International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Physico-Chemical Parameters and Hptlc Qualitative Analysis of Leaf Extracts of *syzygium Cumini*

Bhagwan Singh[1*], Rajasekaran. S[2]

[1]Department of Pharmacology, Research Scholar, Institute of Pharmaceutical sciences and Research Centre, Bhagwant University, Sikar Road, Ajmer, Rajasthan, India – 305004 (Corresponding author)

Email**:** *bhagwanjaisalmer[at]gmail.com[at]gmail.com*
Mobile: +91 89044 49201

[2]Department of Pharmacology, Institute of Pharmaceutical Sciences and Research Centre, Bhagwant University, Sikar Road, Ajmer, Rajasthan, India – 305004

Abstract: ***Objective: This study aimed to evaluate the physico-chemical parameters and hptlc qualitative analysis of leaf extracts of syzygiumcumini. Methods: The shade dried parts of the plant leafs were subjected for the study of physico-chemical parameters and accurate HPTLC method by using Rutin, Gallic acid and quercetin as a standard marker compound with mobile phase of Toluene-Ethyl Acetate-Formic acidMethanol (3: 6: 1.6: 0.4). The detection of Rutin, Gallic acid and quercetin were performed at 254 nm respective. Results: The effect of Syzygiumcumini having significant foreign matter2%, Moisture content3%, Total ash5-6%, Acid insoluble ash0.85%, Ethanol soluble extractive value1.45% and Water soluble extractive value1.35%. The detection of Rutin, Gallic acid and quercetin were performed at 254 nm respectively. Conclusion: It is concluded from this study of physico-chemical parameters and hptlc qualitative analysis of syzygiumcumini possess significant effects.***

Keywords: *Syzygiumcumini*, physico-chemical parameters, hptlc, Rutin, Gallic acid and quercetin

1. Introduction

India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value [1, 2]. Hence, natural products from medicinal plants need to be investigated by scientific methods for their anti-oxidant activity. The plant Acalypha communis is a synonym of Ricinocarpus communis (Müll. Arg.) belonging to the family of Euphorbiaceae. It includes herbs, shrubs and small trees, Shrubs or suffrutex frequently with resinous bright droplets on leaves and inflorescences; indumentum of simple or glandular hairs. Inflorescences spicate, usually unisexual [3].

2. Methods

Chemicals and reagents

All chemicals, reagents and solvents used in the study were of analytical grade.

Collection, Identification and Authentication of the Plant.

The leaves of *Syzygiumcumini*, were collected from the malappuramdistric, kerala, India, during the month of October 2013. The plant materials were identified and authenticated by Dr. Pradeep, Botanist, Calicut University, Kozhikode. The voucher herbarium specimen (SyzygiumcuminiLinn.2013/7) has been deposited in the department of botany.

Loss on drying

Loss on drying is the loss of mass expressed as percent w/w, the prescribed quantity of the fresh plant materials of the two plants were shade dried and the percentage of loss on drying the fresh plant materials was calculated, individually. The results were tabulated in Table no.1

Table 1: The percentage of loss on drying of the fresh plant materials

S. No	Name and parts of the plant	Fresh (kg)	Dried (kg)	Percentage loss on drying
1.	*Syzygiumcumini*	8	6	25

Physico-Chemical Parameters [4-10]

The shade dried parts of the plants were subjected for the study of physico-chemical parameters like:

1) Foreign matter,
2) Moisture content,
3) Total ash value,
4) Acid insoluble ash value
5) Ethyl alcohol soluble extractive value and
6) Water soluble extractive value.

1) Determination of foreign matter

Medicinal plant materials should be entirely free from visible sign of contamination by moulds or insects, and other animal contamination, including animal excreta. No abnormal odour, discoloration, slime or signs of deterioration should be detected. It is seldom possible to obtain marketed plant materials that are entirely free from harmful foreign matter or residue.

Macroscopic examination can conveniently be employed for determining the presence of foreign matter in whole or cut plant materials. However, microscopy is indispensable.

Procedure: 100 g of the plant sample to be examined was weighed and spread out in a thin layer. The foreign matter was detected by inspection with the unaided eye. Separated and weighed and calculated the percentages present.

2) Determination of moisture content

Determination the amount of volatile matter (i. e., water drying off from the drug) in the drug is a measure of loss on drying for substances appearing to contain water as the only volatile constituent, the procedure given below was followed.

Procedure: Accurately weighed drug was placed (without preliminary drying) in a tarred evaporating dish. The drug was dried at 105˚C for 5 hours, and weighed; percentage moisture content was calculated with reference to initial weight.

The percentage of moisture was calculated using the following formula:

$$\text{Moisture percentage} = \frac{Pw-Fw \times 100}{w}$$

Where Pw = pre weight of the sample
Fw = final constant weight of the sample
W = total weight of the sample

3) Ash values

Ash values were determined to estimate the total amount of the inorganic salts present in the drug. This includes total ash, and acid insoluble ash.

The ash remaining followed on ignition of plant materials is determined by two different methods, which measure total ash and acid insoluble ash.

Total ash

The method is designed to measure the total amount of material remaining after ignition. This includes both 'physiological ash', which is derived from the plant tissue itself, and 'non-physiological ash' which is the residue of the extraneous matter (e. g. sand and soil) adhering to the plant surface.

Procedure: 2 gm of ground air-dried material was accurately weighed in a previously ignited and tarred crucible. The material was spread as an even layer and ignited by gradually increasing the heat to 500-600˚C until it became white, indicating the absence of carbon, cooled in a desiccator and weighed. If carbon free ash could not be obtained in this manner, the crucible was cooled and the residue moistened with about 2 ml of water or saturated solution of ammonium nitrate, dried on a water bath and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes & weighed then without any delay. The content of total ash in mg/g of air dried material was calculated. The percentage total ash was calculated according to the following formula.

$$\text{Total ash percentage} = \frac{Pw-Fw \times 100}{W}$$

Where Pw = Pre weight of the crucible
Fw= Final weight of the crucible
W= Total weight of powdered plant material.

4) Acid insoluble ash

The residue was obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measured the amount of silica present, especially as sand and siliceous earth that may be added at the time of collection.

Procedure: To the crucible containing total ash, 25 ml of hydrochloric acid was added, covered with a watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes and then weighed without delay. The content of acid-insoluble ash in mg/g of air-dried material was calculated.

The percentage of acid insoluble ash was calculated according to the formula:

$$\text{Acid insoluble ash percentage} = \frac{Pwb-Fwa \times 100}{W}$$

Where Pwb = Final weight of the crucible with total ash
Fwa = Final weight of the crucible with acid insoluble ash
W = Total weight of powdered plant material.

5) Extractive values

These are used to determine the amount of matter soluble in the solvents used, which include alcohol soluble extractives and water-soluble extractives. Percentage of alcohol and water-soluble extractives were calculated and used as standards.

6) Determination of alcohol-soluble extractive

***Procedure*:** 5 gm of the air dried coarsely powdered drug was macerated with 100 ml of alcohol in a closed conical flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. This was filtered rapidly, taking precautions against loss of solvent evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and dried at 105˚C to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

7) Determination of water-soluble extractive

***Procedure*:** 5 gm of the coarsely powdered air-dried drug was macerated with 100 ml of chloroform water (0.1%) in a closed flask for twenty-four hours, shaking frequently for six hours and allowing to stand aside for eighteen hours. It was filtered rapidly, taking precautions against loss of solvent.25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish and dried at 105˚C to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug. All the tests were done in triplicate.

The results of the data were tabulated in table no: 2

Preparation of the Ethanolic extract of dried leaves of *Syzygiumcumini*:
The granulated dried leaves of *Syzygiumcumini* (500 g) was packed in a Soxhlet apparatus and subjected to continuous hot percolation for using 450 ml of ethanol (95 % v/v) as solvent. The extract was concentrated to dryness under reduced pressure and controlled temperature and dried in a desiccator (yield 75 g, 15 % w/w). The extract was suspended in 5 % gum acacia and used for further experiments.

Hptlc Studies for Qualitative Analysis [11, 12]

Instrumentation
In the present work Camag HPTLC system equipped with Linomat 5 applicator, twin trough chamber (20x10cm, 0.2 mm thick) size, TLC scanner 3, Reprostar 3 with 12bit CCD camera for photo documentation, controlled by WinCATS- 4 software were used. All the solvents used were of high grade obtained from MERCK. All weighing were done on Precisa XB 12A digital balance.

Preparation of standards and sample solution:
2mg in 10 ml ➔ 0.2 mg /ml ➔ 200µg/1000µl ➔0.2µg/µl

Mobile phase
The organic solvents such as toluene**:** ethylacetate**:** formic acid**:** methanol (3: 6: 1.6: 0.4) was used as a mobile phase

Chamber used for mobile phase
Camag twin trough chamber (20 x 10 cm)

Chamber saturation
Chamber saturation was done for 5 minutes.

Stationary phase
TLC aluminum sheet precoated with silica gel 60 F254, (20x10cm) was used as stationary phase, obtained from MERCK.

Procedure
The Ethanolic extract (sample) solutions were prepared. The TLC plate was activated by heating at 1200C for about 30 min prior to use. Ethanolic extract solutions (2 µl), standard solutions (0.2µg/µl) were applied in duplicate, as tracks 8, with a band length of 6.0 mm each on a precoated silica gel 60 F254 TLC plate, with Linomat V applicator using a Hamilton syringe (100µl). Mobile phaseusedtoluene: ethylacetate: formicacid: methanol (3: 6: 1.6: 0.4). No prewashing of the plate was done. Chamber saturation time was 5 minutes. The TLC plate was kept for development to a migration distance of 77 mm. Post derivatization had been done with vanillin-phosphoric acid. The derivatized plate was dried in hot air oven at 60 ^{0}c for 5 minutes and scanned at 254 nm, band length 6.0 mm, slit dimension 6.00x0.45mm, micro, scanning speed (20mm/s) and source of radiation was Deuterium and Tungsten lamps respectively. The Rf and peak area of the spots were interpreted by using software. The derivatized plate was photo documented under 254 nm light using CamagReprostar 3, equipped with 12-bit CCD camera

3. Results and Discussion

Table 2: Physico-chemical parameters (after shade drying) of the plant

S. No	Name of the plant and part used	Foreign matter	Moisture content	Total ash	Acid insoluble ash	Et. oH soluble extractive value	Water soluble extractive value
1.	*Syzygiumcumini (leaves)*	2%	3%	5-6%	0.85%	1.45%	1.35%

Table 3: Observation of Rf values and % area of the chromatogram of rutin gallic acid at 254nm (std)

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	- 0.01	22.8	0.02	89.9	8.13	0.07	1.4	4.57	4.57	Unknown*
2	0.08	0.3	0.13	147.1	13.30	0.21	14.2	1.26	14.26	**Rutin**
3	0.21	14.4	0.24	45.0	4.07	0.25	43.0	1.84	1.84	Unknown*
4	0.25	43.5	0.26	54.1	4.89	0.32	0.0	1.82	1.82	Unknown*
5	0.37	9.1	0.38	10.5	0.95	0.41	0.1	0.30	0.30	Unknown*
6	0.45	3.2	0.46	12.3	1.11	0.49	1.6	0.33	0.33	Unknown*
7	0.55	1.6	0.59	19.7	1.79	0.60	19.2	0.66	0.66	Unknown*
8	0.64	14.3	0.70	320.3	28.96	0.74	123.4	22.06	22.06	**Gallic acid**
9	0.74	124.4	0.84	391.1	35.36	0.96	0.1	53.99	53.99	**Quercetin**
10	0.97	0.8	0.98	15.9	1.43	0.98	3.7	0.18	0.18	Unknown*

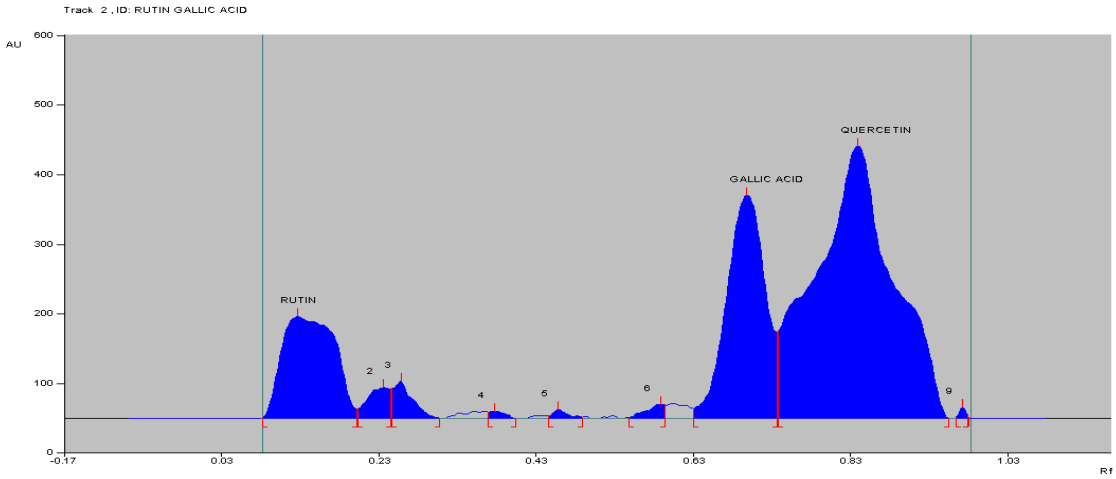


Figure 1: Observation of Rf values and %area of the chromatogram of rutin gallic acid at 254nm (std)

Table 4: Observation of Rf values and %area of the chromatogram of Quercetin at 254nm (std)

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	- 0.01	4.5	0.02	117.0	13.98	0.07	40.7	3590.9	13.87	Unknown*
2	0.18	17.3	0.19	19.4	2.32	0.24	0.0	417.1	1.61	Unknown*
3	0.28	3.2	0.30	16.1	1.93	0.32	0.9	171.7	0.66	Unknown*
4	0.32	2.2	0.33	18.6	2.22	0.34	0.0	90.3	0.35	Unknown*
5	0.38	0.0	0.40	12.1	1.44	0.41	2.9	128.5	0.50	Unknown*
6	0.41	3.1	0.44	20.3	2.43	0.47	12.5	396.1	1.53	Unknown*
7	0.47	13.6	0.47	23.1	2.76	0.49	2.5	161.9	0.63	Unknown*
8	0.50	0.6	0.51	19.0	2.27	0.54	0.0	199.5	0.77	Unknown*
9	0.55	0.7	0.56	22.0	2.63	0.58	2.6	236.9	0.91	Unknown*
10	0.70	16.3	0.74	37.2	4.44	0.75	26.7	656.5	2.54	Unknown*
11	0.75	16.3	0.85	532.0	63.57	0.96	0.2	19845.0	76.64	**Quercetin**

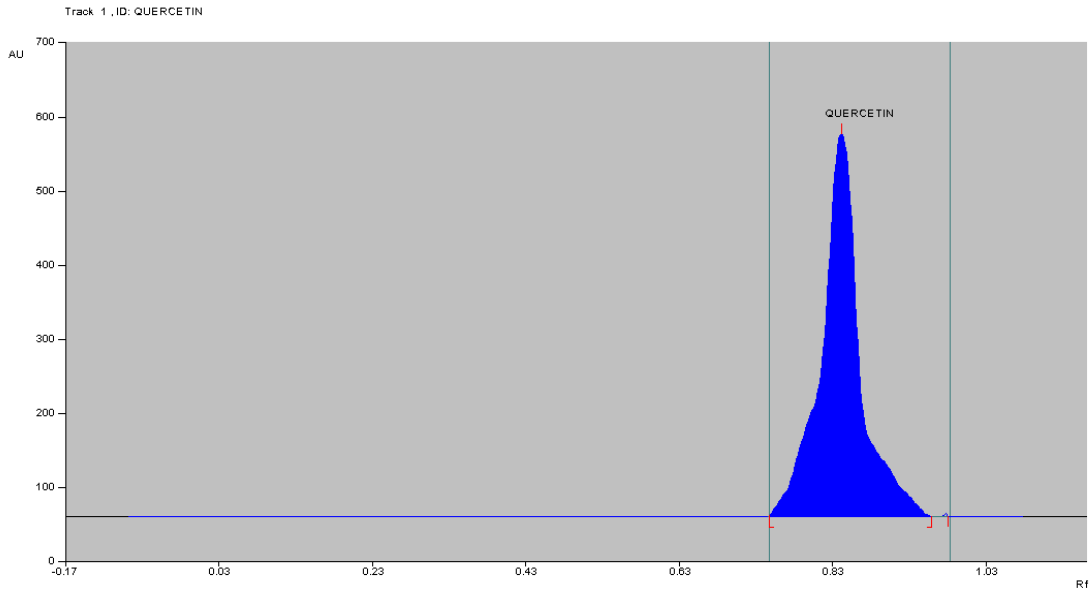


Figure 2: Observation of Rf values and %area of the chromatogram of Quercetin at 254nm (std)

Table 5: Observation of Rf values and %area of the chromatogram of ethanolic extract of *Syzygiumcumini*

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	- 0.01	1.6	0.02	155.3	21.94	0.04	143.0	3375.4	17.12	Unknown*
2	0.04	143.8	0.05	153.4	21.68	0.11	71.6	4915.2	24.93	Unknown*
3	0.12	71.6	0.13	79.4	11.23	0.15	64.6	1452.0	7.36	**Rutin**
4	0.15	64.7	0.19	98.4	13.91	0.25	30.7	4073.8	20.66	Unknown*
5	0.27	27.0	0.29	29.9	4.22	0.33	16.6	1003.7	5.09	Unknown*
6	0.34	15.6	0.35	17.5	2.47	0.38	4.5	279.7	1.42	Unknown*
7	0.39	5.1	0.44	34.8	4.91	0.49	4.5	1202.6	6.10	Unknown*

8	0.77	8.4	0.82	60.0	8.48	0.83	52.5	1169.7	5.93	Unknown*
9	0.83	52.3	0.86	67.1	9.48	0.93	7.8	2091.2	10.60	**Quercetin**
10	0.93	8.1	0.95	11.9	1.68	0.97	0.0	156.2	0.79	Unknown*

Figure 3: Observation of Rf values and %area of the chromatogram of ethanolic extract of *Syzygiumcumini*

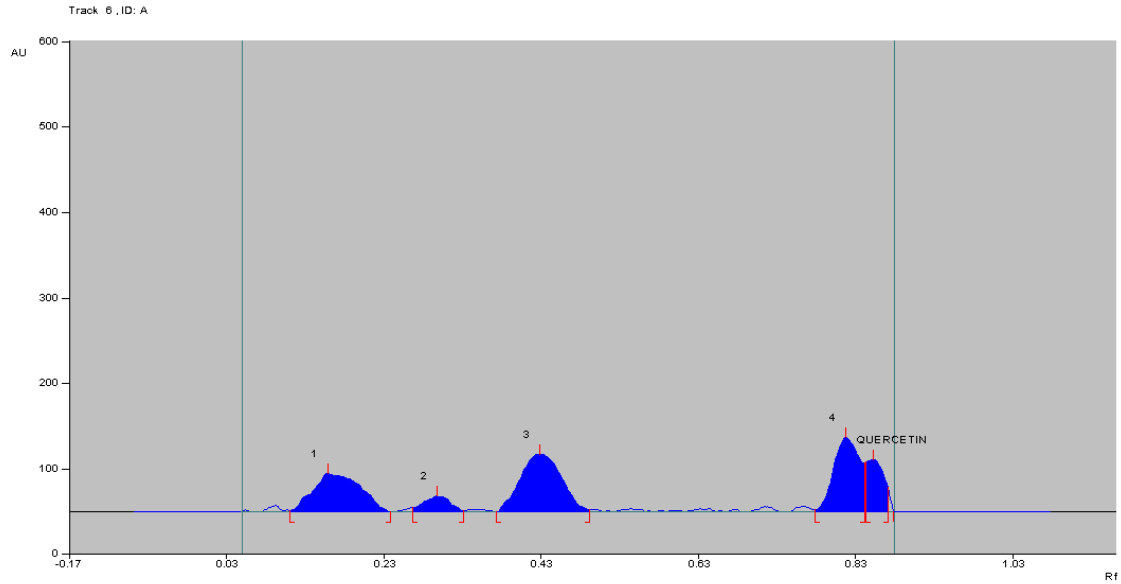
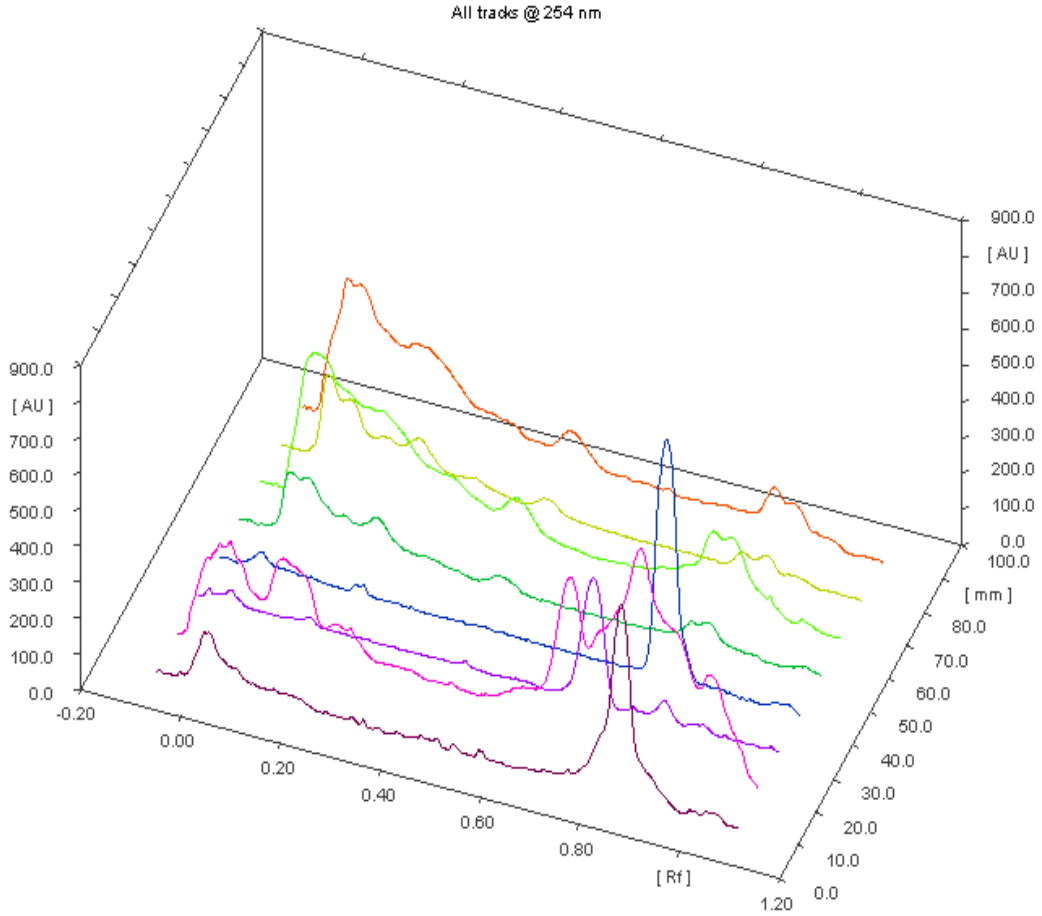


Figure 4: 3D HPTLC Chromatography (Std and test extract)



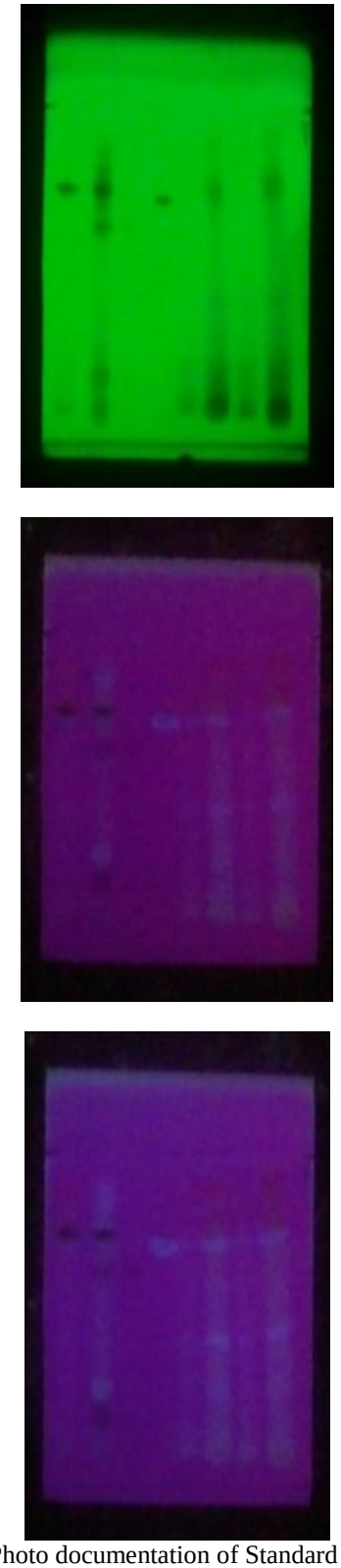


Figure 5: Photo documentation of Standard and samples

4. Conclusion

The present study, the ethanol extract of *Syzygiumcumini* plant leaves gave the evidence of the rutin, quercetion, and galic acid bands are identified and confirmed by comparing the chromatogram obtained from the reference standard flavonoid markers

References

[1] Sandhu DS, Heinrich M (2005). The use of health foods, spices and other botanicals in the sikh community in london. Phyto-therapy res.19: 633-42.)
[2] Gupta, M. P., Solis P. N., Calderon, A. I., Guionneau Sinclair, F., Correa, M., Galdames, C. Guerra, Espinosa, A., Alvenda, G. I., Robles, G., Ocampo, R (2005) Medicinal ethnobotany of the teribes of bocasdel toro, panama. J Ethnopharmacol, 96, 389-401.
[3] Hayden, W. J. & Hayden, S. M.2000. Wood anatomy of Acalyphoideae (Euphorbiaceae). I. A. W. A. Bull.21: 213–235
[4] Ghildiyal Shivani, Gautam MK, Goel RK, Joshi VK. Qualitative phytochemical screening and pharmacognostic standardization of Solanum anguivi Lam root. Asian Pacific Journal of Tropical Biomedicine 2012, 1-4.
[5] Khandelwal KR. Practical Pharmacognosy, Techniques and Experiments, Niraliprakashan, Pune.16th Edn 2003, 157-166.
[6] Evans WC Trease, Evans Pharmacognosy. Elsevier Pub., New Delhi, India.15th Edn, 2006, 538-547.
[7] Wallis DE. Textbook of Pharmacognosy. CBS Publisher and Distributors, New Delhi.5th Edn 1996, 299-301.
[8] European Commission. London: The Stationary Office on Behalf of the Medicines and Healthcare Products Regulatory Agency; 2010. Anonymous. The British Pharmacopoeia Commission. British Pharmacopoeia; p. A-2.88-2.89.
[9] Geneva: Office of the Publications, World Health Organization; 1998. Anonymous. Quality Control Methods for Medicinal Plant Materials; pp.8–46.
[10] 1st ed. Vol.3. New Delhi: Ministry of Health and Family Welfare; 2001. Anonymous. Government of India, Ministry of Health and Family Welfare. The Ayurvedic Pharmacopoeia of India. Part-1; pp.233–51.
[11] Rajasekaran. A, Arivukkarasu. R. and Archana. D. * HPTLC Method for estimation of Gallic acid and Rutin in Haritaki-An Ayurvedic Formulation.2011; Vol.3, No.2, pp 986-999.
[12] P. Deepa*, S. Murugesh, R. Arivukkarasu HPTLC analysis of Dolichandrone atrovirens (sprague) plant bark.2013; vol3 (1) 1019-1029