC performed on the ethanolic fruit and leaves extract of *Luffa acutangula* showed the presence of various phytoconstituents in different concentrations as illustrated in figures and tables. Figure 5 represents the 3-dimensional overlay of the chromatogram of fruit extract of all tracks, at all measured wavelengths. The chromatogram scanned at 254 nm (Fig. 4) represents 11 and 10 peaks for track 2,3 and track 1,4, 5 respectively, whereas the chromatogram of leaves extract scanned at 254 nm (Fig. 7) indicates 10, 11, 12 and 13 peaks for track 4, track 3,5,track 1 and track 2, respectively. The number of peaks shows the presence of various phytoconstituents in the sample. The Rf values (Tables 5 and 6) calculated for the phytoconstituents present in the tested sample which helps in the identification of the unknown compounds by comparing it with reference standards.

Table no. 5 HPTLC data of Ethanolic fruit extract of L. acutangula

Peak	2 μL		4 μL		6 μL		8 μL		10μL	
	R_f	% Area	R_f	% Area	R_f	% Area	R_f	% Area	R_f	% Area
1	0.05	14.77	0.05	18.38	0.05	17.90	0.05	19.86	0.05	19.44
2	0.11	3.97	0.09	5.16	0.09	4.93	0.08	4.95	0.08	4.87
3	0.20	1.73	0.16	3.24	0.18	8.30	0.18	10.79	0.17	11.74
4	0.27	6.83	0.18	0.22	0.23	0.36	0.24	0.39	0.24	.37
5	0.30	7.59	0.22	0.23	0.33	4.57	0.34	4.47	0.35	4.67
6	0.45	6.50	0.34	10.09	0.40	9.45	0.43	10.77	0.35	9.33
7	0.53	9.20	0.40	6.67	0.43	3.56	0.50	6.69	0.44	5.99
8	0.58	5.71	0.44	3.30	0.50	5.37	0.57	4.90	0.50	5.94
9	0.80	39.83	0.55	11.22	0.55	6.37	0.84	36.22	0.85	37.18
10	0.89	0.23	0.81	38.56	0.82	37.57	-	-	-	-
11	-	-	0.92	0.33	0.90	0.18	-	-		

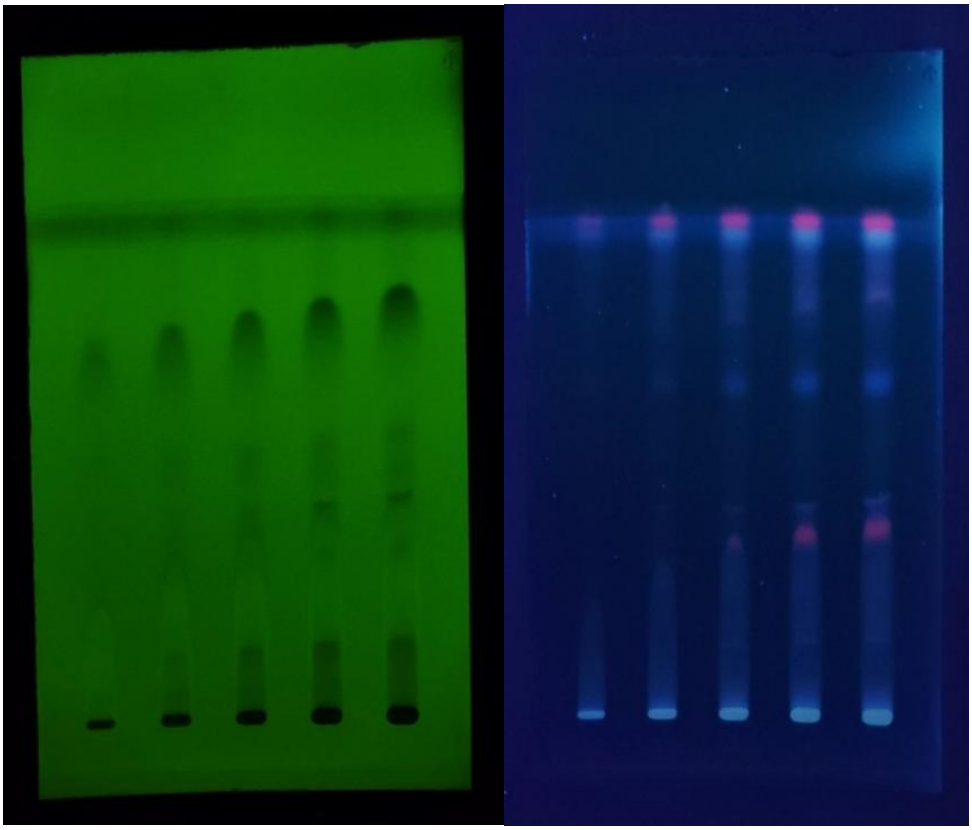


Fig. no. 4 Under UV short Under UV long

Solvent System: [Ethyl Acetate (2.5): n hexane (2.5)]
Track 1: Luffa sample-2 μL
Track 2: Luffa sample 4 μL
Track 3: Luffa sample 6 μL
Track 4: Luffa sample 8 μL
Track 5: Luffa sample 10μL

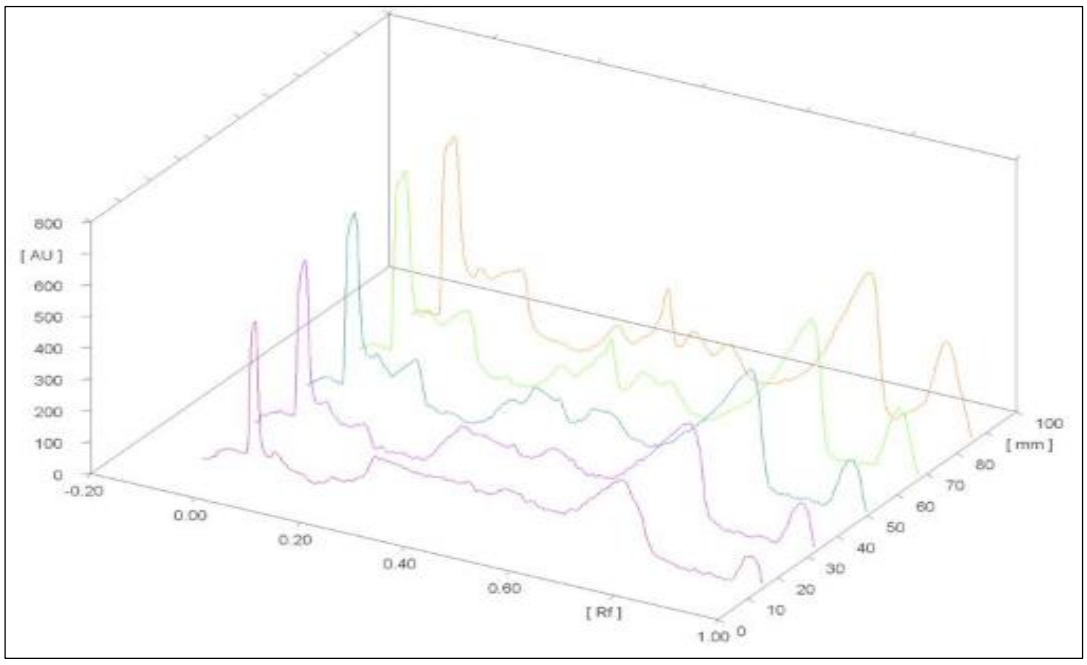
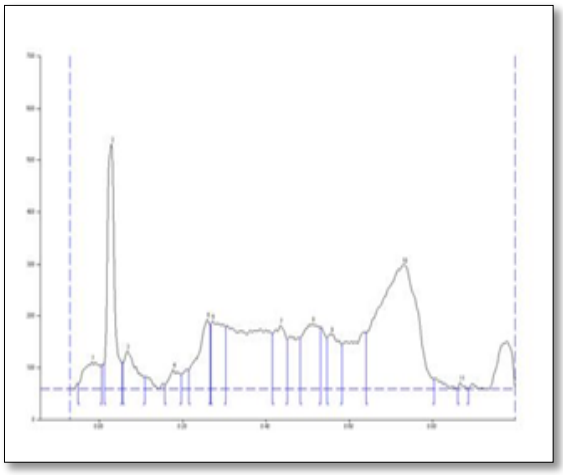
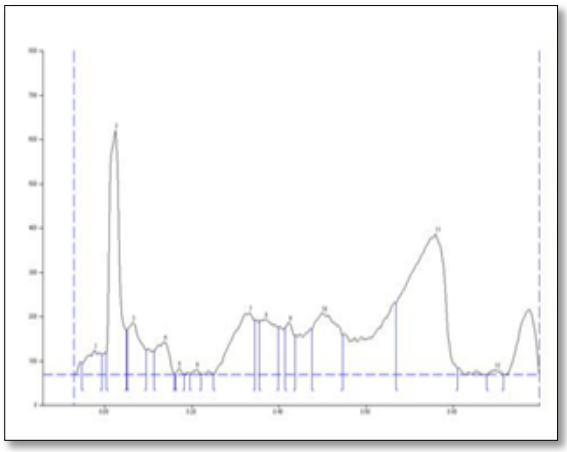


Fig no. 5 3D overlay of HPTLC chromatogram of all tracks, at 254nm



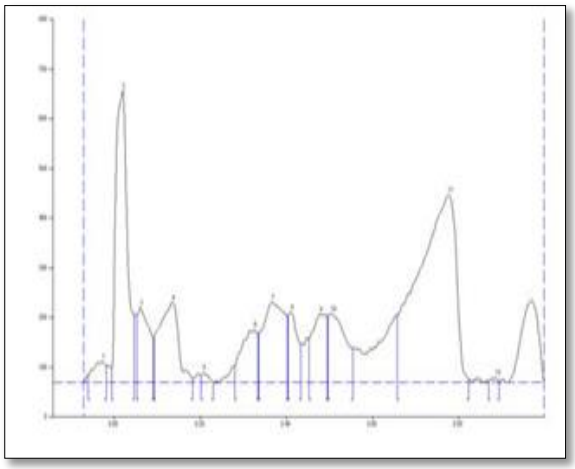
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.05	11.3	-0.02	51.9	3.45	0.00	44.9	1627.5	3.64
2	0.01	46.3	0.03	471.5	31.34	0.05	51.9	6601.7	14.77
3	0.06	52.0	0.07	73.0	4.85	0.11	23.5	1772.6	3.97
4	0.16	8.3	0.18	38.0	2.52	0.20	28.8	773.5	1.73
5	0.21	35.5	0.26	133.4	8.87	0.27	124.8	3053.1	6.83
6	0.27	125.4	0.27	131.5	8.74	0.30	120.5	3392.8	7.59
7	0.42	107.1	0.44	121.6	8.08	0.45	95.4	2907.1	6.50
8	0.48	98.7	0.51	125.6	8.35	0.53	118.5	4110.4	9.20
9	0.55	99.2	0.56	106.5	7.08	0.58	85.6	2553.2	5.71
10	0.64	109.8	0.73	239.4	15.91	0.80	19.2	17801.1	39.83
11	0.86	0.5	0.87	12.1	0.81	0.89	1.3	104.4	0.23

A. HPTLC Fingerprint Profiles and Rf Tables of 2 µL fruit Extract at 254nm



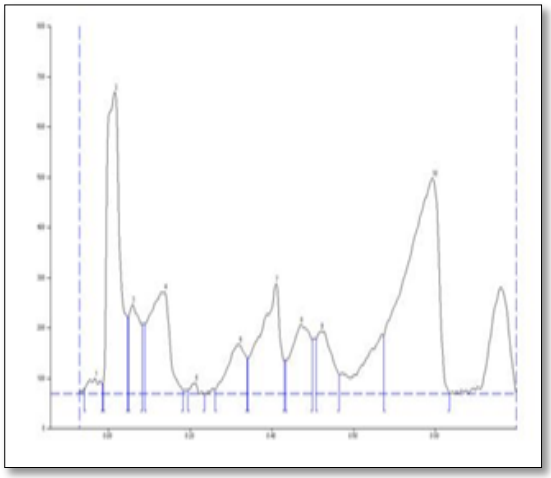
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.05	26.6	-0.02	55.5	3.31	-0.01	46.1	1434.7	2.61
2	0.00	48.9	0.02	552.0	32.90	0.05	102.0	10101.7	18.38
3	0.05	102.9	0.06	117.5	7.00	0.09	56.5	2835.9	5.16
4	0.11	53.6	0.14	74.6	4.45	0.16	2.8	1778.6	3.24
5	0.16	3.6	0.17	14.4	0.86	0.18	0.6	118.8	0.22
6	0.20	2.5	0.21	12.1	0.72	0.22	1.6	127.7	0.23
7	0.25	1.4	0.33	139.1	8.29	0.34	124.1	5543.5	10.09
8	0.36	122.0	0.37	125.9	7.50	0.40	107.2	3666.7	6.67
9	0.41	108.3	0.42	118.5	7.06	0.44	89.9	1813.3	3.30
10	0.48	103.0	0.50	139.7	8.32	0.55	88.7	6165.8	11.22
11	0.67	163.0	0.76	318.1	18.95	0.81	14.3	21195.9	38.56

B. HPTLC Fingerprint Profiles and Rf Tables of 4 µl fruit Extract at 254nm



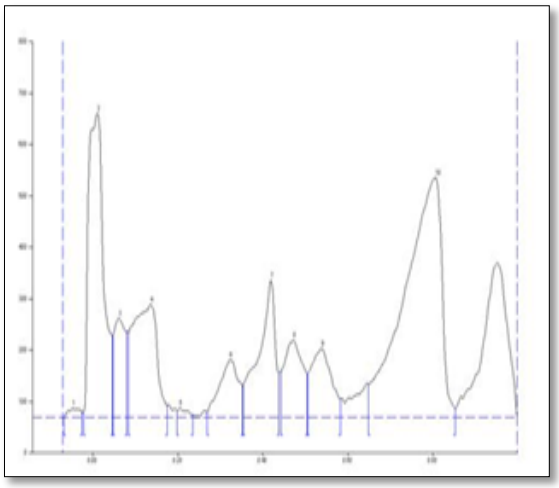
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.06	14.5	-0.03	42.7	2.09	-0.02	33.8	1013.5	1.44
2	-0.01	27.7	0.02	586.9	28.77	0.05	138.0	12641.4	17.90
3	0.05	136.0	0.06	151.7	7.44	0.09	90.5	3477.4	4.93
4	0.09	92.6	0.13	162.0	7.94	0.18	8.9	5861.0	8.30
5	0.20	14.5	0.21	20.1	0.99	0.23	0.8	256.5	0.36
6	0.28	33.1	0.33	107.3	5.26	0.33	99.7	3229.4	4.57
7	0.34	100.1	0.37	162.0	7.94	0.40	135.7	6670.1	9.45
8	0.40	136.0	0.41	142.5	6.98	0.43	77.7	2513.4	3.56
9	0.45	89.1	0.48	137.3	6.73	0.50	134.3	3788.4	5.37
10	0.50	134.7	0.50	138.7	6.80	0.55	69.1	4500.4	6.37
11	0.66	135.4	0.78	377.7	18.51	0.82	6.6	26527.4	37.57

C. HPTLC Fingerprint Profiles and Rf Tables of 6 µl fruit Extract at 254nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.06	10.6	-0.03	30.4	1.49	-0.01	20.3	755.3	0.98
2	-0.01	20.5	0.02	601.3	29.39	0.05	153.9	15351.4	19.86
3	0.05	154.4	0.06	178.9	8.74	0.08	139.2	3829.7	4.95
4	0.09	138.7	0.14	203.7	9.95	0.18	7.2	8339.2	10.79
5	0.19	8.4	0.21	21.3	1.04	0.24	0.5	300.0	0.39
6	0.26	8.4	0.32	98.8	4.83	0.34	71.2	3453.0	4.47
7	0.34	71.5	0.41	219.0	10.70	0.43	66.7	8326.0	10.77
8	0.43	67.6	0.47	138.1	6.75	0.50	108.9	5172.2	6.69
9	0.51	110.0	0.52	125.3	6.12	0.57	38.6	3786.0	4.90
10	0.67	116.3	0.79	429.6	20.99	0.84	0.7	28003.9	36.22

D. HPTLC Fingerprint Profiles and Rf Tables of 8 µl fruit Extract at 254nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.07	0.7	-0.05	18.3	0.84	-0.03	11.1	408.2	0.46
2	-0.02	13.3	0.01	592.3	27.26	0.05	159.7	17116.4	19.44
3	0.05	160.5	0.06	192.7	8.87	0.08	168.0	4287.3	4.87
4	0.08	166.4	0.14	220.4	10.14	0.17	24.8	10335.3	11.74
5	0.20	13.4	0.20	18.6	0.85	0.24	4.6	328.4	0.37
6	0.27	10.7	0.32	111.4	5.13	0.35	63.7	4113.2	4.67
7	0.35	64.6	0.42	266.5	12.26	0.44	86.8	8217.3	9.33
8	0.44	91.5	0.47	150.9	6.94	0.50	85.5	5275.9	5.99
9	0.51	86.0	0.54	134.8	6.20	0.58	36.8	5225.1	5.94
10	0.65	65.1	0.81	467.2	21.50	0.85	18.7	32730.3	37.18

E. HPTLC Fingerprint Profiles and Rf Tables of 10 µl fruit Extract at 254nm

Fig.no.6 HPTLC Fingerprint Profiles and Rf Tables Ethanolic fruit Extract at 254nm

Table no. 6 HPTLC data of Ethanolic leaves extract of L. acutangula

Peak	2 μL		4 μL		6 μL		8 μL		10μL	
	R_f	% Area	R_f	% Area	R_f	% Area	R_f	% Area	R_f	% Area
1	0.07	1.60	0.05	1.08	0.09	0.68	0.09	0.77	0.08	0.55
2	0.12	4.92	0.11	2.79	0.18	1.09	0.28	11.50	0.17	1.01
3	0.20	2.93	0.16	1.17	0.26	2.01	0.30	0.38	0.21	1.04
4	0.23	2.69	0.22	3.01	0.30	0.25	0.34	0.56	0.24	1.36
5	.026	2.19	0.28	1.06	0.43	4.95	0.41	5.09	0.30	0.43
6	0.31	2.23	0.33	0.91	0.50	3.40	0.50	2.85	0.35	0.74
7	0.36	3.01	0.41	3.36	0.60	7.17	0.60	7.90	0.41	5.84
8	0.42	4.23	0.51	4.57	0.72	28.23	0.72	25.40	0.44	1.31
9	0.52	8.00	0.61	10.00	0.79	12.56	0.79	11.68	0.50	2.61
10	0.57	7.93	0.73	26.95	0.85	3.64	0.99	22.50	0.61	9.99
11	0.62	7.30	0.79	10.74	0.99	25.53	-	-	0.73	22.95
12	0.74	31.82	0.84	2.54	-	-	-	-	-	-
13	-	-	0.99	22.56	-	-	-	-	-	-

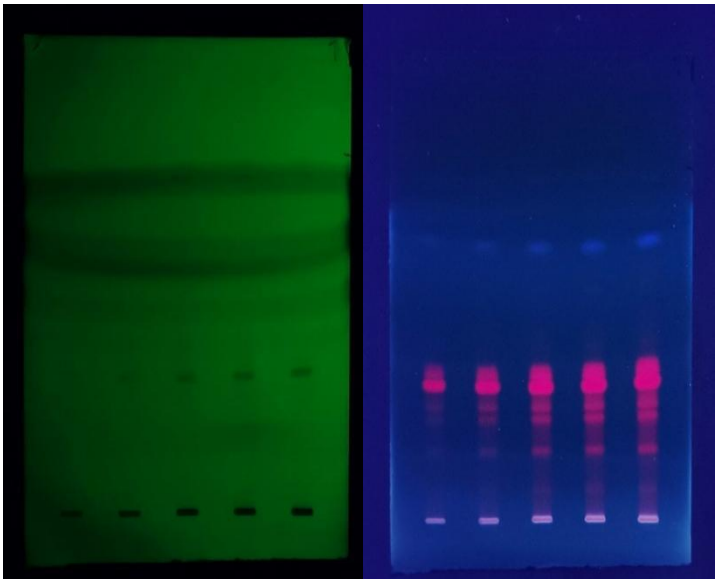


Fig. no. 7 Under UV short Under UV long

Solvent System: [Ethyl Acetate (2.5): n hexane (2.5)]

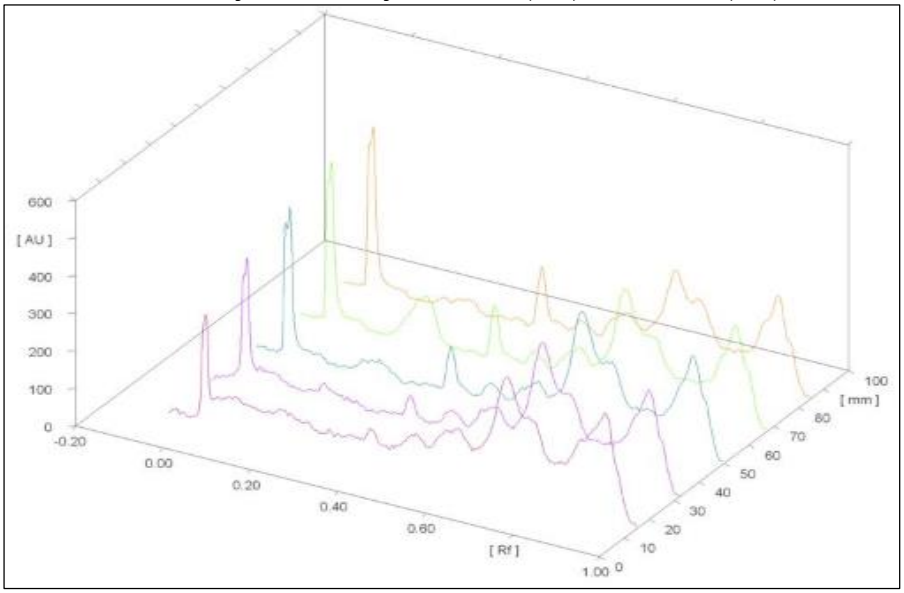
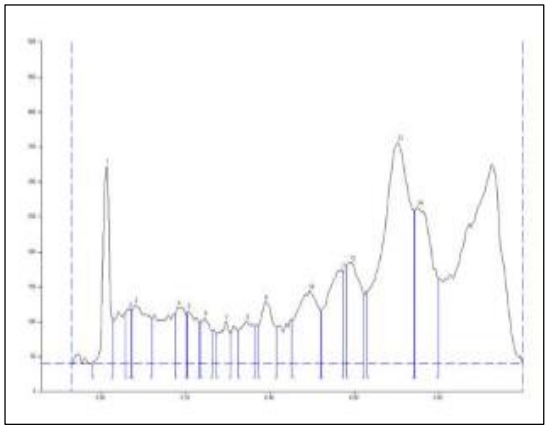
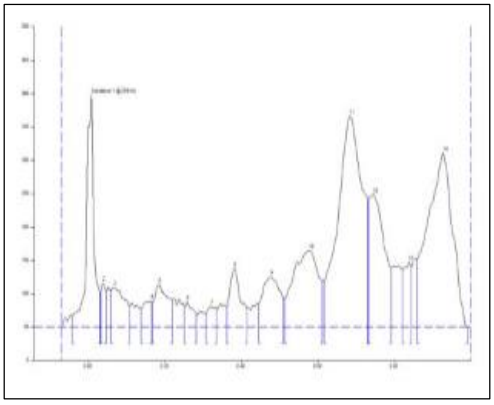


Fig. no. 8 3D overlay of HPTLC chromatogram of all tracks, at 254nm



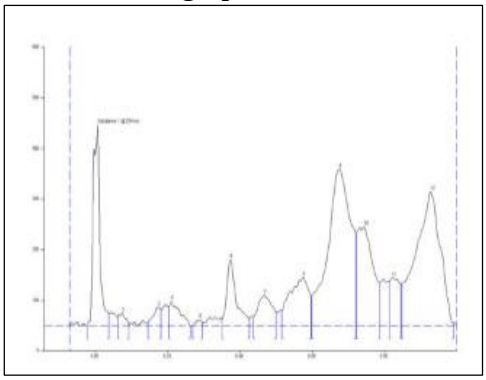
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	0.3	0.01	283.7	15.68	0.03	61.4	3565.7	6.71
2	0.06	73.0	0.07	78.9	4.36	0.07	78.0	849.5	1.60
3	0.07	77.6	0.08	84.8	4.68	0.12	65.4	2614.2	4.92
4	0.17	70.9	0.18	81.7	4.51	0.20	70.0	1555.2	2.93
5	0.20	72.4	0.21	76.3	4.22	0.23	59.8	1429.1	2.69
6	0.24	61.0	0.25	66.0	3.65	0.26	47.6	1163.8	2.19
7	0.27	43.5	0.29	60.7	3.36	0.31	43.4	1185.6	2.23
8	0.32	47.5	0.34	61.3	3.39	0.36	54.5	1599.5	3.01
9	0.37	55.0	0.39	89.2	4.93	0.42	52.8	2246.8	4.23
10	0.45	63.4	0.49	105.1	5.81	0.52	76.4	4249.6	8.00
11	0.52	76.8	0.57	134.9	7.46	0.57	130.7	4211.8	7.93
12	0.58	141.1	0.59	145.5	8.04	0.62	97.7	3876.7	7.30
13	0.63	103.1	0.70	317.0	17.51	0.74	219.7	16902.4	31.82

A. HPTLC Fingerprint Profiles and Rf Tables of 2 µL leaves Extract at 254nm



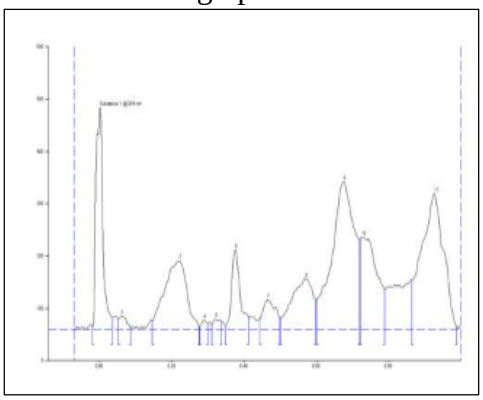
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.04	15.9	0.01	348.5	19.39	0.03	52.3	5615.8	9.24
2	0.03	53.5	0.04	66.0	3.67	0.05	53.5	657.3	1.08
3	0.06	56.6	0.07	59.3	3.30	0.11	31.2	1698.0	2.79
4	0.14	28.8	0.16	40.0	2.23	0.16	39.3	713.5	1.17
5	0.17	37.2	0.18	63.3	3.52	0.22	41.3	1827.7	3.01
6	0.25	30.5	0.26	38.0	2.12	0.28	19.0	646.5	1.06
7	0.31	19.6	0.32	30.5	1.69	0.33	29.4	553.7	0.91
8	0.36	31.4	0.38	87.9	4.89	0.41	27.9	2045.8	3.36
9	0.45	35.2	0.48	75.5	4.20	0.51	44.3	2780.0	4.57
10	0.51	42.9	0.58	115.2	6.41	0.61	70.2	6083.4	10.00
11	0.62	69.5	0.68	315.9	17.58	0.73	192.2	16387.2	26.95
12	0.73	192.6	0.75	198.8	11.06	0.79	89.3	6531.7	10.74
13	0.82	86.0	0.84	97.7	5.44	0.84	90.1	1545.6	2.54
14	0.86	102.1	0.93	260.8	14.51	0.99	0.2	13720.6	22.56

B. HPTLC Fingerprint Profiles and Rf Tables of 4 µL leaves Extract at 254nm



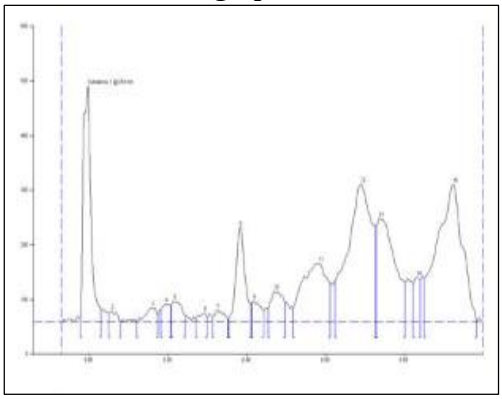
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	0.0	0.01	397.0	23.79	0.04	24.1	5955.2	10.48
2	0.06	18.6	0.07	24.7	1.48	0.09	1.6	389.1	0.68
3	0.15	7.9	0.17	36.5	2.19	0.18	33.5	619.1	1.09
4	0.20	37.4	0.21	48.2	2.89	0.26	0.6	1144.7	2.01
5	0.27	0.4	0.29	11.4	0.68	0.30	7.2	142.1	0.25
6	0.35	11.7	0.37	130.3	7.81	0.43	15.6	2811.6	4.95
7	0.44	19.0	0.47	59.0	3.54	0.50	26.0	1934.0	3.40
8	0.52	31.1	0.58	95.8	5.74	0.60	58.7	4074.3	7.17
9	0.60	59.7	0.68	309.8	18.57	0.72	183.4	16048.2	28.23
10	0.72	183.6	0.74	195.9	11.74	0.79	85.1	7141.0	12.56
11	0.81	87.0	0.82	94.6	5.67	0.85	82.7	2071.3	3.64
12	0.85	82.7	0.93	265.4	15.91	0.99	4.6	14515.7	25.53

C. HPTLC Fingerprint Profiles and Rf Tables of 6 µL leaves Extract at 254nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	5.1	0.00	425.1	25.82	0.03	22.7	6502.4	11.37
2	0.05	20.9	0.06	25.7	1.56	0.09	2.8	438.7	0.77
3	0.15	18.8	0.22	132.7	8.06	0.28	5.7	6576.4	11.50
4	0.28	6.6	0.29	17.3	1.05	0.30	10.8	215.5	0.38
5	0.31	10.6	0.32	19.8	1.20	0.34	14.6	319.8	0.56
6	0.35	7.9	0.38	152.0	9.23	0.41	25.7	2911.7	5.09
7	0.44	17.9	0.46	57.4	3.49	0.50	21.7	1629.3	2.85
8	0.50	22.8	0.57	96.9	5.89	0.60	57.4	4515.0	7.90
9	0.60	57.5	0.68	282.9	17.18	0.72	172.2	14525.5	25.40
10	0.72	173.4	0.73	176.2	10.70	0.79	78.5	6676.7	11.68
11	0.86	93.7	0.93	260.2	15.81	0.99	6.2	12867.5	22.50

D. HPTLC Fingerprint Profiles and Rf Tables of 8 µL leaves Extract at 254nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	5.3	-0.00	431.6	24.95	0.03	23.3	6785.8	12.65
2	0.05	15.6	0.06	19.4	1.12	0.08	0.9	294.9	0.55
3	0.12	0.2	0.16	25.8	1.49	0.17	12.2	542.3	1.01
4	0.18	22.2	0.19	32.7	1.89	0.21	28.1	556.6	1.04
5	0.21	29.5	0.21	37.3	2.16	0.24	11.2	731.6	1.36
6	0.27	3.8	0.29	16.4	0.95	0.30	8.4	229.3	0.43
7	0.31	8.4	0.32	21.3	1.23	0.35	4.8	397.2	0.74
8	0.35	6.2	0.38	172.2	9.95	0.41	33.7	3131.2	5.84
9	0.41	33.3	0.42	38.1	2.21	0.44	20.7	705.2	1.31
10	0.45	24.3	0.47	55.7	3.22	0.50	36.3	1399.2	2.61
11	0.52	26.8	0.58	106.7	6.17	0.61	71.9	5359.2	9.99
12	0.62	74.6	0.69	251.8	14.56	0.73	174.9	12311.3	22.95
13	0.73	174.3	0.74	189.2	10.94	0.80	73.0	7224.2	13.46
14	0.82	69.5	0.83	80.2	4.64	0.84	76.5	1079.5	2.01
15	0.85	80.5	0.92	251.2	14.52	0.98	1.3	12904.7	24.05

E. HPTLC Fingerprint Profiles and Rf Tables of 10 µL leaves Extract at 254nm

Fig.no. 9 HPTLC Fingerprint Profiles and Rf Tables Ethanolic leaves Extract at 254nm

4. Conclusion

The study concluded that the fruit and leaves extract of *Luffa acutangula* contain a rich variety of phytochemicals. In the present study, the phytochemical screening of of extracts of *Luffa acutangula* indicated presence of major bioactive compounds which are resultant for the varied pharmacological and traditional properties of the plant.
The FT-IR analysis identified characteristic functional groups such as carboxylic acid, ethers, alcohols, phenols, alkanes, ketones. Further, HPTLC analysis confirmed the presence of 10 to 14 compounds at 254 nm UV light.

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