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Phytochemical Profiling, Antioxidant, Anticariogenic, and Biocompatibility Evaluation of *Syzygium cumini*- an in vitro study

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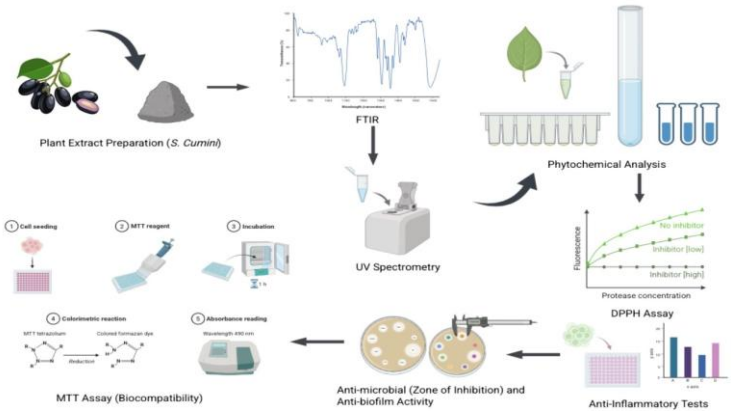
GRAPHICAL ABSTRACT:



ABSTRACT

BACKGROUND: Dental caries is one of the most prevalent health conditions globally affecting individuals across all age groups. To minimize the dental caries, it is necessary to seek anticariogenic agents to develop effective and preventive strategies. With increasing interest in plant-based anticariogenic agents, *Syzygium cumini* (Jamun) has emerged as a promising candidate due to its traditional medicinal use.

AIM: This study aims to investigate the phytochemical composition of Jamun leaves and to evaluate their antioxidant, anti-inflammatory, anticariogenic, and biocompatibility property. **MATERIALS AND METHODS:** The leaves of *S. cumini* were shade dried for 7 days and ground coarsely and extracted with methanol at room temperature. Then it was analyzed for presence of flavonoids, alkaloids, tannins, glycosides, steroids, phenol, saponins, terpenoids according to standard protocol. The antioxidant activity was assessed by DPPH radical method. Zone of inhibition test with minimum inhibitory concentration was conducted for antimicrobial activity. MTT assay was performed for biocompatibility assessment. FTIR spectroscopy and UV visible spectroscopy was done to analyze functional groups and chemical compounds.

RESULTS: The methanolic extract exhibited strong antioxidant activity, with a concentration dependent increase reaching 49.18% at 100 µg/ml. UV Visible spectroscopy confirmed phenolics and anthocyanins, while FTIR revealed hydroxy and carbonyl groups. In antimicrobial tests, the extract produced a zone of inhibition against *Streptococcus mutans* and *Shigella sonnei*. Anti inflammatory evaluation via BSA denaturation assay showed up to 85.78% inhibition at 50 µg/ml. MTT assay confirmed high biocompatibility, and minimal cytotoxicity observed even at higher doses. UV Visible analysis showed a prominent absorption peak around 340 nm indicating phenolic and polyphenolic compounds, while FTIR confirmed functional groups such as hydroxyl and carbonyl, suggesting the presence of flavonoids and related phytoconstituents.

CONCLUSION: The study showed that *Syzygium cumini* leaf extract exhibits strong antioxidant activity and effective inhibition of cariogenic pathogens in a dose dependent manner. The bioactive components such as tannins, phenolics, and flavonoids, may be responsible for the therapeutic potential. These findings show that *Syzygium cumini* promise as a natural, biocompatible anticariogenic agent.

KEYWORDS: Antioxidant, Dental Caries, Flavonoids, Phytochemicals, *Syzygium*

INTRODUCTION

Dental caries is widely regarded as a universal disease, which affects people across the countries, continents, races, cultures, ethnicities and genders. If timely intervention is not done, it can progressively affect the tooth, invade the pulp and eventually lead to damage of the surrounding tissues. (1) It significantly affects the quality of life and psychological well being. (2) Oral microbiota actively contributes to dental caries therefore research and novel antibacterial approaches should be emphasized to address the growing challenge of microbes. (3)

Plants have been a source of medicines, with a wide array of bioactive molecules which helps in disease prevention. The growing interest in plant-based treatments is driven by their potential to offer safe and sustainable alternatives to synthetic antibiotics. These remedies exhibit antimicrobial properties effectively while minimizing the risk of adverse reactions. Moreover, the bioactive compounds in plants are biocompatible, allowing their prolonged use without disrupting the natural microbiota. *Syzygium cumini*, commonly known as the black plum or Jamun, is extensively utilized in traditional medicine systems across various cultures. (4)

Although ***Syzygium cumini*** is well known for its general medicinal properties, but its specific applications in oral health remain unexplored. There is a lack of comprehensive research evaluating its **effectiveness against cariogenic bacteria, antioxidant.** No previous study has combined **phytochemical profiling, antioxidant analysis, anticariogenic properties**, and **biocompatibility assessment** of *S. cumini* extract in a single dental focused investigation, highlighting a significant gap in the existing literature. This study aims to bridge that gap by analyzing the phytochemicals present in *S. cumini*, analyzing its antioxidant activity, test its ability to inhibit pathogens like *Streptococcus mutans*, and evaluate its biocompatibility. The findings are expected to contribute important relevations for the potential use of *S. cumini* as a **natural, safe, and effective agent for the prevention and management of dental caries**, contributing to the development of green alternatives in dental care.

MATERIALS AND METHODS

Plant Material Extract Preparation: The leaves (40 g) were washed with sterile water and shade-dried for 7 days and ground coarsely and extracted with methanol at room temperature. The dried leaves were coarsely ground using a mechanical grinder. The ground material was extracted with methanol using maceration. The plant material was soaked three times in 500 ml of methanol at room temperature for 2 days. The extracts from each maceration were combined and concentrated using a rotary evaporator at 30°C until a crude extract was obtained. The residue of 12.5 g was diluted with 50 ml water and partitioned against petroleum ether, ethyl acetate and n-butanol and were concentrated to dryness. The extract was then analysed for presence of flavonoids, alkaloids, tannins, glycosides, steroids, phenol, saponins, trepenoids, cardiac glycosides according to standard protocol.(5)

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Phytochemical Analysis: The various fractions obtained from **Petroleum Ether fraction**, **Ethyl Acetate fraction**, **n-Butanol fraction** was subjected to qualitative phytochemical screening for the presence of different metabolites. The screening was performed using standard protocols for the detection of compounds (Table no 1).

FTIR Analysis: Fourier-transform infrared (FTIR) spectroscopic analysis of *Syzygium cumini* extract was performed using a PerkinElmer spectrophotometer to identify characteristic absorption peaks within the spectral range of 500–4000 cm^{-1} and to determine the corresponding functional groups present in the extract.

UV Visible Spectroscopy: UV Visible spectrophotometric analysis of the Jamun (*Syzygium cumini*) extract was performed using a JASCO UV visible spectrophotometer at room temperature with a scanning interval of 1 nm. The absorbance of the extract was recorded over the wavelength range of 300–700 nm, covering both the ultraviolet and visible regions for phytochemical characterization.

Antioxidant activity: The antioxidant activity of the extract was determined using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay. The assay was performed with various concentrations of the plant extract. 1 ml of the extract at each concentration was mixed with 3.9 ml of DPPH solution (0.025 g/l in methanol). The mixture was incubated at room temperature for 30 minutes in dark. The decrease in absorbance was measured at 517 nm using a UV–Visible Spectrophotometer at 1-minute intervals. Ascorbic acid was used as a standard. (7)

Percentage of inhibition = Absorbance of control - Absorbance of test sample /Absorbance of control × 100

Anti inflammatory Assay : The plant extract fractions were first dried using a vacuum oven and then re dissolved in isotonic saline solution. Different concentrations of the fractions were prepared. 1.8 ml of 1% BSA solution was added to each concentration of the extract, followed by pH adjustment to 6.5 using 1N HCl. The mixture was incubated at room temperature for 20 min to allow interaction with BSA. The mixture was then heated to 57 °C for 10 min to induce protein denaturation. The absorbance was measured at 660 nm using a UV Visible Spectrophotometer. Diclofenac sodium was used as a standard positive control, while a solution without extract served as a negative control (7).

Anti-denaturation activity (%) = Absorbance of control - Absorbance of sample/Absorbance of control × 100

Antimicrobial Activity: The antimicrobial activity of the plant extract *(S. cumini*) was evaluated using the agar well diffusion method against *Streptococcus mutans and Shigella sonnei*. Sterile MHA plates were prepared, and wells were punched using a sterile corn borer with a thickness of 9mm was used. Each well was filled with different concentrations of the plant extracts (25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml). The plates were inoculated with bacterial strains using the spread plate method and incubated at 37 °C for a day. After incubation, the diameter of the zone of inhibition around each well was measured in millimetres. Appropriate antibiotics were used as positive controls, and a solvent control was included for comparison.

Anti Biofilm Activity: Using a 96-well microtiter plate assay, test compounds were assessed for their anti-biofilm activity against *Shigella sonnei* and *Streptococcus mutans* at concentrations of 25 µg, 50 µg, 75 µg, and 100 µg. Untreated biofilms were used as control group. Bacteria were first cultivated in vinyl U-bottom 96-well plates, where they were left to develop into biofilms for 24 to 48 hours at the ideal growth temperature of 37°C. Only the biofilm that clung to the well surface survived the incubation period when the planktonic (free-floating) bacterial cells were carefully removed using a mild rinsing buffer like phosphate-buffered saline (PBS). The extracellular polymeric substance (EPS) matrix of the biofilm is specifically bound by crystal violet dye, which was used to stain the wells in order to visualise the biofilm and provide a visual representation of the biofilm density. After staining, the excess dye was wiped off, and then the wells were rinsed once more to get rid of any remaining crystal violet. The stained biofilms were solubilised using ethanol or a solvent equivalent to dissolve the crystal violet in order to quantify the results. After that, the solubilised biofilms were moved to a 96-well plate with a flat bottom and optical clarity so that a precise spectrophotometric measurement at 570 nm could be made. This technique allows quantifying the mass of biofilm based on readings of absorbance. (8)

Biocompatibility assay: MTT assay is a colorimetric technique for measuring cell metabolic activity. Following a 24 hour incubation in which the cells were exposed to the test material, cell viability was evaluated. The L929 mouse fibroblast cell line was chosen for the biocompatibility and cytotoxicity investigations. The foetal bovine serum (FBS) and growth factors were added to Dulbecco's Modified Eagle Medium (DMEM) during the L929 cell culture process and the cells were kept at 37°C and 5% CO2. The metabolically active cells convert the tetrazolium salt MTT into formazan crystals. After solubilising formazan product, the absorbance measurements were done using a microplate reader at 570 nm in wavelength. The metabolic activity of the cells, indicates their viability, is directly correlated with the absorbance values. Greater metabolic activity and cell health are indicated by higher absorbance, which suggests that the material is non cytotoxic, lower absorbance would suggest possible cytotoxic effects. (9)

RESULTS

FTIR Analysis: The FTIR spectrum revealed a band around 3299 cm^{-1} corresponding to O–H vibrations, suggesting the hydroxyl groups which is associated with phenols, alcohols, and flavonoids. The peaks near 2924 and 2582 cm^{-1} is associated with C–H vibrations, suggesting the presence of aliphatic chains and organic compounds such as lipids and terpenoids. The peaks at 1596 and 1520 cm^{-1} correspond to C=O vibrations of carbonyl groups are found in aromatic compounds. The bands at 1429 cm^{-1} and 1362.3 cm^{-1} represent C–H vibrations which is associated with phenolic or flavonoid components. The absorption bands around 1229 cm^{-1} correspond to C–O vibrations of alcohols, esters, or ethers, while the peak at 1029 cm^{-1} represents C–O bonds suggests the presence of polysaccharides or glycosides. The bands at 891, 831, and 775 cm^{-1} correspond to aromatic C–H vibrations, confirms the existence of aromatic structures in extract (Figure 1 (a)).

UV Visible Spectroscopy : The UV Visible spectrum exhibited a prominent absorption peak in the range of 320–360 nm, with highest peak observed at approximately 340 nm, followed by a gradual decrease in absorbance up to 700 nm (Figure 1(b)). The absorption band between 300–380 nm indicates the presence of aromatic rings and carbonyl groups, indicating the presence of bioactive phytoconstituents like phenolic compounds, flavonoids, tannins, and anthocyanins. The broad peak suggests the presence of conjugated systems and polyphenolic compounds. The progressive decline beyond 400 nm indicates lesser chromophoric groups, which is characteristic of plant extracts rich in phenolic constituents.

Anti Oxidant Assay : A popular technique to assess a compound's capacity to scavenge free radicals, which is correlated with its antioxidant potential, is the DPPH Assay. Plotting indicates that the percentage of radical scavenging activity increases with as test substance concentrations (measured in µg/ml) rise (Figure1(c)). The percentage of RSA is roughly 9% at the lower end of the concentration spectrum, or about 20 µg/ml. The %RSA gradually rises to almost 50% as the concentration reaches 100 µg/ml. The equation's linear increase indicates a strong dose-dependent response, which means that the antioxidant activity correlates with the dosage of test substance used.

Anti Inflammatory activity: The BSA Denaturation Assay evaluates a material's capacity to prevent protein denaturation, a crucial step in the inflammatory process. Proteins become denatured as a result of inflammation frequently, and preventing this process may have anti-inflammatory effects. The bar graph indicates a distinct reduction in protein denaturation that is dose-dependent and increases as sample concentrations rise from 10 to 50 µg/ml (Figure 1 (d)). The test substance exhibits approximately 22.33% inhibition atv10 µg/ml. The inhibition rises steadily with increasing dose: At 20 µg/ml, 42.77%. The inhibition was 74.912% at 40 µg/ml and peaked at 85.78% with the highest concentration of 50 µg/ml(Table 2).

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Anti Biofilm Activity: The anti biofilm activity was tested for *Enterococcus faecalis, Staphylococcus aureus, Streptococcus mutans* and *Shigella sonnei*.The biofilms were stained to make them visible, with darker staining (deeper blue/purple) indicating thicker or more mature biofilm formation. Lighter staining represents reduced biofilm formation. Thick, dense biofilms with a deeper purple/blue staining are visible, particularly for certain bacterial species. (Figure 2) This illustrates the bacteria's innate ability to form biofilms in the absence of treatment. Treating Rows (25 µg/ml to 100 µg/ml). There is a dose-dependent decrease in biofilm formation as the test substance concentration rises. The biofilm's colour gradually lightens, a sign of decreased bacterial adhesion and matrix formation. At 25 µg/ml: Biofilms are still present, but their formation is slightly less than in the untreated control. The reduction in biofilm formation is more noticeable at 50 and 75 µg/ml. The staining gets lighter as the bacteria's ability to form dense biofilms decreases. At 100 µg/ml: The test compound has significantly inhibited biofilm development at this highest concentration, as indicated by the much lighter staining, which shows that the formation of biofilms in most bacterial species is drastically reduced.

Antimicrobial activity: *Syzygium cumini* demonstrated notable antibacterial activity for *Streptococcus mutans* and *Shigella sonnei*. The zone of inhibition increased in concentration related manner of the extract (25–100 µg/ml). At lowest concentration (25 µg/ml), both organisms showed a moderate inhibition zone of 13 mm, indicating initial sensitivity. With increasing concentration, the inhibition zone progressively enlarged, reaching 17 mm for both strains at 100 µg/ml, suggesting strong antibacterial potential. The extract showed comparable efficacy against both Gram-positive (*S. mutans*) and Gram-negative (*S. sonnei*) bacteria, with slightly higher inhibition with *S. sonnei* at intermediate concentrations (50–75 µg/ml).The small standard deviations (±0.1–0.3 mm) indicate high reproducibility and reliability of the results. (Figure 3 (a)).

Biocompatibility Assay: MTT assay results and the phase contrast microscopy images collectively suggest that the *cumini* extract is biocompatible with L929 fibroblast cells. The extract does not significantly reduce cell viability even at higher concentrations. The *cumini* treated cells retain their normal morphology, similar to the control cells. This indicates that the plant extract is non toxic to these cells and could be considered safe. Cell viability was assessed at 24 hours. The absorbance readings indicate their viability (Figure 1(e)).

Herbal extract at lower concentrations showed no significant cytotoxicity, with cell viability compared with the control group. Higher concentrations exhibited reduced cell viability, indicating potential cytotoxic effects at these levels (Figure 3(b)). Short term exposure (24 hrs) showed minimal effects on cell viability while longer exposure times (48 and 72 hours) revealed a more pronounced effect on cell viability, particularly at higher concentrations.

DISCUSSION

Syzygium cumini, most commonly known as Jamun, is rich in bioactive compounds such as anthocyanins, flavonoids, tannins, and glycosides. The fruit, leaves, seeds, and bark, reveals significant amounts of phenolic compounds which might contribute to its antioxidant properties (10).

The present study demonstrated that *Syzygium cumini* extract exhibited significant, dose-dependent antibacterial activity against both *Streptococcus mutans* and *Shigella sonnei*, with maximum inhibition (17 mm) at 100 µg/ml. Abdelgadir Elfadil Abdelgadir et al (11) conducted antimicrobial assay of the extract using different solvents (petroleum ether, methanol, and water) reported variable antimicrobial activity against a broader range of bacteria and fungi, with *E. coli* consistently sensitive. While methanol extract showed strong activity against *B. subtilis* at higher concentrations, petroleum ether extract was only effective against *E. coli*. Mohamed A A (12) assessed antimicrobial activities of *Syzygium cumini* leaves and found methanolic extracts exhibited higher inhibition zones (18-24 mm) compared to methylene chloride extracts, particularly against *S. aureus* and *E. faecalis.* In another study by Shyamala and Vasantha (13) stated that this activity can be related to the presence of bioactive compounds such as tannins and phenolic constituents. The present study showed comparable activity against both Gram-positive and Gram-negative strains, with slightly higher efficacy against *S. sonnei*. This variation may be due to different extraction methods, test organisms, and phytochemical composition of the extracts.Haque et al studied different concentrations of *Syzygium cumini*a against *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus*, and *E. coli*, and found it exhibited strong antimicrobial activity by reducing bacterial growth by 1 to 7 log units. (14)

According to Banerjee et al (15), the diverse phenolic compounds present in jamun may contribute to antioxidant activity and regulating oxidative processes and the study results showed that *Syzygium cumini* fruit skin extract demonstrated excellent antioxidant activity, exhibiting approximately 85–90% DPPH radical scavenging at 100 µg/ml. Similarly, Ruan (16), study reported antioxidant activity in *Syzygium cumini* leaf extracts, with the ethyl acetate fraction s about 82–85% DPPH radical scavenging at 100 µg/ml. Both the study also identified phenolic compounds as the possible contributorfor the antioxidant effect. In comparison, the present study demonstrated dose-dependent antioxidant activity with 49.18% inhibition at 100 µg/ml. The current investigation likewise showed concentration dependent antioxidant activity, with 49.18% radical scavenging at 100 µg/ml. FTIR and UV-visible (340 nm) examination revealed the presence of phenolic and flavonoid chemicals, confirming that *Syzygium cumini*'s antioxidant potential is mostly attributed to its polyphenolic components.

The current study, showed excellent biocompatibility in the MTT experiment, with negligible cytotoxicity at higher doses and cell viability remaining above 80% at lower values (10–25 µg/ml). Silva et al (17) studied acute oral toxicity in mice and have shown that the methanolic leaf extract is non-toxic up to 3.5 g, with an LD_{50} of 3.873 mg/kg body weight. Similarly, oral administration of the hydroalcoholic leaf extract did not produce any toxic effects even at doses up to 6 g/kg body weight. However, when the methanolic leaf extract was administered intraperitoneally, it exhibited toxicity with an LD_{50} of 489 mg/kg body weight in mice. These investigations consistently indicate that *Syzygium cumini* shows minimal toxicity and good biocompatibility, suggesting their potential therapeutic applications.

LIMITATIONS

The present study is an in vitro design, which may not fully replicate clinical outcomes. While the *Syzygium cumini* extracts revealed promising activities. The cytotoxicity, variability in phytochemical composition, and unexplored molecular pathways still needs to be explored. Moreover, the lack of detailed toxicological, pharmacokinetic, and teratogenic studies, restricts its immediate clinical translation and calls for systematic safety evaluation.

SCOPE OF FUTURE RESEARCH

In future the aim should be to study the molecular mechanisms of *Syzygium cumini* and validate its antioxidant, anti-inflammatory, antimicrobial, antiproliferative, and anticariogenic properties in clinical settings. Special emphasis should be placed on its role on advanced biofilm and enamel studies. The development of safe and effective formulations will be essential for translating these observations into practical dental applications.

CONCLUSION

The study confirmed the presence of flavonoids, phenolic acids, tannins, terpenoids, alkaloids compounds in *Syzygium cumini plant extract*. The extract showed strong antioxidant activity with dose dependent free radical scavenging, with significant anti inflammatory effects with notable antimicrobial and antibiofilm activity against *Streptococcus mutans* and *Shigella sonnei with* good biocompatibility at lower concentrations. These findings suggest that *Syzygium cumini* has promising potential for dental applications.

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Figures

Figure 1

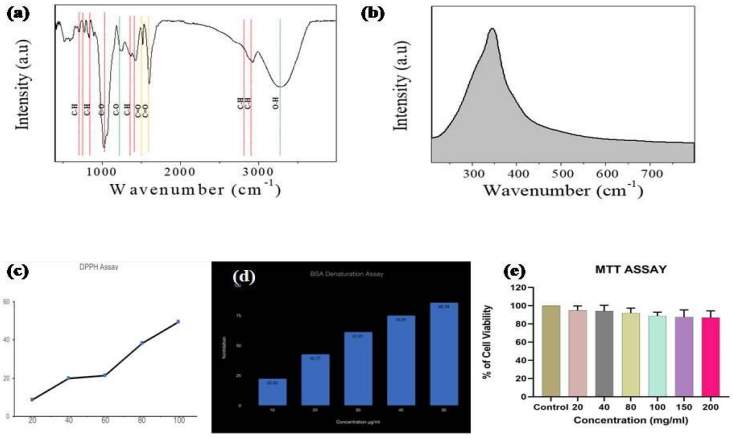


Figure 2

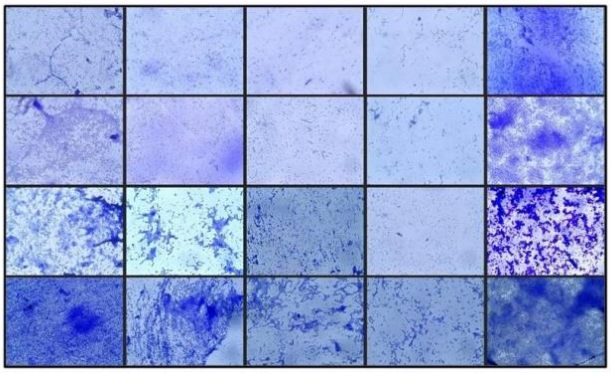
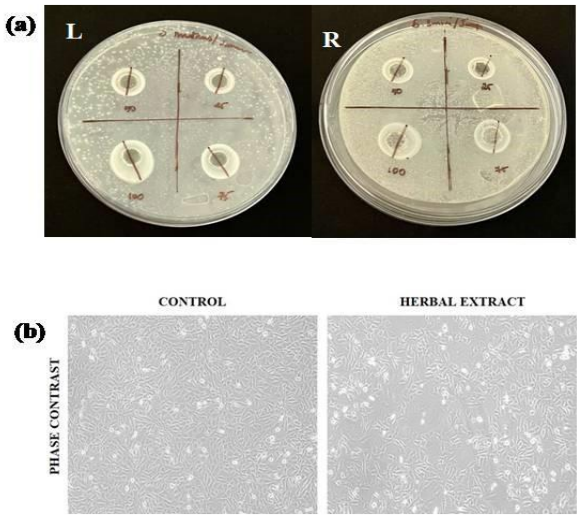


Figure 3



LEGENDS:

Figure 1. (a) Graph depicting the FTIR spectrum of *Syzygium cumini* extract. It demonstrates characteristic absorption bands corresponding to functional groups. (b) UV Visible spectrum of *Syzygium cumini* extract. The spectrum shows a prominent absorption peak around 340 nm indicating the presence of phenolic and polyphenolic compounds. (c) Graph representing DPPH Assay and radical scavenging activity on various concentrations. (d) Graph representing BSA Denaturation Assay at various concentrations. (e) Graph representing cytocompatibility of control and *cumini* groups on L929 fibroblast cells.

Figure 2: Anti Biofilm activity against *Enterococcus faecalis, Staphylococcus aureus, Streptococcus mutans, and Shigella sonnei* at 25µg, 50µg, 75µg and 100µg vs Control group

Figure 3: (a) Zone of Inhibition against *S. mutans* (L) and *S. Sonnei* (R) of the *S. cumini* extract. (b) Morphological evaluation of L929 fibroblast cells. Control and cumini extract treated groups. All the images were captured at a magnification of 100µ (10X)

Table 1: Table representing procedure of phytochemical analysis of the compounds

Table 2: Table representing percentage of inhibition at various concentrations.

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Table 1: Table representing procedure of phytochemical analysis of the compounds

Compound	Test Conducted	Procedure	Observation	Components
Alkaloids	Wagner's Test	Add a few drops of Wagner's reagent (iodine in potassium iodide) to 2–3 mL of the extract.	Formation of a reddish-brown precipitate indicates the alkaloids presence.	Achilleine
Flavonoids	Shinoda Test	Add magnesium turnings and a few drops of concentrated hydrochloric acid to 2–3 mL of the extract.	Development of a pink or red colour indicates the flavonoids presence.	Apigenin, Luteolin, Quercetin, Rutin, Kaempferol
Tannins	Ferric Chloride Test	Few drops of 0.1% ferric chloride solution to 2–3 mL of the extract.	Blue black or greenish colour confirms the presence of tannins.	Catechins
Phenolic Acids	Ferric Chloride Test	Add 1% ferric chloride solution to a small volume of the extract.	Dark green or blue colour indicates phenolic acids	Caffeic Acid, Chlorogenic acid, Salicylic acid
Saponins	Froth Test	Shake the extract vigorously with water in a test tube.	Persistent froth formation indicates the presence of saponins.	Saponin Compounds
Terpenoids	Salkowski Test	Mix 2 mL of extract with 2 mL of chloroform, then carefully add 2 mL of concentrated sulfuric acid along the side of the test tube.	Reddish-brown layer at the interface confirms the presence of terpenoids.	Alpha-Selinene, Beta-Selinene, Germacrene-D, Bornyl Acetate, Borneol, Sabinol
Glycosides	Keller-Kiliani Test	Add glacial acetic acid, a few drops of ferric chloride, and concentrated sulfuric acid to 2 mL of extract.	Formation of a brown ring at the interface indicates the presence of glycosides.	Apigenin Glycosides
Essential Oils	Steam Distillation + GC-MS	Subject powdered plant material to steam distillation. Collect the distillate for GC-MS analysis to identify individual components.	GC-MS reveals the chemical composition of essential oils through chromatographic peaks and mass spectra.	Chamazulene, Beta-Pinene, Eucalyptol, Sabinene, Camphor, Alpha-Terpineol

Table 2: Table representing percentage of inhibition at various concentrations.

Sample Concentration (µg/ml)	Standard (Ascorbic Acid)	Sample	% Inhibition	Standard deviation
10	2.539	1.972	22.33	25.35
20	2.539	1.453	42.77	44.43
30	2.539	0.985	61.21	62.70
40	2.539	0.637	74.91	75.42
50	2.539	0.361	85.78	84.55

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