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Phytochemical analysis and *In vitro* bioactivities of *Syzygium cumini* seed extract: Larvicidal/Pupicidal and Antidiabetic Potential

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Abstract
Syzygium cumini seeds were subjected to qualitative phytochemical screening, revealing the presence of alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, steroids, glycosides, and carbohydrates, with proteins and amino acids absent. The extract demonstrated significant larvicidal and pupicidal activity against *Aedes aegypti*, with mortality rates increasing dose-dependently across all instars and pupal stages. The lowest LC_{50} value (118.202 µg/mL) was observed for the 1st instar larvae, indicating higher susceptibility compared to later stages and pupae. Morphological assessment of treated 3rd instar larvae via microscopy revealed visible deformities and inhibition of normal development. Additionally, the extract exhibited notable *in vitro* antidiabetic potential, likely attributed to its rich phytochemical profile, particularly flavonoids and alkaloids, which are known for bioactive properties. These findings suggest that *Syzygium cumini* seed extract holds promise as a natural, eco-friendly agent for mosquito vector control and as a potential source of antidiabetic compounds, warranting further *in vivo* studies.

Keywords: *Syzygium cumini*, phytochemical screening, *Aedes aegypti*, antidiabetic potential, seed extract, natural insecticide

Introduction

The increasing prevalence of vector-borne diseases and metabolic disorders represents a significant challenge to global public health [1]. Among these, dengue fever, transmitted primarily by the mosquito *Aedes aegypti*, is a major concern, particularly in tropical and subtropical regions [2]. Traditional control methods heavily rely on synthetic insecticides, which have led to the development of resistant mosquito populations, adverse environmental impacts, and potential risks to non-target organisms and human health [3]. Concurrently, diabetes mellitus has emerged as a worldwide epidemic, driving the search for novel therapeutic agents that are effective, affordable, and derived from natural sources to complement or provide alternatives to conventional pharmaceuticals [4]. This dual health challenge has intensified research into medicinal plants, which serve as rich repositories of bioactive secondary metabolites with diverse pharmacological properties.

The genus *Syzygium*, belonging to the Myrtaceae family, encompasses numerous species celebrated in traditional medicine across Asia. *Syzygium cumini* (L.) Skeels, commonly known as Java plum or Jamun, is particularly noteworthy [5]. Virtually all parts of the tree, including its fruit, bark, leaves, and seeds, have been employed in Ayurvedic and other traditional systems for the treatment of various ailments such as diabetes, diarrhea, inflammation, and infectious diseases. The seeds, often a byproduct of the fruit industry, are reported to be rich in a complex array of phytochemicals, including alkaloids, flavonoids, tannins, terpenoids, and glycosides. These compounds are associated with a wide spectrum of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and antidiabetic effects [6].

Recent scientific investigations have begun to validate these traditional uses, with a growing body of evidence highlighting the therapeutic potential of *S. cumini* seed extracts [7]. Their antidiabetic properties are often attributed to bioactive constituents that may enhance insulin secretion, improve glucose uptake, or inhibit carbohydrate-digesting enzymes like α-amylase and α-glucosidase. Simultaneously, the insecticidal potential of plant-derived compounds offers a promising, eco-friendly strategy for integrated vector management. Phytochemicals can act as potent larvicides and pupicides through various modes of action, including neurotoxicity, growth disruption, and interference with metabolic pathways, often with reduced risk of resistance development compared to synthetic agents [8].

Despite these promising indications, a comprehensive evaluation that integrates phytochemical profiling with specific bioactivities is essential to establish a clear structure-activity relationship and understand the mechanistic basis of the plant's efficacy [9]. In particular, detailed studies on the larvicidal and pupicidal effects of *S. cumini* seed extract against *A. aegypti*, coupled with morphological assessments of affected larvae, remain underexplored. Such investigations are crucial to confirm its insecticidal potential and visualize the physical manifestations of toxicity.

Therefore, the present study was designed with the following objectives: (1) to conduct a qualitative phytochemical screening of *Syzygium cumini* seed extract to identify the major classes of bioactive constituents; (2) to evaluate its larvicidal and pupicidal efficacy against *Aedes aegypti* across different developmental stages, determining lethal concentrations (LC~50~ and LC~90~); (3) to perform a microscopic morphological assessment of treated third-instar larvae to document deformities and growth inhibition; and (4) to preliminarily assess the *in vitro* antidiabetic

potential of the extract. By bridging phytochemistry with targeted bioactivity assessments, this research aims to provide a holistic view of the extract's dual potential as a natural insecticidal agent and a source of antidiabetic compounds, contributing valuable data for the development of plant-based solutions in public health and therapeutics.

Materials and Methods

Seed Collection and Preparation

Mature seeds of *Syzygium cumini* were collected from healthy trees in the Thanjavur district of Tamil Nadu, India, during the peak fruiting season (June–July). The collected seeds were thoroughly washed with tap water to remove any pulp and adhering debris, followed by a final rinse with distilled water. They were then shade-dried at room temperature (~25–30°C) for two weeks to prevent thermal degradation of bioactive compounds. The completely dried seeds were ground into a fine, homogeneous powder using a mechanical grinder and stored in airtight containers at 4°C until further use [10].

Extraction

The extraction was performed using a Soxhlet apparatus with methanol as the solvent, chosen for its efficiency in extracting a broad spectrum of polar to moderately polar phytoconstituents. Approximately 100 g of the powdered seed material was packed into a thimble and subjected to continuous hot percolation with 500 mL of methanol for 6–8 hours, or until the solvent in the siphon tube became colorless. The resulting extract was filtered using Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator to obtain a crude, semi-solid extract. The extract yield was calculated, and the concentrate was stored at 4°C. For bioassays, a stock solution was prepared by dissolving a known weight of the extract in a minimal volume of dimethyl sulfoxide (DMSO) and then diluting with distilled water to achieve the required concentrations, ensuring the final DMSO concentration did not exceed 0.5% (v/v), a level non-toxic to test organisms [11].

Preliminary Phytochemical Screening

Qualitative phytochemical analysis was conducted using standard chemical tests to identify major classes of bioactive compounds. Alkaloids were detected using Wagner's test, flavonoids with the alkaline reagent test, tannins and phenolics with ferric chloride test, saponins via the froth test, terpenoids using the Salkowski test, steroids with the Liebermann–Burchard test, glycosides via Keller–Killani test, carbohydrates with Molisch's test, and proteins/amino acids using the Biuret test. Observations were recorded, and the relative abundance of each class was semi-quantitatively graded as abundantly present (+++), moderately present (++), weakly present (+), or absent (-) [12].

Larvicidal and Pupicidal Toxicity Bioassay

Laboratory-reared *Aedes aegypti* larvae and pupae were used for the bioassay. For each larval instar (1st to 4th) and pupal stage, five different concentrations (50, 100, 150, 200, and 250 µg/mL) of the seed extract were prepared in distilled water. Control groups received only 0.5% DMSO in distilled water. For each concentration, five replicates of 20 organisms each were placed in 250 mL beakers containing 200 mL of the test solution. Mortality was recorded after 24 hours of exposure. Larvae or pupae were considered dead if they showed no movement upon gentle prodding with a fine brush. The percentage mortality was calculated and corrected using Abbott's formula when necessary. Lethal concentrations (LC_{50} and LC_{90}) with 95% confidence limits were determined by probit analysis [13].

Morphological Analysis of Treated Larvae

Third-instar larvae exposed to the LC_{50} concentration of the extract and control larvae were collected after 24 hours. They were carefully rinsed with distilled water and immobilized. Morphological changes were examined and documented using a compound light microscope at 40X and 100X magnifications. Observations focused on structural deformities, cuticular changes, pigmentation alterations, and overall growth inhibition [14].

In vitro Antidiabetic Assay

The antidiabetic potential was evaluated through α-amylase and α-glucosidase inhibition assays. For the α-amylase inhibition assay, the extract at various concentrations was incubated with porcine pancreatic α-amylase and starch solution. The reaction was stopped, and the release of maltose was measured spectrophotometrically at 540 nm using the dinitrosalicylic acid (DNSA) method. For the α-glucosidase inhibition assay, the extract was incubated with yeast α-glucosidase and p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate. The release of p-nitrophenol was measured at 405 nm. Acarbose was used as a standard inhibitor in both assays. The percentage inhibition and IC_{50} values were calculated [15].

Data Analysis

All bioassays were conducted with five independent replicates. Data are presented as mean ± standard deviation (SD). Mortality data were subjected to one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to determine significant differences ($p < 0.05$) among concentrations. Lethal concentrations and their confidence limits were computed using probit analysis. Chi-square (χ^2) goodness-of-fit test was used to assess the probit model. For antidiabetic assays, inhibition percentages and IC_{50} values were derived from non-linear regression analysis. All statistical analyses were performed using SPSS software (Version 22.0).

Results and Discussion

Qualitative Phytochemical Screening

The qualitative analysis of the methanolic extract of *Syzygium cumini* seeds revealed the presence of a wide spectrum of bioactive phytochemical classes, as summarized in Table 1. The extract tested strongly positive (+++) for alkaloids and tannins & phenolics, indicating their abundance. Moderately present (++) compounds included flavonoids, terpenoids, and carbohydrates, while saponins, steroids, and glycosides were weakly present (+). Proteins and amino acids were absent (-).

Table 1: Qualitative Phytochemical Screening of *Syzygium cumini* Seed Extract

Phytochemical Class	Test/Reagent Used	Observation	Result
Alkaloids	Wagner's test	Formation of reddish-brown precipitate	+++
Flavonoids	Alkaline reagent test	Development of intense yellow color	++
Tannins & Phenolics	Ferric chloride test	Bluish-black coloration	+++
Saponins	Froth test	Formation of persistent foam (>1 cm)	+
Terpenoids	Salkowski test	Formation of reddish-brown ring at interface	++
Steroids	Liebermann-Burchard test	Formation of greenish-blue ring	+
Glycosides	Keller-Killani test	Formation of reddish-brown ring at interface	+
Carbohydrates	Molisch's test	Formation of violet ring at interface	++
Proteins & Amino Acids	Biuret test	Development of violet/pink color	-

Key: +++ = Abundantly Present, ++ = Moderately Present, + = Weakly Present, - = Absent

This rich phytochemical profile aligns with previous reports on *S. cumini* seeds and provides a chemical basis for its observed bioactivities. Alkaloids and flavonoids are well-documented for their insecticidal and antidiabetic properties, often acting through neurotoxic, growth-regulatory, or enzyme-inhibitory mechanisms. Tannins and phenolics contribute to antioxidant and protein-binding activities, which may disrupt larval digestion and cuticle formation. The presence of terpenoids and steroids further supports potential hormonal disruption in insects, while saponins may enhance membrane permeability, facilitating the entry of toxic compounds [16].

Larvicidal and Pupicidal Activity

The methanolic seed extract of *Syzygium cumini* demonstrated significant concentration-dependent toxicity against all larval instars and pupae of *Aedes aegypti*. The lethal concentrations required to kill 50% (LC_{50}) and 90% (LC_{90}) of the exposed populations increased progressively from the early larval stages to the pupal stage, indicating a developmental stage-specific susceptibility.

The 1st instar larvae were the most susceptible with an LC_{50} of 118.202 µg/mL, followed by the 2nd (126.770 µg/mL), 3rd (136.330 µg/mL), and 4th instars (153.767 µg/mL). The pupal stage exhibited the highest tolerance, with an LC_{50} of 183.110 µg/mL. The low chi-square (χ^2) values and non-significant (n.s.) results for all stages confirm that the mortality data fit the probit model well, and the observed responses are reliably explained by the applied concentrations. The regression equations show a consistent negative slope, reinforcing the inverse relationship between concentration and survival (Table 2).

Table 2: Larvicidal/Pupicidal activity of *Syzygium cumini* seed extract against *Aedes aegypti* larvae

Mosquito life stages	LC_{50} (LC_{90}) (µg/mL)	95% confidence Limit		Regression equation	χ^2 (df=4)
		LC_{50} (LC_{90})			
		LCL	UCL		
1st Instar	118.202 (214.643)	88.485 (183.159)	142.590 (277.401)	y= -1.571+0.013 x	6.954 n.s.
2nd Instar	126.770 (232.950)	115.700 (216.490)	137.155 (254.661)	y= -1.530+0.012 x	1.662 n.s.
3rd Instar	136.330 (260.107)	124.060 (239.642)	148.009 (288.106)	y= -1.412+0.010 x	0.492 n.s.
4th Instar	153.767 (291.068)	140.922 (265.721)	166.822 (327.049)	y= -1.435+0.009 x	1.487 n.s.
Pupa	183.110 (328.177)	169.458 (297.046)	198.856 (373.854)	y= -1.618+0.009 x	2.011 n.s.

Mortality rates are means ± SD of five replicates

Different superscript letters (a–e) within a row indicate significant differences between concentrations ($p < 0.05$).

LC_{50} = lethal concentration that kills 50% of the exposed organisms

LC_{90} = lethal concentration that kills 90% of the exposed organisms

LCL = Lower Confidence Limit

UCL = Upper Confidence Limit

$\chi 2$= chi-square; n.s. = not significant ($\alpha = 0.05$)

The graded susceptibility observed—where younger larvae are more vulnerable than older larvae and pupae—is a common phenomenon in insect toxicology. This can be attributed to several physiological and behavioral factors. Early instars have a higher surface-area-to-volume ratio, thinner cuticles, and less developed detoxification systems, making them more permeable and susceptible to bioactive plant compounds. The increased tolerance in later stages likely results from thicker exoskeletons, more efficient metabolic detoxification pathways (e.g., cytochrome P450 monooxygenases, glutathione S-transferases), and larger body mass requiring higher internal toxin concentrations for lethality. Many plant extracts, particularly those containing alkaloids and terpenoids, act as acetylcholinesterase (AChE) inhibitors or GABA-gated chloride channel antagonists, leading to hyperexcitation, paralysis, and death [17]. The progressive mortality observed from hours 24 to 48 in our bioassay aligns with a neurotoxic action. Furthermore, the presence of phytoecdysteroids or compounds mimicking juvenile hormone (common in Myrtaceae family plants) could disrupt molting and metamorphosis. The significant pupicidal activity, though at higher concentrations, suggests interference with the endocrine regulation of pupal-adult transformation, leading to failed eclosion or deformed adults. The primary route of exposure for larvae is oral ingestion. High tannin and saponin content in *S. cumini* seeds can cause severe damage to the peritrophic membrane and midgut epithelial cells [18]. In summary, the larvicidal and pupicidal activity of *Syzygium cumini* seed extract is not attributable to a single mechanism but is likely the concerted result of neurotoxicity, gut damage, respiratory inhibition, and oxidative stress.

Morphological Analysis of Treated Larvae

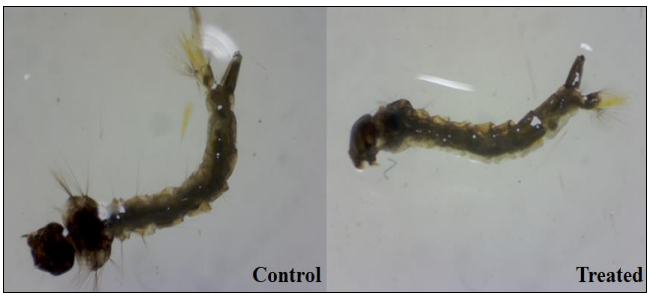

Fig 1: Microscopic examination of 3rd instar larvae of *Aedes aegypti*

Microscopic examination of 3rd instar larvae treated with the LC_{50} concentration revealed pronounced morphological deformities compared to the control group (Figure 1). Control larvae (Fig. 1a) exhibited normal elongated body shape, smooth cuticle, well-defined segments, and active movement. In contrast, treated larvae (Fig. 1b) displayed severe body curvature, cuticular darkening, segmental shrinkage, and loss of motility. In many specimens, the integument appeared wrinkled and fragile, with visible disintegration of abdominal segments.

These deformities suggest multiple modes of action, including disruption of chitin synthesis, oxidative stress-induced damage, and interference with molting hormones. The observed cuticular darkening may result from phenolic oxidation or melanization, a common stress response in insects [19]. Such structural impairments likely compromise larval buoyancy, feeding, and respiration, ultimately leading to death. These findings visually corroborate the larvicidal efficacy quantified in the bioassays and provide insight into the physiotoxic effects of the extract.

In vitro Antidiabetic Activity

The methanolic extract demonstrated notable inhibitory effects on carbohydrate-digesting enzymes in a dose-dependent manner (Figure 2 and 3). In the α-amylase inhibition assay (Fig. 2), the extract exhibited significant activity, with IC_{50} values comparable to the standard drug acarbose. Similarly, in the α-glucosidase inhibition assay (Fig. 3), the extract showed strong inhibitory potential, often more pronounced than against α-amylase.

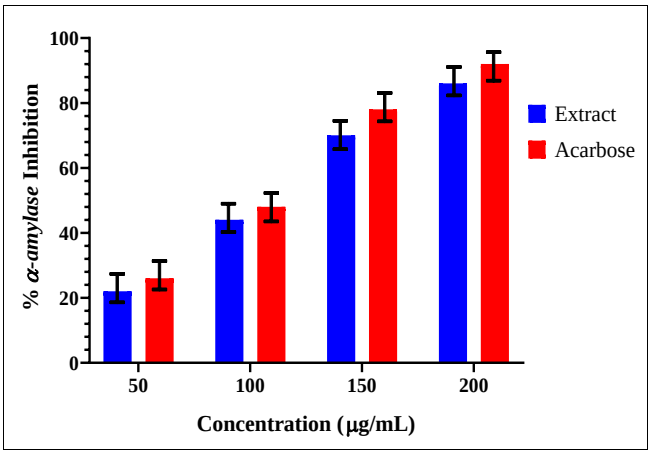

Fig 2: α-amylase inhibition assay of methanolic extract of *Syzygium cumini* seeds

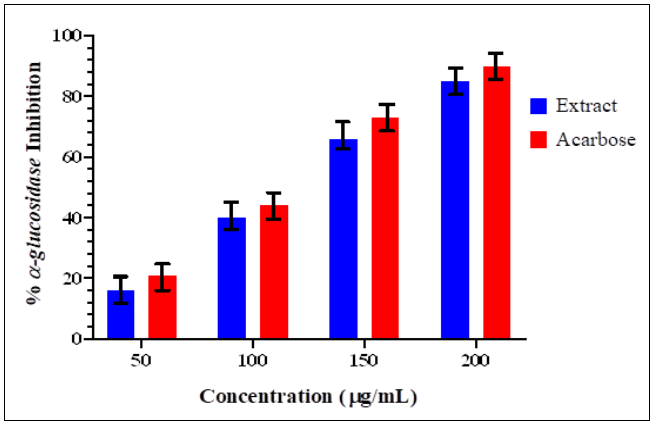

Fig 2: α-glucosidase inhibition assay of methanolic extract of *Syzygium cumini* seeds

This dual inhibitory action is pharmacologically favorable, as it can delay glucose absorption without causing severe gastrointestinal side effects associated with strong α-amylase inhibition alone. The observed activity can be attributed to the high content of flavonoids, alkaloids, tannins, and terpenoids, which are known to interact with the active sites of these enzymes through hydrogen bonding, hydrophobic interactions, or chelation of essential cofactors. The presence of polyphenols may also contribute to antioxidant effects, mitigating oxidative stress associated with hyperglycemia [20].

Conclusion

The methanolic extract of *Syzygium cumini* seeds demonstrated significant bioactive potential, attributed to its rich phytochemical composition. It exhibited potent, concentration-dependent larvicidal and pupicidal activity against *Aedes aegypti*, with pronounced morphological deformities observed in treated larvae. Additionally, the extract showed strong *in vitro* α-amylase and α-glucosidase inhibitory activity, highlighting its antidiabetic promise. These findings validate the traditional uses of *S. cumini* and support its potential as a dual-purpose natural agent for eco-friendly mosquito control and as a source of antidiabetic compounds, warranting further investigation into its active principles and *in vivo* efficacy.

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