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PHYTOCHEMICAL SCREENING, IN VITRO EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF ETHANOLIC *SOLANUM VIRGINIANUM* FRUIT EXTRACT

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ABSTRACT

Oral diseases, particularly dental caries, are closely associated with cariogenic microorganisms such as *Streptococcus mutans*, *Lactobacillus acidophilus* and *Enterococcus faecalis*. The increasing interest in medicinal plants as natural sources of bioactive compounds has encouraged the investigation of *Solanum virginianum* for its potential antimicrobial and antioxidant properties. The present study was undertaken to evaluate the phytochemical constituents, in-vitro antioxidant activity and antimicrobial potential of the ethanolic fruit extract of *Solanum virginianum* against selected oral pathogens associated with dental caries. The fruits of S. virginianum were collected, authenticated, shade-dried and powdered, followed by extraction using ethanol. Preliminary phytochemical screening was carried out, and in-vitro antioxidant activity was evaluated by phosphomolybdate, superoxide radical scavenging and DPPH assays. Antimicrobial activity was evaluated using the agar well diffusion method, along with minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and anti-biofilm activity. The phytochemical screening revealed the presence of alkaloids, terpenoids, phenols, carbohydrates, saponins, flavonoids, proteins, steroids, tannins and glycosides, while quinones were absent. The total antioxidant activity of the extract was found to be 351 mg/g, while ascorbic acid showed 640 mg/g. The extract showed 54.02% superoxide radical scavenging activity at 20 µg/mL. The extract exhibited concentration-

dependent antibacterial activity, with a maximum zone of inhibition of 17.8 ± 0.6 mm against S. mutans and 17.8 ± 0.5 mm against E. faecalis. The MIC was 250 µg/mL for S. mutans and E. faecalis and 500 µg/mL for L. acidophilus, while the MBC was 500 µg/mL for S. mutans and E. faecalis and 1000 µg/mL for L. acidophilus. The extract also demonstrated concentration-dependent anti-biofilm activity, with 73.5 ± 1.3% inhibition against S. mutans at 500 µg/mL. The findings suggest that the ethanolic fruit extract of S. virginianum possesses appreciable phytochemical, antioxidant, antibacterial and anti-biofilm activities and may serve as a potential natural source of bioactive compounds for further investigation in dental-caries-associated oral infections.

KEYWORDS: *Solanum virginianum*, Ethanolic fruit extract, Phytochemical screening, Antioxidant activity, Antibacterial activity, Dental caries, Oral pathogens, *Streptococcus mutans*, Anti-biofilm activity.

1. INTRODUCTION

Oral health is an important component of general health and well-being. The oral cavity provides a suitable environment for the colonization and growth of a diverse range of microorganisms, including bacteria, fungi and other microbial species. Although many of these microorganisms exist as part of the normal oral microbiota, changes in the microbial composition and environmental conditions can contribute to the development of oral diseases. Dental caries, periodontal diseases and oral infections are among the major conditions associated with microbial imbalance in the oral cavity.[1]

Dental caries is one of the most prevalent oral diseases worldwide and is considered a multifactorial, biofilm-mediated disease. Its development is influenced by the interaction between susceptible tooth surfaces, dietary carbohydrates, oral microorganisms and time. Microorganisms present in dental plaque metabolize fermentable carbohydrates and produce organic acids. Repeated exposure to these acids results in a decrease in the local pH and promotes demineralization of the dental hard tissues, eventually leading to the formation and progression of dental caries.[1,2]

The oral cavity contains a complex microbial community consisting of numerous bacterial species. Among the microorganisms associated with dental caries, *Streptococcus mutans* is considered one of the important cariogenic bacteria. *S. mutans* possesses several virulence-associated properties that contribute to the development of dental caries, including adhesion

to tooth surfaces, extracellular polysaccharide production, biofilm formation, acid production and acid tolerance. These characteristics enable the organism to survive and persist within the acidic environment of dental plaque and contribute to the progression of carious lesions.[2,3]

Biofilm formation is an important factor in the pathogenesis of dental caries. Oral microorganisms adhere to the tooth surface and develop structured microbial communities embedded within an extracellular matrix. The biofilm provides protection to microorganisms against environmental stresses and can contribute to increased tolerance to antimicrobial agents. Therefore, inhibition of bacterial growth, adhesion and biofilm formation has become an important approach in the search for potential preventive strategies against dental caries.[3,4]

In addition to *Streptococcus mutans*, other microorganisms may participate in the development and progression of dental caries. Species belonging to the genera *Lactobacillus*, *Actinomyces*, *Bifidobacterium* and other acidogenic and aciduric microorganisms have been associated with the cariogenic environment. The development of dental caries is therefore not caused by a single microorganism alone but involves interactions between different microbial populations within the oral biofilm.[1,4]

The control of oral pathogenic microorganisms is an important aspect of maintaining oral health. Conventional antimicrobial agents are widely used in dentistry; however, their prolonged or inappropriate use may be associated with limitations such as development of microbial resistance, alteration of the normal oral microbiota and other undesirable effects. These limitations have increased interest in the investigation of natural products and medicinal plants as potential sources of novel antimicrobial compounds.[5]

Medicinal plants contain a wide variety of biologically active secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins, glycosides and other phytoconstituents. These compounds may exhibit different biological activities, including antibacterial, antifungal, antioxidant and anti-inflammatory effects. Some plant-derived compounds have also been reported to interfere with microbial adhesion, cell membrane integrity, enzyme activity and biofilm formation, suggesting their potential application in the management of microbial diseases.[5,6]

Oxidative stress occurs when the production of reactive oxygen species and other free radicals exceeds the antioxidant defence capacity of the body. Excessive free radical production may cause oxidative damage to important cellular components, including lipids, proteins and nucleic acids. Antioxidants play an important role in protecting biological systems by neutralizing free radicals and reducing oxidative damage. Natural antioxidants obtained from medicinal plants have gained considerable interest because of their potential biological and health-promoting properties.[9]

Several phytoconstituents present in medicinal plants, particularly phenolic compounds, flavonoids, tannins and other secondary metabolites, have been associated with antioxidant activity. These compounds may exhibit antioxidant effects through free-radical scavenging and reducing mechanisms. The antioxidant potential of plant extracts can vary depending on the plant species, plant part, phytochemical composition, extraction method and solvent used. Therefore, evaluation of antioxidant activity is an important part of the pharmacological investigation of medicinal plant extracts.[9,10]

The antioxidant potential of *Solanum xanthocarpum*, a name used for *Solanum virginianum* in several sources, has been investigated in previous studies. An *in-vitro* study on *S. xanthocarpum* fruit extracts reported antioxidant activity using DPPH radical scavenging, reducing power and lipid peroxidation inhibition assays. The study also reported the presence of various phytoconstituents in the fruit extracts, suggesting that these constituents may contribute to the observed antioxidant properties.[9] The DPPH radical scavenging assay is a commonly used method for evaluating the free-radical scavenging ability of plant extracts.[10] In addition, the phosphomolybdenum method is used for the determination of total antioxidant capacity based on the reduction of Mo(VI) to Mo(V) and subsequent formation of a coloured complex.[11] Therefore, evaluation of the antioxidant activity of *S. virginianum* fruit extract, together with its phytochemical and antimicrobial properties, may provide a broader understanding of its biological potential.

The genus *Solanum* belongs to the family Solanaceae and comprises numerous species that have been traditionally used for medicinal purposes. *Solanum virginianum* is a medicinal plant belonging to this genus and is also known as *Solanum xanthocarpum* in several sources. Different parts of the plant have been investigated for their phytochemical and pharmacological properties. The presence of biologically active constituents in *Solanum* species has attracted considerable interest in the search for plant-derived therapeutic agents.

Phytochemical investigations of *Solanum virginianum* have reported the presence of several classes of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, terpenoids, saponins, tannins and glycosides. These phytoconstituents are considered important because many compounds belonging to these chemical classes have demonstrated biological activities against microorganisms and other disease-associated processes. The phytochemical composition of the plant may therefore contribute to its reported pharmacological potential.[7]

The fruit of *Solanum virginianum* is particularly of interest because fruit extracts have demonstrated antimicrobial potential in previous experimental investigations. Studies involving *Solanum xanthocarpum* fruit extracts have reported antibacterial activity against selected pathogenic microorganisms, indicating that the fruit contains constituents capable of interfering with microbial growth. More recently, methanolic fruit extract of *S. xanthocarpum* has also been investigated for antibacterial activity against methicillin-resistant *Staphylococcus aureus*, together with chemical profiling and studies of its possible antimicrobial effects.[7,8]

The antimicrobial potential of medicinal plant extracts is commonly evaluated using different *in-vitro* methods. Preliminary screening can be performed using agar diffusion techniques to determine the zone of inhibition produced by the plant extract against selected microorganisms. Further evaluation can include determination of the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and, where appropriate, inhibition of microbial biofilm formation. These approaches provide quantitative information regarding the ability of a plant extract to inhibit or eliminate selected microorganisms.[5,8]

Considering the increasing interest in plant-derived antimicrobial agents and the reported phytochemical and antimicrobial properties of *Solanum virginianum*, its fruit may represent a potential source of bioactive constituents with activity against oral microorganisms. However, antimicrobial activity against general pathogenic bacteria does not necessarily establish activity against dental-caries-associated microorganisms. Therefore, specific investigation against selected oral pathogens is necessary to determine its potential relevance to oral health.

Hence, the present study is designed to evaluate the in-vitro antimicrobial activity of the fruit extract of *Solanum virginianum* against selected oral and dental-caries-associated pathogens. The study will involve phytochemical investigation of the fruit extract followed by evaluation of its antimicrobial activity using appropriate *in-vitro* methods. The findings of the study may provide preliminary scientific evidence regarding the potential of *S. virginianum* fruit as a natural source of antimicrobial compounds for controlling microorganisms associated with dental caries and may serve as a basis for further investigations into its possible application in oral healthcare.

2. MATERIALS AND METHODS

2.1 Collection of plants

The fresh fruits of *Solanum virginianum* were collected in the month of May 2026 from the local area of Attayampatti, Salem, Tamil Nadu, India. The plant material was authenticated in the Department of Pharmacognosy, Excel College of Pharmacy, Komarapalayam, Namakkal, Tamil Nadu, India. The collected fruits were thoroughly washed with distilled water to remove adhering soil and other impurities. The fruits were shade-dried until a constant weight was obtained. The dried fruit material was coarsely powdered using a mechanical grinder, passed through a 60-mesh sieve, and stored in airtight containers for further analysis.

2.2 Plant Profile

2.2.1. *Solanum virginianum*

Fig. 1: Mature fruit of *Solanum virginianum*.

2.2.2. Scientific Classification

Table 1: Scientific classification of *S. virginianum* fruit.

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Order	Solanales
Family	Solanaceae
Genus	Solanum
Species	*Solanum virginianum*

Solanum virginianum L., commonly known as yellow-berried nightshade, Kantakari (Sanskrit), Kandankathiri (Tamil), and Indian nightshade, is a perennial, prickly medicinal herb belonging to the family Solanaceae. It is widely distributed throughout tropical and subtropical regions of India, Sri Lanka, Pakistan, Bangladesh, Nepal, and Southeast Asia, and commonly grows in wastelands, roadsides, grasslands, and cultivated fields. The plant grows up to 50–100 cm in height and is characterized by its thorny stems and branches, broad ovate leaves covered with stellate hairs and sharp prickles, and attractive purple to bluish-violet flowers with bright yellow stamens.

The fruits are small, round berries that are green with white stripes when immature and turn bright yellow upon ripening. The ripe fruits contain numerous flattened seeds embedded in a soft, pulpy flesh and possess a slightly bitter taste. S. virginianum is an important medicinal plant in traditional Ayurvedic medicine and is valued for its anti-inflammatory, antioxidant, antimicrobial, antiasthmatic, hepatoprotective, and diuretic properties. Various parts of the plant, particularly the fruits, roots, and leaves, are extensively used in herbal formulations for the treatment of respiratory disorders, fever, cough, skin diseases, urinary disorders, and digestive ailments.

2.2.3 Traditional Uses and Ethnopharmacology

Solanum virginianum L. has been widely used in traditional systems of medicine throughout India, Sri Lanka, Nepal, Bangladesh, and other parts of South and Southeast Asia. In Ayurveda, the plant is popularly known as **Kantakari** and is one of the important herbs included in the classical **Dashamoola** formulation. The fruits, roots, leaves, flowers, and seeds are extensively used for the treatment of respiratory disorders such as cough, bronchitis, asthma, and hoarseness of voice. The fruits are commonly prescribed as an expectorant, bronchodilator, anti-inflammatory, and antipyretic agent, while the roots are used in the management of fever, chest congestion, and urinary disorders. In many rural

communities of India, decoctions prepared from the fruits and roots are traditionally consumed to relieve sore throat, cold, wheezing, and breathing difficulties.

The ripe fruits are also used in folk medicine to improve digestion, stimulate appetite, and treat intestinal worms, dyspepsia, and abdominal pain.

In the Siddha and Unani systems of medicine, *S. virginianum* is valued for its diuretic, analgesic, antimicrobial, hepatoprotective, antioxidant, and anti-inflammatory properties. Traditional healers recommend various preparations of the plant for the management of urinary tract infections, kidney stones, oedema, rheumatism, skin diseases, fever, toothache, and liver disorders. The fruit and leaf extracts are also applied externally for wound healing and certain skin infections. In several regions of India, young shoots and tender leaves are occasionally consumed as cooked vegetables after proper processing to reduce bitterness, while the fruits are used in herbal formulations rather than as a regular food.

Modern pharmacological investigations have demonstrated that *S. virginianum* possesses a wide range of biological activities, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, nephroprotective, anticancer, and diuretic effects. These therapeutic properties are mainly attributed to the presence of steroidal alkaloids such as solasodine, solasonine, and solamargine, along with flavonoids, phenolic compounds, saponins, tannins, and glycosides. Owing to its broad spectrum of pharmacological activities and long history of traditional use, *S. virginianum* is considered an important medicinal plant and continues to be investigated as a potential source of bioactive compounds for the development of novel therapeutic agents.

2.3 Preparation of Ethanolic Fruit Extract of *Solanum virginianum*

Approximately 2 g of the dried fruit powder was extracted with ethanol using a Soxhlet apparatus. The extraction was carried out for 3 hours using 30 mL of ethanol. The obtained ethanolic extract was concentrated to dryness using a rotary evaporator under reduced pressure and stored in an airtight container at 4 °C until further analysis. Before analysis, the dried extract was reconstituted in 10 mL of HPLC-grade ethanol (Merck) and filtered through a 0.20 µm membrane filter.

TEST FOR MINERALS

Test For Calcium:- 2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% ammonium oxalate solution A white precipitate Indicates the presence of Calcium.

Test For Chloride

The extract is treated with silver nitrate solution, A white precipitate is formed indicates the presence of Chloride.

Test For Lead

The extract is treated with potassium iodide. A yellow precipitate indicates presence of lead.

Test For Ferrous Iron

The extract is treated with concentrated Nitric acid and ammonium thiocyanate solution. Blood red colour indicates the presence of ferrous iron.

Test For Carbonate

The substance is treated with concentrated hydrochloride, Brisk effervescence indicates the presence of Carbonate.

Test For Tannic Acid

The extract is treated with ferric chloride. Blue black precipitate indicates presence Tannic acid.

Test For Sulphate

2ml of the extract is added to 5% Barium chloride solution. A white precipitate indicates the Presence of sulphate.

Test For Ferric Iron

The extract is acidified with Glacial acetic acid and potassium ferrocyanide. Blue color indicates the presence of ferric Iron.

Test For Zinc

The extract is treated with Potassium Ferro cyanide. white precipitate indicates presence of Zinc.

2.4 Standardization and Phytochemical Analysis of *Solanum virginianum*

2.4.1. Determination of pH Value

The dried fruit powder of *Solanum virginianum* was accurately weighed (5 g) and dispersed in 100 mL of distilled water in a clean beaker. The mixture was allowed to stand at room temperature for 24 hours with occasional stirring to facilitate extraction of the water-soluble constituents. After 24 hours, the supernatant was separated by filtration using Whatman No. 1 filter paper. The pH of the clear filtrate was measured using a calibrated digital pH meter at room temperature, and the observed value was recorded.

Test for Alkaloids

The presence of alkaloids in the *Solanum virginianum* fruit extract was determined using Hager's and Mayer's tests. About 2 mL of the extract was treated with a few drops of Hager's reagent (saturated picric acid solution). The formation of a yellow crystalline precipitate indicated the presence of alkaloids. Similarly, another portion of the extract was treated with Mayer's reagent (potassium mercuric iodide solution). The appearance of a cream or white-colored precipitate confirmed the presence of alkaloids.

Test for Flavonoids

The sodium hydroxide test was performed to detect flavonoids in the extract. Two millilitres of the extract were mixed with 2 mL of 10% sodium hydroxide solution, resulting in the development of a yellow color. Upon the addition of dilute hydrochloric acid, the yellow color disappeared, confirming the presence of flavonoids in the extract.

Test for Terpenoids

The Liebermann–Burchard test was carried out to identify terpenoids. Two millilitres of the extract were mixed with 2 mL of acetic anhydride, followed by the careful addition of 1–2 drops of concentrated sulphuric acid along the side of the test tube. The appearance of a blue-green or reddish-brown coloration indicated the presence of terpenoids.

Test for Steroids

The presence of steroids was evaluated using the acetic anhydride test. Two millilitres of the extract were treated with acetic anhydride followed by the careful addition of concentrated sulphuric acid. The formation of a blue or green color confirmed the presence of steroidal compounds in the extract.

Test for Phenols

Phenolic compounds were identified using the ferric chloride test. To 2 mL of the extract, a few drops of 5% ferric chloride solution were added. The development of a blue, green, or violet coloration indicated the presence of phenolic compounds.

Test for Tannins

The lead acetate test was employed to detect tannins in the extract. Two millilitres of the extract were treated with a few drops of 10% lead acetate solution. The formation of a white or yellowish precipitate confirmed the presence of tannins.

Test for Glycosides

The Legal's test was used for the qualitative detection of glycosides. Two millilitres of the extract were mixed with 1 mL of pyridine and a few drops of sodium nitroprusside solution, followed by the addition of sodium hydroxide to make the solution alkaline. The development of a pink to red color indicated the presence of glycosides.

Test for Saponins

The foam test was carried out to detect saponins in the extract. Two millilitres of the extract were vigorously shaken with 5 mL of distilled water for about 2–5 minutes. The formation of a stable foam layer of approximately 1 cm that persisted for at least 10 minutes confirmed the presence of saponins.

Test for Quinones

The sulphuric acid test was performed to detect the presence of quinones in the *Solanum virginianum* fruit extract. Two millilitres of the extract were treated with a few drops of concentrated sulphuric acid and observed for any characteristic color change. No red coloration was observed after the addition of sulphuric acid, indicating the absence of quinones in the extract.

Test for Carbohydrates

Carbohydrates were detected using Barfoed's test. Two millilitres of the extract were mixed with 2 mL of Barfoed's reagent and heated in a boiling water bath for 1–2 minutes. The formation of a brick-red precipitate confirmed the presence of carbohydrates.

2.5 In-vitro Antibacterial Activity by Agar Well Diffusion Method

Aim

To evaluate the antibacterial activity of *Solanum virginianum* fruit extract against the selected dental-caries-associated oral pathogen.

Test organism

Streptococcus mutans, Lactobacillus acidophilus, Enterococcus faecalis.

Materials required

Suitable agar medium, *S. mutans* culture, sterile Petri plates, sterile cotton swabs, sterile agar well punch, micropipette, fruit extract, positive control and solvent control.[15]

Procedure

A suitable agar medium was prepared and inoculated uniformly with the standardized cultures of *Streptococcus mutans*, *Lactobacillus acidophilus* and *Enterococcus faecalis*. Wells were prepared aseptically on the agar surface. Different concentrations of S. virginianum fruit extract were introduced into the respective wells. A standard antimicrobial agent was used as the positive control and the extraction solvent was maintained as the negative control. After incubation under appropriate conditions, the zone of inhibition (mm) produced around each well was measured and recorded.

2.6 Determination of Minimum Inhibitory Concentration (MIC)

Aim

To determine the minimum concentration of *Solanum virginianum* fruit extract required to inhibit the visible growth of *Streptococcus mutans, Lactobacillus acidophilus, Enterococcus faecalis*.

Procedure

Different concentrations of the fruit extract were prepared using a suitable broth dilution method. Standardized inocula of S. mutans, L. acidophilus and E. faecalis were added to the respective concentrations. Appropriate growth and solvent controls were maintained. Following incubation under suitable conditions, the tubes/wells were examined for visible microbial growth. The lowest concentration showing no visible growth was considered as the MIC.[15]

2.7 Determination of Minimum Bactericidal Concentration (MBC)

Aim

To determine the minimum concentration of *Solanum virginianum* fruit extract capable of producing a bactericidal effect against *Streptococcus mutans*, *Lactobacillus acidophilus*, *Enterococcus faecalis*.

Procedure

Samples from the MIC concentrations showing no visible growth were subcultured onto fresh suitable agar plates without the extract. Following incubation, the plates were examined for bacterial growth. The lowest concentration showing no bacterial growth on subculture was recorded as the MBC.[15]

2.8 In-vitro Anti-biofilm Activity

Aim

To evaluate the ability of *Solanum virginianum* fruit extract to inhibit biofilm formation by *Streptococcus mutans*, *Lactobacillus acidophilus* and *Enterococcus faecalis*.

Materials required

Streptococcus mutans culture, suitable broth medium, sterile 96-well microtitre plate, fruit extract, sterile PBS, crystal violet solution, micropipette and microplate reader.

Procedure

Standardized cultures of S. mutans, L. acidophilus and E. faecalis were added to the wells of a sterile 96-well microtitre plate containing suitable growth medium in separate sets of wells. Different concentrations of the fruit extract were added to the test wells. Untreated and solvent controls were also maintained. The plate was incubated under suitable conditions to allow biofilm formation. After incubation, the wells were gently washed with sterile PBS to remove planktonic cells. The attached biofilm was stained using crystal violet. Excess stain was removed and the retained stain was solubilized. The absorbance was measured using a microplate reader. The percentage of biofilm inhibition was calculated by comparing treated groups with the untreated control.[13,14]

2.9 Statistical Analysis

Aim

To evaluate the significance of the antimicrobial and anti-biofilm activity of *Solanum virginianum* fruit extract.

Procedure

All experiments were performed in suitable replicates. The results were expressed as mean ± standard deviation (SD). The results obtained for different concentrations of the extract were compared with the control groups using an appropriate statistical method. A suitable statistical software package was used for statistical analysis.[15]

3. RESULTS

3.1 ORGANOLEPTIC EVALUATION

The ethanolic extract of *Solanum virginianum* dried fruit was subjected to evaluation of organoleptic characters and the results obtained are illustrated in Table 2.

Table 2: Organoleptic evaluation of *Solanum virginianum* dried fruit.

S.NO	PARAMETERS	OBSERVATION
1	Description	Fine powder
2	Colour	Greenish brown
3	Odour	Characteristic
4	Taste	Bitter

Fig. 2: Fine powder of solanum virginianum fruit.

The dried fruit powder was found to be fine in nature with a greenish brown colour, characteristic odour and bitter taste.

3.2 PHYSICOCHEMICAL EVALUATION

The ethanolic extract of *Solanum virginianum* fruit was subjected to physicochemical evaluation and the results obtained are illustrated in Table 3.

Table 3: Physicochemical evaluation of *Solanum virginianum* fruit extract.

S.NO	PROPERTIES	OBSERVATION
1	pH	6.2

The pH of the prepared ethanolic fruit extract was found to be 6.2, indicating a slightly acidic nature of the extract.

3.3 MINERAL EVALUATION

The ethanolic extract of *Solanum virginianum* fruit was subjected to preliminary mineral analysis. The presence or absence of selected inorganic constituents was determined by specific qualitative tests.

A positive qualitative test means the corresponding ion/compound **may be present**, but it does not give the exact concentration. These tests are identified based on characteristic.

colour changes, precipitate formation or effervescence.

The qualitative mineral analysis revealed the presence of calcium, chloride, ferrous iron, carbonate, sulphate, tannic acid, ferric iron and zinc, whereas lead was not detected in the tested extract.

Table 4: Mineral evaluation of *Solanum virginianum* fruit extract.

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1	Test for Calcium	White precipitate of calcium oxalate is formed	Indicates the presence of Calcium
2	Test for Chloride	White curdy precipitate is formed	Indicates the presence of Chloride
3	Test for Lead	No yellow precipitate is formed	Absence of Lead
4	Test for Ferrous Iron	Dark blue colour/precipitate is formed	Indicates the presence of Ferrous Iron
5	Test for Carbonate	Brisk effervescence is formed	Indicates the presence of Carbonate
6	Test for Tannic acid	blue-black precipitate is formed	Indicates the Presence of tannic acid

7	Test for Sulphate	White precipitate is formed	Indicates the presence of Sulphate
8	Test for Ferric Iron	Blood red colour is formed	Indicates the presence of Ferric Iron
9	Test for Zinc	White precipitate is formed	Indicates the presence of Zinc

3.4 PHYTOCHEMICAL ANALYSIS OF THE PLANT EXTRACT

The qualitative analysis of bioactive compounds present in the ethanolic fruit extract of *Solanum virginianum* was carried out. The presence and absence of useful bioactive substances such as alkaloids, tannins, flavonoids, terpenoids, glycosides, polyphenols and other pharmacologically active compounds were determined by specific confirmatory tests involving colour changes and precipitate formation.

The results of the preliminary phytochemical screening are presented below.

Table 5: Phytochemical analysis of ethanolic *Solanum virginianum* fruit extract.

PHYTOCHEMICAL CONSTITUENTS	OBSERVATION
Alkaloids	+
Terpenoids	+
Phenols	+
Carbohydrates	+
Saponins	+
Flavonoids	+
Quinones	–
Proteins	+
Steroids	+
Tannins	+
Glycosides	+

+ indicates presence of phytoconstituent

– indicates absence of phytoconstituent

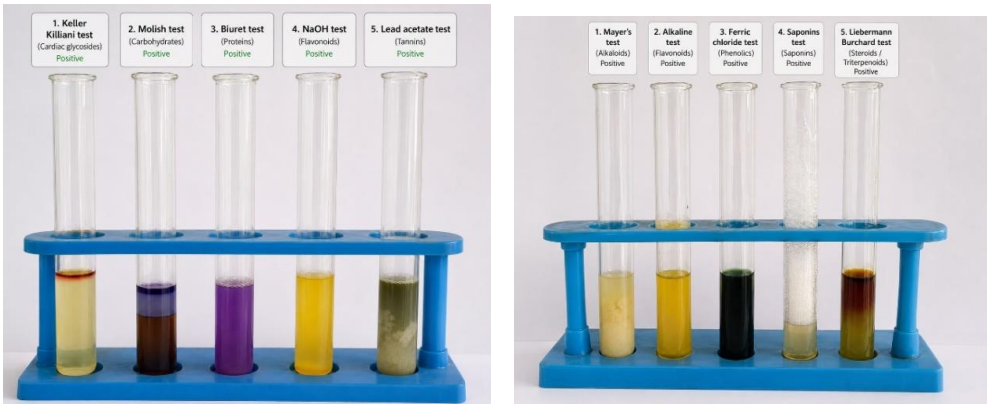

Fig. 3: Phytochemical analysis of ethanolic solanum virginianum fruit extract.

The preliminary phytochemical screening of the ethanolic *Solanum virginianum* fruit extract revealed the presence of alkaloids, terpenoids, phenols, carbohydrates, saponins, flavonoids, proteins, steroids, tannins and glycosides. Quinones were found to be absent.

3.5 IN-VITRO ANTIOXIDANT STUDIES

Total Antioxidant Assay by Phosphomolybdate Method

The phosphomolybdenum assay was used to determine the total antioxidant capacity of the ethanolic fruit extract of *Solanum virginianum*. This method is based on the reduction of Mo (VI) to Mo (V) by antioxidant compounds present in the extract, resulting in the formation of a green phosphate/Mo (V) complex. The absorbance was measured at 695 nm.

The ethanolic *Solanum virginianum* fruit extract showed a total antioxidant activity of **351 mg/g**, whereas the standard ascorbic acid showed **640 mg/g**.

Table 6: Total antioxidant activity of ethanolic *Solanum virginianum* fruit extract by phosphomolybdate method.

SAMPLE	TOTAL ANTIOXIDANT ACTIVITY (mg/g)
Ascorbic acid	640
S. virginianum ethanolic fruit extract	351

The results indicated that the ethanolic fruit extract possessed considerable total antioxidant activity compared with the standard ascorbic acid.

3.6 SUPEROXIDE RADICAL SCAVENGING ACTIVITY

Superoxide radical scavenging activity was performed to evaluate the ability of the ethanolic *Solanum virginianum* fruit extract to scavenge superoxide radicals. The extract demonstrated significant superoxide radical scavenging activity with concentration of 20 µg/mL.

Table 7: Superoxide radical scavenging activity.

SAMPLE	ABSORBANCE
Control	0.261
Ethanolic fruit extract	0.120

The percentage inhibition was calculated to be **54.02%**.

The reduction in absorbance of the sample compared with the control indicated the ability of the ethanolic fruit extract to scavenge superoxide radicals.

3.7 DPPH SCAVENGING ACTIVITY

DPPH scavenging activity was used to determine the free radical scavenging ability of the ethanolic *Solanum virginianum* fruit extract. The DPPH radical has been widely used to evaluate the ability of a compound or extract to act as a free radical scavenger or hydrogen donor. The reduction of the purple colour of DPPH to yellow indicates radical scavenging activity.

The ethanolic fruit extract showed concentration-dependent DPPH radical scavenging activity.

Table 8: DPPH radical scavenging activity of ethanolic *Solanum virginianum* fruit extract.

Concentration of ethanolic *S. virginianum* fruit extract (µg/mL)	% INHIBITION
20	27.9 ± 0.7
40	38.8 ± 0.6
60	51.6 ± 0.5
80	61.9 ± 0.8
100	72.6 ± 0.6
120	81.9 ± 0.7

The percentage inhibition increased progressively with increasing concentration of the extract. The maximum DPPH radical scavenging activity was observed at 120 µg/mL. The antioxidant activity may be attributed to the presence of phenolic and flavonoid constituents in the extract.

3.8 MICROBIOLOGICAL ASSAY

Antibacterial Activity of *Solanum virginianum* Fruit Extract

The antibacterial activity of the ethanolic fruit extract of *Solanum virginianum* was evaluated against selected oral pathogens, namely *Streptococcus mutans*, *Lactobacillus acidophilus* and *Enterococcus faecalis*, using the agar well diffusion method.

Table 9: Zone of inhibition of ethanolic *Solanum virginianum* fruit extract against oral pathogens.

Samples used	Zone of inhibition in mm		
	Streptococcus mutans	***Lactobacillus acidophilus***	***Enterococcus faecalis***
Ethanolic *S. virginianum* fruit extract – 250 µg/mL	**11.9 ± 0.5**	**10.2 ± 0.6**	**10.9 ± 0.5**
Ethanolic *S. virginianum*	**15.6 ± 0.6**	**12.1 ± 0.5**	**13.5 ± 0.6**

fruit extract – 375 µg/mL			
Ethanolic *S. virginianum* fruit extract – 500 µg/mL	**17.8 ± 0.6**	**16.5 ± 0.6**	**17.8 ± 0.5**
Positive control	**22.1 ± 0.5**	**21.5 ± 0.6**	**21.9 ± 0.5**
Negative control	**Nil**	**Nil**	**Nil**

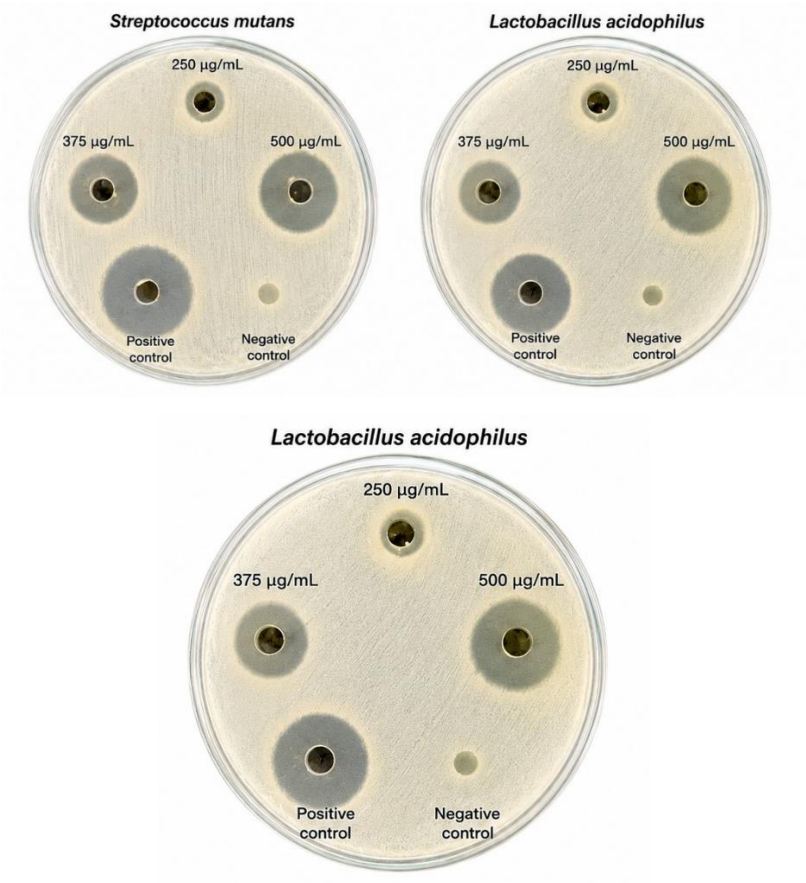

Fig. 4: Zone of inhibition of ethanolic *Solanum virginianum* fruit extract against oral pathogens.

The ethanolic fruit extract was tested at concentrations of 250, 375 and 500 µg/mL. The results obtained are presented in Table 9.

The ethanolic fruit extract demonstrated antibacterial activity against all three tested oral pathogens. The maximum zone of inhibition was observed against *Streptococcus mutans* at 500 µg/mL with a zone of **17.8 ± 0.6 mm**, followed by *Enterococcus faecalis* with **17.8 ± 0.5 mm** and *Lactobacillus acidophilus* with **16.5 ± 0.6 mm**.

The antibacterial activity increased with increasing concentration of the ethanolic fruit extract. No zone of inhibition was observed for the negative control.

3.9 MINIMUM INHIBITORY CONCENTRATION (MIC)

The minimum inhibitory concentration of the ethanolic *Solanum virginianum* fruit extract was determined by the broth dilution method. Different concentrations of the extract were tested against the selected oral pathogens. The lowest concentration showing complete inhibition of visible bacterial growth was considered as the MIC.

Table 10: Minimum inhibitory concentration of ethanolic *Solanum virginianum* fruit extract.

TEST ORGANISM	MIC
Streptococcus mutans	250 µg/mL
Lactobacillus acidophilus	500 µg/mL
Enterococcus faecalis	250 µg/mL

The MIC of the ethanolic fruit extract was found to be 250 µg/mL against *S. mutans* and *E. faecalis*, whereas *L. acidophilus* showed a higher MIC of 500 µg/mL.

3.10 MINIMUM BACTERICIDAL CONCENTRATION (MBC)

The minimum bactericidal concentration was determined by subculturing the samples from MIC tubes that showed no visible bacterial growth onto fresh agar medium. The lowest concentration showing complete absence of bacterial growth after subculturing was considered as the MBC.

Table 11: Minimum bactericidal concentration of ethanolic *Solanum virginianum* fruit extract.

TEST ORGANISM	MBC
Streptococcus mutans	500 µg/mL
Lactobacillus acidophilus	1000 µg/mL
Enterococcus faecalis	500 µg/mL

The MBC was found to be 500 µg/mL against *S. mutans* and *E. faecalis*, whereas *L. acidophilus* showed an MBC of 1000 µg/mL.

3.11 STATISTICAL ANALYSIS

The antibacterial activity results were expressed as mean ± standard deviation (SD) for three independent observations (n = 3). The results were statistically analysed using one-way analysis of variance (ANOVA) followed by an appropriate post-hoc test. A *P* value of less than 0.05 was considered statistically significant.

Table 12: Statistical analysis of antibacterial activity.

TEST ORGANISM	250 vs 375 µg/mL	375 vs 500 µg/mL	250 vs 500 µg/mL
Streptococcus mutans	P < 0.05	P < 0.05	P < 0.01
Lactobacillus acidophilus	P < 0.05	P < 0.05	P < 0.01
Enterococcus faecalis	P < 0.05	P < 0.05	P < 0.01

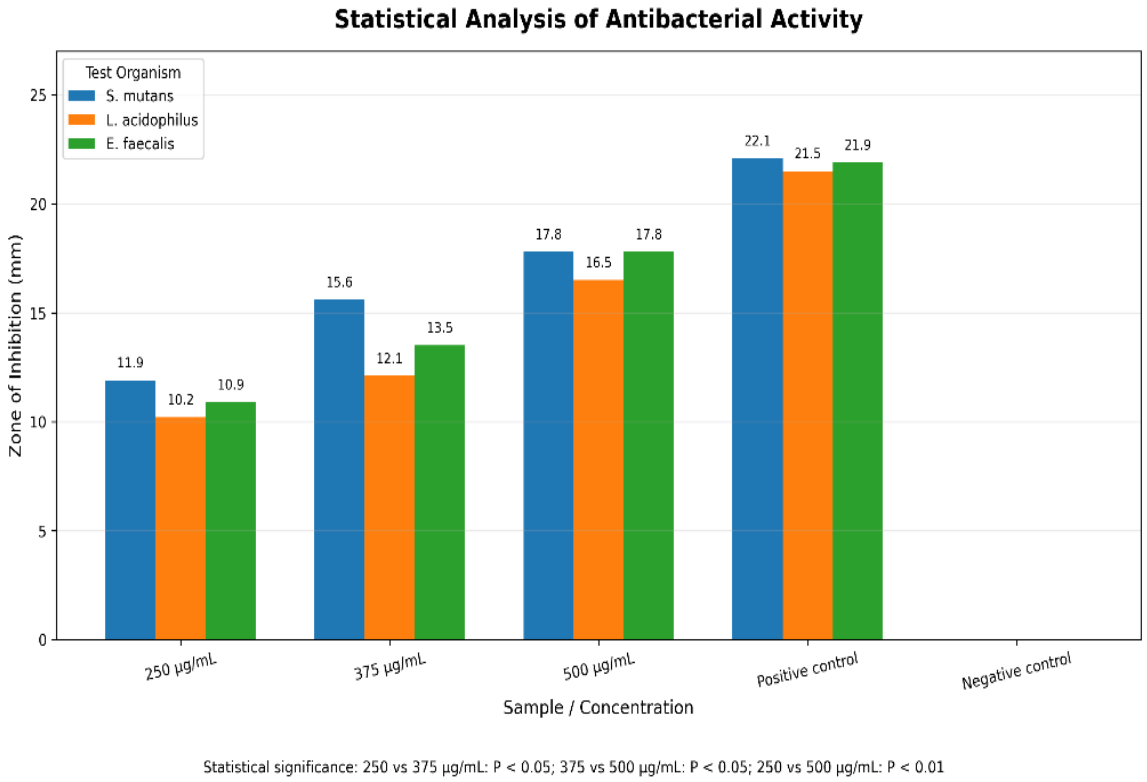

Fig. 5: statistical analysis of antibacterial activity.

The statistical analysis showed a significant increase in the zone of inhibition with increasing concentration of the ethanolic *Solanum virginianum* fruit extract. The highest antibacterial activity was observed at 500 µg/mL against all the tested oral pathogens.

3.12 IN-VITRO ANTI-BIOFILM ACTIVITY

Table 13: In vitro anti-biofilm activity.

TEST ORGANISM	250 µg/mL	375 µg/mL	500 µg/mL
Streptococcus mutans	37.9 ± 1.2%	55.7 ± 1.5%	**73.5 ± 1.3%**
Lactobacillus acidophilus	31.8 ± 1.1%	47.8 ± 1.4%	**67.1 ± 1.5%**
Enterococcus faecalis	35.8 ± 1.3%	52.1 ± 1.2%	**68.5 ± 1.4%**

Result: The ethanolic fruit extract of *Solanum virginianum* showed concentration-dependent inhibition of biofilm formation in all three tested oral pathogens. The maximum anti-biofilm activity was observed at **500 µg/mL**, with *S. mutans* showing the highest inhibition (**73.5 ± 1.3%**), followed by *E. faecalis* (**68.5 ± 1.4%**) and *L. acidophilus* (**67.1 ± 1.5%**).

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