

Yellow Berried Nightshade: A Potential Source of Oral Care Compounds with Antibacterial, Antioxidant, and Anti-Inflammatory Properties- An In-Vitro Investigation

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ABSTRACT

Introduction Yellow berried nightshade (*S. sisymbriifolium*) has long been used in traditional medicine for its antimicrobial, antioxidant, anti-inflammatory, antipyretic, and analgesic properties. Due to potential side effects of conventional chemical-based oral products, there is increasing interest in natural plant-derived alternatives for oral health.

Materials and Methods Leaves were collected from a botanical garden in Chennai, Tamil Nadu, India. One gram of plant material was extracted in 10 mL ethanol, shaken for 30 minutes, and centrifuged for 10 minutes. The supernatant was filtered using Whatman No. 1 filter paper and evaporated at room temperature. Antibacterial activity was assessed using the agar well diffusion method against *Lactobacillus* sp., *Streptococcus mutans*, and *Candida albicans*. Antioxidant activity was evaluated using the DPPH assay. Anti-inflammatory activity was assessed using a protein denaturation method with diclofenac sodium as a standard, using a reaction mixture of BSA, egg albumin, and PBS.

Results The extract showed highest antibacterial activity against *Lactobacillus* sp. (19 mm and 22 mm), exceeding gentamicin (17 mm). Activity against *S. mutans* (17 mm) and *C. albicans* (18 mm) was lower than controls. Antioxidant activity increased from 27% to 69%, indicated by a color change from purple to yellow/clear. Anti-inflammatory activity showed concentration-dependent inhibition of protein denaturation, with higher concentrations demonstrating reduced turbidity.

Conclusion *S. Sisymbriifolium* extract exhibits significant antibacterial, antioxidant, and anti-inflammatory properties, highlighting its potential as a natural therapeutic agent for oral health.

Keywords: Antibacterial activity; Anti-inflammatory activity; Antioxidant activity; Innovation; In vitro; Sustainability; Synthesis; Yellow berried nightshade.

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Introduction

Oral health plays a crucial role in overall well-being, with the mouth serving as a gateway to systemic health. Common oral diseases, including dental caries, periodontal disease and gingivitis are primarily driven by microbial imbalances, oxidative stress and inflammation. It is widely accepted that the microbial community dynamics of *S. mutans*, *Lactobacillus* sp, *C. albicans*, *Prevotella* sp, *Veillonella* sp, are closely associated with the etiology and development of dental caries [1].

S. mutans has been studied in detail and considered as one of the major etiological agents driving the mineral

equilibrium breakdown [2]. It plays a vital role in the adhesion and colonization of various microorganisms within the oral cavity. It does so by attaching itself to the dental surfaces covered with salivary protein membranes [3]. *S. mutans* is a gram-positive, facultative anaerobic, and catalase-negative bacterium that generates lactic acid and can lower the environmental pH from seven to 4.2 within approximately 24 hours [4]. It can degrade carbohydrates and synthesize glucan, which is crucial for interacting with dental structures and eventually causing mineral breakdown [5]. It can ferment and create acids from carbohydrates, including glucose,

lactose, and raffinose. This is the very reason for which it is identified as the primary pathogen in the onset of dental caries. Its ability to produce extracellular matrices strengthens its persistence in biofilms. And so, it is thought that *S. mutans* possess competitive advantages within dental biofilms and have a high cariogenic potential [6].

Another organism that has been implicated in the initiation of dental caries is the *Lactobacillus* sp. They were the first micro-organisms implicated in human dental caries over a century ago. It was the main etiological candidate before *S. mutans* became dominant in the literature in the 1950s [7]. This bacteria in specific has been studied in detail in relation to childhood caries. *Lactobacillus* sp. are frequently identified at active carious sites, especially in lesions with advanced caries in adult and paediatric patients [8]. There is also evidence that under conditions like continuous sugar consumption, weak acids and other antimicrobials such as bacteriocins from *lactobacilli* can become detrimental to the microbial community, especially those in its proximity [7] From previous studies, there is no doubt that *lactobacilli* is one of the most cariogenic bacteria in the oral environment. While it is not the initiator of caries, it plays a crucial role in its progression. Some lactobacilli, such as *L. gasseri*, *L. fermentum*, *L. vaginalis*, and *L. casei*, have been reported to be prevalent at the majority of oral sites, such as saliva, tongue, carious lesions, and dental plaques [9].

Candida albicans is an important opportunistic pathogenic fungus found in the oral cavity, which plays an important role in oral microecological balance, and often interacts with other bacteria including *S. mutans* [10]. The association of *S. mutans* and *C. albicans* with the onset of ECC is well established. Additionally, *S. mutans* strains harbor collagen-binding proteins which actively bind to collagen-rich dentin and are linked to increased caries risk [11]. The interaction of these organisms is often mediated via the binding of Glucosyltransferase enzymes secreted by *S. mutans* to *C. albicans* surface proteins Als1 and Hwp1. During these interactions, both *C. albicans* and *S. mutans* exhibit increased gene regulatory activity, leading to the modulation of virulence attributes and adaptation to environmental changes. This results in the strong attachment of the species to tooth surfaces [12].

Keeping all the above studies in mind, it is understood that there is a need for development of a novel product that can arrest the progression of dental caries or at least incorporate certain properties of plant based oral care products that can prevent the initiation of dental caries. Traditional oral care products often include chemical agents that, while effective, may carry the risk of side effects or long-term health consequences.

Consequently, there has been growing interest in the exploration of natural, plant-based compounds that possess multi-targeted therapeutic properties for oral health [13].

Among such plants, *Solanum sisymbriifolium* (Yellow Berried Nightshade), a member of the Solanaceae family, has gained attention for its potential medicinal benefits. Historically, *S. sisymbriifolium* has been utilized in traditional medicine for its antimicrobial, antioxidant, and anti-inflammatory properties [14]. Solanaceae is an important family that comprises both economic as well as medicinal uses, such as potatoes, tomatoes, eggplants, and peppers, being the most important crops that belong to this family. Among *Solanum* species, *Solanum sisymbriifolium* is proven to have an abundant medicinal value, which could aid in curing different ailments [15]. The plant's antipyretic properties have been exploited for centuries to cure fevers, while its analgesic effects have made it a popular remedy for body aches and rheumatism. Furthermore, poultices made from crushed leaves are topically applied to wounds, cuts, and skin infections, leveraging the plant's antiseptic and wound-healing potential [16].

Despite its widespread use, scientific investigation into its efficacy, particularly in the context of oral health, remains limited. This research aims to explore the potential of Yellow Berried Nightshade as a natural source of bioactive compounds that could serve as effective agents for oral care. Specifically, we examine its antibacterial, antioxidant, and anti-inflammatory properties through in-vitro methods to assess its suitability for combating common oral pathogens, reducing oxidative damage, and mitigating inflammation in oral tissues. This research seeks to contribute to the growing body of evidence supporting the use of plant-based alternatives in the development of safer and sustainable oral care products.

Materials and methods:

The present study was waived for ethical clearance by the Institutional Research and Ethics committee being an in-vitro investigation and did not involve any human participants.

Collection of plant sample:

The leaves of plants were collected from the botanical garden in Chennai, Tamilnadu, India. The identity of the plant material was confirmed by a botanist. Collected leaves were dried at room temperature in a well-ventilated room and ground to a fine powder. One gram of each plant was extracted in 10 mL of ethanol, in a polyester centrifuge tube. The tube was vigorously shaken for 30 min on an orbital shaker, then centrifuged for 10 min and the supernatant was filtered using Whatman No.1 filter paper before being transferred into pre-weighed glass containers. This

was repeated thrice on the same plant material and the solvent was removed by evaporation under a stream of air at room temperature to produce the dried extract [17]. The final prepared extract of *Solanum sisymbriifolium* is depicted in Figure 1.

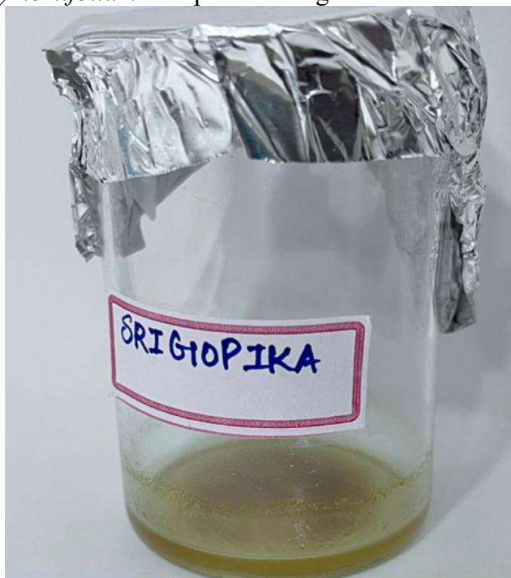


Figure 1- Ethanolic extract of *Solanum sisymbriifolium*

Antibacterial activity:

Fresh bacterial cultures were prepared by inoculating a loopful of each strain into nutrient broth and incubating overnight at 37°C. Two wells were created and were filled with 50 and 100µL of the given samples. The cultures were then adjusted to approximately 10⁷ CFU/mL and uniformly swabbed onto Mueller-Hinton agar (MHA) plates using sterile cotton swabs. Wells of 5 mm diameter were aseptically punched into the agar using a sterile cork borer. Different concentrations of the extract along with a standard antibiotic control were introduced into the wells. The plates were incubated at 37 °C for 24 h. Following incubation, the zones of inhibition around each well were measured to assess antibacterial efficacy with the help of Kirby-Bauer disk diffusion method. The measurement was done using Hiantibiotic Zone Scale [18].

Antioxidant activity

The antioxidant activity of samples was measured in terms of hydrogen donation or radical scavenging capacity using the DPPH (2,2-Diphenyl Picryl-Hydrazyl) method, which involves stable radicals. 0.004g of DPPH was dissolved in 100 mL of ethanol to prepare a 0.004% DPPH solution. The test samples were prepared at different volumes - 100 µL, 200 µL, and 300 µL. For the blank, 2 mL of distilled water was added and for the standard, 1.9 mL of distilled water and 100 µL of ascorbic acid solution was added. Test

samples were added to the corresponding test tubes and 2 mL of the prepared DPPH solution to each test tube. After incubating the mixture in the dark for 30 min at room temperature, the absorbance was measured using a spectrophotometer at 517 nm [19]

Anti-inflammatory activity:

In order to study the anti-inflammatory activity of the plant extract, Diclofenac sodium, an anti-inflammatory drug was used as a reference. The reaction mixture was composed of 0.2 ml of BSA, 0.2 ml of egg albumin, 2.8 ml of phosphate-buffered saline solution (PBS, pH 6.4), and 2 ml of the different samples at varying concentrations (0.2 to 1.2 g/L). Distilled water was used as a negative control. Then the mixtures were incubated at 37 °C for 15 min. The mixture was incubated in a water bath at 70 °C for 45 min. The optical densities were measured at 660 nm using a UV/visible spectrophotometer every 15 min [20].

Minimum Inhibitory Concentration (MIC):

The MIC of *S. sisymbriifolium* extract was evaluated against the individual culture of *S. mutans*, *C. albicans* and *Lactobacillus* in MHB, through micro-dilution method. In brief, *S. sisymbriifolium* (2 mM) was serially diluted with MHB in a 96-well plate. For *S. mutans*, 5 µL of standardized microbial suspensions adjusted to approximately 5 × 10⁵ CFU/mL was used as inoculum. For *C. albicans*, MOPS buffer was used according to the Clinical and Laboratory Standards Institute (CLSI) M27-A4 guideline [21]. The grown *C. albicans* colonies were suspended in 0.85 % saline and adjusted to the turbidity of 0.5 McFarland standard. Then, the suspension was diluted approximately 2.5 × 10³ CFU/mL in RPMI medium, and 5 µL used as inoculum. Then, the plates were incubated at 37 °C for 24 h. The control and blank were maintained without *S. sisymbriifolium* extract and with microbial culture, respectively. Finally, the absorbance of the tests was measured at 595 nm in the microplate reader [22].

Results:

The antibacterial activity of *Solanum sisymbriifolium* extract was evaluated against three oral pathogens: *Lactobacillus sp.*, *Streptococcus mutans*, and *Candida albicans* using the agar well diffusion method. From figure 2, it can be observed that the *S. sisymbriifolium* extract exhibited the highest efficacy against *Lactobacillus sp.*, with zones of inhibition (ZOI) of 19 mm (50µL) and 22 mm (100µL). These results surpassed the inhibitory effect of the standard antibiotic, Gentamicin (17 mm). While active against *S. mutans* (17 mm at 100µL) and *C. albicans* (18 mm at 100µL), the extract was less potent than the respective controls, Amikacin (20 mm) and Fluconazole (30 mm).

From figure 3, it can be observed that maximum inhibition of 95.9% was achieved at the highest

concentration and a sharp decline in efficacy was observed as the extract was diluted, reaching a minimum of 12.3%. Based on the percentage of inhibition, the MIC is effectively represented by the higher concentration ranges where the percentage of inhibition remains above 85%. The control for MIC was set at a value of 0.09, whereas the standard is 0.03. Figure 4 shows the results of antioxidant activity, where the value of control is 0.85 and standard is 0.10 and it is observed that the radical scavenging activity increased significantly with higher concentrations of the extract. Scavenging percentages rose from 27% to 69% and the shift in color from deep purple (control) to varying shades of yellow/clear in the test samples confirms the reduction of DPPH radicals by the extract's antioxidant compounds. Figure 5 depicts the anti-inflammatory potential of *S. sisymbriifolium* extract that was evaluated using the egg albumin denaturation assay. The value for control for anti-inflammatory activity was set at 0.76 and standard at 0.20. The extract demonstrated a clear concentration-dependent inhibitory effect on protein denaturation across a wide range. Higher concentrations showed lower turbidity compared to the lower concentrations, indicating greater protection against albumin denaturation, by effectively stabilizing proteins against thermal denaturation.

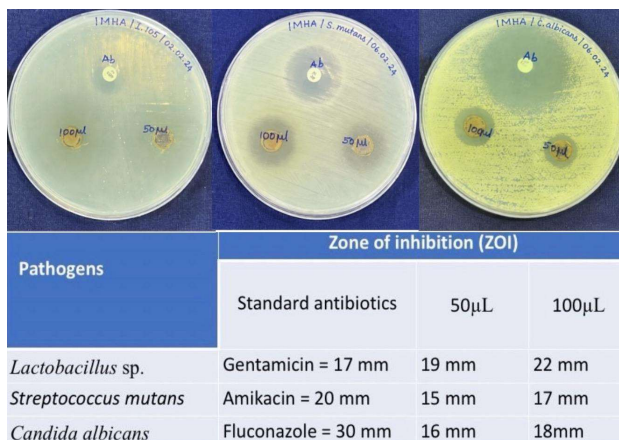


Figure 2 - Antibacterial activity (ZOI) of *Solanum sisymbriifolium* extract and zone of Inhibition of (A) *Lactobacillus* sp., (B) *Candida albicans*, and (C) *Streptococcus mutans* against standard antibiotics.

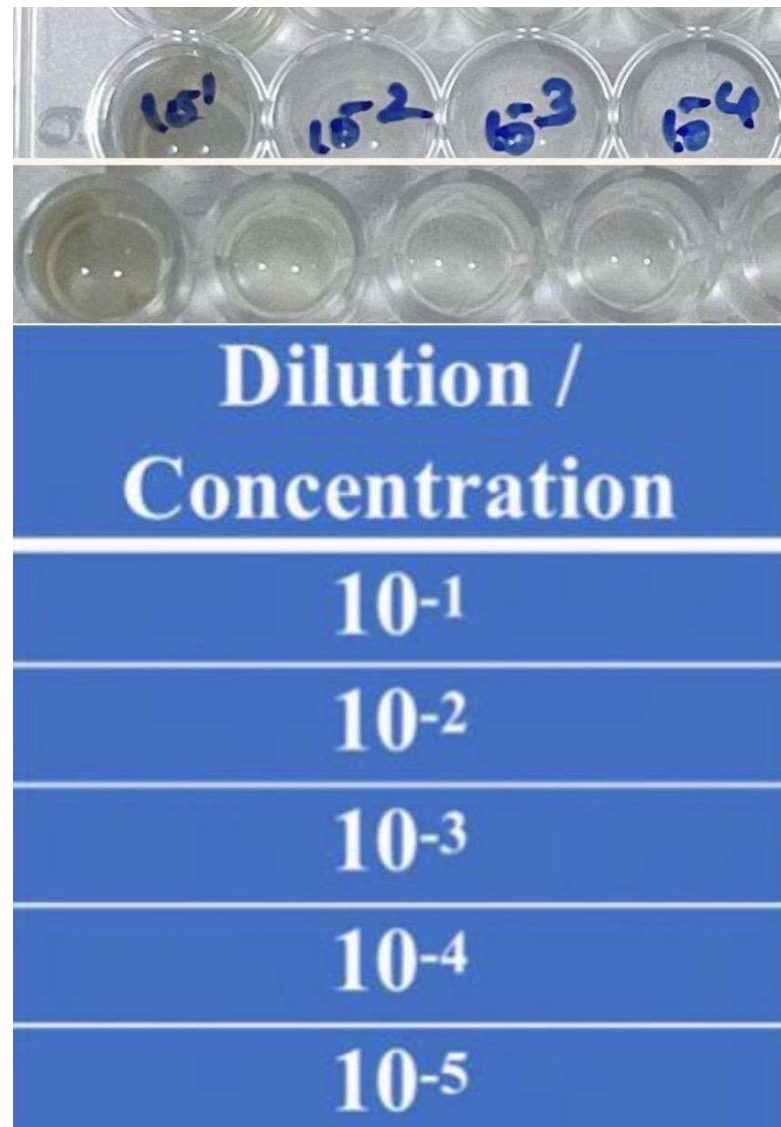


Figure 3 MIC of *S. sisymbriifolium* extract against (A) *Lactobacillus* sp., (B) *C. albicans*, and (C) *S. mutans*



Concentration	Percentage %
100 μ L	27%
200 μ L	48%
300 μ L	69%

Figure 4. Antioxidant activity of *S. sisymbriifolium* extract



Figure 5 - Anti-inflammatory activity of *S. sisymbriifolium* extract

Discussion:

From this study and the results observed above, it can be confirmed that *S. sisymbriifolium* has good antibacterial activity, especially against *Lactobacillus* sp. Apart from this, in high concentrations, the plant extract has proved to have good antioxidant and anti-inflammatory activities.

From other articles that studied different members of the Solanum species, it was observed that they have been known to exhibit various bioactives. Preliminary results suggest that raw fruits of *S. xanthocarpum* exhibit strong anti-inflammatory and anti-oxidation potentiality. Experimental data suggest that either phenolic compounds or alkaloids are likely to be the active pharmaceutical ingredient [23].

Another article concluded that the extract exhibited inhibitory activity against all bacteria. Gram-positive bacteria showed greater susceptibility to extract when compared to Gram-negative bacteria. The extract was effective in inhibiting the mycelia growth of all test fungi with marked activity against *Curvularia* sp. and *Alternaria* sp. [24]. Additionally, this plant is also said to have anti diabetic activity and this was proved in a study and concluded that the extracts of the leaves of *S. xanthocarpum* was efficient anti hyperglycaemic agents at a concentration of 200 mg/kg and possess potent antioxidant activity [25].

In similar research that studied the antioxidant activity of the leaves of *S. guaraniticum*, it was observed that the ethyl acetate fraction showed the better antioxidant activity by the DPPH assay, which can be attributed to its high content of total polyphenols. The presence of high contents of rosmarinic acid in the crude extract and *n*-butanol fraction may support the popular use of the plant to treat liver diseases [26].

In an in vivo study that tried to study the anti-inflammatory activity using a member of the Solanum

sp, it was concluded that the extract of *S.melongena Linn* possesses anti-inflammatory activity in experimental animals. The study also substantiates the traditional use of *S.melongena Linn* for the treatment of various inflammatory ailments. No adverse effect or mortality was detected in Swiss albino mice [27]. In another study that observed anti-inflammatory activity it was noted that the leaves of *Solanum nigrum* possess anti-inflammatory properties and have been used to alleviate inflammation-related conditions. Methanolic extract of the whole plant of *Solanum nigrum* was investigated which demonstrated that plant extract exhibited anti-inflammatory activity [28].

A limitation of the study is that it involves only the fruit of the plant and not the other extracts such as leaves, stem and root, which might have better activities and might also possess anti diabetic and anti-cancer activity. Other drawbacks include the limited species of bacteria and fungi were used in the study and using only an ethanolic extract when other solvents such as methanol or hydroxy alcohol can be used. Additionally, the environment that this study is conducted in, is not similar to the conditions under which the product is intended to be used and this might in turn alter the results. In vivo studies are required to confirm its future therapeutic applications in human

Conclusion

The extract of *Solanum sisymbriifolium* demonstrates significant therapeutic potential as a multi-functional agent with potent antibacterial, antioxidant, and anti-inflammatory properties. It exhibited exceptional efficacy against *Lactobacillus* sp., outperforming the standard antibiotic Gentamicin, while maintaining notable inhibitory effects against *S. mutans* and *C. albicans*. Furthermore, the extract showed a strong concentration-dependent ability to scavenge DPPH radicals and stabilize proteins against thermal denaturation. These findings suggest that *S. sisymbriifolium* could serve as a valuable natural source for developing novel treatments for oral infections, inflammatory conditions, oral potentially malignant disorders as well as dental materials with anti-cariogenic properties. Additional studies are warranted to isolate the specific bioactive compounds responsible for these biological activities.

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Conflict of Interest:

Nil.

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