Phytochemical analysis, nutritional profile and antibacterial studies of *Syzygium cumini* seeds

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Abstract

The current study reveals the phytochemical constituents, Nutritional aspects and anti bacterial properties of *Syzygium Cumini* seeds. *S.Cumini* is a tropical evergreen tree which belongs to the family Myrtaceae. *S.Cumini* is widely used in traditional medicine to treat blisters in mouth, colic, diarrhoea, digestive complaints, dysentery, pimples, stomach-ache etc.. Studies on *Syzygium Cumini* reported the anti diabetic property. Hence it is widely used for treat diabetes in India. The phytochemical analysis detected the seven various metabolites such as Alkaloids, Proteins, Saponins, Quinines, Flavonoids, Phenols and Phytosterol. Ethyl acetate extract of *S.Cumini* seed showed an inhibitory activity against the bacterial strain. The tree is rich in phytochemicals like glycoside jambolin anthocyanins, tannins, terpenoids, Xanthoproteins, Gallic acid and various minerals. These wide ranges of health promoting compounds make them a suitable candidate to be used as a nutraceutical. It has a high source of vitamin A and C. This indicate the abundant nutritional value of *Syzygium cumini* seed extract.

Key words: anthocyanins, nutraceutical, Xantho proteins.

INTRODUCTION

Syzygium cumini (L.) Skeels belongs to the family Myrtaceae commonly known as njaval, jamun, black plum, Indian blue berry etc.. The native home of the *Syzygium* is India and East Indies. It is found throughout in India up to an altitude of 1800 meters and its habitat starts from Myanmar and extended to Afghanistan. This plant is also found in other countries like Thailand, Philippines, Madagascar (Ivan, 2006). *Syzygium cumini* is a medium-sized tree 10-30m high, with a straight to crooked, short, stout trunk, 40-100cm in diameter, sometimes trees with circumferences of 62.5 cm have been reported. It can live up to 80- 100 years and planted in the various regions spontaneous. The entire part of plant has been widely used in the treatment of various diseases in the traditional

and folk medicine (Abdul Aziz and Sabyasachi Banerjee, 2018). Leaves are entire with narrow transparent margin. Flower clusters on old twig at the back of leaves, 5- 6 cm and wide. The fruit is commonly known as jamun (Hindi), java plum, black plum, jambul and Indian black berry. Fruits ovoid-oblong or elliptical berries, numerous, crowded in clusters, almost stalkless along twigs at the back of leaves; often curved, green at first, turning pink and then finally purple-black, 1-2.5 cm (max. 5) long; pulp greyish-yellow, white or pale violet. Ripe fruit is usually eaten fresh; it is juicy, almost odourless, with a pleasant, slightly bitter, astringent taste.

Syzygium cumini is widely used in traditional medicine to treat diabetes in India (Kandan Prabakaran and Govindan Shanmugavel, 2017). A glycoside in the seed, jamboline, is considered to have anti-diabetic properties (Ratsimamanga *et al*., 1973). The seeds have also shown anti-inflammatory effects in rats and antioxidant properties in diabetic rat (Prince and Venon, 1998). Older reports from Indian medical journals suggest jambul seed and bark can be beneficial in humans with diabetes (Sepha GS and Bose SN, 1956). The bark contains tannins and carbohydrates, accounting for its long-term use as an astringent to combat ailments like dysentery (Namasivayam *et al*., 2008). Jamun fruit seeds and pulp have been reported to serve various purposes in diabetic patients. Such as lowering blood glucose levels and delaying diabetic complications including neuropathy and cataracts. Jamun is most often recognized as an adjuvant therapy in type-2 diabetes. Good quality jambolan juice is excellent for sherbet, syrup and “squash”. In India the latter is a bottled drink prepared by cooking the crushed fruits for 5 to 10 minutes at 140^0 F, pressing out the juice, combining it with sugar and water and adding citric acid and sodium benzoate as a preservative. The seeds are claimed to contain alkaloid jambosine and antimellin, which halts the distatic conservation of starch in to sugar. The seeds have been reported to be rich in flavonoids, a well known antioxidant, which accounts for the scavenging of free radicals and protective effect on antioxidant enzymes and also found to have high total phenolics with significant antioxidant activity and are fairly rich in protein and calcium java palms are rich in sugar, mineral salt, vitamin c, which fortifies the beneficial effects of vitamin c, anthocyanins and flavonoids starch, myricyl alcohol in the

unsaponified fraction of seeds and a small quantity of pale yellow essential oil and also present. Fixed oils and fats are absent, proteins, steroids, triterpenoids are present in more quantity. The present paper was the detailed study of phytochemical, anti bacterial and nutritional aspect of *Syzygium cumini* seed.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

The fully matured *Syzygium cumini* seeds were collected in March month of 2021 from Panayarakunnu in Thiruvananthapuram District of Kerala..

BOTANICAL DESCRIPTION

The smooth tree of Myrtaceae family, 4-15 meters in height. Leaves leathery oblong- ovate to elliptical or obovate and 6-12 cm long, the tip being broad and shortly pointed. The panicles are borne mostly from the branchlets below the leaves, often being axillary or terminal and 4-6 cm long. The flowers are numerous, scented, pink or nearly white, without stalks, and borne in crowed fascicles on the ends of the branchlets. The calyx is funnel shaped, about 4mm long, and 4 toothed. The petals cohere and fall together as a small disk. The stamens are very numerous and as long as the calyx. Fruit is oval to elliptic; 1.5-3.5cm long, dark purple or nearly black, luscious, fleshy and edible; it contains single large seed.

SCIENTIFIC CLASSIFICATION

Kingdom : Plantae

Order : Myrtales

Family : Myrtaceae

Genus : Syzygium

Species : Cumini

Binomial name *: Syzygium cumini*

1. PHYOTOCHEMICAL ANALYSIS

QUALITATIVE ANALYSIS

1.1. Preparation of plant sample

The *Syzygium cumini* fruits were first washed well and pulp were removed from the seeds. Seeds were washed several times with distilled water to remove the traces of pulp from the seeds. The seeds were dried at room temperature and crushed into powder.

1.2. Sample extraction

1g of powdered sample were mixed with 10 ml of solvent (hexane, ethyl acetate, acetone, benzene, methanol and distilled water). These organic extract were kept overnight. After that it was centrifuged at 10000 rpm for 10 minutes. The supernatant were used for further analysis.

1.3. Pytochemical analysis

Phytochemical analysis of *Syzygium cumini* seed were carried out using standard method described by Sofowora, 1993.

(a) Detection of protein and aminoacid

5ml of extract was dissolved in 5ml of water and was subjected to the following test.

Ninhydrin test

1ml of the extract was treated with few drops of Ninhydrin solution.

Violet colour appeared suggesting the presence of amino acids and protein.

(b) Detection of carbohydrates

Molisch's test

Few drops of Molisch's reagent were added to 2ml portion of the various extracts. This was followed by addition of 2ml of con: H_2SO_4 down the side of the test tube. The mixture was

then allowed to stand for tow-three minutes. Formation of a red or dull violet colour at the interphase of the two layers were the indication of a positive test.

(c)Detection of fat and oil

Spot test

This was done by preparing spot on the filter paper with the test solution and oil staining on the filter paper indicated the presence of fixed oil and fat.

(d)Detection of phenol and tannins

1ml of extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black colouration indicated the presence of phenols and tannins.

(e)Detection of flavanoid

Alkaline reagent test

1ml of extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

(f)Detection saponin

Foam test

To 2ml of extract were added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

(g)Detection of steroid

Salkowski test

To 2 ml of aqueous extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

(h)Detection of Quinones

1 ml of extract was treated with alcoholic potassium hydroxide solution.

Quinones give colouration ranging from red to blue.

(i)Detection of xanthoprotein

1 ml of extract was treated with a few drops of conc. Nitric acid and ammonia solution. Formation of reddish orange precipitate indicated the presence of xanthoprotein.

(j) Detection of coumarins

1 ml of alcoholic extract was treated with alcoholic sodium hydroxide solution. Production of dark yellow colour indicated the presence of coumarins.

(k) Detection of carboxylic acid

1 ml of extract was treated with few ml of saturated solution of sodium bicarbonate. Observation of effervescence indicated the presence of carboxylic acid.

(l)Detection of Alkaloids

Crude extract were mixed with 2 ml of Wagner's reagent. Reddish brown loured precipitate indicated the presence of alkaloids.

2. NUTRITIONAL STUDY

QUANTITATIVE SCREENING OF PHYTOCHEMICALS

(a) Estimation of carbohydrates (Anthrone's method)

1 g of plant material was ground with 10 ml of 80% acetone. The crude extract was filtered. To 1 ml of the supernatant, 5 ml of anthrone's reagent was added. The test was kept in a water bath for 3 minutes and cooled in an ice bath. The OD was taken at 660 nm.

(b) Estimation of sugar

To 1 ml of the plant extract add 10 ml of distilled water. Filter and centrifuge at 3000 rpm for 10 minutes. Adjust the total volume of 10 ml using distilled water. Take 0.1 ml of the extract and add 1 ml of 5% phenol and 5 ml of concentrated sulphuric acid. The mixture is mixed well. It is hot hence it is cooled for 30 minutes. Measure the OD at 490 nm and extrapolate the value in the standard graph.

(c) Estimation of starch

To 1 ml of extract add 10 ml of distilled water. Filter it using cheese cloth. 0.5 ml of the filtrate add 4 ml of distilled water. Then add 1 ml of iodine. Heat it in the water bath for 10 minutes. Blue colour is developed. Read the OD at 650 nm.

(d) Estimation of protein (Lowery *et al*., 1951)

To 1 ml of the extract add 1 ml of distilled water with an ice bucket filter and centrifuge at 5000 rpm for 5 minutes make up the volume of the supernatant use to 10 ml distilled water. Pipette out 1 ml of extract and add 5 ml of folin ciocalteau phenol reagent. After 10 minutes measure the optical dencity at 500 nm and extrapolate the value in the standard graph.

(e) Estimation of aminoacid

To 1 ml of the extract added 10 ml of 80% ethanol. Make up the volume of the supernatant to 10 ml distilled water. Pipette out 2 ml of extract and added 2 ml of ninhydrin solution shacked well. Heat it in boiling water bath for 15 minutes until bluish purple colour appear. Cool it and added 50% ethanol read OD value at 510 nm and extrapolates the value in the standard graph.

3. ANTIMICROBIAL ACTIVITY OF SYZYGIUM CUMINI SEEDS

The anti bacterial activity of *Syzygium cumini* were evaluated according to the procedure described by Perez *et al*., (1990).

AGAR- WELL DIFFUSION METHOD

PRINCIPLE

The antimicrobials present in the sample is allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in centimeters.

PREPARATION OF EXTRACT

Dried seed powder (12g) was subjected to extraction using Cold extraction method with ethyl acetate as solvent. The extract was immersed in solvent and kept in rotary shaker for 48-72 hours at room temperature. The extract was recovered by filtration using double layer muslin cloth and dried under pressure. 1mg of dried extract was dissolved in 1ml of DMSO (Dimethyl sulfoxide) and used for antibacterial studies.

REAGENTS

1. Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.8 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

3. Streptomycin (standard antibacterial agent, concentration: 10mg / ml)

PROCEDURE

Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *E.coli*, *Pseudomonas aeroginosa* and *Streptococcus mutans* (growth of culture adjusted according to McFards Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and sample of 25, 50, and100 µl concentration were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control.

RESULTS AND DISCUSSION

The present study aims to investigate the preliminary phytochemical, nutritional and antibacterial activity of *Syzygium cumini* seed. The antimicrobial activity determined by using selected pathogenic organisms. The results emerged from these studies are presented below under appropriate headings.

1. PHYTOCHEMICAL ANALYSIS (QUALITATIVE ANALYSIS)

Preliminary qualitative phytochemical evaluation was performed for hexane, acetone, methanol and distilled water. The results obtained were represented in the table 3. Analysis of plant extracts revealed the presence of phytochemicals such as proteins, alkaloids, phenols, flavonoids, phytosterols, quinones, xanthoprotein, coumarin, saponins except carbohydrates. These results were supported by Ayyanar and Subhash-Babu, (2012) in *Syzygium cumini* seeds.

The result obtained in the case of hexane extract of *Syzygium cumini* seed only showed the presence of alkaloids. Acetone extract showed the presence of proteins, alkaloids and xanthoprotein. The proteins, alkaloids, phenols, flavonoids, phytosterol, quinone and saponin present in the case of methanol extract, while carbohydrate, fat, xanthoprotein, coumarin, and carboxylic acids are absent. Azra kamal, (2014) also reported the presence of preliminary phytochemical analysis of methanolic extract revealed the presence of alkaloids, proteins, saponins, flavonoids except carbohydrates in the extract of *Syzygium cumini* seeds. Alkaioids have been associated with medicinal use for centuries and one of their common biological properties is their cytotoxicity (Nobori *et al*., 1994). Saponins has the property of precipitating and coagulating red blood cells. Flavonoids were found in the extracts and are potent water soluble antioxidants (Borhade, 2012). Flavonoids have been referred to as nature's biological response modifiers. Aqueous extract of seed showed the presence of phenol, xanthoprotein, coumarin and carboxylic acid. The medicinal properties of *Syzygium cumini* seed extracts may be due to the presence of above mentioned phytochemicals.

2. NUTRITIONAL ANALYSIS (QUANTITATIVE ANALYSIS)

Table 2 showed the result of quantitative analysis of *syzygium cumini* seeds. *Syzygium cumini* seed rich in protein (2.502 %), sugar (2.502 %) followed by amino acid (0.582 %), carbohydrate (0.544 %) and starch (0.034 %). Bhatnagar *et al*., (2002) revealed that the *Syzygium cumini* seed contains protein (3.38 %), carbohydrate (1.3 %) and starch (1.82 %).

3. ANTIBACTERIAL ACTIVITY

The agar well diffusion method was used to evaluate the antibacterial activity of the *Syzygium cumini* seed extract by measuring the zone of inhibition against the test organisms. **Figure 6** showed the diagrammatic representation of antibacterial activity of ethyl acetate extract of *Syzygium cumini* in the concentration of 25, 50, and 100µl against the bacterial strains *Escherichia coli, Pseudomonas aeroginosa,* and *Streptococcus mutans*. Streptomycin was used as a positive control. The results were recorded in the Table 3, 4, and 5. The antibacterial activities of the selected bacterial strains are examined by the presence or absence of inhibition zones. The zone of inhibition was high in *Streptococus mutans*, and *Pseudomonas aeroginosa* when compared to *Escherichia coli*. Ruthurusamy *et al*., (2015) also reported ethyl acetate extract of *Syzygium cumini* seed inhibit the growth of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeroginosa*. Mubassara *et al*., (2015) antibacterial activity of various extracts of *Syzygium cumini*. The leaf and bark extracts were found to be more potent antimicrobial than the pulp and seed extracts. The results obtained from the study suggest a potential application of *Syzygium cumini* plant for treatment of infectious diseases.

PLATE - 1

(A)

(B)

(C)

(D)

A –*Syzygium cumini* fruits, B – *Syzygium cumini* seeds, C - Shade dried seeds of *Syzygium cumini*, D – *Syzygium cumini* seed powder

Table 1: Phytochemical Screening Of *Syzygium cumini* Seed

SL NO	Test	Method	Hexane	Acetone	Methanol	Distilled water
1	Carbo hydrate	Molisch's Test	-	-	-	-
2	Proein	Ninhydrin Test	-	+	+	-
3	Fat and oil	Spot Test	-	-	-	-
4	Alkaloid	Wagner's Test	+	+	+	-
5	Phenol	Ferricchloride Test	-	-	+	+
6	Flavanoid	Alkaline Regent Test	-	+	+	-
7	Phytosterol	Salkowski Test	-	-	+	-
8	Quinone	KOH Test	-	-	+	-
9	Xantho Protein	Nitric acid Test	-	+	-	+
10	Coumarin	Made alkaline	-	-	-	+
11	Carboxylic acid	NaOH Test	-	-	-	+
12	Saponin	Form Test	-	-	+	-

PLATE – 2

(A)

(B)

(C)

(D)

Phytochemical analysis of various solvent extracts (Hexane, Acetone, Methanol and Distilled water) of *Syzygium cumini* seed.

(A) Hexane (B) Acetone

(C) Methanol (D) Distilled water

Table 2: Quantitative analysis of *Syzygium cumini* seeds

Sl. No	Phytochemicals	Quantity(%)
1	Carbohydrate	0.544
2	Protein	2.502
3	Amino acid	0.582
4	Sugar	2.502
5	Starch	0.034

Table 3: Antibacterial activity of *Syzygium cumini* seed extract (Ethyl acetate) against *Escherichia coli*

Sample	**Concentration (µl)**	**Zone of inhibition (cm)**
Seed powder extract	Streptomycin (20µl)	3.7
	25	1.0
	50	1.1
	100	1.5

Table 4: Antibacterial activity of *Syzygium cumini* seed extract (Ethyl acetate) against *Pseudomonas aeroginosa*

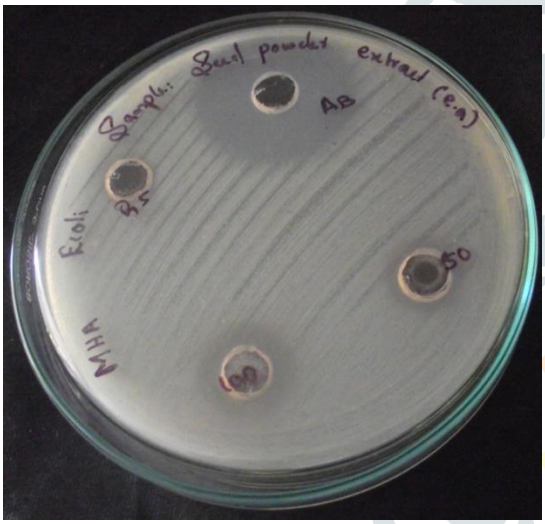
Sample	**Concentration (µl)**	**Zone of inhibition (cm)**
Seed powder extract	Streptomycin (20µl)	3.7
	25	1.0
	50	1.3
	100	1.6

Table 5: Antibacterial activity of *Syzygium cumini* seed extract (Ethyl acetate) against *Streptococcus mutans*

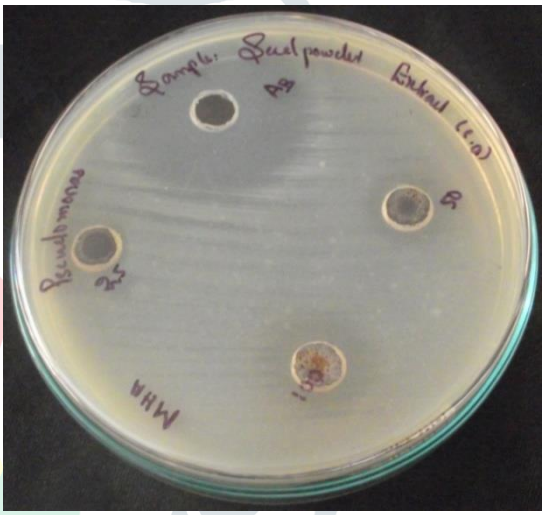
Sample	Concentration (µl)	Zone of inhibition (cm)
Seed powder extract	Streptomycin (20µl)	3.8
	25	1.2
	50	1.6
	100	2.1

Note: Concentration of Sample Stock: 1mg/ml DMSO

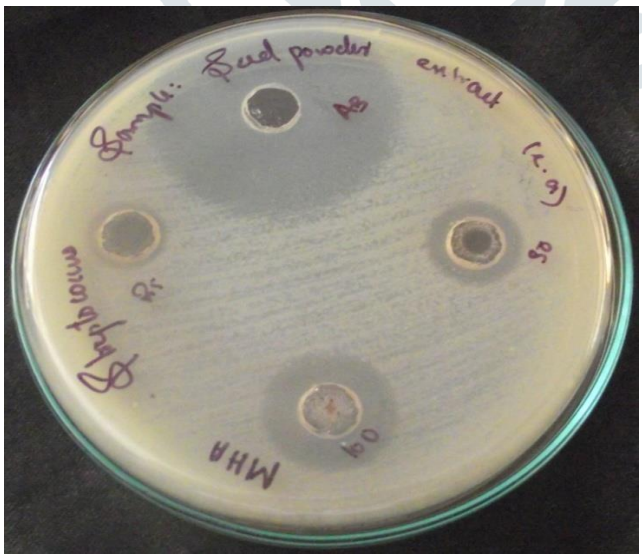
PLATE – 3



(A)



(B)



(C)

Antibacterial activity of *Syzygium cumini* seed

(A) Escherichia coli

(B) Psudomonas aeroginosa

(C)Streptococcus mutans

CONCLUSION

Syzygium cumini has been traditionally used in medicine for various purposes. A number of studies have been conducted to elucidate the therapeutic and nutritional activity of its different plant parts. Antidiabetic activity of the plant is well established but there are also published data on potent activity against other diseases. The seed of this plant are pharmacologically the best studied but others also posses pharmacological potential.

Based on the traditional uses and literature review of earlier studies, the plant was selected. In the present study, an attempt has been made to identify the phytochemical, nutritional and antibacterial activity of *Syzygium cumini* seed. Phytochemical analysis conducted on the plant extracts has revealed the presence of phytochemicals such as alkaloids, proteins, saponins, quinines, flavonoids, phenols and phytosterol. Plants having antimicrobial compounds have enormous therapeutic potential as they can act without any side effect as often found with synthetic antimicrobial products. In the present study, the ethyl acetate extract of *Syzygium cumini* seed showed inhibitory activity against the bacterial strains. Hence it can be concluded that most of the biologically active phytochemicals were present in *Syzygium cumini* seed extracts and show better antibacterial activity.

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