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Phytochemical screening and antimicrobial activity of *Syzygium cumini* leaf extract: An experimental laboratory study

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Abstract:

Herbal antimicrobials are increasingly being explored as safer alternatives for managing resistant microorganisms in dentistry and medicine. Therefore, it is of interest to evaluate the phytochemical composition and antioxidant and antimicrobial potential of the ethyl acetate leaf extract of *Syzygium cumini* using phytochemical screening, paper chromatography and agar-based antimicrobial assays. The extract contained alkaloids, flavonoids, tannins, saponins, terpenoids, proteins/amino acids and starch, while chromatography confirmed the separation of bioactive constituents. Complete inhibition of bacterial growth was observed on extract-incorporated agar plates after 24 hours of incubation, showing strong antimicrobial activity. Thus, data shows that *Syzygium cumini* leaves as a promising natural source of bioactive compounds for developing plant-based oral healthcare products, including mouthwashes, irrigants and dentifrices.

Keywords: *Syzygium cumini*, medicinal plants, dentistry, irrigants, flavonoids, Jamun, antimicrobial properties, ethnomedicine, *Streptococcus mutans*, mouthwash, periodontal diseases

Background:

Earth's biodiversity provides a vast reservoir of medicinal flora, representing a rich legacy of global therapeutic significance. Phytochemicals, or plant secondary metabolites, are primarily responsible for the medicinal benefits attributed to medicinal plants. These organic compounds contribute to plant defence mechanisms and confer a wide range of positive health effects in humans [1]. Major chemical categories of phytochemicals include terpenoids, alkaloids, carbohydrates, flavonoids, tannins and steroids [2, 3]. *Syzygium cumini* (*Eugenia cumini*, *Syzygium jambolanum*; commonly known as Jamun, Malabar plum, Java plum, black plum, jambul, or jambolan) has long been valued for its diverse therapeutic properties, including hypoglycaemic, anti-diarrhoeal, antibacterial, anti-inflammatory, anti-diabetic and anti-HIV effects. Among the major bioactive constituents reported are acetyl-oleanolic acid, triterpenoids, ellagic acid, isoquercetin, quercetin, kaempferol and myricetin [4]. The taxonomic hierarchy of *Syzygium cumini* is as follows: it belongs to the kingdom Plantae, within the subkingdom Viridiplantae and is classified under the infrakingdom Streptophyta. It is placed under the division Tracheophyta, subdivision Spermatophytina and infradivision Angiospermae. Its class is Magnoliopsida and it falls under the superorder Rosanae. The plant is assigned to the order Myrtales and is part of the family

Myrtaceae. Its genus is *Syzygium* and the species is *Syzygium cumini* [5]. Humankind has long been fascinated by natural compounds and has extensively investigated their potential as sources for drug discovery. These bioactive molecules are derived from a variety of origins, including pre-biotic, microbial, plant and animal sources [6]. Plants, in particular, contain numerous bioactive constituents such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols, terpenoids and flavonoids, many of which exhibit therapeutic properties [7, 8]. Among these, phenolic phytochemicals play a crucial role as antimicrobial agents, exerting their effects through degradation of microbial cell wall protein components, interference with enzyme function and inhibition of DNA and RNA replication [9]. Therefore, it is of interest to report the phytochemical profile and antimicrobial activity of *Syzygium cumini* leaf extract, with emphasis on its potential applications in dental and medicinal formulations.

Methodology:

This experimental laboratory study was designed to evaluate the phytochemical and antimicrobial properties of *Syzygium cumini* (Jamun) leaves. The study was carried out in three sequential phases: (i) collection of Jamun leaves and preparation of plant extracts, (ii) qualitative phytochemical evaluation of bioactive

compounds and (iii) evaluation of antimicrobial activity. Standard analytical procedures described in published phytochemical research were adopted, with appropriate modifications to suit the available laboratory facilities.

Collection of Jamun leaves and preparation of plant extracts:

Fresh leaves of *S. cumini* were collected from neighbourhood trees and a garden source. The initial fresh weight of the leaves recorded was 480g. The leaves were wrapped in newspaper and shade-dried under ambient conditions for 3–4 days, after which the dry weight was 150g. The dried leaves were ground and crushed in a domestic mixer-grinder, yielding a fine powder of 120g, which was stored in an airtight container at room temperature until further use. The extracts were prepared by mixing the leaf powder with solvent and with distilled water, respectively, to obtain a solvent extract and an aqueous extract. Both extracts were transferred to conical flasks, covered with aluminium foil and cotton plugs to prevent contamination and filtered through What man filter paper. The filtrates were collected in labelled, sterile test tubes for subsequent analysis. Paper chromatography was carried out to confirm the separation of compounds in the extracts. Two spots, one for the aqueous extract and one for the solvent extract, were applied on the baseline of a chromatography paper. The paper was then placed in a developing chamber containing the solvent system n-butanol : acetic acid : water and the chromatogram were allowed to develop until the solvent front reached the upper limit. Retention factor (Rf) values were subsequently calculated. For preparation of culture media, yeast extract, peptone, sodium chloride and agar were dissolved in 100 mL of distilled water and the pH was maintained at 7.8 to support optimal microbial growth. The medium was autoclaved for 30–45 minutes and incubated for 24 hours. After incubation, the molten agar medium was poured into sterile Petri plates and allowed to solidify. A 100 μ L aliquot of bacterial culture was withdrawn from liquid broth and transferred to the agar plates, where it was spread uniformly using a sterile spreader. The plates were then incubated at 37°C for 24 hours. For liquid broth preparation, both agar-containing and agar-free solutions were prepared and incubated. The agar-free broth was distributed into two halves in a conical flask and microbial colonies were collected from streaking plates using a sterile inoculation loop and mixed thoroughly with the broth. The flasks were then incubated in a BOD incubator at 37°C with shaking at 120 rpm for 48 hours.

Alkaloid test:

A reddish-brown precipitate was observed on addition of Wagner's reagent to the plant extract, confirming the presence of alkaloids.

Flavonoid test:

An intense yellow colouration developed in the test tube on addition of dilute sodium hydroxide, indicating the presence of flavonoids in the extract.

Tannin test:

Development of a green colour following addition of 5% ferric chloride confirmed the presence of tannins in the plant extract.

Saponin test:

Vigorous shaking of the plant extract with distilled water produced persistent froth, indicating the presence of saponins.

Terpenoid test:

A bluish-red precipitate was formed on addition of chloroform and concentrated sulphuric acid to the extract, confirming the presence of terpenoids.

Ninhydrin test for amino acids:

A violet-coloured ring was observed in the test tube following the Ninhydrin reaction, indicating the presence of proteins and amino acids in the leaf extract.

Starch test:

A canary-yellow colour was observed on addition of 5% potassium hydroxide to the aqueous extract, indicating the presence of starch.

Evaluation of bioactive compounds:

The Jamun leaf extract was subjected to qualitative screening for the presence of alkaloids, tannins, flavonoids, saponins, terpenoids, proteins/amino acids (Ninhydrin test) and starch, using standard published protocols. For detection of alkaloids, Wagner's reagent was prepared by dissolving 1 g of iodine and 1.5 g of potassium iodide in 25 mL of distilled water. To 2 mL of plant extract in a test tube, a few drops of Wagner's reagent were added; the formation of a reddish-brown precipitate was taken as indicative of the presence of alkaloids. For flavonoids, 1 mL of plant extract was taken in a test tube and 1 mL of dilute sodium hydroxide solution was added. The appearance of a yellow colouration in the sample was considered indicative of the presence of flavonoids. For tannins, 2 mL of plant extract was mixed with 0.5 mL of 5% ferric chloride (FeCl_3) solution in a test tube. Development of a green colour was taken as confirmation of the presence of tannins. For saponins, 1 mL of plant extract was mixed with 5 mL of distilled water in a test tube and shaken vigorously. The formation of persistent froth was considered indicative of the presence of saponins. For terpenoids, 1 mL of plant extract was mixed with 1 mL of chloroform and 1 mL of concentrated sulphuric acid (H_2SO_4). The formation of a bluish-red precipitate confirmed the presence of terpenoids. For proteins and amino acids, the Ninhydrin test was performed by adding 1 mL of 0.2% ninhydrin reagent to 1 mL of plant extract. The test tubes were heated in a water bath on a heating mantle and observed for the development of a violet ring or colour, indicating the presence of proteins/amino acids. For starch, 1 mL of aqueous plant extract was mixed with 1 mL of 5% potassium hydroxide (KOH) solution and the development of a canary-yellow colour was recorded as a positive test for starch.

Evaluation of antimicrobial properties:

Antimicrobial evaluation was performed using the agar-incorporation method. Nutrient agar was melted and approximately 30 mL was poured into each sterile Petri plate. Using a micropipette, 500 µL of the previously prepared solvent extract was mixed with the agar medium prior to solidification. After the medium had set, the bacterial suspension prepared in liquid broth was poured onto the plates and uniformly distributed with a sterile glass spreader. The plates were then incubated at 37°C for 24 hours. Control plates without plant extract were prepared in parallel. Microbial growth, or the absence thereof, was assessed visually after the incubation period. Descriptive observations were recorded for each phytochemical test (presence or absence of the diagnostic colour change or precipitate) and for the antimicrobial assay (presence or absence of bacterial growth on the extract-incorporated plates). Ethical considerations were limited to standard laboratory biosafety and waste-disposal practices, as the study did not involve human or animal subjects.

Results:

The phytochemical screening and antimicrobial evaluation of *Syzygium cumini* leaf extracts yielded consistent and reproducible observations across all assays. Paper chromatography and qualitative phytochemical tests are summarised in (Table 1) and the antimicrobial findings are

presented in (Table 2). Paper chromatography was used for the detection of amino acids in the extracts. Two distinct spots were observed on the chromatogram, with retention factor (Rf) values of 0.9 for spot A (representing the less polar fraction) and 0.5 for spot B (representing the more polar fraction). The Rf values confirmed successful separation of compounds based on their polarity and solubility differences in the chosen solvent system. Petri plates containing the plant extract incorporated into nutrient medium were inoculated with bacterial culture and incubated at 37°C. After 24 hours of incubation, no visible bacterial growth was observed on the extract-incorporated plates, whereas control plates without extract showed normal microbial growth. This finding clearly indicates that the leaf extract of *S. cumini* exhibits significant antimicrobial activity under the tested experimental conditions. As shown in Table 1, all seven major classes of secondary metabolites tested (alkaloids, flavonoids, tannins, saponins, terpenoids, proteins/amino acids and starch) were detected in the leaf extract of *S. cumini*, indicating a rich phytochemical profile. The data in Table 2 show complete inhibition of bacterial growth on the extract-incorporated plate after 24 hours, while the control plate without extract supported normal bacterial proliferation, confirming the antimicrobial efficacy of the leaf extract under the tested conditions.

Table 1: Qualitative phytochemical screening of syzygium cumini leaf extract

Phytochemical Test	Reagent / Method	Observation	Inference
Paper chromatography	n-butanol : acetic acid : water	Two spots (Rf 0.9 and 0.5)	Separation based on polarity
Alkaloids	Wagner's reagent	Reddish-brown precipitate	Present
Flavonoids	Dilute NaOH	Intense yellow colour	Present
Tannins	5% FeCl ₃	Green colour	Present
Saponins	Distilled water (frothing)	Persistent froth	Present
Terpenoids	Chloroform + conc. H ₂ SO ₄	Bluish-red precipitate	Present
Proteins / Amino acids	Ninhydrin (0.2%)	Violet-coloured ring	Present
Starch	5% KOH (aqueous extract)	Canary-yellow colour	Present

Table 2: Antimicrobial activity of syzygium cumini leaf extract on agar-incorporation assay

Group	Plant Extract Added	Incubation (37 °C, 24 h)	Observation
Control plate	No	Yes	Visible bacterial growth
Test plate (solvent extract)	Yes (500 µL)	Yes	No bacterial growth observed

Discussion:

The widespread use of herbal medicines has renewed interest in *Syzygium cumini* because of its antimicrobial, antioxidant, anti-inflammatory and therapeutic properties. Owing to its long history in traditional medicine and favourable safety profile, *S. cumini* has emerged as a promising source of bioactive compounds for pharmaceutical and dental applications. Dubale *et al.* [10] comprehensively reviewed the pharmacological potential of *S. cumini* and reported that its leaves, seeds, bark and fruits contain abundant phenolics, flavonoids, tannins, alkaloids, terpenoids and glycosides that contribute to antimicrobial, antioxidant, antidiabetic and anti-inflammatory activities. Likewise, Plaskova and Mlcek [11] highlighted that the fruit and leaf extracts of *S. cumini* are rich in polyphenols and anthocyanins with potent free radical scavenging activity, supporting their use as natural therapeutic agents. The phytochemical profile observed in the present study is in

agreement with these findings. Qualitative screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, proteins/amino acids and starch in the leaf extract, while paper chromatography demonstrated effective separation of phytochemical constituents according to their polarity. These compounds are well recognised for their ability to inhibit microbial growth, neutralise reactive oxygen species and reduce inflammatory responses, thereby contributing to oral and systemic health. Bakrim *et al.* [12] similarly reported that *S. cumini* possesses a diverse phytochemical composition with significant antioxidant, antimicrobial, anticancer and cardioprotective properties, primarily attributed to its high phenolic and flavonoid content. The antimicrobial findings of the present study further reinforce previous evidence. Complete inhibition of bacterial growth on extract-incorporated agar plates after 24 hours indicates strong antibacterial activity of the ethyl acetate leaf extract. Comparable observations were reported by

Shamsudin *et al.* [13], who showed that plant-derived phytochemicals, particularly flavonoids, tannins and terpenoids, exert antimicrobial effects through disruption of microbial cell walls, inhibition of essential enzymes and interference with nucleic acid synthesis. These mechanisms may explain the pronounced antibacterial activity observed in the present investigation. Furthermore, Rauf *et al.* [14] emphasized that the therapeutic efficacy of *S. cumini* is closely linked to its rich phytochemical composition and highlighted its potential in developing evidence-based herbal formulations for chronic inflammatory and infectious diseases. The present findings support these conclusions by confirming both the phytochemical richness and antimicrobial efficacy of *S. cumini* leaves. Collectively, the available evidence indicates that *S. cumini* leaf extract represents a promising natural source of bioactive compounds for future dental applications, including mouthwashes, dentifrices, root canal irrigants, periodontal therapeutics and other plant-based oral healthcare products.

Conclusion:

The leaf extract of *Syzygium cumini* (Jamun) showed the presence of important phytochemicals such as alkaloids, flavonoids, tannins, saponins and terpenoids. It also exhibited significant antimicrobial activity, indicating its potential dental and medicinal value. Thus, Jamun leaf extract may be useful in

developing natural oral care products and reducing dependence on synthetic antimicrobials.

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