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Primary phytochemical and pharmacognostic studies on Syzygium cumini Linn. (Jambhul)

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Abstract

From the ancient periods most of the people use lots of herbal medicine. The Syzygium cumini Linn plant has a many medicinal properties. The S. cumini belong to the Myrtaceae family and occurs in tropical and subtropical region of world. This plant origin is South Asia. In India plant found all over the country and it is used for food and medicine. The present study was conducted to investigate the primary phytochemical and pharmacognostic study of S. cumini. The leaves, stem-bark and fruits this plant parts used for the study of primary phytochemical and pharmacognostic examination. The pharmacognostic study based the parameter of macroscopic, microscopic and physico-chemical analysis of plant. The characteristic microscopic features of leaf and stem were observed as stomata, trichomes, multicellular trichomes, phloem cells, xylem cells, collenchyma, spongy parenchyma, vascular bundles and palisade cells. The plant parts like stem, leaves, fruits and seeds of S. cumini were collected from local forest of Nanded. The moisture content, swelling index, foaming index and fluoresces analysis of powder were characterised. Fluoresces analysis of S. cumini crude powder of leaf, stem and fruits under visible and UV light were recorded. Investigation of primary phytochemical analysis were carried out by the qualitative phytochemical test which showed the presence of alkaloids, tannin, flavonoids, saponin, steroids, triterpenoids, phenol, carbohydrate etc. The results obtained in present study will helps to understand importance of S. cumini in medicinal field.

Keywords: Pharmacognostic study, Syzygium cumini L, Eugenia jambolana, phytochemical analysis, Jamun plant etc.

Introduction

Syzygium cumini Linn. (syn. Eugenia jambolana) Plant is commonly kwon as the Jamun plant in Hindi and Jambhul in Marathi, which is distributed along tropical and sub-tropical regions of world. In India the Jamun plant is distributed in all over the country, it is cultivated and sometime occurs wild. The plant has many medicinal properties, which is used in folk medicine from the ancient periods. This plant belongs to the Myrtaceae family. Although it is a tropical tree but it grows easily in subtropical climates also. S. cumini is a fast-growing plant; it can grow up to a height of 30 m and can live for approximately 100 years. Its dense foliage provides shade and is grown just for their fruits and ornamental values (Krushna murti et al. 2012) ^[3]. Jamun plant has reported to contain vitamin C, alkaloids, Saponin, gallic acid, tannins, anthocyanins, glucoside, phenolic compound and other components (Suradkar et al. 2015)^[4]. The plant used in many diseases, it is well known for its medicinal and curative properties like hepatoprotective, antidiuretic, antioxidant, antimicrobial, antifungal and various other properties. Their beautiful purple to black colour of the fruit is due to the presence of anthocyanin and is responsible for high antioxidant property (Ruchi Sharma et al, 2020) ^[5]. A variant of the tree produces green coloured young fruit and after ripen the fruit converted as purple to black coloured. The fruit has a combination of fragrant sweet, astringent flavour and mildly sour tends to colour the tongue purple (Madhulika et al. 2016) [6]. Jamun plant start flowering from the month of March to April. The flowers are small, about approximately 5 mm in diameter. The fruits are fully developed and mature in May or June. The fruit is oblong, ovoid in shape and used for food and medicine.

Particularly interesting is the quest for anti-inflammatory, hypoglycaemic, and cancer-fighting bioactive compounds in various herbal plant foods such vegetables, fruits, spices, teas, and medicine. Several of these compounds have been credited with antioxidant and free radical scavenging properties (Semwal *et al.* 2021) ^[13]. Almost all parts of jamun tree including leaves, seeds, fruit pulp, kernels and stem bark possess therapeutic efficacy (Akhila 2018) ^[11]. *S. cumini*. has wide range of medicinal properties, which have been reported as various bioactive compounds presence in different parts of plant.

The leaves, stem-bark, fruit and seeds are used in many disease treatments. Leaves, bark and fruits are used for the treatment of diabetes, sore throat, astringent and diarrhoea. The plant powder used for headache, stomach-ache, roughness loss of skin coloration. Seed are used for diabetes, the mature fruit used for indigestion (More *et al.* 2023)^[7].

The pharmacognostic study has got its special importance in identifying the genuine drugs. In this we have studied macroscopic, microscopic and organoleptic features of the plant. Phytochemical studied we are doing here will be useful for identifying different bioactive compounds. According to Helmstadter, As there is an increasing scientific interest in antidiabetic herbal medicinal plants, it might be useful to summarize those early research results, to compare them with recent studies and to see, which conclusions might be drawn (Helmstadter 2008) ^[8].

Materials and Methods

Collection and Authentication of plant material

The plant parts like stem, leaves, fruits and seeds of *S. cumini* were collected from local forest of Nanded. The collected material were brought to laboratory. The dust and other adherent were removed by washing thoroughly with clean fresh water and air dried in a shaded area. The collected plant material was stored for further use. The collected and fresh plant material were used for calculation of stomatal index and anatomical studies. The plant material were identified and Authenticated from Department of Botany, B.A.M.U. University, Aurangabad with accession no. 00898.

Extraction of material

Plant material like leaves, stem and fruits were shade dried and made in to powder by using the electrical grinder. The coarse powder (100 g) was extracted successively with methanol, each 250 ml in a Soxhlet apparatus for 24 hrs (Tresinha *et al.* 2014) ^[1]. The methanolic extracts of plants were collected in test tube and stored in a refrigerator for further use.

Macroscopic Study

Macroscopic examination is the study of external morphology of plants and plant part seen by naked eyes. Which included the study of habitat, root, stem, leaf, flower, fruits, seed of plant. This study was done partly in field and laboratory and the characteristic feature of the plant were recorded.

Microscopic Study

In microscopic study anatomical observation of plant sections were made. The free hand sections of leaves and stem were taken in the laboratory and stained using safranin and fast green. After staining the sections were fixed in glycerine and were observed under the microscope and photographed using camera.

Stomatal Number and Stomatal index

The study of stomatal number and stomatal index was done by taking of epidermal peels of leaves. For the study of stomatal number and index leaves epidermal peel was removed and stained it by Safranin and observed under the 10X eye-piece and 45X objective of light Microscopes. The total number of stomata at specific area of objective per peel were counted. The number of stomata at specific area of objective were counted and the stomatal index were determined by using following formula (Shyam Baboo *et al.*).

Stomatal index =
$$\frac{S}{S+E} \ge 100$$

Where, S = Stomatal cells, E = epidermal cells.

Organoleptic study of powder

In organoleptic evaluation, the characters like colour, odour, taste and textures of leaf, stem and fruits powder were studied are recorded in table (Shanthi 2014)^[14].

Physico-chemical property of powder

In physico-chemical studies we have analysed leaves, stembark and fruit powder for fluorescence analysis, moisture content, swelling index, foaming index etc including primary phytochemistry.

Fluorescence analysis

The Jamun leaves, stem-bark and fruits powder were treated with various chemical reagents and passing through the visible light and UV light. The powder of leaves, stem and fruits was prepared after passing it through mesh 40 and its fluorescence characters were studied both in daylight and in UV light (254 and 366 nm) using different solvents like sulphuric acid, hydrochloric acid, ferric chloride acetic acid etc. (Tanwar *et al.* 2012)^[2].

Moisture content

The studies on moisture content of powder were performed by the loss on drying methods. The moisture present in jamun leaves stem and fruits were determined by drying the sample in hot air oven at 110 °C till constant weight. Following equation was followed to determine the moisture content (Ahmad Raza, 2015)^[10].

Moisture % = $\frac{\text{Initial weight}-\text{Final weight}}{\text{Initial weight}}$

Swelling index

According to WHO guideline swelling index was decided by following methods with slight modification. Accurately weighed 1 gram of fine leaf, stem and root powders were introduced in 100 ml measuring cylinder and marked in 0.2 ml divisions from 0-10 ml in an upwards direction on cylinder. 100 ml of distilled water was added to the powders and shake it to mix thoroughly. One gram powder occupied initial volume were marked. Then the mixture were allowed to stand for 12 hours at room temperature. After that the final volume occupied by the plant material was measured (in ml). For the calculation of swelling index following formula were used.

Swelling index = final volume – initial volume (in ml).

Foaming index

According to WHO guideline studies on foaming index were done by following method with minor modification. One gram of fine powder (leaf, stem and fruits) were taken into separate conical flask (500 ml). 100 ml of boiling water was poured into the flask and maintained in the temperature at 80-90 °C by heating for 30 minutes. Then allowed to cool at room temperature and sufficient amount of water added into the decoction to make the volume up to 100 ml. 10 clean test tubes were taken and marked with 1 to 10. The successive portions of 1 ml, 2 ml up to 10 ml of powder was taken in separate tubes and adjusted remaining volume with the distilled water up to 10 ml in each tube. Tubes were shaked for 15 seconds and allowed to stand for 15 min. The height of the foam were measured. If the height of the foam were less than 1cm in each tube, the foaming index is considered as less than 100 (not significant). If the foam is more than 1cm height after the dilution of plant material in the 6th tube, then corresponding number of the test tube was the index sought (significant). If the height of the foam in every tube is more than 1cm, the foaming index is more than 1000 (more significant). Foaming Index was calculated by using the following formula,

Foaming Index = 1000/a

Where, a = Volume (ml) of decoction used for preparing the dilution in the tube where exactly 1 cm or more foam was observed.

Preliminary phytochemical screening

The preliminary phytochemical screening of leaves, stem and fruit was done by the following test for secondary metabolites as described by Hasanuzzaman (2016)^[12].

1) Alkaloids

Mayer's Test: In 1ml of plant extract 1ml of Mayar's reagent were added by the side of test tube. Formation of white or creamy precipitate indicates the positive result for alkaloids.

2) Flavonoids

Alkaline Reagent Test: For the detection of flavonoids 1 ml of plant extract were treated with few drops of 5% NaOH solution. The colour turns to yellow. After addition of few drops of 10% HCl solution become colourless, which indicates the presence of flavonoids.

3) Steroids

Libermann-Burchard's test: 1ml acetic anhydride solution was added into the filtrate then 1ml of concentrated sulphuric acid, a brown ring is formed at the junction of two layers. The upper layer turned into green or blue colour indicates the presence of steroids.

4) Triterpenoids

Libermann-Burchard's test: 1ml acetic anhydride solution was added into the filtrate then add 1ml of concentrated sulphuric acid, a brown ring is formed at the junction of two layers. The formation of deep red colour in lower layer indicates the presence of triterpenoids.

5) Saponin

Froth Test: for the detection of Saponins few ml of extracts was mixed with the same amount of distilled water. The suspension is shaked for 15 to 30 seconds and allowed to stands. The foam formation in test tube indicates presence of saponin.

6) Tannins

Ferric Chloride Test: About 0.5g of plant extract was boiled in 20 ml of distilled water in a test tube, then filtered it. After that 1ml of 0.1% ferric chloride solution into the filtrate. Appearance of brownish green or blue-black colour indicates presence of tannins.

7) Glycosides

Keller-Kiliani test: For the detection of glycosides, 1ml of filtrate was treated with 1ml of glacial acetic acid, few drops

of ferric chloride solution and few drops of concentrated sulphuric acid was added. Appearance of green blue colour indicates the positive result of cardiac glycosides.

8) Anthraquinones

Sanker-Nahar test: One ml of filtrate was treated with the same volume of aqueous base NaOH solution. Appearance of pink or violet colour in the base layer of solution indicates presence of anthraquinones.

9) Coumarins

A little amount of extract is dissolved in methanol and 3-4 ml alcoholic KOH was added to it. Formation of a yellow colour which disappeared on adding concentrated HCl indicates the presence of coumarins.

10) Oil and fats/lipid

Spot test: For this detection, few drops of filtrate was pressed between two filter paper indicates the presence of fixed oil.

11) Phenol

Ferric chloride test: In 1ml of plant extract, few drops of diluted ferric chloride solution was added. Formation of violet or blue, green and red colour indicates the presence of phenol.

12) Protein

Millions reagent test: In 1 ml of plant extract, 1 ml of million's reagents was added in test tube. Formation of radish brown colour indicate the presence of protein.

13) Carbohydrates

Iodine test: 1ml of plant extract treated with few drops of iodine solution. Formation of blue colour indicates presence of carbohydrates.

Results and Discussion Macroscopic / Morphological characters



Fig 1: A) S. cumini tree, B) S. cumini flower.

S. cumini Linn. is widely distributed in India and widely cultivated in tropical and sub-tropical region of world. It is an evergreen perennial tree grows up to 30 to 32 meters, with adventitious roots. Stem is unbranched, greyish white in colour. The stem bark is coarse and discoloured the lower bark. The leaves are simple, opposite, the shape is elliptic to oblong. The venation is reticulate, the midrib is prominent

Journal of Pharmacognosy and Phytochemistry

and yellowish. The leaf blades have many lateral veins closely parallel, exstipulate with acute apex and entire margin. The flower colour is white to pinkish and about 1 cm in size. Many small flowers arise on the branches during the April-May. The fruits are ovoid, 1-seeded berry, with a length of 2 centi-meter (0.8 inch), dark purple red, shiny, with white to lavender flesh.

Microscopic character Stomatal Numbers and Stomatal Index

As shows in observation table 1. and image A, B, C, D and E of fig.2, the stomata are present only on lower epidermis (adaxial) of leaf whereas upper epidermis (abaxial) is devoid of stomata. Lower epidermis has several anisocytic type of stomata.

Sr.no.	Epidermis	Stomatal no.	Stomatal Index
1	Upper epidermis	Devoid	Devoid
2	Lower epidermis	13-18	16%

Table 1: Observation, Stomatal number and Stomatal index



Fig 2: A and B shows the abaxial surface of leaf with stomata, C and D shows the Adaxial surface of leaf, D show the surface view of S, cumini leaf.

Microscopic study of *S. cumini* was done by taking free hand sectioning of Stem and leaf

T.S. of leaf

Upper epidermis and lower epidermis are small and single cell layered. Under microscope epidermal layer is found to be covered by the thin cuticle. Epidermal layer is followed by single elongated palisade cells. Mesophyll tissue is composed of 7 to 8 layers of spongy parenchymatous cells. The spongy cells are disrupted by secretory canals. Endodermis is a single layered and encloses vascular bundles. It is followed by the small single layered cell of pericycle. The vascular bundle is of Meri stele type which is composed from protoxylem towards outer side and metaxylem towards inside. Phloem also presents in meristele.



Fig 3: T. S. of leaf (midrib).

T. S. of Stem

The section of young stem consists of single layered epidermal cells layer but soon in secondary growth it is found to be replaced by cork. Epidermal layer is followed by the elongated palisade cells in young stage. Cortex composed by many layers of polygonal parenchymatous cell. Cortex followed by the single layer of endodermis. Bicollateral type of vascular bundle present in centre of stem. Outer side of vascular bundle band of sclerenchyma tissues presents. Cortex disrupted the long medullary ray. Phloem tissue followed the xylem and centrally parenchyma cell present in form of pith.



Fig 4: A, B, traverse section of stem of *S. cumini* stem. Where ep-epidermis, ct-cuticle, pa-parenchyma, co-collenchyma, ph-phloem, xy-xylem, vb-vascular bundle, en-endodermis, pi-pith.

Organoleptic study: Organoleptic study was done by

physical observation of crude powder of Stem, Leaves and Fruits.

Sn no	Character	Plant Parts				
Sr. 110.	Character	SC-L	SC-S	SC-F		
1	Colour	Deep green	Greyish	Light purple		
2	Odour	Pungent	Odourless	Pungent		
3	Taste	Not significant	Tasteless	Light sweet		
4	Texture	Smooth	Friable	Smooth and Granular		

Table 2: Organoleptic study of powder



Fig 5: A, B, C, Powder of fruit, leaves and stem respectively. Where, SCL-S. cumini Leaf, SCS-S. cumini stem and SCF-S. cumini fruits.

Physico-chemical studies

Moisture content: The results of moisture content of leaves, stem and fruit powder of *S. cumini* are represented in table 3.

Sr. no.	Plant part	Moisture content%
1	SC-L	6.279±0.6
2	SC-S	4.651±0.8
3	SC-F	5.231±0.7

Swelling index

Fable 4:	Swelling	index	of S.	cumini	powder
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Sr. no.	sample	Quantity of sample	Initial volume	Final volume	Swelling index
1	SC-L	1 g	6	18	12
2	SC-S	1 g	8	16	08
3	SC-F	1 g	5	10	05

(Value in ml)

Foaming index

Table 5: Foaming index of S. cumini powder.

Sr. no.	Sample	Foaming index		
1	SC-L	333.33	Significant	
2	SC-S	250.00	Significant	
3	SC-F	Less than 100	Not significant	

Fluorescence analysis of powder: The S. cumini powder treated with various chemical and observed under day light

and fluorescence light (UV light 245 nm), the results are mention the table 6.

Table 6: Fluorescence analysis of S. cumini L. powder under visible light and UV light (254nm)

Sn no	Descent with newdor	SC-L		SC-S		SC-F	
51. 110.	Keagent with powder	Visible light	UV Light	Visible light	UV Light	Visible light	UV Light
1	P+Alone	Greenish light	Herbage green	Light green	Green	Pale brown	Light green
2	P + water	Light green	Umber light	Light yellow	Green	Light green	Yellowish green
3	P + ethanol	Light green	Brown	Pale yellow	Green	Pale yellow	Dark green
4	P + methanol	Green	Brown	Light yellow	Deep green	Brown	Light green
5	P + NaOH	Light orange	Dark brown	Greenish yellow	Green	brown	Dark green
6	P + HCl	Buff	Brownish black	Greenish yellow	Green	Light green	Yellowish green
7	P + H2SO4	Dark orange	Radish brown	Greenish yellow	Black	Brown	Dark green
8	P + HNO3	Orange	Dark green	Radish brown	Brown	Radish orange	Fluorescent green
9	P + KOH	Orange	Dark red	Light green	Green	Light green	Dark green
10	P+ acetic acid	Yellow	Orange	Pale yellow	Red	Light green	Yellowish green

Preliminary phytochemical screening of extracts: The results of preliminary

phytochemical analysis of S. cumini are shown the table 7.

Sm No	Chamical constituent	Test	Р	Plant parts		
Sr. No.	Chemical constituent	Test	SC-L	SC-S	SC-F	
1	Alkaloids	Mayers test	+	+	+	
2	Flavonoids	NaOH and HCl test	+	+	+	
3	Tannin	Ferric chloride test	+	+	+	
4	Steroids	Lebermann- Burchards test	+	+	+	
5	Triterpenoids	Lebermann- Burchards test	+	+	+	
6	Saponins	Foam test	+	+	+	
7	Glycosides	Keller-Kiliani test	-	-	+	
8	Anthraquinones	Sanker-Nahar test	-	-	-	
9	Coumarins	KOH, HCl test	-	-	-	
10	Fixed oil and fats	Spot test	-	-	-	
11	Phenol	Ferric chloride test	+	+	+	
12	Protein	Million's reagent test	-	-	-	
13	Carbohydrates	Iodine test	+	+	+	

Discussion

The S. cumini belong to the myrtaceae family. This plant is mostly used in food and diet for their medicinal properties. In the present investigation the pharmacognostic, phytochemical, macroscopic and microscopic studies was done. The microscopic examination of S. cumini shows stomatal index is 16 ± 1 and the number of stomata were found to be approximately 13 to 18. The transvers sections of leaves and stem shows the epidermal layer, mesophyll cells, cortex and bicollateral vascular bundles with starch crystal. Secretory duct was also present in leaves. Organoleptic study of powder of Syzygium cumini Linn mention in table 2. This study shows that the powder of leaf, stem and fruits have characteristic colour, odour, taste and texture which support inclusion of powder form of drugs in different medicines. The moisture content of Syzygium cumini powder of leaves, stem-bark and fruit is 6.279±0.6, 4.651±0.8 and 5.231±0.7 respectively. Moisture content of stem-bark is slightly less than leaves and fruit powder.

Swelling index is medicinally important properties. The presence of gum and mucilage plant powder shows the

swelling properties. *S. cumini* shows the moderate properties of swelling index. The maximum swelling index is shown by leaf powder 12 ml in 100 ml volume, followed by stem powder and fruits powder. The presence of saponin in plant powder shows the foaming properties. Foaming index is the most significant phenomena of pharmacognostic study. The leaves and stem sample shows the significant foaming index than the fruit sample.

Fluorescence studies were done under the visible light and UV light. The wavelength of UV light was 254 nm. All the sample shows different features and colour which are mention in the table 3. The investigation of preliminary phytochemical analysis of *S. cumini* shows presence of alkaloids, tannin, flavonoids, saponin, steroid, triterpenoids, phenol and carbohydrates. The glycosides, protein, fixed oil and anthraquinone were found to be absent in extract.

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