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Research Article

PHARMACOGNOSTICAL STANDARDIZATION AND ANTIMICROBIAL ACTIVITY OF LEAVES OF *SYZYGIUM CUMINI* (LINN.) FROM VARIOUS REGION OF NORTH INDIA

Deepak Kumar[1]*, Shefali Arora[2] and Muneer Alam[1]
[1]Department of Pharmaceutical Chemistry, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun (UK) India
[2]Department of Chemistry, University of Petroleum and Energy Studies, Dehradun (UK) India
*Corresponding Author Email: deepsingh2304@gmail.com

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ABSTRACT
Syzygium cumini is one of the widely used medicinal plants in the treatment of various diseases in particular diabetes. In present investigation, the pharmacognostic study of *Syzygium cumini* leaf is carried out from different region of north India. The study includes determination of foreign matter, microscopy and macroscopy examination, determination of ash, determination of total alcoholic extractives and loss on drying. Preparation of different leaves extract, their phytochemical analysis and antimicrobial activity of *Syzygium cumini* were also carried out. Results from the above studies were comparable with different regions of India. From antimicrobial study, ethyl acetate extract showed maximum antimicrobial activity at a concentration 200 mg/ml.
Keywords: *Syzygium cumini,* Pharmacognostical Standardization, Antimicrobial, Chloramphenicol, Antifungal, Antibacterial.

INTRODUCTION

Syzygium cumini Linn (family Myrtaceae), commonly known as Jamun (Hindi), is a medicinal plant and utilizable species. Common names are Java plum, Black plum, Jambul and Indian Blackberry[1]. The original home of Jamun is India, distributed throughout India, in forest up to 1800 m usually along the bank and moist localities. The sprouts are refrigerant, carminative and astringent to bowels. Powdered seeds are used as a remedy in diabetes and in metrorrhagia[2]. The tree fruits once in a year and the berries are sweetish sour to taste. The ripe fruits are used for health drinks, making preserves, squashes, jellies and wine[3]. In association to its dietary use, all parts of the tree and importantly the seeds are used to treat a range of ailments, the most important being diabetes mellitus[4]. Different parts of the Jamun were also reported for its antioxidant, anti-inflammatory, neuropsycho-pharmacological, anti-microbial, anti-bacterial, anti-HIV, anti leishmanial and antifungal, nitric oxide scavenging, free radical scavenging, anti-diarrheal, anti fertility, anorexigenic, gastro protective and anti ulcerogenic and radio protective activities[4]. The major phytoconstituents are reported to contain vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidinglucoside and other components[5,6]. The objective of present study was too standardized and evaluates antimicrobial activity of leaves of *Syzygium cumini* Linn. from the different region of North India.

MATERIALS AND METHODS

Collection and Identification of plant materials

The plant leaves were collected from different regions from North India (i.e. 4 different cities or states) likes Nagina, Bijnor (Uttar Pradesh), Dehradun (Uttarakhand), Pinjor (Punjab), Kanpur (Uttar Pradesh) and Mandi (Himachal Pradesh), India and Identified by Dr. Vidit Tyagi, Botanist, Dept. of Botany, Dolphin PG Institute of Biomedical and Natural Sciences, Dhradun, Uttarakhand, India.

Pharmacognostical Evaluation of leaves

The pharmacognostical evaluation or standardization of the raw material were done according to the guidelines mentioned by WHO in Quality control of Herbal Drugs[7,8]. Following parameters were evaluated.

Determination of Foreign Matter

50 g of leaves of *Syzygium cumini* leaf were taken and were looked for foreign matter by naked eyes and with the help of magnifying glass of 10x power.

Macroscopic and Microscopic Examination

The leaves of *Syzygium cumini* leaf were examined for their macroscopic and microscopic properties. Prior to the visual examination, leaves were softened with a cotton swab moistened with water.

Determination of ash

Total ash

2 g each of air dried leaves of *Syzygium cumini* leaf were taken in a pre-weighed and pre-ignited silica crucible. Then covered with a lid and kept in muffle furnace for 4 – 6 hours to heat up to 500 – 600°C. It was then cooled in desiccators and weighed again to determine the total Ash value, result shown in Table 1.

Acid-insoluble ash

To the total ash obtained from above, 25 ml of dilute hydrochloric acid (5.93 ml of conc- HCl diluted to 100 ml with water), was added and boiled for 5 minutes and then filtered with an ash-less filter paper. The insoluble matter was washed with hot water and then placed in the crucible and ignited again for about 6 – 8 hours and then weighed again, result shown in Table 1.

Water soluble ash

To the crucible of total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter filtered on an ash less filter paper. The residue was washed with hot water and

ignited in a crucible for 15 minutes at 450°C. The weight of the residue (in mg) from the weight of total ash, result shown in Table 1

Total Alcohol extractive

4 g of air-dried leaves of *Syzygium cumini* leaf were taken in a 250 ml glass stoppered conical flask and 100 ml ethanol was added. It was then shaken frequently for about 6 hours and then left standing overnight (~18 h). Ethanol then was filtered and 25 ml of it was transferred in to a pre-weighed china dish and evaporated to complete dryness and then weighed again. The weight of alcohol extractive was determined for 25 ml and was then multiplied by 4 to calculate the total alcohol extractive for 100 ml (4 g of sample) result shown in Table 1.

Total Water extractive

4 g of air-dried leaves of *Syzygium cumini* leaf were taken in a 250 ml glass stoppered conical flask and 100 ml distilled water was added. It was then shaken frequently for about 6 hours and then left standing overnight (~18 h). Water then was filtered and 25 ml of it was transferred in to a pre-weighed china dish and evaporated to complete dryness and then weighed again. The weight of water extractive was determined for 25 ml and was then multiplied by 4 to calculate the total water extractive for 100 ml (4 g of sample) result shown in Table 1.

Loss on Drying

2 g leaves, each of *Syzygium cumini* leaf were placed in a previously weighed weighing bottle and then kept in the oven, maintained at 105°C for about 2 hours and then weighed again to give weight of water and volatile matter in the sample, result shown in Table 1.

Preparation of extracts

The collected plant Material was washed with water to remove other undesirable material and dried under shade. The air-dried leaves (350 g) of *Syzygium cumini* were crushed. The crushed leaves extracted with Methanol by cold percolation method using percolator. The extract was evaporated till dryness to obtain a residue of 85 g (24.28 %). From total methanol extract, preparation of different fractions by cold percolation method using increasing polarity of solvents by separation technique i.e. Petroleum Ether, Chloroform, Ethyl Acetate, Butanol[9] were done.

Qualitative Phytochemical Tests

All extracts of leaves of *Syzygium cumini* were subjected to evaluate the presence of different phytoconstituents such as alkaloids, carbohydrates, steroids, proteins and amino acids, saponin and phenolic compounds[10,11].

Antimicrobial Activity of Leaves Extracts

The antimicrobial activity of the leaves of *Syzygium cumini* was carried out. The leaves extract were screened for antibacterial and antifungal activities at a dose of 200 mg/ml.

Antibacterial activity of leaves extract

The antibacterial activity was studied against the micro organism and the bacterial cultures used in the study were: *Escherichia coli, Klebsiella pneumoniae, Bacillus cereus, Staphylococcus aureus* and *Salmonella typhii.* These bacteria were provided by department of microbiology, Dolphin (PG) institute of biomedical and natural sciences, Manduwala, Dehradun, India checked for purity by convention biochemical method. The well diffusion method used for antibacterial activity. The media used for antibacterial activity Muller- Hinton Agar (MHA) and Nutrient broth (NB). About 100 µl of each leaves extracts individually was loaded in each well. Petri plates were incubated for overnight at $37^0C \pm 0.5^0C$ in the incubator. After incubation, the diameter of clear zone of incubation produced around the well or holes were measured in mm by ESR Tube and compared with standard drug.

Antifungal activity of leaves extract

The antifungal activity was against the micro organism and the fungi cultures used for this study were: *Aspergillus niger, Penicillum crysogenum, Saccharomyces cerevisiae* and *Candida albicans.* The cultures were obtained from the standard cultures maintained in the Dolphin (PG) Institute of Biomedical and Natural Sciences, Manduwala, Dehradun, India. These cultures were maintained on sabouraud dextrose agar (SDA) at first being incubated at 30^oC for about 32-48 hours and then stored at 4^0C at stock cultures for further anti fungal activities. The well diffusion method was used for antifungal activity. About 100 µl of each leaves extracts individually was loaded. After incubation, the diameter of clear zone of inhibition produced around the well or a hole was measure in mm by ESR tube and compared with standard drug.

RESULTS

Table 1: Standardization

PLACE	LOD (%)	ASH (%)	Acid-insoluble ASH (%)	Water-soluble ASH (%)	Alcohol Extractive	Water Extractive	Foreign matter (%)
NAGINA (UP)	6.6	5.82	1.0	1.55	7.30	13.90	2.0
DEHRADUN (UK)	11.2	5.55	1.25	4.33	10.25	8.40	1.44
PINJOR (HARYANA)	10.50	2.92	1.0	0.85	9.60	14.10	3.22
KANPUR (UP)	7.95	13.57	2.25	1.35	9.05	22.10	3.14
MANDI (HIMACHAL)	3.8	9.98	1.85	2.0	7.00	8.63	2.54

Macroscopic Study

The leaves measure about 10 to 15 cm long and 4 to 6 cm wide. These are entire, ovate-oblong, sometimes lanceolate and also acuminate, coraceous, tough and smooth with shine above. The fragrant flowers of Jamun are small, nearly 5 mm in diameter. These are arranged in terminal trichotomous panicles greenish white in color.

Microscopy

Transverse section of *S. cumini* leaves showed following features-

Epidermis

Two to three layered epidermis.

Mesophyll

It is composed of isodiametric thin walled parenchymatous ground cells which are packed with simple starch grains. In the mid-rib region, the vascular bundles show xylem, towards upper epidermis and phloem on the lower side. Starch grains, oil globules, tannin cells and stone cells are also visible.

The antibacterial activity of different extracts of *Syzygium cumini* and standard drug chloramphenicol were tested for different strains of bacteria and zone of Inhibition was recorded in mm.

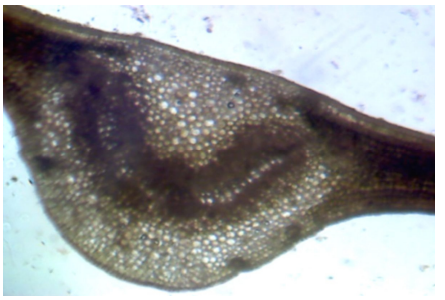

Figure 1: TS of leaf of *S. cumini*

Table 2: Antibacterial activity of different extracts of *Syzygium cumini* and standard drug chloramphenicol

S. No	Test organism	Inhibition Zone (in mm)					
		Methanol	Pet. Ether	Chloroform	Ethyl Acetate	Butanol	Standard drug (Chloramphenicol)
1	*E. coli*	20	15	16	26	17	30
2	*K. pneumonia*	18	13	0	27	18	31
3	*B. cereus*	29	19	13	35	19	28
4	*S. typhii*	18	18	0	21	19	28
5	*S. aureus*	32	19	24	28	21	26

Antifungal activity of different extracts of *Syzygium cumini* and standard drug chloramphenicol were tested for different strains of fungus and recorded the zone of inhibition in mm.

Table 3: Antifungal activity of different extracts *Syzygium cumini* and standard drug Chloramphenicol

S. No	Test Organism	Inhibition zone (in mm)					
		Methanol	Pet. Ether	Chloroform	Ethyl Acetate	Butanol	Standard drug (Chloramphenicol)
1	*P. chrysogenum*	32	0	19	38	17	-
2	*A. niger*	33	33	18	30	30	-
3	*C. albicans*	26	30	20	31	30	20
4	*S. cerevisiae*	27	0	19	20	27	28

DISCUSSION

The standardization results showed in Table 1, the LOD (Loss on Drying) result is maximum in the Dehradun, India leaf 11.2 %, it means that the maximum moisture content found in the leaf of Dehradun, India and low moisture content in Mandi (H.P), India leaf 3.8 % and other Nagina (U.P), India leaf 6.6 % Pinjor (Haryana), India leaf 10.5 % Kanpur (U.P), India leaf 7.95 %. The ash value maximum for the Kanpur (U.P), India leaf 14 % and lower for the Pinjor (Punjab), India leaf 3 % its show that the Kanpur leaf found their low toxic substance as compare to pinjor (Haryana), India leaf and other Nagina (U.P), India leaf 5.9 % Dehradun (U.K), India leaf 5.25 % Mandi (H.P), India leaf 10 %. Acid insoluble ash is equal (lower) for Nagina (U.P), India and Pinjor (Haryana), India 1 % and the maximum for the Kanpur leaf 2.25 % and other leaf Dehradun (U.K), India leaf 1.25 % Mandi (H.P), India 1.85 %. Water soluble ash maximum for the Dehradun leaf 4.33 % and minimum for the Pinjor (Haryana), India leaf 0.85 % and other leaf Nagina (U.P), India leaf 1..55 % Kanpur (U.P), India 1.35 % Mandi (H.P), India 2 %. Alcohol extract is maximum for the leaf of Dehradun (U.K), India 10.2 % and minimum for the Mandi (H.P), India leaf 7.0 % and other Nagina (U.P), India leaf 7.3 % Pinjor leaf 9.6 % Kanpur (U.P), India leaf 9.05 % .Water extract is maximum for the leaf of Kanpur (U.P), India leaf 22.10 % and minimum for the Dehradun (U.K), India (8.40 %) leaf and other leaf Nagina (U.P), India 13.9 % Pinjor (Haryana), India 14.1 % Mandi (H.P), India 8.62 %. Foreign matter is maximum for the leaf of Pinjor (Haryana), India 3.22 % and minimum for the Dehradun (U.K), India leaf 1.44 % and other leaf Nagina (U.P), India 2 % Kanpur (U.P), India 3.14 % Mandi (H.P), India 2.54 %. From Phytochemical analysis results we can find out that methanol extract was the richest extract for phytoconstituents except tannins of phenolic compounds, carbohydrates and flavonoids. It contains all tested phytoconstituents viz. Alkaloids, glycosides, proteins and amino acid, triterpinoids of sterols, fats and fixed oil and saponins. Ethyl Acetate extract contain protein and amino acid, and fats and fixed oil except triterpenoids of sterols. Petroleum Ether, chloroform extracts contains only saponin sterols and fats and fixed oil. Antibacterial activity of total methanol extract and its fractions against tested microorganism were done and compared with standard drug Chloramphenicol. All extracts showed antibacterial activity against *Escherichia coli* in which maximum inhibition zone showed by the Ethyl Acetate (26 mm) and minimum inhibition zone by petroleum ether (15 mm) and other chloroform (16 mm), Butanol (17 mm), and methanol (20 mm). Ethyl Acetate extract showed maximum inhibition zone (27 mm) against *K. pneumonia* and

minimum inhibition zone showed by petroleum ether (13 mm) and other Methanol and Butanol both showed (18 mm) inhibition zone. Against *Bacillus cereus* bacterial strain Ethyl Acetate showed maximum inhibition zone (35 mm) and minimum inhibition zone showed by chloroform (13 mm). The inhibition zone against *Bacillus cereus* by methanol extract (29 mm) and Petroleum ether and Butanol both showed (19 mm). Against *Salmonella typhii*, Ethyl acetate extract showed maximum inhibition zone (21 mm), Butanol extract (19 mm) and methanol and petroleum ether extract both showed inhibition zone (18 mm). Against *S. aureus*, Methanol extract showed maximum inhibition zone (32 mm) and minimum inhibition zone showed by Petroleum ether (19 mm). Chloroform extract showed inhibition zone against *S. aureus* (24 mm), Ethyl acetate (28 mm), and Butanol (21 mm). All extracts showed Antifungal activity against *Penicillium chrysogenum* in which the maximum inhibition zone showed by the Ethyl Acetate (38 mm) and minimum inhibition zone showed by Butanol (17 mm) among other extract such as chloroform showed inhibition zone (19 mm) methanol extract (32 mm) except Petroleum Ether. All extracts showed Antifungal activity against *Aspergilus niger* in which Methanol extract and Petroleum ether showed maximum inhibition zone (33 mm), minimum inhibition zone showed by chloroform (18 mm) among other extract such as Ethyl acetate and Butanol both showed inhibition zone (30 mm). Against *Candida albicans* the maximum inhibition zone showed by Ethyl Acetate (31 mm) and minimum inhibition zone by Chloroform (20 mm) and among other extracts methanol showed (26 mm), Petroleum Ether and Butanol showed inhibition zone (30 mm); against *Saccharomyces cerevisiae* maximum inhibition zone showed by Methanol (27 mm) and Butanol (27 mm). Chloroform extract and ethyl acetate extract showed inhibition zone (19 mm) and (20 mm) respectively against *Saccharomyces cerevisiae*.

CONCLUSION

Finally we can find out the Pharmacognostical Standardization result of leaf of *Syzygium cumini* from North India the best result showed by the Pinjor (Haryana), India Plant region this mean the soil and nature are best for the *Syzygium cumini*. The standardization is thus a very crucial part of establishing its correct identity. The present study could therefore serve as important data for proper identification, collection and investigation of the *Syzygium cumini* leaves. However, the variation on reported values and estimated values in the present study may be expected due to the various ecological factors. Evaluation of antimicrobial activity of this plant in which Ethyl acetate showed maximum activity against both antibacterial and antifungal strains. Further Study needed for the isolation of active constituents.

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