



Characterization and Therapeutic Potential of *Syzygium Cumini*: Review of Sources, Morphology and Future Research

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Abstract Java plum is widely recognized as a plant with valuable medicinal properties, originating from Indonesia and India and distributed globally in the tropic and sub-tropic regions of the world. The plant is rich in alkaloids, flavonoids, phenylpropanoids, terpenes, tannins, and lipids. The phytoconstituents of the plant seeds possess various vital pharmacological activities and clinical effects including their antidiabetic potential. The bioactive phytoconstituents of Java plum seeds include jambosine, gallic acid, quercetin, β -sitosterol, ferulic acid, guaiacol, resorcinol, p-coumaric acid, corilagin, ellagic acid, catechin, epicatechin, tannic acid, 4,6 hexahydroxydiphenoyl glucose, 3,6-hexahydroxy diphenoylglucose, 1-galloyl glucose, and 3-galloyl glucose. Considering all the potential beneficial effects of the major bioactive compounds present in the Jamun seeds, in the current investigation, the specific clinical effects and the mechanism of action for the major bioactive compounds along with the extraction procedures are discussed.

Keywords *Syzygium Cumini*, Java plum

1. Introduction

Syzygium Cumini (L.) Skeels., more popularly referred to as black Jamun or Java plum, belongs to the family Myrtaceae, and is a vital indigenous plant with medicinal applications originally from India and Indonesia; it is distributed in the tropics and subtropics around the globe [1,3]. The plant is fast-growing and can grow 30 m or more in height and its lifespan is more than 100 years [4]. The plant is treated as economically important as all of its parts, starting from the seeds and leaves to the wood, have great medicinal and economical values [2]. The plant possesses various phytoconstituents and has high antioxidant potential, which is very much beneficial for our bodies. It possesses phytoconstituents that include glucoside, anthocyanin, steroids, phenols, flavonoids, and terpenoids [5]. The fruit is rich in carbohydrates, vitamins, and minerals; the fruit pulp contains some important minerals including manganese, calcium, potassium, iron, zinc, and sodium [6,7]. Its purple-to-blackish colour is the result of the anthocyanins present within the plant. Other than the fruits, leaves and bark also have medicinal properties [2]. They are used in diabetes, ringworm, and diarrhoea [1,2]. The bark is used as a digestive anthelmintic and diuretic [4]. In addition, seeds are used in various traditional and oriental systems of medicine such as in Ayurvedic, Unani, and Chinese medicines as a natural substitute for the treatment of hyperglycemia, ulcers, dysentery, asthma, glycosuria, and bronchitis [2].



2. Geographical source

The jamun is native to India, Burma, Ceylon and to the Andaman Islands and available throughout Indian plains as well as in Kumaon hills up to 1,600 m. It is found grown as a wild and semi-wild in tropical and subtropical parts of India viz., Punjab, Haryana, Uttar Pradesh, Maharashtra, Rajasthan, Gujarat, Madhya Pradesh, Bihar, Chhattisgarh, Jharkhand, Karnataka, Kerala, Tamil Nadu and Andhra Pradesh. It is widely distributed in Sri Lanka, Malaysia, Thailand, Philippines and Australia.

Species	Common Name and Use	Distribution
<i>S. aromaticum</i>	Clove, dried flower buds commercially important	Evergreen trees cultivated in Tamil Nadu and Kerala
<i>S. jambos</i>	Rose-apple	Many parts of India
<i>S. fruticosum</i>	Wild jamun	Avenue Tree
<i>S. claviflorum</i>	Fruits edible	The Andamans
<i>S. javanica</i>	Water apple	South India and West Bengal
<i>S. aquem</i>	Watery rose-apple, Fruits edible	A small tree distributed in Assam and Meghalaya
<i>S. samarangense</i>	Wax Jambu, Fruits edible	The Andamans and many parts of India
<i>S. zeylanicum</i>	Fruits edible	Maharashtra, Karnataka, Orissa, Kerala and Andamans
<i>S. mappaceum</i>	Ornamental plant	Assam, Meghalaya, Arunachal Pradesh and Tamil Nadu
<i>S. amottlanum</i>	Fruits edible	Western Ghats, The Nilgris, Palani and Anamalai hills
<i>S. zeylanicum</i>	Fruits edible	Western ghats of India

Syzygium Cumini (Java plum, Jamun):

Large, evergreen beautiful tree of the Indian subcontinent but has also naturalised throughout Southeast Asia and the Pacific Islands. It is widely cultivated in Haryana as well as the rest of the Indo-Gangetic plains on a large scale. Fruits are generally ovoid to oblong in shape, deep purple or bluish in colour, having juicy, sweet pulp and a small stone.

***Syzygium jambos* (Rose apple):** Trees are medium, evergreen and grown in Assam, Bihar, Andhra Pradesh, Tamil Nadu, West Bengal, coastal areas of Maharashtra and Gujarat. Leaves have very small petioles and calyx persistent. Fruits are light yellow- white in colour, rose scented and seeds are polyembryonic.

***Syzygium uniflora* (Surinam cherry or Pitanga cherry):** A small tree and bears small-sized fruits having bright red colour and aromatic flavour. The tree is found in South India.

***Syzygium fruticosum*:** Trees are suitable for windbreak and have straight growth habit. Fruits are edible and small. *Syzygium densiflora*: Suitable for use as rootstock for *Syzygium Cumini*. It is resistant to termites attack.

3. Morphology

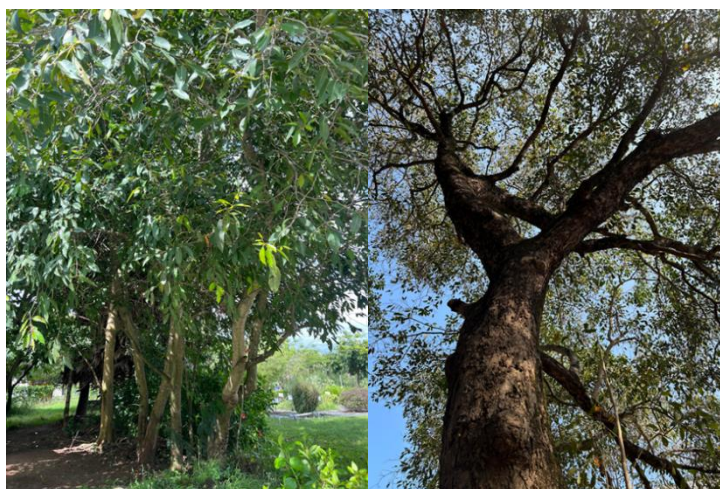
Java plum grows quickly and forms dense foliage, providing abundant shade. The rough, dark bark near the tree's base lightens to a smoother grey higher up, and its water-resistant wood is used for construction, like railway sleepers and motor installations for wells.

The young leaves start out pinkish and mature to a leathery, glossy dark green, providing nutritious fodder for livestock. The tree flowers between March and April, producing small, fragrant blossoms. The fruits mature from May to June, ripening in stages from green to pink, crimson, and finally deep black (or occasionally white, depending on the variant). Known for their mix of sweet, mildly sour, and astringent flavours, these fruits often stain the tongue purple. This tree's ornamental appeal and versatile uses make it valuable in both rural and urban environments [25].



Botanical Description:

- a) Kingdom: Plantae
- b) Subkingdom: Viridiaeplantae
- c) Infrakingdom: Streptophyta
- d) Division: Tracheophyta
- e) Subdivision: Spermatophytina
- f) Infra Division: Angiospermae
- g) Class: Magnoliopsida
- h) Superorder: Rosanae
- i) Order: Myrtales
- j) Family: Myrtaceae
- k) Genus: Syzygium
- l) Species: Cumini
- m) Scientific Name: *Syzygium cumini*.



The morphology of the fruit of *Syzygium Cumini* (commonly known as Jamun, Jambolan, or Java plum) includes the following features:

General Description

- Type: The fruit is a berry.
- Shape: The fruit is typically oval to oblong, sometimes ovoid.
- Size: It usually ranges from about 1–2 cm in length and 1–1.5 cm in diameter, though size can vary with the cultivar

Colour and Texture

- Colour: The fruit is green when immature and turns dark purple to almost black when ripe. Some may appear magenta or reddish-purple in certain stages of ripening.
- Texture: The outer skin is smooth and shiny, and it can appear waxy.

Flesh and Seed

- Flesh (Pulp): The pulp is juicy and can be pinkish to purple. It has a slightly astringent, sour-sweet taste, which becomes sweeter as the fruit ripens.
- Seed: Each fruit typically contains a single, large, polyembryonic seed that is either round or oblong, with a hard, greenish-brown coating. In some cases, the seed may split into two halves.

Arrangement and Clustering

- Arrangement: The fruits grow in clusters on the tree, hanging from short stems.



- Clustering: Clusters often have several berries, though each berry remains distinct [25,26].

Other Features

- Juice: The juice is often dark purple and can stain, commonly used for natural dyes.
- Aroma and Flavor: The fruit has a mildly sweet fragrance with an astringent, sweet-tart flavour profile, becoming less astringent as it ripens.



The morphology of *Syzygium Cumini* leaves (commonly known as Jamun or Java plum) includes the following features:

General Description

- Shape: The leaves are typically elliptical, ovate to oblong, sometimes lanceolate.
- Size: They range from about 5–12 cm in length and 2.5–6 cm in width.
- Texture: The leaves are leathery and smooth, with a glossy surface on the top side.

Margins and Venation

- Margins: The edges of the leaves are entire, meaning they lack serration or lobes.
- Venation: They exhibit a pinnate venation pattern with a prominent midrib. Secondary veins run parallel, arching towards the margins and converging near the leaf tip.

Petiole

- Length: The petiole is short, typically around 0.5–1.5 cm.
- Colour and Texture: It is green, firm, and sometimes exhibits a slight pinkish hue in young leaves.



Arrangement and Phyllotaxy

- Arrangement: Leaves are opposite and decussate, meaning they are paired at each node and arranged in a cross pattern.
- Phyllotaxy: Opposite.

Other Features

- Aroma: Crushed leaves emit a faint, turpentine-like aroma.



- Glands: Tiny, glandular dots may be visible under magnification, which sometimes give the leaves a slightly dotted appearance.

Adaptation

The leathery and glossy nature of the leaves helps reduce water loss and adapt to subtropical to tropical climates, where *Syzygium Cumini* commonly grows.

The bark of *Syzygium Cumini* (Jamun or Java plum) has distinctive morphological features:

General Description

- Colour: The bark is dark grey to brownish-grey, with an overall rough texture.
- Texture: Mature bark is rough and fissured, sometimes with vertical cracks and scaly patches.

Structure and Layers

- Outer Bark: The outer layer is thick and corky, providing protection against environmental stressors.
- Inner Bark: The inner bark, when exposed, has a reddish-brown to pinkish hue.
- Pattern and Shedding
- Pattern: The bark has a rough, ridged, and grooved appearance, with vertical fissures that may become deeper and more pronounced with age.
- Shedding: Older trees may shed bark in small, irregular patches, which can reveal smoother, lighter-coloured patches underneath.



Other Features

- Lenticels: Young stems and branches often have visible lenticels (small, raised pores) that help in gas exchange.
- Aromatic Properties: When scraped, the inner bark has a slight aromatic quality, similar to the leaves, due to essential oils.

The roots of *Syzygium Cumini*, commonly known as jamun or black plum, exhibit several distinct morphological characteristics:

- Type: The root system is typically fibrous, consisting of many thin, branching roots that spread widely in the soil.
- Main Roots: The main taproot is relatively deep and robust, helping anchor the plant and access deeper soil moisture.
- Lateral Roots: Numerous lateral roots emerge from the main taproot, increasing the surface area for nutrient and water absorption.
- Root Hairs: These fine, hair-like extensions increase the absorptive capacity of the roots, aiding in nutrient uptake.
- Adaptation: The roots often exhibit adaptations to local soil conditions, such as being more shallow in compact or rocky soils.
- Mycorrhizal Associations: *Syzygium Cumini* may form symbiotic relationships with mycorrhizal fungi, which enhance nutrient absorption, particularly phosphorus [25,26,27].

4. Conventional Use of the Plant for Therapeutic Activity

All parts of the jambolan can be used medicinally and it has a long tradition in alternative medicine. From all over the world, the fruits have been used for a wide variety of ailments, including cough, diabetes, dysentery,



inflammation and ringworm [8]. It is also an ancient medicinal plant with an illustrious medical history and has been the subject of classical reviews for over 100 years. It is widely distributed throughout India and ayurvedic medicine (Indian folk medicine) mentions its use for the treatment of diabetes mellitus. Various traditional practitioners in India use the different parts of the plant in the treatment of diabetes, blisters in mouth, cancer, colic, diarrhoea, digestive complaints, dysentery, piles, pimples and stomachache [9]. During the last four decades, numerous folk medicinal reports on the antidiabetic effects of this plant have been cited in the literature. In Unani medicine various parts of jambolan act as liver tonic, enrich blood, strengthen teeth and gums and form good lotion for removing ringworm infection of the head [10].

Malayalis in South India

Paste of seeds is prepared with the combination of leaves of *Momordica charantia* and flowers of *Cassia auriculata* and taken orally once a day for 3 months to treat diabetes. [30].

Seeds are taken orally for generations as the centerpiece of an effective therapy for counteracting the slow debilitating impacts of diabetes [14].

Siddis in Karnataka, India

The juice obtained from the stem bark is mixed with butter milk and taken orally every day before going to bed to treat constipation. The same recipe, when taken early in the morning on an empty stomach, is claimed to stop blood discharge in the faeces [15].

Rural population in Brazil

Leaves of jambolan are taken orally in the treatment of diabetes [16].

Lakher and Pawi in North east India

Infusion of fruit or mixture of powdered bark and fruit is given orally to treat diabetes. Juice obtained from the seeds is applied externally on sores and ulcers. Powdered seeds are mixed with sugar are given orally 2–3 times daily in the treatment of dysentery. The juice of leaves is given orally as an antidote in opium poisoning and in centipede bite [12].

The plant has been viewed as an antidiabetic plant since it became commercially available several decades ago. In the early 1960s to 1970s, some preliminary reports on the antidiabetic activity of different parts of jambolan in diabetic animals were reported. Most of these studies have been conducted using crude preparation of the plant without pointing out their chemical profile and antidiabetic action in animals is not fully understood. A number of herbal formulations were also prepared in combination with this plant available in the market which showed potential antidiabetic activity and are used regularly by diabetic patients on the advice of the physicians. Different parts of the jambolan were also reported for its antioxidant, anti-inflammatory, neuropsychopharmacological, antimicrobial, anti-bacterial, anti-HIV, antileishmanial and antifungal, nitric oxide scavenging, free radical scavenging, anti-diarrheal, antifertility, anorexigenic, gastroprotective and anti-ulcerogenic and radioprotective activities [17].

Reported medical actions

Jamun is rich in essential nutrients, including vitamins (especially vitamin C), minerals (like iron and calcium), and antioxidants, particularly anthocyanins, which give the fruit its characteristic deep colour. It also contains bioactive compounds like polyphenols, tannins, and flavonoids, which contribute to its health benefits.

The extracts of jamun is known to possess a wide range of pharmacological activities including antidiabetic, antihyperlipidemic, antihypercholesterolemic, anti-inflammatory, antimicrobial, anticancer, neuroprotective, cardioprotective, hepatoprotective and antioxidant activities as established by scientific studies.

Antidiabetic activity

Various *in vivo* studies revealed the antidiabetic activity of the extracts of jamun seeds. The hypoglycemic effect of the flavonoid rich extract from the jamun seeds was studied in streptozotocin induced diabetic rats. The flavonoid rich extracts from the jamun seeds have shown to reduce fasting blood glucose level in severe and mild diabetic rats. Further, the flavonoids stimulated the *in vitro* insulin release from the pancreatic islets by 16% [33]. The ethanolic extract of jamun seeds was used alone and in combination with glimepiride in rats. It significantly reduced the blood glucose level in rats [34].



A human study has been conducted on patients with type 2 diabetes mellitus and uncontrolled blood sugar level. The patients were on oral hypoglycemic agents [OHA] and were administered with the powder of jamun seeds at a dose of 10g/day, it resulted in decrease in the fasting plasma glucose and postprandial blood sugar. Supplementation with jamun seed powder improved glycemic control significantly [35].

Antihyperlipidemic and antihypercholesterolemic activity

Occurrence of several bioactive compounds in the jamun seeds help to regulate the blood lipid profile. Alcoholic jamun seed extract was administered orally to diabetic rats at a dose of 100mg/kg body weight. The extract seemed to reduce blood glucose & urine sugar and serum lipids in alloxan diabetic rats [36]. The ethanolic extract of jamun seeds exhibited hypolipidemic effect as evident from fall in total serum cholesterol (TC)/ high density lipoprotein (HDL) cholesterol ratio, serum low density lipoprotein (LDL) cholesterol level and decreased activity of HMG-CoA reductase [37]. The plasma lipoprotein cholesterol (HDL-, LDL-, and VLDL-C) and fatty acid composition were altered in streptozotocin-induced diabetic rats when administered the ethanolic jamun seed extract [38].

Anti-inflammatory activity

Several *in vivo* studies confirmed that the jamun seed extracts are capable of exhibiting anti-inflammatory effects. It is effective in both acute and chronic inflammation. The inhibition of carrageenan induced paw edema in rats was reported on treatment with chloroform fraction of *Syzygium cumini*. Migration of leukocytes into the pleural fluid was inhibited. In addition, the reduction of the weight of cotton pellet-induced granuloma was observed [39]. Ethyl acetate and methanol extracts of jamun seeds when administered orally to Wistar rats with carrageenan induced paw edema elicited anti-inflammatory effect [40].

Antimicrobial activity

Bag *et al.* [2012] reported the antibacterial activity of ethanol and acetone extract of *S. cumini* seeds. Agar well diffusion and microbroth dilution assay methods were used by the author to confirm the antibacterial effect of *S. cumini* seeds. Jasmine *et al.* [2010] have reported on the antibacterial activity of jamun seed extracts against β -lactamase producing drug resistant *Staphylococcus aureus*. The authors reported that the saponins derived from the methanol extract of jamun seeds demonstrated antibacterial activity. The zone of inhibition was in the range of 14–21 mm and a minimum inhibitory concentration (MIC) was in the range of 31.75–62.5 $\mu\text{g/mL}$. Numerous sesquiterpenoids have been isolated from the seeds of *Eugenia jambolana* and their structure were elucidated using NMR and MS spectrometry data analysis. Among the 24 sesquiterpenoids isolated, eight of them showed inhibition against the growth of *Staphylococcus aureus* [43].

Santos *et al.* [2020] reported that the phenolic extracts of jamun seeds exhibited antimicrobial activity against a variety of pathogens, including *Aeromonas hydrophila*, *Chromobacterium violaceum*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* serovar Typhimurium, *Serratia marcescens*, *Listeria monocytogenes*, and *Staphylococcus aureus*. The antimicrobial action of jamun seeds against *Bacillus subtilis* was reported. The methanolic extract of the seeds were used in the agar well diffusion assay.

The antifungal activity of jamun seeds were reported by Chandrasekaran and Venkatesalu [2004] by using the methanolic and water extract of the seeds. The extract was found to be effective against fungi such as *Candida albicans*, *Aspergillus niger* and *Microsporum gypseum*.

Anticancer activity

Jamun seed extract exhibited protection in albino mice against peroxidative damage contributing to skin cancer [44]. The oral intake of extracts reduced tumour burden, number of papilloma cells and their size. In the study by Arun *et al.* [2011], mice were given oral doses of jamun seed extract (500 - 1500 mg/kg body weight) prior to exposure to genotoxic carcinogens (7,12- dimethylbenz (α) anthracene and urethane). The treatment effectively prevented pBR322 DNA breakage, significantly reduced chromosomal abnormalities during metaphase, and lowered the formation of micronuclei in polychromatic erythrocytes.

Neuroprotective properties

Alikatte *et al.* [2012] evaluated the anti-amnesic effects caused by the methanolic extract of *S. cumini* seeds. The authors reported on the anti-amnesic effects of the seed extract on rat models. The extract administration (200 and 400 mg/kg) improved the impairment of short term or working memory induced by scopolamine and reversed



cognitive impairments in rats. The activity of acetylcholinesterase in the brains of the rats treated with the extract was inhibited, further the level of lipid peroxidation was lower and the activities of superoxide dismutase and catalase higher than in the control group.

Cardio and hepatoprotective properties

Oral administration of methanolic extract of jamun seeds to mouse models at a dose of 200 mg/kg body weight exhibited the protective and recovery ability of the cardiac tissues from damage. The recovery ability was due to the capability of the extract to reduce the biomarkers causing myocardial necrosis. The biomarkers include aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid, creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) [45].

The methanolic extract of jamun seeds administered orally to mice (200 mg/kg body weight) has shown hepatoprotective effect. It was studied that the methanolic extract of *S.cuminiseeds* helped in the recovery of liver damage. This revealed the ability of the extract to reduce serum AST, ALT, total protein and bilirubin levels [45]. Carbon tetrachloride is a widely used experimental hepatotoxicant. Administration of methanolic extract of *Eugenia jambolana* (doses 100, 200 and 400 mg/kg) to carbon tetrachloride treated rats revealed its hepatoprotective effect. The extract significantly reversed the elevated marker enzymes (glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), acid phosphatase (ACP) and bilirubin), which were comparable even with those of the Liv.52®-treated group.

Antioxidant activity

The antioxidant potential of jamun seeds was analysed by several in vitro methods, using various techniques for the extraction of bioactive compounds. The phenolic constituents present in the fruit parts of the underutilised indigenous black jamun landraces exhibited significant antiradical activity [46]. Bitencourt et al. [2017] conducted an in vivo experiment that demonstrated the antioxidant effect of jamun seed extract. *Candida albicans*-infected diabetic rats were treated with an aqueous seed extract and the same extract in the form of nanoparticles with a daily dose of 100 mg/kg for three weeks. The crude extract and its nanoparticle form were able to decrease levels of thiobarbituric acid reactive substances (TBARS) in serum, kidney, liver, and pancreas of the treated groups when compared to control animals.

5. Isolation and Characterization of Various Parts of *Syzygium Cumini*

The bioactive phytoconstituents of Java plum include jambosine, gallic acid, quercetin, β -sitosterol, ferulic acid, guaiaicol, resorcinol, p-coumaric acid, corilagin, ellagic acid, catechin, epicatechin, tannic acid, 4,6 hexahydroxydiphenoyl glucose, 3,6-hexahydroxy diphenoylglucose, 1-galloyl glucose, and 3-galloyl glucose. Hence, isolation and analysis is of crucial importance.

Considering all the potential beneficial effects of the major bioactive factors such as the extraction methods of Jamun seeds, solvents used in extraction, and duration of extraction cause significant changes in extracted bioactive constituents and reaction kinetics. This naturally leads to the potential health benefits, which are medicinal properties, attributed to Jamun seeds. The most common and important methods used to extract the major bioactive components of Jamun seed are Soxhlet extraction (SJE) and gas chromatography–mass spectroscopy (GC-MS) are the main methods in Jamun seed oil analysis.[47]

Methodology:

1. Collection:

Fresh leaves, bark, seeds, or fruit of *Syzygium Cumini* are collected, washed, and dried. These parts are then ground into a fine powder.

2. Extraction:

Syzygium Cumini extracts can be prepared using various solvents like methanol, ethanol, acetone, or aqueous solutions, depending on the targeted compounds. The powdered plant material is subjected to Soxhlet extraction or maceration, where the solvent is allowed to interact with the plant material to dissolve the phytochemicals. After extraction, the mixture is filtered, and the solvent is evaporated using a rotary evaporator, leaving behind the crude extract.



3. Isolation of Active Compounds:

Thin Layer Chromatography (TLC) and Column Chromatography are commonly employed to separate the compounds in the crude extract. Fractions from column chromatography can be analysed and purified further. High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS) can be used to identify and quantify the bioactive compounds.

4. Characterization of Phytochemicals:

- UV-Visible Spectroscopy: Used for preliminary analysis of phenolic and flavonoid content.
- Fourier-Transform Infrared Spectroscopy (FTIR): Helps identify functional groups present in the isolated compounds.
- Nuclear Magnetic Resonance (NMR) Spectroscopy: Provides detailed structural information about the isolated compounds.
- Mass Spectrometry (MS): Helps determine the molecular weight and composition of isolated compounds.

Phytochemical screening [20,21]

Alkaloids

- Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
- Mayer's Test: To a few ml of plant sample extract, two drops of Mayer's reagent are added along the sides of the test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.
- Wagner's Test: A few drops of Wagner's reagent are added to few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive.[18]

Flavonoids

- Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless in addition to dilute acid, indicates the presence of flavonoids.
- Froth Test: The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins.

Tannins

- Ferric Chloride Test: The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral ferric chloride solution are added. A dark green colour indicates the presence of phenolic compounds.

Glycosides

For 50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests:

- Legal's Test: 50 mg of extract is dissolved in pyridine; sodium nitroprusside solution is added and made alkaline using 10% NaOH. Presence of glycoside is indicated by the pink colour.

Phenols

- Lead Acetate Test: The extract (50 mg) is dissolved in distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Proteins

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatman No. 1 filter paper and the filtrate is subjected to a test for proteins.

- Millon's Test: To 2 ml of filtrate few drops of Millon's reagent are added. A white precipitate indicates the presence of proteins.

Carbohydrates

- Molisch's Test: To 2 ml of plant sample extract, two drops of alcoholic solution of naphthol are added. The mixture is shaken well and a few drops of concentrated sulphuric acid is added slowly along the sides of the test tube. A violet ring indicates the presence of carbohydrates.

Phytosterols

- Libermann-Burchard's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Con. Sulphuric acid was added.



Antioxidant assay

The antioxidant assay was performed using the potassium ferrocyanide reducing method by Oyaizu using Ascorbic Acid (1%) as standard. Aliquots of the extract were taken in a test tube and made up to a volume of 1 ml with distilled water. Then 2.5 ml of Phosphate buffer and 2.5 ml of potassium ferricyanide were added to each of the tubes, and incubation was carried out for 20 min at 50°C in a water bath. 2.5 ml of 2.5 ml + FeCl₃ = 2.5 ml³. The concentration of the sample was calculated by using the following formula, (O.D = Optical Density)

$$\text{Concentration of the sample (mg/ml)} = \frac{\text{concentration of sample} \times \text{O.D of sample}}{\text{O.D of sample}}$$

Extraction:

Dried leaf powder (50 g for each solvent) was soaked separately in 250ml of each solvent namely: distilled water, ethanol, methanol, ethyl acetate, in a flask. The five flasks were covered and then kept at room temperature for around one week. After that, the separate solutions were filtered by Whatman filter paper (no.1). Each filtrate was collected in a round bottom flask and was subjected to evaporation to achieve a gummy appearance. Then the gummy substance of each solvent was dried at room temperature. The powdered extracts were weighed and stored at 40°C for further work. From 50 g dried leaf powder, 7 g (14%) of extract was finally obtained from aqueous extract; 7.8 g (15.6%) of extract from ethanolic extract; 7.3 g (14.6%) from methanolic extract; 6.9 g (13.8%) from hexane extraction.[22]



Figure: extraction by decoction method

TLC of gallic acid: This separation process consists of two phases: a stationary phase and a mobile phase. The mobile phase consists of the mixture to be separated which percolates through the stationary phase. These two phases can be solid-liquid, liquid-liquid or gas-liquid.

Thin Layer Chromatography (TLC) is a solid-liquid form of chromatography where the stationary phase is a polar adsorbent and the mobile phase can be a single solvent or combination of solvents.

Principle: Thin Layer Chromatography (TLC) is a type of chromatography which is based upon the distribution of biomolecules between two immiscible phases. In TLC the stationary phase is a polar adsorbent, like finely ground alumina (Al₂O₃) or silica (SiO₂) particles which are coated on a glass slide or plastic sheet to create a thin layer of the particular stationary phase.

The solvent (mobile phase) is allowed to move up the plate by capillary action through the adsorbent at its own rate and as a result differential partitioning occurs between the components of the mixture dissolved in the solvent and the stationary adsorbent phase. The more strongly a given component of a mixture is adsorbed onto the stationary phase, the less time it will spend in the mobile phase and the more slowly it will migrate up the plate. When the solvent front has moved to within about 1 cm of the top end of the adsorbent, the plate should be removed from the developing chamber. If the components of the sample are coloured, they can be observed directly. [22]



Mobile phase used

Ethyl acetate, formic acid, glacial acetic acid, and water in varying ratios was tried. The mobile phase toluene: ethyl acetate: formic acid: methanol (2.5:2:0.9:0.3)

Sr. NO.	Chemical	Solvent /Mobile phase	RF value
1	Galic acid	phase toluene: ethyl acetate: formic acid: methanol (2.5:2:0.9:0.3)	0.35/10=0.35
2	Ellagic acid	phase toluene: ethyl acetate: formic acid: methanol (2.5:2:0.9:0.3)	0.19\10=1.9

Extraction Java plum containing gallic acid by decoction method. For verification of that gallic acid TLC is performed in which RF value. From which identification of Gallic acid from java plum and Extraction of Gallic Acid from Java Plum Leaves has been done.

Different methods are used in the analysis of the bioactive components of Jamun seed. While the most common traditional methods are the conventional solvent extraction method and SJE, modern methods include UJE or MJE, ultrafiltration, solid-phase microextraction, and supercritical fluid extraction.

Carmo Brito *et al.* emphasised that ethanol 95% with 1% of HCl (v/v) is the most efficient extraction method to extract anthocyanins from Jamun fruits. In another study, as a result of analysis using different extraction methods, the total phenolic contents (mg GAE/100 g), flavonoid content (mg quercetin equivalents (QE)/100 g), and total anthocyanin contents (mg cyanidin-3-glucoside equivalent (CYE)/g) were obtained with the highest amount in the ethanol extracts of both Jamun fruit and seeds. This was followed by methanol and water extracts. In addition to the extraction technique and the characteristics of the solvent used, pH is also one of the important factors in the analysis of bioactive components; especially anthocyanins.[22]

**GC-MC method of analysis:**

Black plum seed powder is subjected to the Soxhlet extraction process using hexane as a solvent to obtain black palm seed extract. GC-MS is a unique method for the analysis and measures the quantity of organic volatile and semi-volatile compounds. GS is employed to separate mixtures into individual components employing a temperature-controlled capillary column. MS is applied to recognize a variety of components from their mass spectra. In the present study, black plum seed powder is used for extraction and analysis to study the constituents. Black plum seed extract is extracted by solvent extraction technique using Soxhlet extractor with hexane as a solvent.[23]



SPECIFICATIONS AND CONDITIONS DURING GC-MS ANALYSIS

• Run time (min)	54.09
• Injection volume(μ l)	1.00
• Scans	6439
• Low mass (m/z)	40
• High mass (m/z)	400
• Gas	Helium
• Solvent	Hexane

The work presented relates to the study of GC-MS analysis of the extracts of black plum seed obtained using solvent extraction with hexane as a solvent. The extract is found to contain many medicinally active compounds. Ten compounds are identified and details presented, five of which are found to exhibit antimicrobial activity against different diseases. The medicinally active compounds can be isolated and considered for the preparation of medicine.[24]

Areas to be explored

Reviews detailing the chemical constituents of the *Syzygium cumini* have been reported by several researchers. The widespread uses of *Syzygium Cumini* in traditional medicines have resulted in considerable chemical analysis of the plant, and active principles which attribute the plant to its medicinal properties have been identified and isolated. The widespread use of *Syzygium Cumini* in traditional medicine reflects its pharmacological importance. The edible pulp of the plant forms 75% of the whole fruit. Various minerals and vitamins were reported like Ca, Mg, P, Fe, Na, K, Cu, S, Cl, vitamin C, vitamin A, riboflavin, nicotinic acid, choline and folic acid. Glucose and fructose are the principle source of sweeteners in ripe fruit with no trace of sucrose [28]. Maleic acid is the major acid (0.59% of the weight of fruit) [28,29]. Small quantity of Oxalic acid has also been reported [CSIR et al,2002]. Tannins mainly Gallic acid is responsible for the astringency effect of the fruits [28]. The astringency activity is due to efficiency to combine with tissues and proteins and precipitate them. Tannins are also efficient for gastroprotective and antiulcerogenic activity [30]. The purple colour of the fruit is due to the presence of one or two cyanidin diglycosides [29].

The plant leaves contain an essential oil with pleasant odour. The oil contains terpenes, limonene and dipentene (20%), sesquiterpenes of cadalane type (40%), and sesquiterpenes of azulene type (10% or less). Yield and physical characteristics of the oil varies according to the season of collection. This essential oil is reported to be responsible for the antibacterial activity of the leaves. Flower of plant contains Oleanolic acid and other three triterpenoids also reported in the flowers are acetyl oleanolinacid (0.3%) melting point (260-262oC), Eugenia- triterpenoid A (0.5%) and Eugenia triterpenoid B (0.3%). Flowers also contain ellagic acid (0.01). er potential applications in pharmaceuticals, nutraceuticals, and even sustainable materials. Ellagic acid arises from lactonization of B hexa-hydroxydiphenic acid during chemical hydrolysis of tannins.

The plant seeds are rich in protein and calcium. The seeds contain tannins (19%), ellagic acid, gallic acid (1-2%). A glycoside- Jamboline, starch, Myricyl alcohol in the unsaponified fraction of seeds and a small quantity (0.05%) of pale-yellow essential oil are also present [32].

Although extensive research has been conducted on various parts of *Syzygium Cumini* including its fruit, leaves, stem, and bark, relatively little attention has been given to its seeds. The seeds of *Syzygium Cumini* hold promising potential, as they are known to contain bioactive compounds with possible health benefits, particularly for managing diabetes and promoting antioxidant activity. Future studies could focus on exploring these medicinal properties in greater depth, along with investigating others.

Expanding research on *Syzygium Cumini* seeds could open new avenues for utilising this underexplored part of the plant.

While *Syzygium Cumini* (Jamun) has been extensively studied for its medicinal properties, there are several areas that are less researched or need further exploration when compared to other plants like Moringa, Turmeric, or



Ashwagandha because these have similar properties. Here are some of the under-researched aspects of *Syzygium Cumini*:

1. Neuroprotective Effects:

Current Status: Limited research exists on the neuroprotective effects of *Syzygium Cumini* seeds and other parts. While the fruit and seeds have shown potential for antioxidant and anti-inflammatory properties, the specific effects on brain health, neurodegenerative diseases like Alzheimer's and Parkinson's, and cognitive enhancement are not well studied.

Research Gap: Compared to plants like Bacopa monnieri (Brahmi) and Turmeric (*Curcuma longa*), which have been extensively researched for their neuroprotective potential, *Syzygium Cumini* needs more exploration in this area.

2. Mechanisms of Anti-Diabetic Action:

Current Status: While *Syzygium Cumini* is known for its anti-diabetic effects, the precise molecular mechanisms behind how it regulates blood glucose levels are not well understood.

Research Gap: Other plants like Moringa and Fenugreek have well-documented mechanisms of action related to insulin sensitivity and glucose metabolism. *Syzygium Cumini* would benefit from more in-depth research into its active compounds, receptors, and pathways involved in diabetes management.

3. Antimicrobial Activity:

Current Status: Some antimicrobial properties of *Syzygiumcumini* seeds and bark have been reported, but this research is relatively limited and lacks detailed studies on their spectrum of activity against various pathogens.

Research Gap: While plants like Neem and Garlic have been extensively researched for their broad-spectrum antimicrobial properties, *Syzygium Cumini* requires further studies to identify its potential as a natural antimicrobial agent, especially against antibiotic-resistant strains.

4. Cardiovascular Benefits:

Current Status: Although *Syzygium Cumini* is known to offer some benefits in managing cholesterol and blood pressure, more focused studies are needed to understand its full potential in cardiovascular health.

Research Gap: Compared to well-studied plants like Garlic, Pomegranate, and Moringa, which have demonstrated clear cardiovascular benefits, *Syzygium Cumini* requires more research to fully establish its effectiveness in managing heart disease and improving blood vessel function.

5. Cosmetic and Skincare Applications:

Current Status: While there is some evidence of *Syzygiumcumini*'s antioxidant and antimicrobial properties that could benefit skin health, detailed studies on its role in anti-aging, skin regeneration, and wound healing are minimal.

Research Gap: Plants like Turmeric and Neem have been widely researched for their skin benefits, and there is a growing body of evidence supporting their use in skincare products. *Syzygiumcumini* could potentially serve as a natural ingredient for skin health, but more targeted research is needed.

6. Biodegradable and Sustainable Materials:

Current Status: The potential for *Syzygium Cumini* seeds to be used in eco-friendly materials like biodegradable plastics or packaging has not been thoroughly investigated.

Research Gap: Other plants, such as Moringa and Hemp, have seen growing interest for their applications in sustainable materials and bioplastics. *Syzygium Cumini* could also contribute to this field, but it lacks sufficient research on its bio-based material applications.

7. Toxicological Studies:

Current Status: There are very few toxicological studies regarding the safe consumption and long-term use of *Syzygium Cumini* extracts, especially at higher doses.

Research Gap: While other plants, such as Ashwagandha and Moringa, have been tested for safety and toxicity, comprehensive safety studies are needed for *Syzygium Cumini*, especially in regard to its seeds, which are less studied.



8. Bioavailability of Active Compounds:

Current Status: Research on the bioavailability and absorption of the bioactive compounds in *Syzygium Cumini* is minimal.

Research Gap: Plants like Turmeric and Moringa have had extensive studies on the bioavailability of their active compounds (e.g., curcumin in turmeric), which is crucial for maximising therapeutic effects. *Syzygium Cumini* seeds could benefit from similar studies to understand how well its beneficial compounds are absorbed and utilised by the body.

9. Herbal Synergy and Combinations:

Current Status: There is little research into how *Syzygium Cumini* interacts with other herbs and medicinal plants in combination therapy.

Research Gap: In contrast to well-studied herbs like Ashwagandha and Fenugreek, which are often tested in combination with other herbs for enhanced therapeutic effects, *Syzygium Cumini* could be researched for its synergy with other plants, especially for diabetes, inflammation, and cardiovascular health.

While *Syzygium Cumini* has shown promising medicinal properties, there are several under-explored areas, particularly when compared to other plants with similar health benefits. Future research in these neglected areas could lead to a deeper understanding and broader application of this plant in medicine, industry, and environmental sustainability.

Fields of Study

1. Phytochemistry:

Study of the chemical compounds, especially bioactive components, in different parts of the plant. Investigation into flavonoids, alkaloids, terpenes, anthocyanins, and phenolics.

2. Pharmacology and Toxicology:

Examining the medicinal properties, therapeutic effects, and potential toxicity of extracts. Areas include anti-diabetic, anti-inflammatory, antioxidant, antimicrobial, and anti-cancer effects.

3. Nutritional Studies:

Analysing the fruit's nutritional profile, including vitamin, mineral, and fibre content. Focus on its potential benefits for diet, especially regarding blood sugar regulation.

4. Agricultural and Environmental Studies:

Understanding the cultivation conditions, soil requirements, and pest management. Impact on biodiversity, as Java plum trees support various insect and bird species.

5. Food Science:

Developing products like juices, jams, wines, and dietary supplements from the fruit. Study of shelf-life, preservation techniques, and enhancing palatability.

6. Ethnobotany and Traditional Medicine:

Documenting its traditional uses across cultures for insights into potential pharmacological applications. Studying preparation methods in traditional medicine to explore potential scientific validation.

Additional Areas of Interest

Genetics and Breeding: Breeding for better yield, disease resistance, and nutritional profile. Genetic analysis for understanding adaptation to different climates.

Environmental Impact and Ecosystem Services: Examining its role in supporting wildlife, improving soil quality, and adapting to climate resilience.

Each of these fields offers a vast range of research opportunities, especially for those interested in the multifaceted uses of Java plum.

Specific research areas on the Java plum (*Syzygium Cumini*) often focus on its unique bioactive properties, ecological impact, and applications in food and medicine. Here are some of the targeted research areas:

1. Anti-Diabetic Research

- Area: Studying the seed and fruit extracts for managing blood sugar levels.
- Focus: Effects on insulin sensitivity, glucose metabolism, and glycemic control.



- Methods: In vitro (lab) and in vivo (animal/human) studies to understand active compounds like jamboline and ellagic acid.

2. Antioxidant and Anti-Inflammatory Studies

- Area: Investigating the high antioxidant content, particularly in the fruit peel and pulp.
- Focus: Analysis of anthocyanins, polyphenols, and flavonoids in reducing oxidative stress and inflammation.
- Methods: Using chemical assays (e.g., DPPH, FRAP) and cell culture studies to measure antioxidant capacities.

3. Antimicrobial and Antiviral Studies

- Area: Exploring leaf and bark extracts for antimicrobial and antiviral properties.
- Focus: Testing effectiveness against bacteria (e.g., *E. coli*, *Staphylococcus aureus*) and potential use as a natural preservative.
- Methods: Microbiological assays and comparing extract effectiveness with synthetic antimicrobials.

4. Cancer Research

- Area: Examining the potential anticancer effects of Java plum compounds.
- Focus: Investigating effects on cell proliferation, apoptosis (cell death), and metastasis.
- Methods: In vitro studies using cancer cell lines (e.g., breast, colon) and in vivo models to assess anti-tumor activity.

5. Nutritional Composition and Food Product Development

- Area: Assessing nutrient profiles and creating food products like jams, juices, and wine.
- Focus: Determining vitamin, mineral, and fibre content and developing value-added products.
- Methods: Nutritional analysis, sensory evaluation, and studying preservation techniques.

6. Genetic and Breeding Studies

- Area: Improving cultivars for disease resistance, yield, and nutritional profile.
- Focus: Genomic studies to understand variations and breed for specific traits.
- Methods: Genotyping, genome sequencing, and traditional breeding combined with genetic markers.

7. Traditional Medicine and Ethnobotanical Research

- Area: Documenting traditional uses across various cultures.
- Focus: Investigating applications in treating diabetes, respiratory conditions, and digestive disorders.
- Methods: Ethnobotanical surveys, interviews, and collaboration with indigenous practitioners.

8. Ecological Impact and Climate Adaptability

- Area: Studying the Java plum's role in ecosystems, its adaptability, and climate resilience.
- Focus: Assessing its benefits in soil improvement, carbon sequestration, and as a food source for wildlife.
- Methods: Field studies in diverse ecosystems, soil and biodiversity assessments, and climate modelling.

9. Toxicological Assessment

- Area: Ensuring safety in medicinal or supplemental use of Java plum extracts.
- Focus: Determining safe dosages, potential toxicity, and side effects, especially for prolonged use.
- Methods: Toxicity assays, animal testing, and dose-response studies for pharmacological safety.

Each of these areas combines advanced lab methods with practical applications, offering diverse avenues for scientists across fields like pharmacology, nutrition, environmental science, and ethnobotany.

6. Conclusion and Future Prospective Directions

According to the review, the seed of the jamun fruit has a richly-varied composition of bioactive compounds, include-terpenoids, phenolic compounds and saponins with high contents of gallic acid, ellagic acid and hydrolysable tannins.

These compounds are responsible for the extensive biological activities of jamun seeds and their extracts. Jamun seeds appear to be a low-cost source of a natural antidiabetic agent, although their antioxidant, anti-inflammatory antimicrobial potential is becoming more and more appreciated. As modern techniques for the extraction, separation and purification of jamun seeds bioactives are developed, it seems that powder, extracts and fractions may soon be



harnessed in the production of functional foods and nutraceuticals intended for people at risk of diabetes, cancer, cardiovascular, hepatic and neurodegenerative diseases, and bacterial and microbial infections.

Range of pharmacological properties is possessed by various extracts of jamun which include antidiabetic, antihyperlipidemic, antihypercholesterolemic, anticancer, cardioprotective, hepatoprotective, neuroprotective, anti-inflammatory, antioxidant, and antimicrobial activities.

Nevertheless, more research is needed to elucidate the molecular mechanisms of the of health-beneficial activities of jamun seed compounds. The biological activity of saponins and lignans from jamun seeds, and the bioavailability of bioactive compounds are still poorly understood. In addition, the nutritional value of jamun seeds seems to be still little known, especially in terms of proteins, their amino acid composition and biological value. Clinical trials of jamun seed-based goods on humans, taking into account all safety concerns, will increase the value of jamun seeds for use in the food and non-food industries.

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