



Research Article

Potential of Siddha and Ayurvedic herbs in oral healthcare: A comparative study of *Terminalia Chebula* and *Withania Somnifera*

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Abstract

The increasing prevalence of antibiotic resistance and oral infections has spurred interest in plant-based alternatives with antimicrobial and antioxidant properties. Traditional Indian medicinal systems, such as Siddha and Ayurveda, have long utilized *Terminalia chebula*, (*T.chebula*) (*Kadukkai*) and *Withania somnifera* (*W.somnifera*) (*Ashwagandha*) for their therapeutic benefits. Objectives To assess and compare the antimicrobial activity of ethanolic extracts of *Terminalia chebula* (*T. chebula*) and *Withania somnifera* (*W. somnifera*) against *Streptococcus mutans* (*S.mutans*) and *Candida albicans* (*C.albicans*), and to evaluate their antioxidant capacity using the DPPH assay. Methods: Ethanolic extracts of *T. chebula* and *W. somnifera* were prepared and tested for antimicrobial activity using the agar well diffusion method. Minimum inhibitory concentration (MIC) was determined using serial dilution. Antioxidant activity was assessed via DPPH free radical-scavenging assay at various concentrations. Results: *T.chebula* showed superior antimicrobial activity compared to *W. somnifera*, with larger zones of inhibition and lower MIC values against both *S. mutans* and *C. albicans*. Both extracts demonstrated dose-dependent antioxidant activity, with *T. chebula* exhibiting greater radical-scavenging potential. The strong antimicrobial and antioxidant effects of *T. chebula* are attributed to its high content of tannins, gallic acid, and phenolic compounds. Conclusion: *T.chebula* and *W.somnifera* exhibit significant antimicrobial and antioxidant properties, supporting their traditional use in oral healthcare. *T. chebula* in particular demonstrates strong potential as a natural agent in the prevention and management of oral infections. Further research, including phytochemical isolation, cytotoxicity studies, and clinical evaluation, is warranted to develop effective herbal formulations for dental applications.

Keywords: *Terminalia chebula*, *Withania somnifera*, Antimicrobial, Antioxidant, *S. mutans*, *C. albicans*, Oral health, Siddha, Ayurveda.

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Introduction

The resurgence of interest in herbal medicine has brought renewed attention to the therapeutic potential of single-herb formulations. In the Siddha system of traditional medicine, this practice is embodied in the concept of Yegha Mooligai Prayogam—the clinical application of a single herb for specific ailments. Compared to polyherbal remedies, which may contain metals and minerals, single-herb therapies are considered safer, more economical, and more accessible, making them particularly relevant in modern integrative healthcare (1).

Among these, *Terminalia chebula* (*T.chebula*) (commonly known as *Kadukkai*) holds a revered position in both Siddha and Ayurvedic systems of medicine. Often praised as being "superior to the nourishing mother," *T. chebula* is notable for encompassing

five of the six tastes (rasas) described in classical Siddha texts, underscoring its wide therapeutic range. Rich in tannins, flavonoids, and phenolic compounds, it exhibits significant antioxidant, antimicrobial, anti-inflammatory, and rejuvenating properties (2,4).

The global burden of oral diseases including dental caries, periodontitis, and oral fungal infections highlights the need for effective, natural alternatives to conventional treatments. *Streptococcus mutans* (*S.mutans*) is a key bacterial pathogen associated with dental caries, forming biofilms and producing acids that erode tooth enamel. In children, *S. mutans* is the primary cause of early childhood caries, a condition with long-term effects on nutrition, speech, and overall quality of life. Additionally, under certain conditions, *S. mutans* can enter the bloodstream and contribute to infective endocarditis, posing a systemic threat (5,6). Similarly, *Candida albicans* (*C.albicans*), an opportunistic fungal pathogen, is responsible for a range of oral conditions including oral candidiasis, angular cheilitis, denture stomatitis, and in immunocompromised individuals, even esophageal or systemic candidiasis (7).. These infections are typically managed with antibiotics and antifungal agents, which

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are increasingly challenged by the rise of drug resistance and adverse side effects.

Concurrently, the rise in multidrug resistance due to the overuse of antibiotics in humans and animals, alongside the stagnation in the development of new antimicrobials, has driven global interest in plant-based antimicrobial alternatives (9,10). The natural agents like *T. chebula*, with their broad-spectrum antimicrobial and antioxidant properties, present a holistic and safer approach to managing oral infections and maintaining oral health.

Incidentally, India's traditional systems of medicine offer a rich repository of plant-based therapies. One such herb is *Withania somnifera* (*W.somnifera*) (L.) Dunal, commonly known as *Ashwagandha*, Indian Ginseng, or Winter Cherry. A foundational herb in Ayurvedic medicine, it has been used for over 3,000 years to enhance physical and mental resilience. As an adaptogen, *Ashwagandha* supports stress resistance and physiological balance. It has been extensively studied for its anti-inflammatory, neuroprotective, immunomodulatory, antidiabetic, and anticancer effects (3,8)

Ashwagandha's antioxidant activity is of particular reference, which is critical in protecting against oxidative stress a central mechanism in the development of chronic conditions such as cancer, cardiovascular disease, and neurodegenerative disorders. This activity is primarily attributed to its bioactive withanolides, which possess potent free radical-scavenging abilities and enhance the activity of endogenous antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase(4).

The comparative evaluation of antimicrobial and antioxidant properties of *W.somnifera* with *T.chebula* particularly and their roles in oral health against key pathogens like *S. mutans* and *C. albicans* remain unexplored. Hence, the present study aims to evaluate the antioxidant and antimicrobial potential of two prominent medicinal plants, *T.chebula* and *W.somnifera*, with a specific focus on their activity against oral pathogens: *S.mutans* and *C.albicans*. The findings may support the development of effective, plant-based alternatives for the prevention and management of common oral infections.

Materials and Methods

Plant Material Collection and Preparation

Dried fruits of *T.chebula* and roots of *W.somnifera* were collected from a certified Ayurvedic herb supplier. The plant materials were authenticated by a botanist and voucher specimens were deposited in the institutional herbarium for future reference. The collected materials were cleaned, shade-dried, and ground into a fine powder using a mechanical grinder. The powdered samples were stored in airtight containers at room temperature until extraction.

Preparation of Plant Extracts

The powdered samples were subjected to cold maceration using ethanol (70%) as the solvent. For each plant, 50 g of powdered material was soaked in 250 mL of ethanol and left for 72 hours with intermittent shaking. The mixtures were then filtered using Whatman No.1 filter paper. The filtrates were concentrated using a rotary evaporator under reduced pressure and the crude extracts were stored at 4°C for further use.

Microbial Strains

The standard *S.mutans* (ATCC 25175) and *C.albicans* (ATCC 10231) microbial strains were used. Both organisms were

obtained from a certified microbial culture collection center and were maintained on brain heart infusion (BHI) agar and Sabouraud dextrose agar (SDA), respectively.

Antimicrobial Activity Assay

The antimicrobial activity of the plant extracts was evaluated using the agar well diffusion method. Mueller-Hinton agar (for *S. mutans*) and Sabouraud dextrose agar (for *C. albicans*) plates were prepared. A standardized inoculum (0.5 McFarland standard, approx. 1.5×10^8 CFU/mL) of each organism was swabbed evenly onto the respective agar surfaces. Wells of 6 mm diameter were punched into the agar using a sterile cork borer. 100 µL of each plant extract (concentrations: 25 mg/mL, 50 mg/mL, and 100 mg/mL) was introduced into the wells. Plates were incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters using a digital caliper. Positive control used were Chlorhexidine (0.2%) for *S. mutans* and *Nystatin* (100 IU/mL) for *C. albicans* Negative control: 70% ethanol

Minimum Inhibitory Concentration (MIC)

The MIC was determined using the broth microdilution method in 96-well microtiter plates according to CLSI guidelines. Serial two-fold dilutions of the extracts were prepared (ranging from 0.156 mg/mL to 10 mg/mL). Each well was inoculated with microbial suspension and incubated at 37°C for 24 hours. MIC was defined as the lowest concentration showing no visible growth.

Antioxidant Activity Assay

The antioxidant potential of the extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. 1 mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of plant extract at various concentrations (10–100 µg/mL). The mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer.

The percentage of DPPH radical scavenging activity was calculated using the formula:

$$\text{Inhibition\%} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{Control}}} \times 100$$

Where A_{control} is the absorbance of the control (DPPH + methanol), and A_{sample} is the absorbance of the test extract. Ascorbic acid was used as a reference antioxidant standard.

Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean \pm standard deviation (SD). Statistical significance was evaluated using one-way ANOVA followed by Tukey's post hoc test. A p-value of <0.05 was considered statistically significant.

Results

Antimicrobial Activity

Agar Well Diffusion Assay

Both *T.chebula* and *W.somnifera* extracts exhibited concentration-dependent antimicrobial activity against *S. mutans* and *C. albicans*. The zones of inhibition increased with higher concentrations of each extract.

At 100 mg/mL, *T.chebula* showed a maximum zone of inhibition of 18.6 ± 0.3 mm against *S. mutans* and 16.2 ± 0.3 mm against *C. albicans*, respectively. *W.somnifera* at the same concentration

exhibited zones of 14.7 ± 0.4 mm (*S. mutans*) and 13.9 ± 0.4 mm (*C. albicans*). Positive controls (Chlorhexidine and Nystatin) showed zones of 23.5 ± 0.3 mm and 20.4 ± 0.2 mm, respectively. No zone of inhibition was observed in the negative control (70% ethanol).

Table 1: Zone of Inhibition (mm) of Plant Extracts against Oral Pathogens

Plant Extract	Concentration (mg/ml)	S.mutans (mm)	C.albicans (mm)
T.Chebula	25	10.6±0.3	8.9±0.4
	50	14.3± 0.4	11.6±0.3
	75	16.5±0.2	12.9±0.4
	100	18.6±0.3	16.2±0.3
W.Somnifera	25	8.7±0.3	7.2±0.2
	50	10.4±0.2	9.4±0.3
	75	12.9±0.3	11.6±0.3
	100	14.7±0.4	13.9± 0.4
Ethanol (control)		0	0
Chlorhexidine		23.5 ±0.3	-
Nystatin		-	20.4±0.2

Minimum Inhibitory Concentration (MIC)

The MIC values indicated that *T. chebula* exhibited stronger antimicrobial activity compared to *W. somnifera*. MIC of *T. chebula* was found to be 1.25mg/ml against *S.mutans* and 2.5 mg/ml against *C.albicans* whereas, MIC of *W. somnifera* against *S.mutans* and *C. albicans* were 2.5mg/ml and 5.0mg/ml respectively

Antioxidant Activity (DPPH Assay)

Both extracts demonstrated effective DPPH radical scavenging activity in a concentration-dependent manner (Table 2)

Table 2: Antioxidant activity of Plant Extracts Against Oral Pathogens

Concentration (µg/ml)	DPPH scavenging activity(% inhibition)		
	T.chebula	W.somnifera	Ascorbic acid
20	33.5±1.4	22.6±1.3	47.3±1.1
40	46.3±1.5	34.7±1.2	63.2±1.3
60	63.2±1.1	46.3±1.3	77.2±1.2
80	72.3±1.2	59.3±1.2	86.3±1.3
100	85.2±1.2	73.6±1.3	92.9±1.1
IC50 value	58.7±1.3	49.4±1.1	68.4±1.2

At 100 µg/mL concentration *T. chebula* exhibited 85.2% inhibition and *W. somnifera* 73.6% inhibition. Ascorbic acid have shown 92.9% inhibition

Statistical Significance

All results were statistically significant ($p < 0.05$) when compared with negative controls. Differences between the extracts were also significant, with *T. chebula* consistently demonstrating superior activity in both antimicrobial and antioxidant assays.

Discussion

The present study highlights the promising antimicrobial and antioxidant properties of *T.chebula* and *W.somnifera*, two well-known medicinal plants in traditional Indian medical systems such as Siddha and Ayurveda. Both plant extracts demonstrated inhibitory activity against *S.mutans* and *C.albicans*, two key oral pathogens, and showed strong dose-dependent free radical-scavenging activity in the DPPH assay.

Among the two, the ethanolic extract of *T. chebula* showed superior antimicrobial efficacy, reflected by larger zones of inhibition and lower minimum inhibitory concentrations (MICs). This finding aligns with prior studies attributing its antimicrobial effects to a rich composition of tannins, gallic acid, chebulinic acid, and other polyphenolic compounds that can disrupt microbial membranes and inhibit biofilm formation, interfering with bacterial adhesion (11-16). The activity against *S. mutans*, a primary causative agent in dental caries, is particularly relevant. This pathogen contributes to tooth decay through acid production and biofilm formation. Inhibiting its growth suggests potential for *T. chebula* to be developed into herbal oral hygiene products, such as toothpaste, rinses, or gels. Such alternatives may offer advantages over synthetic agents like chlorhexidine, which are known to cause staining and taste disturbances with prolonged use.

Candida albicans, a commensal fungus that can become pathogenic under immunosuppressed conditions, also responded to both plant extracts, with *T. chebula* again demonstrating greater antifungal activity. This suggests utility not only in preventive oral care, but also in managing oral candidiasis, especially among vulnerable populations such as denture wearers and immunocompromised individuals.

In terms of antioxidant activity, both extracts demonstrated concentration-dependent radical scavenging. *T. chebula* again showed greater activity, likely due to its higher polyphenolic and vitamin C content. Oxidative stress is a major contributor to the pathogenesis of periodontal disease and oral mucosal conditions, making these findings relevant for broader applications in oral health maintenance (17-18).

While *W. somnifera* displayed comparatively moderate antimicrobial activity, it showed notable antioxidant potential. This is supported by previous research linking its bioactive withanolides to enhanced expression of endogenous antioxidant enzymes such as SOD, catalase, and GPx. These actions are beneficial in reducing oxidative tissue damage in the oral cavity, especially under chronic inflammatory conditions (19-26).

The antimicrobial effects observed in this study can be attributed to the phytochemicals that include Tannins that will precipitate microbial proteins, inhibit key enzymes, and bind to bacterial cell walls, flavonoids which alters microbial cell permeability and disrupt enzymatic activity. Additionally phenolic acids can cause membrane disruption, leading to cell lysis. These underlying mechanisms with ie the observed efficacy of *T. chebula* and *W. somnifera* against *S. mutans* and *C. albicans*, supports the therapeutic claims found in traditional medical texts.

The increasing antibiotic resistance, initiates a growing need for safe, effective, and natural antimicrobial agents. The specificity of *T. chebula*'s action, particularly against *S. mutans*, suggests its potential in targeted oral healthcare strategies. Its broad-spectrum activity combined with antioxidant benefits could position it as a multifunctional agent in both preventive and therapeutic oral

products. Moreover, the study found that *T. chebula* exhibited lower MIC values against *S. mutans* compared to *C. albicans* indicating pathogen-specific potency. This could be clinically beneficial by reducing the disruption of beneficial oral microbiota, unlike many broad-spectrum antibiotics that may cause dysbiosis.

To further validate and expand upon these findings, future studies should isolate and characterize individual bioactive constituents using techniques such as HPLC, LC-MS/MS, or GC-MS and conduct cytotoxicity and biocompatibility assessments on oral epithelial cells. Further, in vivo studies and clinical trials to establish efficacy, dosage, and safety in real-world oral care scenarios should be performed along with other herbs to explore the synergistic effects for enhanced therapeutic outcomes

Conclusion

The present study demonstrates that both *T.chebula* and *W.somnifera* possess significant antimicrobial and antioxidant properties, validating their traditional use in oral healthcare. Among the two, *T. chebula* exhibited notably stronger activity against *S.mutans* and *C.albicans*, as well as superior free radical-scavenging potential in antioxidant assays. These findings sustains the potential of *T. chebula* as a powerful, plant-based alternative to synthetic antimicrobial agents in the prevention and management of oral infections, including dental caries and candidiasis. Its long-standing use in Ayurveda and Siddha medicine, combined with its safety profile, further enhances its value as a candidate for incorporation into natural oral care formulations such as mouthwashes, toothpastes, or gels.

Moreover, the antioxidant activity observed in both extracts may contribute to reducing oxidative stress in oral tissues, which is vital in the progression of periodontal and mucosal diseases. These herbs may serve as holistic agents in promoting oral and systemic health.

Future studies should focus on the isolation and characterization of active phytoconstituents, evaluation of cytocompatibility with oral tissues, and validation through in vivo and clinical trials to establish safety, efficacy, and optimal formulations for therapeutic use.

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