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SCREENING OF PHYTOCONSTITUENTS, HPTLC AND FT-IR ANALYSIS IN *LUFFA CYLINDRICA* (L.) M. ROEM FRUITS

Kallootharamadam Vishnu Namboothiri Megha *, Vinitha Saseendra Babu and Puthuparambil Madhavan Radhamany

Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram - 695581, Kerala, India.

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Correspondence to Author:
K. V. Megha

Plant Reproductive Biology Laboratory, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram - 695581, Kerala, India.

E-mail: meghakv073@gmail.com

ABSTRACT: The present investigation deals with the preliminary phytochemical, High Performance Thin Layer Chromatography and Fourier Transform Infrared Spectral analysis of *Luffa cylidrica* (L.) Roem (Cucurbitaceae) fruits. The results revealed that most of the phytochemicals evaluated were present in ethanolic extract as compared to the petroleum ether extract. Quantitative analysis revealed that the fruits were rich in phenolic compounds. As the ethanolic extract resolved most of the phytoconstituents studied, it was subjected to High Performance Thin Layer Chromatographic (HPTLC) studies. HPTLC data of extract shows ten different peaks confirming that ten compounds are present in the ethanolic extract of *Luffa cylindrica*. The FT-IR spectrum showed the presence of alcohols, phenol, alkanes, alkenes and ketones. The results confirm the fact that *L. cylindica* possess important bioactive constituents useful for our health, so further, scientific investigation is needed.

INTRODUCTION: Plants have been used since time immemorial as they are the most abundant source of drugs for traditional systems of medicine. The man began using plant extracts to protect him against several ailments and also to improve his health and lifestyle. Plants contain different phytoconstituents like flavonoid, alkaloid, phenol and tannins, carboxylic acids, terpenes, amino acids and inorganic acids. These phytoconstituents give specific distinctiveness and properties to plants [1]. Hence, the analysis of these chemical constituents would help in analyzing biological properties in plants. There are a variety of techniques that can be used to determine and estimate the presence of such phytoconstituents in medicinal plants.

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Chromatography and spectroscopic techniques are the most useful and popular tools used for this purpose. The Fourier Transform Infrared Spectrophotometer (FT-IR) was perhaps the most powerful tool for identifying the types of chemical bonds/functional groups present in the phyto-chemicals. 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 an established time-saving method to characterize and identify functional groups [2]. On the other hand, High-Performance Thin Layer Chromatography (HPTLC) analysis enables the most complicated separation of the phyto-constituents and allows more accurate quantitative measurements.

Luffa cylindrical (L.) Roem (Sponge gourd), an underutilized vegetable with medicinal property, is a fibrous plant that belongs to the family Cucurbitaceae. It is widely distributed in the tropics and subtropics, as a cultivated and naturalized plant.

It is a fast-growing annual climber with alternate and palmately lobed leaves and bright yellow-colored flowers. The flowers are monoecious, with five petals that are united below into a bell-shaped corolla. Anthers cohering in a mass, the ovary is two-celled, style is slender with three stigmas. Both male and female flowers are on the same plant and are pollinated by bees.

The pollinated flowers develop into cylindrical or elliptical ovate fleshy and dehiscent green fruits, which grew up to 60 cms in length contain seeds in a system of many inter-twined cellulose fibers. The green fruits are turned to brown when mature and dry on the vine to develop an inedible sponge-like structure.

As the fruit turns old and dry, the endocarp becomes a persistent fibrous vascular network that is used in various ways. The young fruit is eaten fresh or cooked as a vegetable, but it has to be picked before the fibrous vascular bundles harden and before the purging compounds develop. The leaves are also eaten as a vegetable.

The fruits and leaves are browsed by goats and bees feed on the flowers. *Luffa cylindrica* finds wide applications in the Ayurvedic system of medicine. The young fruit juice is used to cure vitated kapha, pitta, jaundice, infective hepatitis, constipation, skin diseases, piles, bronchitis, *etc.* [3]. Hence, the present study was done to evaluate the preliminary phytochemical profiling, HPTLC analysis, and FTIR spectrum profile of *L. cylindrica*.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material: The fruits of the *L. cylindrica* were collected from Thiruvananthapuram district of Kerala from its natural habitat during November 2018. The botanical identities were verified by the Curator, Department of Botany, University of Kerala, and a voucher specimen were deposited at the Department herbarium (KUBH 7075).

Preparation of Extract: The fruits of *L. cylindrica* were shade dried and made into a coarse powder (Sieve No. 40) and stored in a well-closed airtight container. The powdered fruits of the plant (50 gm.) were packed in a Soxhlet apparatus and continuously extracted with petroleum ether (40-60 °C) till complete the extraction. After completion of extraction, the solvent was removed by distillation, and the concentrated extract obtained was dried under reduced pressure using a rotatory evaporator at a temperature not exceeding 40 °C and then give moderate heating on a water bath. From the drug, petroleum ether was removed, and the defatted drug was extracted with ethanol (95%) to complete the extraction.

After completion of extraction, the solvent was removed by distillation and then concentrated extract obtained dried under reduced pressure at a temperature not exceeding 40 °C and then give moderate heating on water bath [4].

Preliminary Phytochemical Investigations: The preliminary phytochemical investigations were carried out on the petroleum ether and ethanolic extracts of fruits of *Luffa cylindrica*for qualitative and quantitative identification. Tests were carried out by following standard procedures [5]. All the chemicals and reagents used were of analytical grade.

High Performance Thin Layer Chromatography (HPTLC): For the HPTLC analysis, ethanolic extract of *L.cylindrica*fruits was applied as bands on the plate with Camag microliter syringe attached with automatic TLC sampler 4 (ATS4).

The TLC plate was aluminum sheet precoated with silica gel 60 F_{254} and applied 5 µL and 10 µL of the extract separately on the TLC plate as 10 mm bands in two tracks of 10 mm distance.

The plate was developed in the solvent system (Toluene: ethyl acetate: formic acid in the ratio 5: 2: 0.2). The plate was developed using a Camag twin trough chamber. Photo documentation was done using Camag visualizer.

Camag TLC scanner installed with WINCATS software was used for fingerprint development in the UV and visible region, after derivatization with vanillin-sulphuric acid. The developed plate was air-dried and visualized under UV 254 and 366 nm.

The fingerprints were recorded, and the plate was derivatized using vanillin-sulphuric acid. The chromatograms under white light and fingerprint profile at 575 nm were recorded.

Fourier Transform Infra-red (FTIR): About 1 mg of the dried methanolic extract was encapsulated in 10 mg of KBr pellet, in order to prepare translucent sample discs.

The powdered sample of the extract was loaded in an FTIR spectroscope (Shimadzu, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The spectral data was taken on Agilent Cary 630 FTIR spectrometer that is based on the ATR (Attenuated total reflection) method. This was done to find out the functional groups present in the plant extract.

RESULTS AND DISCUSSION:

Preparation of Extract: The nature and percentage yield of the extract in both petroleum ether and ethanol is depicted in **Table 1**.

TABLE 1: NATURE AND PERCENTAGE YIELD OF EXTRACT

S. no.	Name of the extract	Nature	Colour	% yield of extracts in (gm)
1	Petroleum ether	Thick oil	Greenish brown	5.87 gm
2	Ethanol	Syrupy	Dark yellow	7.13 gm

Preliminary Phytochemical Screening: Phyto-chemical characters are the indicators of chemical constituents of the plant. The study of phytochemical variation is very important in medicinal plants since the medicinal property of any plant is the result of action of such chemicals.

Analysis of phytochemical variability between accessions will help to identify them in terms of their difference in phytochemical constitution [6]. Previous reports showed that ethanolic extracts of *L. cylindrica* had major metabolites [7] the preliminary. Phytochemical analysis showed that most of the phytochemicals evaluated were present in the ethanolic extract as compared to the petroleum ether extract **Table 2**.

TABLE 2: PRELIMINARY PHYTOCHEMICAL TESTS OF PETROLEUM ETHER EXTRACT AND ETHANOLIC EXTRACT

Metabolites	Petroleum Ether	Ethanol
Alkaloids	-	+
Carbohydrate	-	+
Glycosides	-	+
Cardiac glycosides	+	+
Tannins	-	+
Phenolic compounds	-	+
Protein and free amino acids	-	+
Flavanoids	-	+
Reducing sugar	-	+
Saponins	-	+
Phytosterols	-	+
Fatty acid	-	+
Carotenoids	-	+
Terpenoids	-	+
Steroids	-	+
Quinines	-	+
Catecholins	-	+
Coumarins	-	+
Gum and mucilage	+	+
Anthraquinones	-	+

Quantitative analyses of phytochemicals were carried out in the ethanolic extract. Secondary metabolites like phenols, flavonoids, saponin, amino acid, protein, and starch were estimated, and the mean value of triplicated experiments is depicted in **Fig. 1**.

The extract contained these phytochemicals in significant quantity, and phenolic content was found to be the highest. As the ethanolic extract resolved most of the phytoconstituents studied, it was subjected to High-Performance Thin Layer Chromatographic (HPTLC) studies.

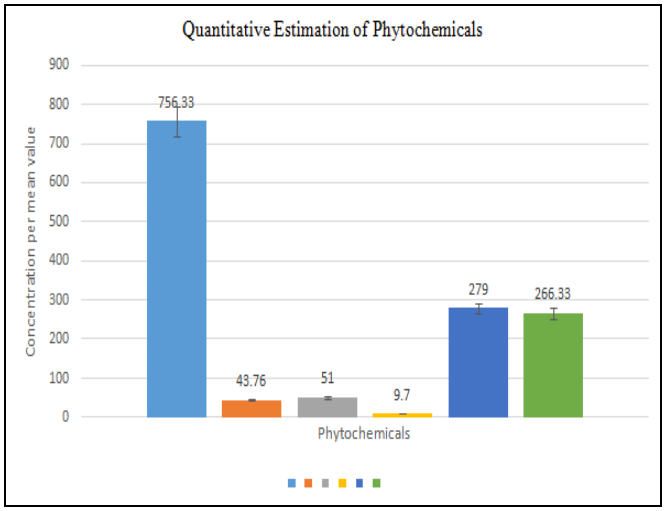


FIG. 1: QUANTITATIVE ESTIMATION OF PHYTO-CHEMICALS

HPTLC Analysis: Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines since they might represent the chemical ingredients of the herbal medicines and its products 8 appropriately. HPTLC method is commonly applied for the identification and testing

of purity, stability, dissolution, or content uniformity of raw materials and formulated products. HPTLC is a valuable tool for the investigation of herbal products concerning different aspects of their quality.

The majority of plant ingredients react with vanillin-sulphuric acid with colored zones; therefore, this spray reagent was used to detect the presence of phytocompounds through HPTLC of *L. cylindrica* extract.

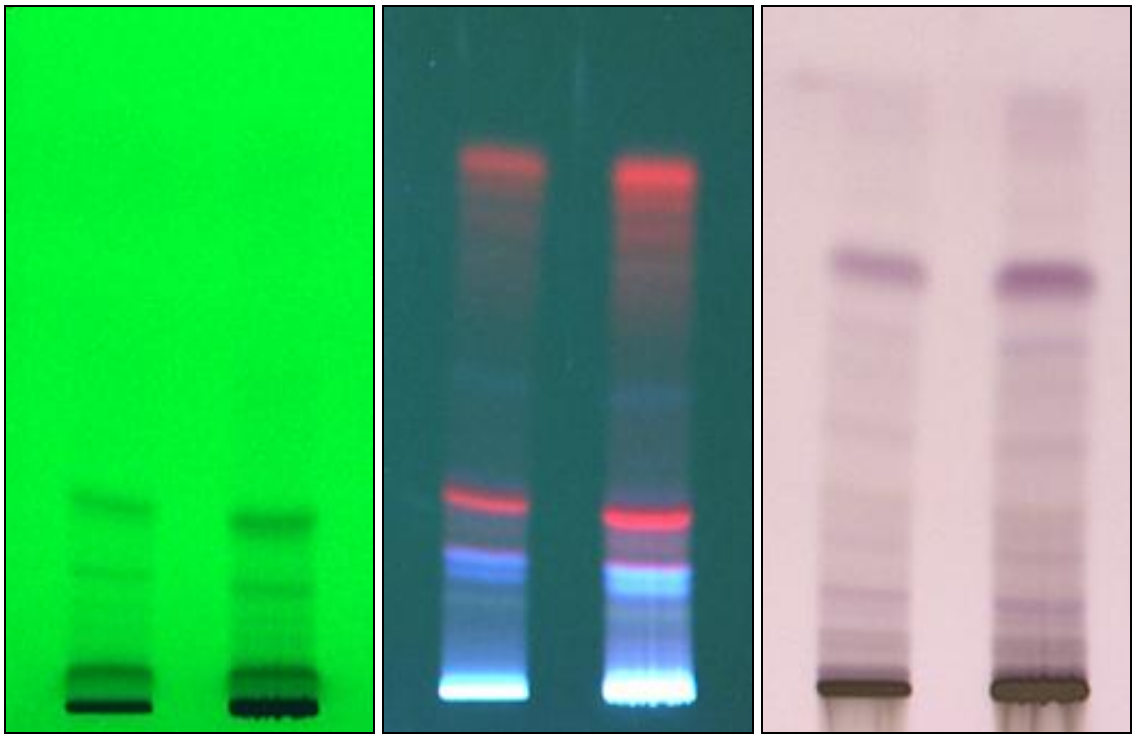
The HPTLC chromatogram of ethanolic extract of *L. cylindrica* recorded at 254 nm, 366 nm, and after derivatization with vanillin sulphuric acid at 575 nm is depicted in **Fig. 2**. The HPTLC fingerprint profiles, R_f values, and area obtained for extracts after scanning at UV 254 nm, 366 nm, and after derivatization with vanillin sulphuric acid are given in **Fig. 3, 4, 5**.

The R_f value and color reactions of various compounds were given in **Table 3**. The solvent system used for this particular study was toluene: ethyl acetate: formic acid (5: 2: 0.2), and there was a good separation of the compound in this solvent system.

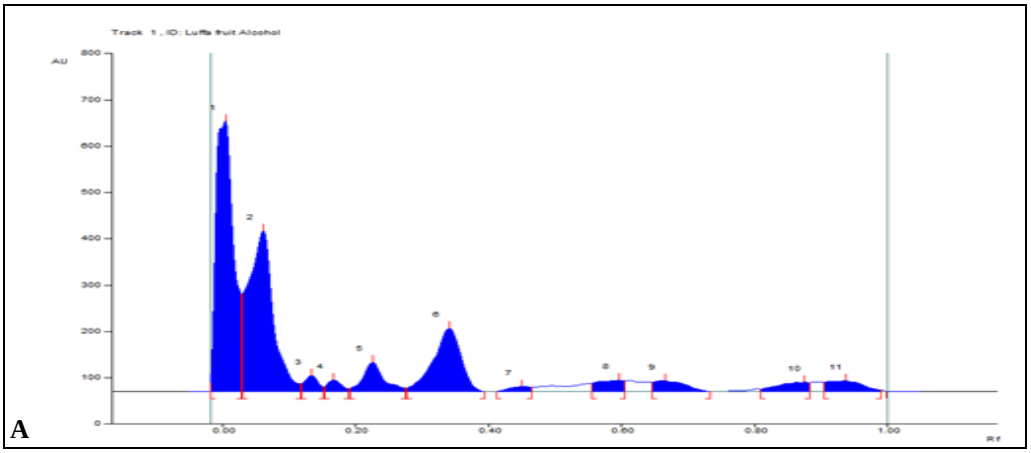
In our present study, the highest number of peaks was obtained at 575 nm (10), which was followed by 254 nm (9).

TABLE 3: HPTLC DATA OF ETHANOLIC EXTRACT SHOWING RF VALUES AND AREA OF MAJOR COMPOUNDS

Peak	254 nm		366 nm		575 nm	
	R_f	Area (%)	R_f	Area (%)	R_f	Area (%)
1	0.10	22.64	0.15	1.40	0.11	3.64
2	0.13	1.04	0.23	18.93	0.19	4.27
3	0.16	0.82	0.31	8.54	0.26	3.07
4	0.25	5.87	0.37	1.62	0.34	4.28
5	0.37	22.74	0.74	3.29	0.46	3.91
6	0.44	1.49	0.95	9.22	0.55	5.19
7	0.55	3.48	-	-	0.63	9.25
8	0.70	2.06	-	-	0.78	38.60
9	1.00	6.87	-	-	0.87	1.02
10	-	-	-	-	0.97	1.61

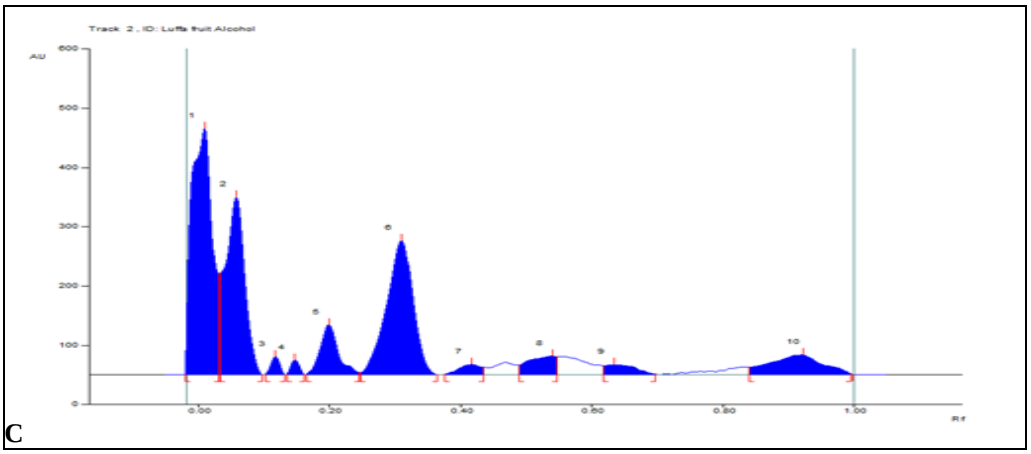


Under UV short **Under UV long** **Under white light after derivatization**



A

	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
	1	-0.02 Rf	18.3 AU	0.01 Rf	584.2 AU	45.19 %	0.03 Rf	10.6 AU	10699.1 AU	36.77 %
	2	0.03 Rf	210.6 AU	0.06 Rf	347.7 AU	26.90 %	0.12 Rf	16.6 AU	9093.1 AU	31.25 %
	3	0.12 Rf	17.1 AU	0.14 Rf	35.0 AU	2.71 %	0.15 Rf	10.1 AU	495.3 AU	1.70 %
	4	0.16 Rf	10.5 AU	0.17 Rf	25.4 AU	1.96 %	0.19 Rf	5.9 AU	351.9 AU	1.21 %
	5	0.19 Rf	6.4 AU	0.23 Rf	63.2 AU	4.89 %	0.28 Rf	7.3 AU	1377.2 AU	4.73 %
	6	0.28 Rf	7.7 AU	0.34 Rf	136.5 AU	10.56 %	0.40 Rf	0.3 AU	3870.7 AU	13.30 %
	7	0.41 Rf	0.6 AU	0.45 Rf	10.3 AU	0.80 %	0.47 Rf	9.3 AU	233.2 AU	0.80 %
	8	0.56 Rf	17.8 AU	0.60 Rf	24.1 AU	1.87 %	0.61 Rf	22.6 AU	681.0 AU	2.34 %
	9	0.65 Rf	20.8 AU	0.67 Rf	23.1 AU	1.79 %	0.74 Rf	0.3 AU	771.3 AU	2.65 %
	10	0.81 Rf	5.3 AU	0.88 Rf	20.1 AU	1.55 %	0.88 Rf	19.4 AU	669.6 AU	2.30 %
B	11	0.91 Rf	20.4 AU	0.94 Rf	23.0 AU	1.78 %	0.99 Rf	3.3 AU	852.5 AU	2.93 %



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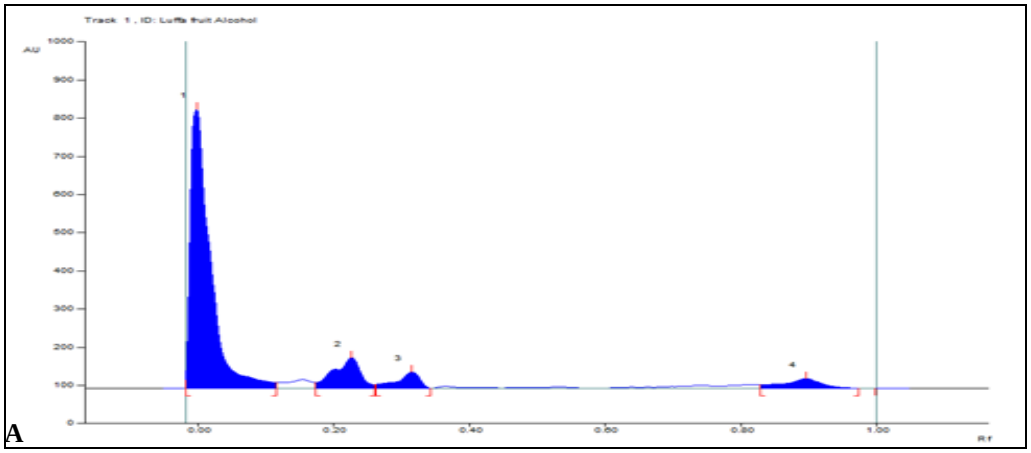
	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
	1	-0.02 Rf	0.0 AU	0.01 Rf	415.8 AU	35.18 %	0.03 Rf	70.7 AU	9023.4 AU	33.00 %
	2	0.03 Rf	171.2 AU	0.06 Rf	300.5 AU	25.43 %	0.10 Rf	0.1 AU	6191.5 AU	22.64 %
	3	0.10 Rf	0.6 AU	0.12 Rf	30.8 AU	2.61 %	0.13 Rf	0.2 AU	284.2 AU	1.04 %
	4	0.14 Rf	1.2 AU	0.15 Rf	24.7 AU	2.09 %	0.16 Rf	0.5 AU	223.1 AU	0.82 %
	5	0.17 Rf	0.5 AU	0.20 Rf	84.1 AU	7.11 %	0.25 Rf	4.0 AU	1604.4 AU	5.87 %
	6	0.25 Rf	4.1 AU	0.31 Rf	226.4 AU	19.16 %	0.37 Rf	0.1 AU	6218.0 AU	22.74 %
	7	0.38 Rf	0.8 AU	0.42 Rf	17.2 AU	1.45 %	0.44 Rf	12.5 AU	408.4 AU	1.49 %
	8	0.49 Rf	16.8 AU	0.54 Rf	31.6 AU	2.67 %	0.55 Rf	30.0 AU	951.3 AU	3.48 %
	9	0.62 Rf	15.3 AU	0.63 Rf	17.0 AU	1.44 %	0.70 Rf	1.0 AU	562.2 AU	2.06 %
D	10	0.84 Rf	12.8 AU	0.92 Rf	33.6 AU	2.84 %	1.00 Rf	1.9 AU	1878.7 AU	6.87 %

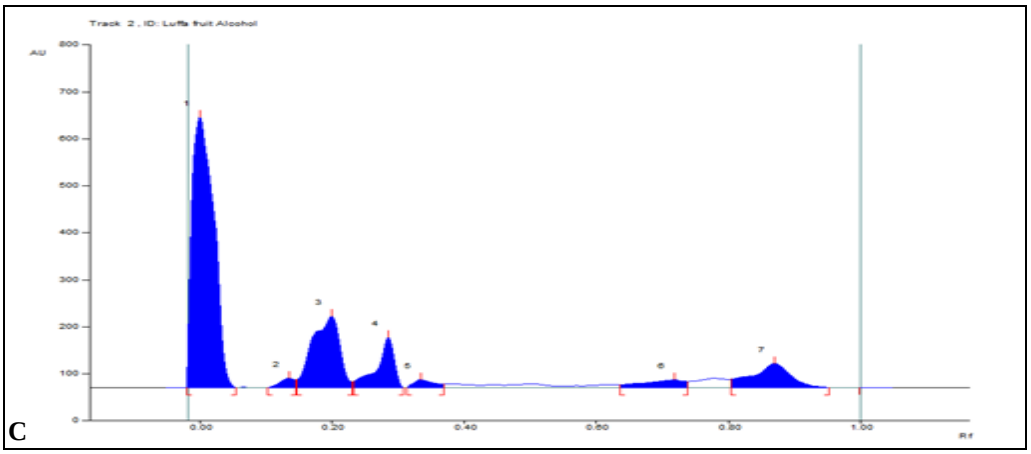
FIG. 2: HPTLC FINGERPRINT PROFILES AND R_f TABLES OF 5 µL AND 10 µL ETHANOL EXTRACT AT 254 nm



A

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	21.6 AU	0.00 Rf	731.2 AU	82.98 %	0.12 Rf	15.1 AU	14716.4 AU	77.72 %
2	0.17 Rf	15.6 AU	0.23 Rf	80.7 AU	9.16 %	0.26 Rf	10.3 AU	2224.7 AU	11.75 %
3	0.26 Rf	10.5 AU	0.32 Rf	43.6 AU	4.95 %	0.34 Rf	0.0 AU	988.4 AU	5.22 %
4	0.83 Rf	9.1 AU	0.90 Rf	25.7 AU	2.91 %	0.98 Rf	0.2 AU	1005.4 AU	5.31 %

B



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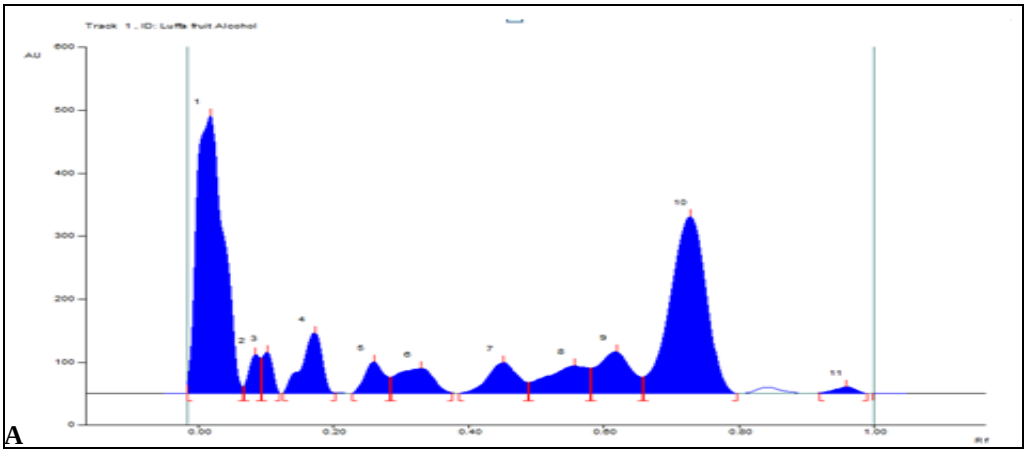
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	0.0 AU	0.00 Rf	576.6 AU	61.29 %	0.05 Rf	0.1 AU	12937.2 AU	57.00 %
2	0.10 Rf	0.1 AU	0.14 Rf	20.2 AU	2.14 %	0.15 Rf	16.5 AU	317.2 AU	1.40 %
3	0.15 Rf	16.6 AU	0.20 Rf	151.8 AU	16.13 %	0.23 Rf	12.9 AU	4297.6 AU	18.93 %
4	0.23 Rf	13.3 AU	0.29 Rf	106.8 AU	11.35 %	0.31 Rf	0.5 AU	1938.7 AU	8.54 %
5	0.31 Rf	0.7 AU	0.34 Rf	17.0 AU	1.81 %	0.37 Rf	7.1 AU	367.6 AU	1.62 %
6	0.64 Rf	6.5 AU	0.72 Rf	16.8 AU	1.78 %	0.74 Rf	13.1 AU	746.1 AU	3.29 %
7	0.81 Rf	17.9 AU	0.87 Rf	51.6 AU	5.49 %	0.95 Rf	0.4 AU	2092.8 AU	9.22 %

D

FIG. 3: HPTLC FINGERPRINT PROFILES AND R_f TABLES OF 5 µL AND 10 µL ETHANOL EXTRACT AT 366 nm



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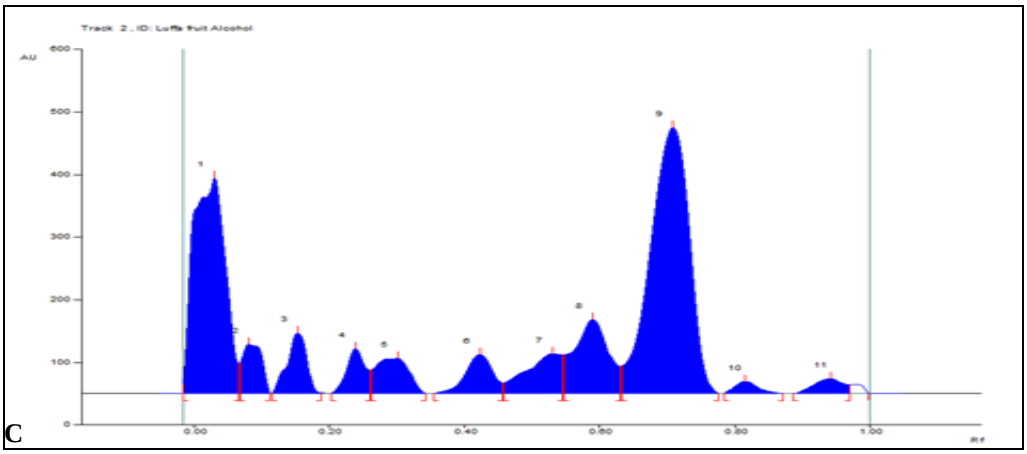
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	12.8 AU	0.02 Rf	441.3 AU	36.60 %	0.07 Rf	10.9 AU	11689.1 AU	34.75 %
2	0.07 Rf	12.1 AU	0.09 Rf	62.0 AU	5.14 %	0.09 Rf	56.3 AU	717.8 AU	2.13 %
3	0.09 Rf	56.5 AU	0.10 Rf	65.4 AU	5.43 %	0.12 Rf	0.2 AU	696.0 AU	2.07 %
4	0.13 Rf	0.9 AU	0.17 Rf	96.2 AU	7.98 %	0.20 Rf	0.8 AU	1912.8 AU	5.69 %
5	0.23 Rf	0.3 AU	0.26 Rf	50.6 AU	4.20 %	0.28 Rf	25.7 AU	1010.5 AU	3.00 %
6	0.29 Rf	26.0 AU	0.33 Rf	39.4 AU	3.27 %	0.38 Rf	1.2 AU	1456.3 AU	4.33 %
7	0.39 Rf	0.2 AU	0.45 Rf	49.0 AU	4.07 %	0.49 Rf	17.5 AU	1484.3 AU	4.41 %
8	0.49 Rf	17.7 AU	0.56 Rf	44.0 AU	3.65 %	0.58 Rf	40.1 AU	1874.2 AU	5.57 %
9	0.58 Rf	40.3 AU	0.62 Rf	66.4 AU	5.50 %	0.66 Rf	26.2 AU	2206.0 AU	6.56 %
10	0.66 Rf	26.6 AU	0.73 Rf	280.8 AU	23.29 %	0.80 Rf	0.1 AU	10358.0 AU	30.79 %
B 11	0.92 Rf	0.9 AU	0.96 Rf	10.6 AU	0.88 %	0.99 Rf	0.0 AU	234.7 AU	0.70 %



C

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	14.5 AU	0.03 Rf	344.2 AU	25.35 %	0.07 Rf	47.4 AU	11202.4 AU	25.15 %
2	0.07 Rf	48.2 AU	0.08 Rf	78.5 AU	5.79 %	0.11 Rf	1.7 AU	1621.0 AU	3.64 %
3	0.12 Rf	0.2 AU	0.16 Rf	96.5 AU	7.11 %	0.19 Rf	0.8 AU	1903.7 AU	4.27 %
4	0.20 Rf	0.1 AU	0.24 Rf	71.6 AU	5.28 %	0.26 Rf	37.0 AU	1365.1 AU	3.07 %
5	0.26 Rf	37.4 AU	0.30 Rf	55.9 AU	4.12 %	0.34 Rf	0.2 AU	1907.4 AU	4.28 %
6	0.35 Rf	0.1 AU	0.43 Rf	62.1 AU	4.57 %	0.46 Rf	16.7 AU	1741.0 AU	3.91 %
7	0.46 Rf	16.7 AU	0.53 Rf	63.2 AU	4.66 %	0.55 Rf	61.3 AU	2311.8 AU	5.19 %
8	0.55 Rf	61.4 AU	0.59 Rf	118.3 AU	8.72 %	0.63 Rf	43.6 AU	4121.9 AU	9.25 %
9	0.63 Rf	43.8 AU	0.71 Rf	424.8 AU	31.29 %	0.78 Rf	0.3 AU	17191.7 AU	38.60 %
10	0.79 Rf	0.2 AU	0.82 Rf	19.0 AU	1.40 %	0.87 Rf	0.1 AU	454.4 AU	1.02 %
D 11	0.89 Rf	0.0 AU	0.94 Rf	23.4 AU	1.72 %	0.97 Rf	13.5 AU	716.6 AU	1.61 %

FIG. 4: HPTLC FINGERPRINT PROFILES AND RFTABLES OF 5 µL AND 10 µL ETHANOL EXTRACT AT 574 nm

FT-IR Analysis: The results of FT-IR peak values and functional groups are represented in **Table 4**. The FT-IR spectrum profile is illustrated in **Fig. 5**. The FT-IR gave a broad peak at 3293.53 and 2649.06 cm^{-1}, which indicated the presence of O-H stretching. It gave a strong peak at 2916.76 cm-1 showing C-H stretching. Peaks at 921.06, 863.03, 820.93, and 779.72 $cm^{-1,}$ which indicated the presence of C-H bending. The peaks obtained at 1709.89 and 1044.64 cm^{-1} indicated the presence of C=O stretching. The peak obtained at 811.56 cm^{-1} indicated the presence of C-H bend out of the plane. The peak obtained at 1415.54 cm^{-1} indicated the presence of C=C stretching, which was medium and weak showing multiple bands. The FT-IR spectrum confirmed the presence of alcohols, phenols, alkanes, ketones, in methanolic extract of *L. cylindrica*. The FTIR analysis revealed the presence of polyphenols and flavonoids due to O-H stretching, terpenes due to the C-H group. The functional groups present in test plant are aldehydes, alkenes, amines, amides, alcohols, phenols, aromatics, carboxylic acids and anhydride, esters and lactones, ethers, quinines, and organic halogen compounds. All these compounds belong to secondary plant metabolites as per researcher explanations [9, 10]. These were confirmed by the FT-IR spectrophotometer study that predicted the presence of the groups: O-H, C=H, C-H, and C=O, and C=C stretching. The presence of characteristic functional groups of carboxylic acids, anhydrides, alcohols, phenols, amines, amides, esters, ethers, sulphur derivatives, glycosides, and carbohydrate could be responsible for the various medicinal properties of *Luffa cylindrica*.

TABLE 4: PEAK FREQUENCIES AND FUNCTIONAL GROUPS IN THE FTIR SPECTRA OF *L. CYLINDRICA*

Peak frequency(cm^{-1})	Functional group	Functional group name	Type of vibration	Intensity
3293.54	O-H	Hydroxyl	Stretch, H bonded	Strong, broad
2916.76	C-H	Alkane	Stretch	Strong, very broad
2649.06	O-H	Hydroxyl	Stretch	Strong
1709.89	C=O	Keto	Stretch	Strong
1415.54	C=C	Alkene	Stretch	Medium-weak, multiple bands
1044.64	C=O	Keto	Stretch	Strong
921.01	=C-H	Alkane	Bending	Strong
863.03	=C-H	Alkane	Bending	Strong
820.93	=C-H	Alkane	Bending	Strong
779.72	=C-H	Alkane	Bending	Strong

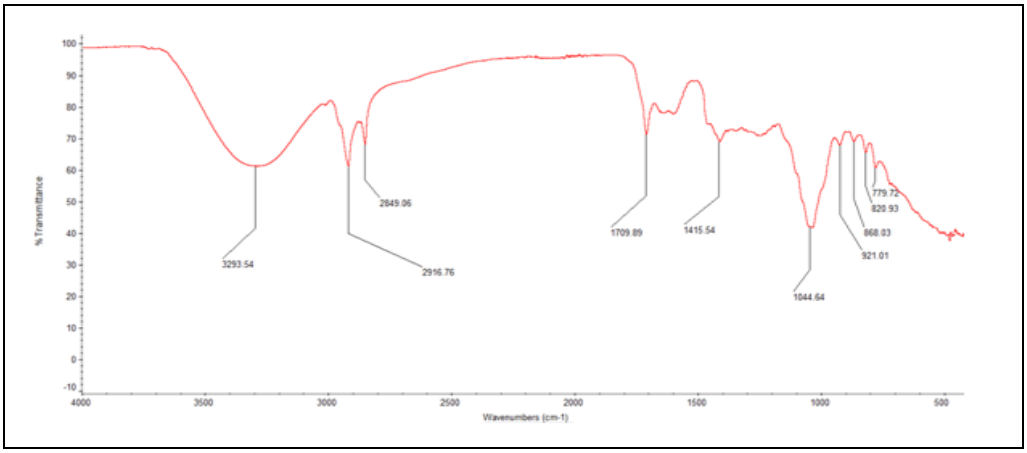


FIG. 5: FTIR PEAK VALUES AND FUNCTIONAL GROUPS OF METHANOLIC EXTRACT OF *LUFFA CYLINDRICA*

CONCLUSION: In the present study, phyto-chemical evaluation of *L. cylindrica*, an underutilized vegetable with medicinal properties, was carried out. From the preliminary phytochemical analyses, it is clear that ethanolic extract contains 19 major compounds. Further, HPTLC analysis confirmed the presence of 10 compounds less than 575 nm UV light. A detailed analysis of ethanolic extract for the characterization of compounds and validation of the pharmacological activities of *L. cylindrica* using the FTIR spectroscopic technique showed that the presence of phenolic compounds and flavonoids, which can be isolated and further screened for

different kind of biological activities depending on their therapeutic uses. Also, research will be needed to find out the structural analysis of flavonoid compounds by the use of different analytical methods such as NMR and Mass Spectrophotometer.

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