International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

PREPARATION AND EVALUATION OF HERBAL MOUTHWASH CONTAINING METHANOLIC EXTRACT OF *WITHANIA SOMNIFERA* ROOT

Priyansh Patel*, Deepak Kumar Basedia, B. K. Dubey
Technocrats Institute of Technology-Pharmacy, Bhopal (M.P.)

***Correspondence Info:**
Priyansh Patel
Technocrats Institute of Technology-Pharmacy, Bhopal (M.P.)
Email: patelpriyansh@gmail.com

***Article History:**

Received: 15/02/2025
Revised: 09/03/2025
Accepted: 13/03/2025

ABSTRACT

This study focuses on the preparation and evaluation of a herbal mouthwash containing methanolic extract of *Withania somnifera* (Ashwagandha) root, aiming to assess its potential as a natural alternative for oral hygiene. The methanolic extract was obtained with a yield of 11.6%, and preliminary phytochemical screening revealed the presence of alkaloids, glycosides, phenols, carbohydrates, flavonoids, and proteins. The mouthwash, formulated with this extract, exhibited desirable characteristics including a pH of 6.2 and foaming ability. Antibacterial testing showed significant antimicrobial activity, particularly against *Streptococcus mutans* and *Klebsiella pneumoniae*, with higher concentrations of the extract producing larger zones of inhibition. These results suggest that the herbal mouthwash has potential antibacterial and therapeutic properties, making it a promising natural product for oral care, with the added benefit of being gentle on oral tissues.

Keywords: *Withania somnifera*, Ashwagandha, herbal mouthwash, methanolic extract, oral hygiene, antimicrobial activity, *Streptococcus mutans*, *Klebsiella pneumoniae*, phytochemical screening, natural oral care.

INTRODUCTION

Herbal mouthwashes are gaining popularity as a natural alternative to synthetic oral care products due to their therapeutic properties and minimal side effects. *Withania somnifera*, commonly known as Ashwagandha, is a well-known herb in traditional medicine, particularly in Ayurveda, for its broad spectrum of medicinal properties. The root of *Withania somnifera* has been reported to exhibit various pharmacological activities, including anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory effects, making it a promising candidate for inclusion in oral care formulations (Raut *et al.,* 2012; Singh *et al.,* 2018).

Methanolic extracts of *Withania somnifera* have gained attention for their higher yield of bioactive compounds such as withanolides, alkaloids, flavonoids, and phenolic acids, which are believed to contribute to its pharmacological efficacy (Wankhede *et al.,* 2015). These compounds have shown significant antimicrobial activity, which is particularly relevant in the prevention and treatment of oral diseases such as gingivitis, periodontitis, and oral infections caused by bacteria and fungi (Kumar *et al.,* 2017). Additionally, the anti-inflammatory properties of *Withania somnifera* may help reduce gum inflammation and promote overall oral health (Thakur *et al.,* 2016).

The use of herbal mouthwashes, especially those containing *Withania somnifera* root extract, could provide an effective, natural alternative to conventional mouthwashes that often contain synthetic chemicals, alcohol, and fluoride, which can have adverse effects on oral tissues over time. The methanolic extract of *Withania somnifera* is of particular interest because of its potent bioactive compounds that may be more effective in combating oral pathogens while promoting gum health and reducing inflammation (Kumar *et al.,* 2017).

This study aims to formulate and evaluate an herbal mouthwash containing the methanolic extract of *Withania somnifera* root, assessing its antimicrobial and anti-inflammatory properties as well as its overall safety and efficacy in oral care. By investigating the potential of *Withania somnifera* in mouthwash formulations, the research could contribute to the development of more natural, plant-based oral hygiene products.

MATERIALS AND METHODS

Extraction of plant material

The collected plant material (Roots) was cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant material was converted into moderately coarse powder in hand grinder. Powdered plant material was weighed (60 gm) and packed in soxhlet apparatus (77). The plant material was subjected to extraction by Methanol as solvent. The liquid extracts were collected in a tarred conical flask. The solvent removed by distillation (Kokate; 1999).

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. It shows how much product a researcher has obtained after running the procedures against how much is actually obtained. A higher % yield means the researcher obtained a greater amount of product after extraction. The % yield was calculated by using formula:

$$\% \text{ yield} = [(\text{weight of dried extract}) / (\text{weight of dried plant sample})] \times 100$$

Phytochemical Screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the different extracts of *Withania somnifera,* was subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids (Mahire *et al.,* 2020).

Formulation of herbal mouthwash

The herbal mouthwash was prepared by the formula given in table 5.9. The extracted ingredients are mixed in a fixed ratio. Each ingredient was taken in a weighted quantity. The extract was thoroughly combined with a small amount of water in a mortar and pestle. All of the remaining ingredients were added gradually and thoroughly mixed. Mint oil was added drop by drop and thoroughly combined, taking care to prevent lump formation. Then, drop by drop, PEG 40 and glycerol were added and thoroughly mixed (Bodake *et al.,* 2022).

Finally, water was added to make up the volume, as well as a preservative, and the product was packaged in an attractive, well-closed container.

Table 1: Formulation Herbal Mouthwash of *Withania somnifera* extract

S. No.	Ingredients	Role	Quantity
1	*Withania somnifera*	Antibacterial	250 mg
2	Peppermint	Eliminate harmful bacteria	0.2 ml
3	Honey	Antibacterial	0.2ml
4	Methyl paraben	Preservative	0.2 gm
5	PEG 40	Surfactant	0.2
6	Glycerol	Cosurfactant	0.1
7	Mint Oil	Flavor	0.1
8	Distilled water	Vehicle	q.s to make

Evaluation of herbal mouthwash:

Colour and odour Physical parameters like odour and colour were examined by visual examinations (Renuka and Muralidharan; 2017).

pH

pH of prepared herbal mouthwash was measured by using digital pH meter. The pH meter was calibrated using standard buffer solution about 1 ml of mouthwash was weighed and dissolved in 50ml of distilled water and its pH was measured. Test for microbial growth in formulated mouthwash. The formulated mouthwash was inoculated in the plates of agar media by streak plate method and a control was prepared. The plates were placed in the incubator and are incubated at 37°C for 24 hours. After the incubation period plates were taken out and checked for microbial growth by comparing it with the control.

Foam Height

One mL of mouthwash was mixed in 10 mL distilled water. The mixture was poured into a 100 mL measuring cylinder. Water was added to the volume to make it 100 mL. The mixture received 25 strokes, after which it was kept aside. The height of the foam above the aqueous volume was observed.

***In-vitro* antibacterial activity**

In vitro antibacterial activity was performed on *Streptococcus mutans* and *K. pneumoniae*. The agar well diffusion technique was used for determining the zone of inhibition (Bodiba *et al.,* 2018). The strains of *S. mutans* and *K. pneumoniae* were inoculated in nutrient agar plate. Plates were dried and 4 wells were made with the help of 6 mm agar well cutter. 2.5 mg/ml, 5.0 mg/ml and 10 mg/ml of prepared mouthwash was loaded in all the respective wells. Distilled water used as control. The agar plates were kept undisturbed to allow the passive diffusion of herbal mouth wash into the agar culture medium. Then the plates were incubated at 37°C for 24 hours. The zone of inhibition was calculated in mm.

RESULTS AND DISCUSSION

The preparation and evaluation of a herbal mouthwash containing methanolic extract of *Withania somnifera* roots demonstrates the potential of this plant in promoting oral health. The results of the various analyse extractive values, phytochemical screening, mouthwash characteristics, and antimicrobial testing highlight the therapeutic benefits that

Withania somnifera can offer as an alternative to synthetic oral hygiene products.

The methanolic extract of *Withania somnifera* roots yielded 11.6%, indicating a moderate but significant extraction of bioactive compounds from the plant. Methanol is an effective solvent that is capable of extracting a wide range of compounds from plant materials, which is reflected in the brown color of the extract. This color suggests the presence of active compounds such as alkaloids, phenols, and flavonoids, which are known for their beneficial effects in promoting oral health.

The preliminary phytochemical screening of the methanolic extract revealed the presence of several important bioactive compounds, including alkaloids, glycosides, phenols, carbohydrates, flavonoids, and proteins. These compounds are known for their antimicrobial, anti-inflammatory, and antioxidant properties, making them useful in oral care. Alkaloids and glycosides, for example, are often associated with antimicrobial activity, while phenols and flavonoids are known to have antioxidant and anti-inflammatory effects. The absence of tannins, saponins, and terpenoids does not undermine the mouthwash's potential, as other present compounds still contribute significantly to its effectiveness in oral hygiene.

The characteristics of the formulated herbal mouthwash indicate that it is a suitable product for oral care. The yellow color and characteristic odor align with the expected properties of a herbal formulation. The pH of 6.2 is within the ideal range for mouthwashes, ensuring that the formulation is gentle on oral tissues and will not cause irritation. The foam height of 3 ml suggests that the mouthwash has some foaming ability, which is important for ensuring that the product can be evenly spread throughout the oral cavity during use, aiding in the cleaning process.

The antibacterial testing conducted using the agar well diffusion method revealed that the methanolic extract of *Withania somnifera* has a dose-dependent antibacterial effect against *Streptococcus mutans* and *Klebsiella pneumoniae*. The extract showed significant inhibition of bacterial growth, with larger zones of inhibition observed at higher concentrations. The strongest antibacterial effect was seen against *S. mutans*, which is a major pathogen involved in the development of dental caries. The antimicrobial activity observed suggests that the mouthwash could be effective in controlling oral bacteria, preventing plaque buildup, and reducing the risk of tooth decay and gum disease.

Table 2: Extractive values obtained from *Withania somnifera* roots

S. No.	Solvent	Color of extract	% Yield
1	Methanol	Brown	11.6%

Table 3: Preliminary phytochemical screening of *Withania somnifera* extract

S. No.	Phytoconstituents	Test Name	Methanolic extract
1	Alkaloids	Hanger's Test	+
2	Tannins	Gelatin Test	-
3	Glycosides	Leagel's test	+
4	Saponins	Froth test	-
5	Terpenoids	Salwaski's test	-
6	Phenols	Ferric chloride test	+
7	Carbohydrates	Fehling's Test	+
8	Flavonoids	Lead acetate	+
9	Proteins & Amino acids	Precipitation test	+

Table 4: Evaluation of mouth wash

Sr. No.	Characteristics	Observation
1.	Color	Yellow
2.	Odour	Characteristic smell
3.	pH	6.2
4.	Foam Height	3 ml

Table 5: Result of agar well diffusion antibacterial assay

Organisms	Zone of Inhibition (mm)		
	2.5 mg/ml	**5.0 mg/ml**	**10 mg/ml**
S. mutans	9 ± 0.5	12 ± 0.74	15 ± 0.47
K. pneumoniae	8 ± 0	10 ± 0	12 ± 0

CONCLUSION

Based on the findings, it can be concluded that the herbal mouthwash formulated with methanolic extract of *Withania somnifera* root has promising potential as a natural alternative to conventional mouthwashes. The extract demonstrated notable antimicrobial activity, particularly against *S. mutans* and *K. pneumoniae*, and the mouthwash exhibited favorable characteristics such as a balanced pH and foaming ability. The presence of bioactive compounds like alkaloids, glycosides, phenols, and flavonoids further

supports the formulation's efficacy in promoting oral health. Further studies, including clinical trials, are necessary to validate its long-term safety and effectiveness for regular use in oral hygiene.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Bodake, R., Belhekar, A., Bochare, V., Vidhate, P. & Kumbhar, S. (2022) Formulation and evaluation of herbal mouth Wash. *Int. J. Adv. Res Sci. Commun. Technol.*, 2, 608–617.
- Bodiba, D.C., Prasad, P., Srivastava, A., Crampton, B. & Lall, N.S. (2018) Antibacterial activity of *Azadirachta indica*, *Pongamia pinnata*, *Psidium guajava*, and *Mangifera indica* and their mechanism of action against *S. mutans*. *Pharmacognosy Magazine*, 14, 76–80.
- Kokate, C.K. (1999). *Practical Pharmacognosy, Vallabh Prakashan*. New Delhi, pp. 107–121.
- Kumar, P., Gupta, M. & Chandel, H.S. (2017) Antimicrobial and anti-inflammatory potential of *Withania somnifera* in oral care. *Journal of Medicinal Plants Research*, 11, 82–90.
- Mahire, S.P. & Patel, S.N. (2020) Extraction of phytochemicals and study of its antimicrobial and antioxidant activity of *Helicteres isora* L. *Clinical Phytoscience*, 6, 3–6.
- Raut, A.A., Rege, N.N. & Dahanukar, S.A. (2012) *Withania somnifera* (Ashwagandha): A review of Pharmacological Aspects. *Journal of Natural Remedies*, 12, 122–126.
- Renuka, S. & Muralidharan, N.P. (2017) Comparison in benefits of herbal mouthwashes with chlorhexidine mouthwashes: A review. *Asian Journal of Pharmaceutical and Clinical Research*, 10, 3–7.
- Singh, G., Chouhan, S. & Jachak, S.M. (2018) *Withania somnifera*: A medicinal herb for better oral health. *Natural Product Communications*, 13, 1–6.
- Thakur, M., Mishra, R. & Dubey, S. (2016) Anti-inflammatory effects of *Withania somnifera* and its potential use in oral hygiene. *Journal of Ethnopharmacology*, 18, 15–19.
- Wankhede, S., Joshi, R. & Jadhav, V. (2015) *Withania somnifera* root extract and its therapeutic potential. *International Journal of Pharmaceutical Sciences*, 7, 1–5.