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Pharmacognostic And Phytochemical Studies Of Leaves Of *Syzgium Cumini*

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Abstract

The aim of the current research is to develop different Pharmacognostic and phytochemical analysis for the evaluation of leaf of this plant. Microscopy, Phytochemical analysis of leaf drug was performed and results were maintained. Phytochemical tests indicated the occurrence of flavonoids, tannins, steroids and phenols etc. The results obtained from standardization of leaf drug recognized the macro and microscopical parameters, physicochemical parameters. Therefore, these techniques are helpful for rapid recognition of crude drug. The outcome of the research study possibly will be helpful in setting investigative indices for the identification and grounding of a monograph of the plant drug.

Keyword: microscopy, morphology, phytochemical screening, Syzgium cumini

INTRODUCTION:

Medicinal plants are one of the sources of natural products for the treatment and management of debilitating diseases. The use of plant extracts and isolated pure compounds has provided the basis for the production of herbal medicines and phytopharmaceutical compounds[1]. For a healthcare product to be globally accepted, it must be scientifically validated to ascertain its level of purity, potency, efficacy and safety[2,3]. The World Health Organization has provided standard parameters to assess the quality, safety, and efficacy of herbal plant including physicochemical and phytochemical evaluation of crude drugs[4]. Various steps are involved in setting these pharmacognostic standards for the purpose of formulating a monograph of a crude drug. The quality assessment of medicinal plants is of great importance in order to justify their acceptability in conventional system of medicine[5]. Uniformity of quality is promoted by the use of standards which are numerical qualities by which the quality of herbs may be assessed.

The genus Syzygium is one of the genera of the myrtle family Myrtaceae which is native to the tropics, particularly to tropical America and Australia. It has a worldwide, although highly uneven, distribution in tropical and subtropical regions[6]. The genus comprises about 1100 species, and has a native range that extends from Africa and Madagascar through southern Asia east through the Pacific. Jambolan is a large evergreen and densely foliaceous tree with greyish-brown thick bark, exfoliating in woody scales[7]. The wood is whitish, close grained and durable; affords brown dyes and a kind of a gum Kino. The leaves are leathery, oblong-ovate to elliptic or obovate-elliptic with 6 to 12 centimeters long (extremely variable in shape, smooth and shining with numerous nerves uniting within the margin), the tip being broad and less acuminate[8].

Plant drugs usually adulterated with similar looking drugs therefore it is mandatory to use plant drugs after standardization for research work. This research paper deals with macroscopical characteristics, microscopy and physiochemical of leaves of jamun plant. In addition, it also consists of phytochemical screening of successive extracts of the Jamun plant [9].

MATERIALS AND METHODS

Plant Collection and Authentication: The leaves of *Syzygium cumini* were collected from plants growing in the Jhansi district during the months of January-February, 2021. It was then authenticated from Central Ayurvedic Research Institute, Jhansi. An herbarium specimen bearing voucher No. NBI/PH/1311 has been deposited in the Department of Pharmacognosy, Institute of Pharmacy, Bundelkhand University, Jhansi, Uttar Pradesh, India. The leaves were dried at 40 °C for 15 days, then it was blended into coarse powder by electrical grinder. The powdered drug was passed through sieve No. 22 to get uniform particle size [10, 11, 12].

Macroscopic Evaluation: Fresh plant leaves of *Syzygium cumini* was subjected to color, odor and taste, determination of shape, size, surface characteristics and appearance etc.

Microscopic evaluation: For microscopical examinations, free hand sections of the leaves of *Syzygium cumini* weas cut, cleared with chloral hydrate solution and water, and stained with a drop of hydrochloric acid and phloroglucinol. Photomicrographic images were taken by using Trino CXR camera [13].

Proximate analysis[14, 15]:

Proximate analysis of powdered plant material of *Syzygium cumini* leaves was carried out using reported methods. Following determinations were done

- ❖ Loss on drying
- ❖ Total ash
- Acid insoluble ash
- Water soluble ash
- ❖ Extractive value
- Alcohol soluble extractives
- Water soluble extractives
- ❖ Foaming index

Loss on Drying: Placed about 2 gm of the air-dried plant material, accurately weighed in a previously tarred flat weighing bottle. Dried the sample by heating in an oven at 100-105º C for 5 hrs. Dried until two consecutive weighing was not differed by more than 5 mg, unless otherwise specified in the test procedure. Then calculate the loss of weight in mg/gm of the air-dried material.

Determination of Ash Value: The ash remaining following ignition of powder plant material was determined by three different methods which measures total ash, acid insoluble ash, water soluble ash.

I) Total Ash: The total ash measures 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 residue of the extraneous matter adhering to the plant surface.

About 4 g of the ground air-dried powdered material was accurately weighed, in previously ignited and tarred silica crucible. The material was spreaded in an even layer and ignited by gradually increasing the heating to 500-600 ˚C until it was white, indicating the absence of carbon. It was allowed to cool in a desiccator and weighed. Content of total ash was calculated in form of mg per gm of air-dried material.

II) Acid Insoluble Ash: Acid insoluble ash measures the amount of silica present, especially as sand and salicaceous earth. In crucible containing the total ash, 25 ml of 2N hydrochloric acid was added, crucible was covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot distilled water and this liquid was added to the crucible. The insoluble matter on an ashless filter paper was washed with hot water until the filtrate was neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hotplate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes and then weigh. Calculate the content of acid insoluble ash in mg per gm of air-dried material.

III) Water Soluble Ash: In crucible containing the total ash, 25 ml of water was added and boiled gently for 5 min. Insoluble matter was collected on ashless filter paper and washed with hot water until the filtrate was neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite in a crucible for 15 min. at a temperature not exceeding 450 ˚C to constant weight. Allow the residue to

cool in a suitable desiccator for 30 min. and then weigh. Weight of the residue was subtracted from weight of total ash. Calculate the content of water-soluble ash in mg per gm of air-dried material.

Extractive Value: Extractive value determines the amount of active constituents extracted with solvents from given amount of herbal material. It is usually calculated as alcohol soluble extractive value and water-soluble extractive value.

i) Alcohol soluble extractive value: About 5.0 g of coarsely powdered air-dried material was weighed, in a glass-stoppered conical flask. Macerate with 100 ml of the alcohol for 6 hours, shaking frequently, and then allow standing for 18 hours. The content was filtered rapidly. Care was taken during filtration to avoid loss of solvent and 25 ml of the filtrate was transferred to a tarred flat-bottomed dish and evaporated to dryness on a water-bath. Dried the extract at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay. Calculated the content of extractable matter in mg per gm of air-dried material.

ii) Water soluble extractive value: About 5 g. of the air-dried drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours shaken frequently during 6 hours and allowed to stand for 18 hours. The solution was filtered rapidly, 25 ml of filtrate was transferred to tarred flat bottom dish and evaporated to dryness on water bath. Extract was dried at 105 C for 6 hours, cooled in desiccator for 30 minutes and weighed without delay. Content of extractable matter was calculated in mg per g of air-dried material.

Determination of foaming index: Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of foaming index.

Accurately 1 gm of the powdered plant was transferred to a 500 ml conical flask containing 100 ml of boiling distilled water. It was boiled moderately for 30 minutes. The filtrate was cooled and filtered in a 100 ml volumetric flask and sufficient distilled water was added through the filter to dilute to volume. The decoction was poured into 10 stoppered test-tubes (height 16cm, diameter 16mm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml, and the volume of the liquid was adjusted in each tube with water to 10 ml. the tubes were shaken in a lengthwise motion for 15 seconds, two shakes per second. Allowed to stand for 15 minutes and the height of the foam were measured.

Foaming index: 1000 / a

Where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed.

Extraction Procedure[16, 17, 18]:

Petroleum Ether Extraction: Coarse powder of *Syzgium cumini* Linn leaves was extracted with 250 ml of petroleum ether using soxhlet apparatus. The extraction was carried out for 72 hours. After extraction, the solvent was distilled out to obtain a concentrated extract. Then the concentrated extract was vacuum dried and the dry extract was stored in an air tight container for further phytochemical and pharmacological studies.

Ethanol Extraction: Coarse powder of *Syzgium cumini* Linn leaves was extracted with 250 ml of ethanol using soxhlet apparatus. The extraction was carried out for 72 hours. After extraction, the solvent was distilled out to obtain a concentrated extract. Then the concentrated extract was vacuum dried and the dry extract was stored in an air tight container for further Phytochemical and pharmacological studies.

Investigation of Preliminary Phytochemical Studies: The various extracts of *Syzygium cumini* obtained were subjected to qualitative analysis to test the presence of various phytochemical constituents like alkaloids, carbohydrates, glycosides, flavonoids, steroids, amino acid, phenols, proteins, tannins etc[19].

Thin Layer Chromatography: The term "thin-layer chromatography", introduced by E. Stahl in 1956, means a chromatographic separation process in which the stationary phase consists of a thin layer applied to a solid substrate or "support". Thin layer chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) now also called planar chromatography.

Thin-layer chromatography or TLC is a solid-liquid form of chromatography where the stationary phase is normally a polar absorbent and the mobile phase can be a single solvent or combination of solvents [20]. TLC is a quick, inexpensive microscale technique that can be used to:

Solvent System

- Stationary phase - Silica gel G
- Mobile phase - Toluene: Ethyl Acetate: Methanol (7:2:1)
- Detecting agent - visual & UV light

The Rf values were calculated using the formula:

Rf = Distance travelled by solute/ Distance travelled by solvent.

High Performance Thin Layer Chromatography: High performance thin layer chromatography (HPTLC) is an enhanced form of thin layer chromatography (TLC). A number of enhancements can be made to the basic method of thin layer chromatography to automate the different steps, to increase the resolution achieved and to allow more accurate quantitative measurements[21].
Mobile Phase: Toluene: Ethyl Acetate: Formic Acid (8: 2: 0.1 v/v/v)
Saturation Time: 20 minutes
Procedure: Apply 5µL, 6 µL and 7 µL of test solution on different tracks i.e., T1, T2 and T3 over pre-coated silica gel $60F_{254}$ TLC plate (Merck) of uniform thickness (0.2 mm) and develop the plate in the mobile phase upto a distance of 7 cm. Dry the plate and visualize at wavelengths 254 nm and 366 nm. For the derivatization step, dip the plate in Anisaldehyde Sulphuric acid reagent (ASR)and heat at 105°C till the color of the spots/bands appears.
Visualization: Observe the plate (pre and post derivatization) under different wavelengths i.e., UV-254 nm, UV-366 nm and white light to record the fingerprint profile. This reveals several prominent bands of different colors as presented in **Figure 7.8.**

RESULTS AND DISCUSSION

Macroscopic and Organoleptic Evaluation: The image of the leaf of *Syzgium cumini* is shown in **Figure 1**. The detail of macroscopic characters and organoleptic characters are shown in **Table 1**. The description of macroscopic studies is as follows.

Figure 1: *Syzgium cumini* leaves

Table 1: Macroscopical and Organoleptic property of *S. cumini* leaves

S. No.	Plant part and characters	Observation
1	Size	5-18 cm long and 2.5 to 8 cm wide
2	Shape	Oblong-oval or elliptic
3	Margin	Entire
4	Apex	Blunt or tapering to a point
5	Base	Slightly unequal
6	Colour	Dark green and light green
7	Odour	Turpentine like
8	Taste	Slightly astringent

Microscopic Studies: The upper epidermis shows the presence of a single layer of wavy epidermal cells with striated cuticle. Palisade is made up of a single layer beneath upper epidermis in the lamina region and contains compact elongated cells. Spongy parenchyma is 5 to 8 layered. Spheraphide is distinctly visible in the lamina region between palisade cells and spongy parenchyma cells. Midrib shows the presence of collenchyma below the upper epidermis and above the lower epidermis. Vascular bundle (Xylem and phloem) was present in the center. Sclerenchyma is present in between the vascular bundle and collenchyma in the midrib region. Powder under a microscope showed different anatomical characters are shown in **Figure 2**.

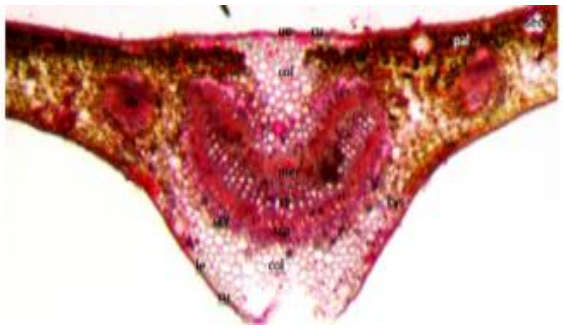

Figure 2: TS of Leaf *Syzgium cumini* (Dorsiventral) Passing Through Midrib at 4X

Upper epidermis (ue) covered with cuticle(cu), followed by single layered elongated palisade layer(pal) disrupted by secretory canal (sec) and spongy cells disrupted by vascular bundle(vb), centrally placed meristele (mer) i.e. forming an arc having lignified xylem(xy) towards upper surface arranged in radiating bands spreading towards lower surface, protoxylem pointing towards upper surface and phloem bands at lower side, vascular bundle covered with sclerenchymatous pericycle bands(scp) and below to that endodermis, lower collenchymas shows deposition of crystal sheath (cry), starch grain (sg), cystolith (cys), below that lower epidermis (le) covered with cuticle(cu).

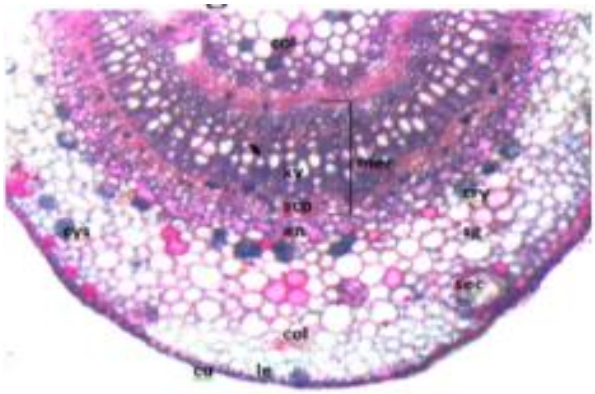

Figure 3: TS of Leaf *(Syzgium cumini)* Passing Through Midrib Lower Region at 10X

Upper collenchyma (col), centrally placed meristele (mer) i. e. forming an arc having lignified xylem (xy) towards upper surface arranged in radiating bands spreading towards lower surface , protoxylem pointing towards upper surface and phloem bands at lower side, vascular bundle covered with sclerenchymatous pericycle bands (scp) and below to that endodermis, lower collenchyma showing deposition of crystal sheath (cry), starch grain(sg), cystolith (cys) and secretory canal (sec), single layered lower epidermis (le) covered with cuticle (cu).

Proximate Analysis: The results obtained from various determinations are compiled in Table **2**. The ash values of a drug gave an idea of the earthy matter, or the inorganic composition and other impurities present along with the drug. The extractive values are primarily useful for the determination of exhausted or adulterated drug.

Table 2: Physicochemical parameters of powder of *Syzgium cumini* leaves

S. No.	Parameters	Values (% w/w)
1	Loss on Drying	7.65
2	Ash Value	
	Total ash	11.76
	Acid insoluble ash	2.67
	Water soluble ash	5.75
3	Extractive value	
	Water soluble extractive	9.6
	Alcohol soluble extractive	5.7
4	Foaming index	<100

The values given here are expressed as percentage of air-dried material. Each value is average of three determinations

Table 3: The extractive values of powder drug

Plant Material	Solvent			
	Water	**Chloroform**	**Ethanol**	**Petroleum ether**
Syzgium cumini	20.65	12.65	15.65	8.86

Table 4: The Ash value of powder drug

Plant Material	Ash Value %w/w		
	Total ash	**Acid insoluble ash**	**Water soluble ash**
Syzgium cumini	10.23	1.09	3.21

The morphological, microscopical and physico-chemical parameters of *Syzgium cumini* leaves can possibly help to differentiate the drug from its other species and the pharmacognostic profile of the plant presented here will assist in standardization viz., quality, purity and sample identification.

Extraction of *Syzgium cumini* Leaves:

Figure 4: Coarse Powder of *Syzgium cumini* Leaves

Table-5: Organoleptic study of Course Powder of *Syzgium cumini* Leaves

S. No.	Parameters	Characteristics features
1	Color	Green
2	Order	Characteristics
3	Taste	Acrid
4	Nature	Coarse powder

Figure.5: Extraction of Drug (*Syzgium cumini*)

Table 6: Extract Characteristics

Types of solvent	Consistency	Colour
Pet. ether	Oily	Dark green
Ethanol	Pasty	Dark green

The percentage yield of ethanolic and Petroleum ether extract of *Syzgium cumini* Linn. leaves was found to be

- Ethanolic extract: 9.65 % w/w.
- Petroleum ether extract: 5.27 % w/w.

Preliminary Phytochemical Study: Preliminary phytochemical screening of Phaseolus vulgaris, revealed the presence of following phytoconstituents.

Figure 6: Phyto-chemical screening of plant extract

Table 7: Test for Carbohydrates

S. No.	Plant constituent's test/reagent used	Pt. ether	Ethanol
1	Molisch's Test	_	+
2	Fehling's	+	+
3	Barfoed's Test	_	_
4	Benedict's test	+	+

Table 8: Test for Alkaloids

S. No.	Plant constituent's test/reagent used	Petroleum ether	Ethanol
1	Meyer's reagent	+	+
2	Dragendroff's reagent	+	+
3	Wagner's reagent	+	+
4	Hager's reagent	_	+
5	Muroxide test	_	+

Table 9: Test for Amino Acids

S. No.	Reagent	Pt. ether	Eethanol
1	Millon's Test	+	+
2	Ninhydrine Test	_	_
3	Biuret Test	+	+
4	Xanthoprotein Test	-	+

Table 10: Test for Saponin

S. No.	Reagent	Pt. ether	Ethanol
1	Foam test	+	-
2	Haemolysis test	_	_

Table 11: Test for Anthraquinone Glycosides

S. No.	Reagent	Pt. ether	Ethanol
1	Baljet' s Test	+	+
2	Legal's Test	_	+
3	Borntrager's Test	+	+

Table 12: Test for Flavonoids

S. No.	Reagent	Pt. ether	Ethanol
1	Shinoda test	_	+
2	II	_	_

Table 13: Test for Gums and Mucilage's

S. No.	Reagent	Pt. ether	Ethanol
1	I	_	+
2	II	_	+

Table 14: Test for Fixed Oils and Fats

S. No.	Reagent	Pt. ether	Ethanol
1	Spot Test	_	_
2	II	_	_

Table 15: Test for Phytosterols

S. No.	Reagent	Pt. ether	Ethanol
1	Liebermann-burchard test	_	_
2	Salkowski reactions	_	+

Table 16: Test for Phenolic Compounds and Tannins

S. No.	Reagent	Pt. ether	Ethanol
1	I	_	_
2	II	+	+
3	III	_	+
4	IV	+	_

+ mean present, -mean absent

Thin Layer Chromatography:

Figure 7: TLC of *Syzgium cumini* leaves extract

The number of spots, R_f value of the same and the colour of the spots under UV light 366nm and visible light is presented in Table 7.17 and the photograph of the plate is presented in Figure 7.5.

Table 17: Phytochemical evaluation of ethanolic extract of *Syzgium cumini* by TLC studies

Solvent system	Detecting agent	No. of spots	Colour of spots	Rf values
Toluene: ethyl acetate: methanol (7: 2 :1)	Under UV at 366nm	6	Orange	0.45
			Dark orange	0.44
			Dark red	0.38
			Light pink	0.37
			Light pink	0.32
			Light pink	0.29
	Under Visible light	6	Yellow	0.49
			Brown	0.42
			Dark green	0.39
			Yellowish green	0.38
			Yellowish green	0.34
			Yellow	0.31

The extract showed 6 spots at 366nm and 6 spots at visible light. The Rf value of 0.45, 0.42 and 0.38 may be due to the presence of flavonoids, phenolic compounds and tannin. When viewed under UV at 366m and visible light after development in the mobile phase namely Toluene: ethyl acetate: methanol (7: 2 :1).

High Performance Thin Layer Chromatography: Observe the plate (pre and post derivatization) under different wavelengths i.e., UV-254 nm, UV-366 nm and white light to record the fingerprint profile. This reveals several prominent bands of different colors as presented in **Figure 8.**

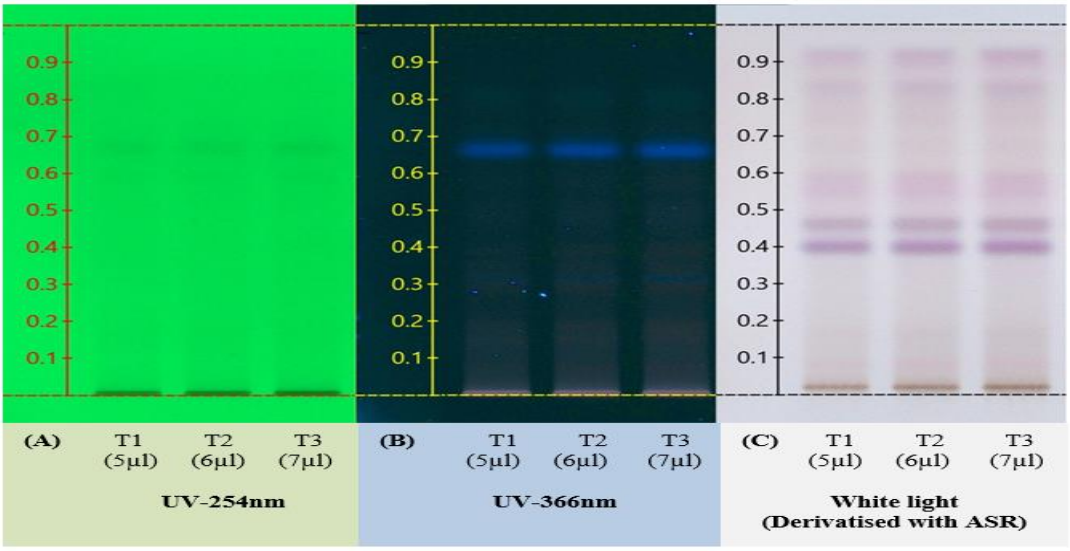

Figure: 8: HPTLC chromatograms of *Syzygium cumini* (Leaves) under UV 254 nm, UV 366 nm, and after derivatization (under white light).

Table 18: Rf value of the spots at 254 and 366nm

S. No.	254 nm			366 nm			White light (Derivatised with ASR)		
	5 µl	6µl	7µl	5 µl	6 µl	7 µl	5 µl	6 µl	7 µl
1.	0.007	0.007	0.007	0.007	0.007	0.007	0.018	0.018	0.018
2.	0.673	0.675	0.78	0.661	0.661	0.660	0.398	0.396	0.398
3.	-	-	-	-	-	-	0.456	0.452	0.453
4.	-	-	-	-	-	-	0.551	0.552	0.554
5.	-	-	-	-	-	-	0.832	0.825	0.827
6.	-	-	-	-	-	-	0.916	0.915	0.915
7.	-	-	-	-	-	-	0.833	0.831	0.829
8.	-	-	-	-	-	-	0.902	0.907	0.905

HPTLC Densitometry Scan Profile and Photo-documentation: The HPTLC densitogram of pre and post derivatized plates under the above-mentioned visualization wavelengths revealed several peaks as shown in

Figure 9-14.

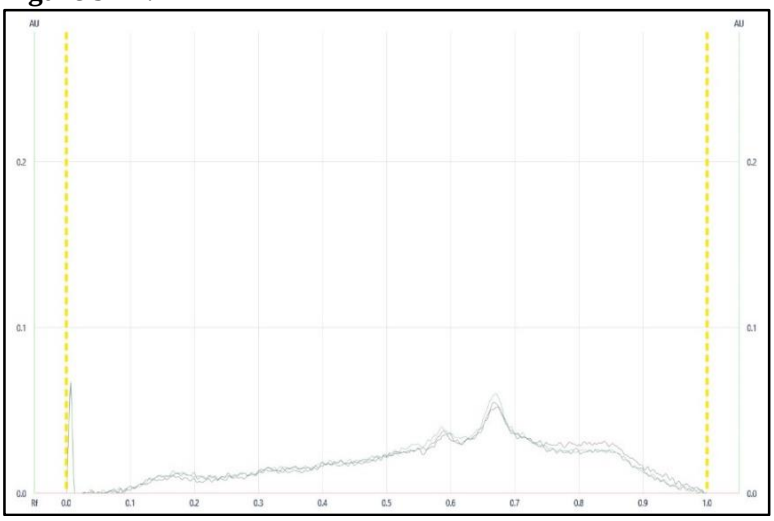

Figure 9: 2D Densitogram at UV 254 nm

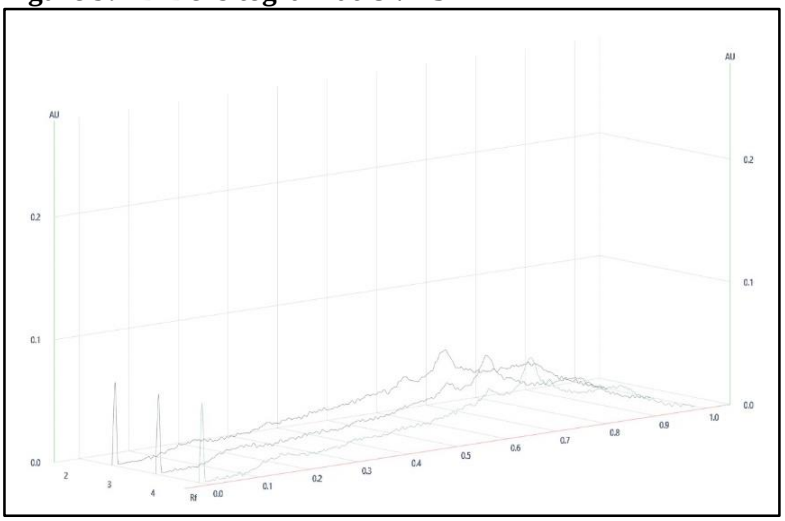

Figure 10: 3D Densitogram at UV 254 nm

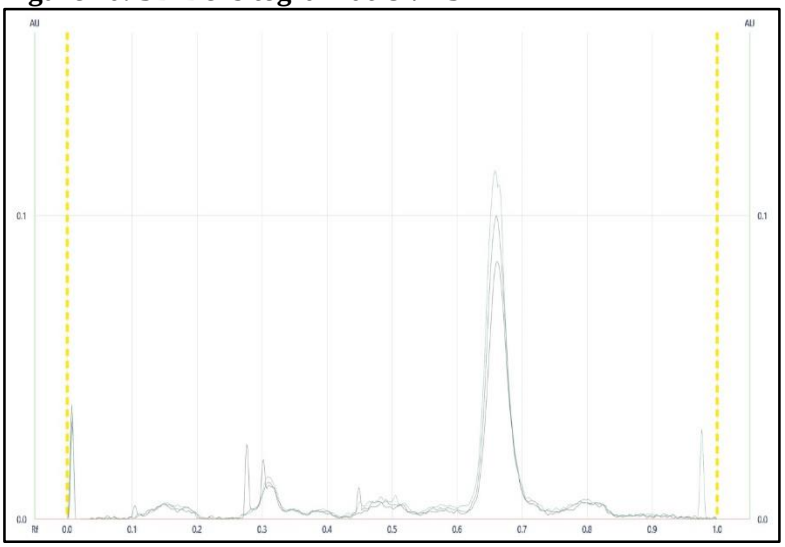

Figure 11: 2D Densitogram at UV 366 nm

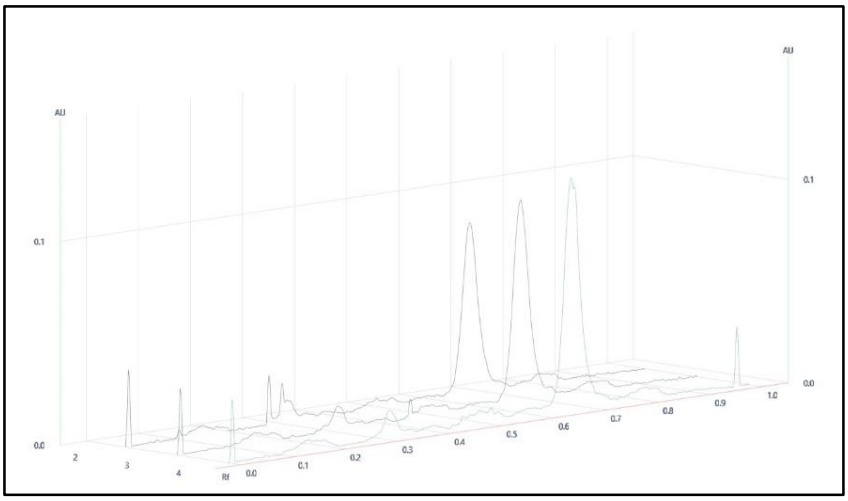

Figure 12: 3D Densitogram at UV 366 nm

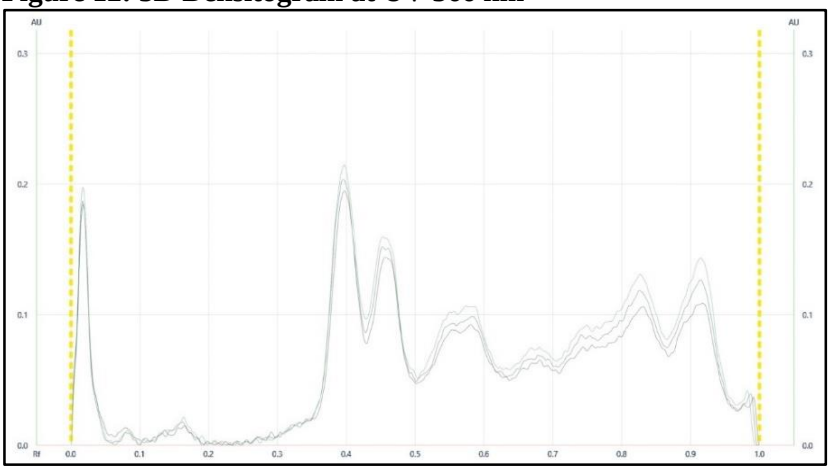

Figure 13: 2D Densitogram after derivatization

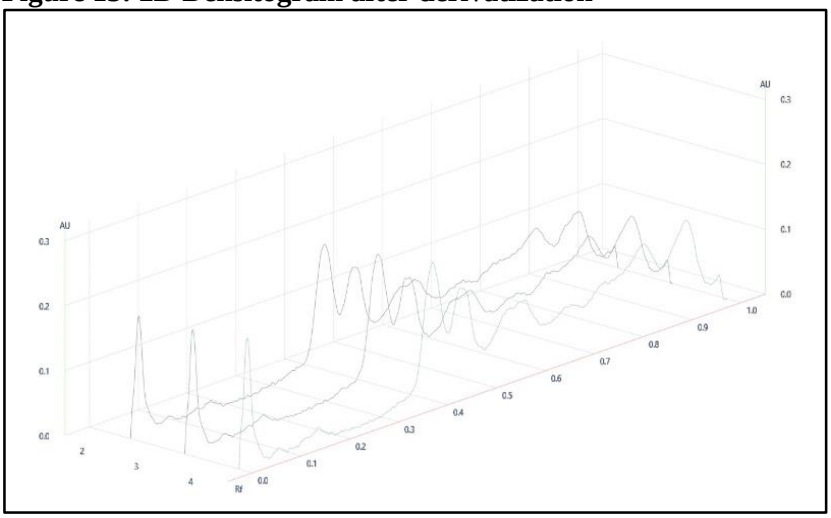

Figure 14: 3D Densitogram after derivatization

The HPTLC fingerprint profile of the ethanolic extract of *Syzgium cumini* Rf values compared with standard quercetin, gallic acid and catechin. The ethanolic extract of *Syzgium cumini* Rf values also coincided with standard Rf values and hence it may be confirmed that the ethanolic extract showed the presence of quercetin, gallic acid and catechin.

CONCLUSION:

Syzgium cumnini Linn is the traditional therapeutic plant with a great potential and acts as remedy to cure diverse ailments. The present research work was focused on performance of standardization parameters for

recognition of original drug. These analytical techniques include macroscopical & microscopical examination idiosyncratic identification of drug. In addition to that physicochemical analysis was performed with parameters like ash values, loss on drying, extractive values. Further, qualitative phytochemical investigation was also performed on successive leaf extracts. These standardization parameters were studied and data obtained possibly will be helpful for the quality assessment of plant drug material, secondly, this data would be helpful for determination of adulterated and substituted drug. The pharmacognostic approach of study of *Syzium cumini* Linn; sets qualitative and quantitative standards. It would be definitely establishing distinctiveness, eminence and purity of this plant drug in intimately correlated species.

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