Phytochemical Screening and Antibacterial Activity study of *Syzygium cumini* (Myrtaceae) Seed Extracts

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ABSTRACT

Syzygium cumini is commonly known as jamun, jambolan, Java plum or black plum, is an evergreen tropical tree in the flowering plant from family Myrtaceae, and is one of the most popular fruits. It is planted in various regions spontaneous. It is native of India, Bangladesh, Sri Lanka, Myanmar, Nepal, China, Australia, Thailand, Kenya, Colombia, Mexico, United States of America, Zambia, and Zimbabwe. The entire part of the plants has been widely used in the treatment of various diseases in the traditional and folk medicine. The edible part of fruits (jamun) contain vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidinglucoside and other components. The seeds of *Syzygium cumini* possess anti-diabetic, antipyretic, anti-inflammatory, hypolipidaemic, psychopharmacological, anti-diarrheal, antioxidant and antibacterial activities.
In this present study, the phytochemical investigation and antibacterial activity studies were carried out with using methanol, petroleum ether and ethanol extracts of the seeds of *Syzygium cumini* from the family Myrtaceae. Preliminary the phytochemical screening of all extracts revealed the presence of phytoconstituents like alkaloids, tannins, saponins, flavonoids, phenols, terpenoids, steroids and amino acids and absence of anthraquinone glycosides. The antibacterial activity of all three extracts was tested against some pathogenic bacteria using the Cup-Plate method. The different extracts of *Syzygium cumini* seeds showed inhibitory activity over Gram negative bacteria such as *Salmonella typhi* and *Escherichia coli* and Gram positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*. The results showed that the methanolic extract was slightly more potent than the other two.

Keywords: Phytochemical Screening, Antibacterial, *Syzygium cumini,* Myrtaceae, Seed Extracts

INTRODUCTION

Almost 25% of globally prescribed drugs obtained from the various plants sources, used as the natural products for diseases prevention and control as well as in drug development. It has also been very well documented in the world forum WHO's report that state about 80% of world's population are dependent on plants to meet their primary health care needs (Ahmadullah and Nayar, 1999). Plant metabolites have been of great interest to human for long time due to their pharmacological relevance. A large proportion of world population, especially in the developing countries depends on traditional system of medicine for various diseases. Plant based drugs constitute a major share of medicine in India, china viz ayurveda, yoga, unani, siddha, homeopathy and naturopathy, except allopathy (Vaidya & Devasagagam, 2007).

Syzygium cumini is commonly known as jamun, jambolan, Java plum or black plum, is an evergreen tropical tree in the flowering plant from family Myrtaceae, and is one of the most popular fruits. It can live up to 80-100 years, and planted in the various regions spontaneous. It is native of India, Bangladesh, Sri Lanka, Myanmar, Nepal, China, Australia, Thailand, Kenya, Colombia, Mexico, United States of America, Zambia, and Zimbabwe. The entire part of the plants has been widely used in the treatment of various diseases in the traditional and folk medicine. The chief active constituents of *Syzygium cumini* are myricetine, β-sitosterol, myricyl alcohol, betulinic acid, friedeanol, epifriedeanol, eugenin, ß- sitosterol-D-glucoside, Kamepferol-3-0-glucoside, quercetin, astragalin, and gallic acid. The edible part of fruits (jamun) contain vitamin C, gallic

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acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidinglucoside and other components. The seeds of *Syzygium cumini* possess anti-diabetic, antipyretic, hypolipidaemic, psychopharmacological, anti-diarrheal, anti-inflammatory, antioxidant and antibacterial activities. It is reported that seed extracts of *Syzygium cumini* given to the animals with the dose of 5gm/kg of bodyweight was more effective than the oral hypoglycaemic or anti-diabetic drug glibenclamide in the case of type 2 diabetes. The preparation of tea from the leaves of *Syzygium cumini* has also a hypoglycaemic effect used in the case of diabetes. The flowers are believed to possess an antipyretic effect to reduce the fever. The seeds are additionally used as an anesthetic in South American cultures. The leaves of *S. cumini* are also used in the treatement of various skin diseases. The Leaves, seeds, fruits and stem of jamun tree have some antimicrobial effect (Sharma S, et al. 2012).

Preliminary phytochemical screening also showed the presences of alkaloids, tannins, saponins, flavonoids, phenols, terpenoids, steroids and amino acids and absence of anthraquinone glycosides in seeds extracts of *syzygium cumini*. The present study was designed to investigate the phytochemical compounds and antibacterial activity of the methanolic, petroleum ether and Ethanolic extracts of *syzygium cumini* seeds.

Taxonomic Classification:
Kingdom - Plantae
Subkingdom - Viridiplantae
Infrakingdom - Streptophyta
Division - Tracheophyta
Subdivision - Spermatophytina
Infradivision - Angiospermae
Class - Magnoliopsida
Superorder - Rosanae
Order - Myrtales
Family - Myrtaceae
Genus - Syzygium
Specie - Cumini

Morphology:
Black plum or jamun is a tropical evergreen tree that grows up to 25-30 meters (80-100 feet) tall, with white gray coloured stems and coarse and discoloured lower bark. The leaves have a characteristic smell like turpentine, and are simple, dark green, opposite, oblong-oval or elliptical, glossy, smooth, leathery in touch and blunt or tapering at the apex point. The leaves are 5-25 centimetres long and 5-10 centimetres wide in size. The midrib of the leaves is prominent and yellowish in colour when mature. The leaf blades have many lateral veins closely parallel. The colours of flowers are white-pinkish, appear in clusters about 4-10 centimetres long, and each being 1-2 cm long and 1.5 cm wide in size across with the four to five united petals and many stamens. The calyx is look like funnel shaped. The fruits appear in the clusters form of 10-50, are ovoid, one seeded berry, 2-4 centimetres long, dark purple, shiny red, dark brown or nearly black in colour. The fruit is generally astringent, sometime unpalatable and flavour varies from sour to fairly sweet (Ayyanar and subhash-babu, 2012).

Figure: *Syzygium cumini* seeds and fruits

MATERIALS AND METHODS

Collection of Plant Materials: The fresh fruits of *Syzygium cumini* were collected from the local region of city Asansol, West Bengal, India in second week of August 2017. The collected fruits were washed under running tap water to remove the dust particles, fruit pulp was separated, seeds were cleaned thoroughly,

dried at room temperature for 1-2 weeks and finally crushed into the powder by using electrical grinder.

Preparation of Plant Extracts: The powdered sample were percolated by using Soxhlet apparatus successively with the organic solvent such as methanol, petroleum ether and ethanol (70% w/v) respectively. The extracts were taken and kept for further studies.

Screening for Phytochemical Compounds:
The seed extracts of *Syzygium cumini* were analysed for the presence of Phytoconstituents such as alkaloids, tannins saponins, flavonoids, phenols, terpenoids, steroids, amino acids and anthraquinone glycosides according to the standard methods **(**Harborne 1998), (Kokate 2001).

Test for alkaloids [Mayer's Test]: 1.36 gm of mercuric chloride dissolved in 60 ml and 5 gm of potassium iodide were dissolved in 10 ml of distilled water respectively. These two solvents were mixed and diluted to 100 ml using distilled water. To 1 ml of acidic aqueous solution of samples few drop of reagent was added. Formation of white or pale precipitates shows the presence of alkaloids.

Test for Tannins:
Ferric Chloride Test: The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green or black colour precipitates indicates the presence of tannins.
Lead acetate Test: In a test tube containing about 5 ml of sample extract, a few drops of 1% solution of lead acetate was added. Formation of bulky white precipitates indicates the presence of tannins.

Test for Saponins: A drop of sodium bicarbonate was added in the test tube containing 50 ml extract of the sample. The mixture was vigorously shaken and kept for two minutes. A honey comb like froth was formed and it showed the presence of saponins.

Test for Flavonoids: In a test tube containing about 0.5 ml of alcoholic extract of sample, 5-10 drops of diluted Hydrochloric acid and small amount of Mg or Zn were added and the solution was boiled for few minutes. Appearance of reddish pink or dirty brown colour indicates the presence of flavonoids.

Test for Phenols: To 1 ml of the alcoholic solution of the sample, 2 ml of distilled water followed by few drops of the 10% aqueous solution of ferric chloride were added. Formation of blue or deep green colour indicates the presence of phenols.

Test for Terpenoids: In a test tube, 2 ml of chloroform and 5-10 drops of conc. H_2SO_4 were added to 1mg of extract and observed for reddish brown colour that indicated the presence of terpenoids.

Test for Steroids [Salkowski's Test]: About 100 mg of dried extract of sample was dissolved in 2 ml of $CHCl_3$. H_2SO_4 was added carefully to form a lower layer. A reddish brown colour at the interface was an indicative of steroidal ring.

Test for Amino acids: 2 ml of extract sample was treated with the 1-2 drops of ninhydrin reagent. Appearance of violet or purple colour indicates the presence of amino acids.

Test for Anthraquinone Glycosides: Borntrager's Test: In a test tube containing 5 ml of extract, 2 ml of dilute H_2SO_4 was added, boil for 5 min and filtered. To the filtrates, equal volumes of $CHCl_3$ was added and mixed well. Organic layer was separated and 10% ammonia solution was added to this. Appearance of brick pink colour of the ammonia layer confirmed the presence of anthraquinone glycosides.

Screening of Antibacterial Activity through Cup-Plate Method: Antibacterial activity studies were carried out by Cup-Plate method. Nutrient agar plates were prepared to screen the bacterial growth for each extracts. The four bacterial strains were spread on the plate separately with the aid of spreader. Through cork borer well were prepared on the agar plate and the sample solution for each extracts (100 µl) was loaded within the well. Those plates were transferred into the incubator at 37^{o}C and kept for 24 hrs. The zone of inhibition (in mm) was calculated. The whole process should run aseptically (Akinpelu, 2006), (Prashanth et.al, 2006).

RESULT AND DISCUSSION

Table I: Phytochemical screening test of *Syzygium cumini seed extracts:*

Phyto-constituents	Organic Solvents		
	Methanolic Extract	Petroleum ether Extract	Ethanolic Extract
Alkaloids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Phenols	+	+	+
Terpenoids	+	-	+
Steroids	+	+	+
Amino acids	+	+	+
Anthra-quinone Glycosides	-	-	-

(+) = Presence of Phytoconstituents
(-) = Absence of Phytoconstituents

***Table II: Antibacterial activity of Syzygium cumini* seed extracts**

Test Organism	Zone of Inhibition (in mm)		
	Methanol	Petroleum ether	Ethanol
Escherichia coli	22.6	21.4	17.0
Salmonella typhi	19.3	17.0	10.4
Staphylococcus aureus	23.6	20.8	10.6
Bacillus subtilis	17.2	16.8	9.4

CONCLUSION

In this present study, the phytochemical screening and antibacterial activities were performed with methanol, petroleum ether and ethanol extracts of the seeds of *Syzygium cumini*. The results of phytochemical screening studies shows the *Syzygium cumini* seeds were rich in alkaloids, tannins, saponins, flavonoids, phenols, terpenoids, steroids and amino acids (as shown in Table I).

The antibacterial studies has been performed against two Gram positive pathogenic bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram negative pathogenic bacteria (*Escherichia coli* and *Salmonella typhi*) with three different extracts viz. methanol, petroleum ether and ethanol extracts using the Cup-Plate method. The zone of inhibition found for each extracts against the tested bacterial strains has been presented in Table II. So it has found that the methanolic extracts have more potent antibacterial activity than the other two.

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