

## Original Research Article

# Efficacy of *Andrographis paniculata* compared to *Azadirachta indica*, *Curcuma longa*, and sodium hypochlorite when used as root canal irrigants against *Candida albicans* and *Staphylococcus aureus*: An *in vitro* antimicrobial study

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## Abstract

**Aim:** The aim is to test the antimicrobial activity of *Andrographis Paniculata*, *Azadirachta indica* (neem), and *Curcuma Longa* (curcumin) as a root canal irrigant, against *Staphylococcus aureus* and *Candida albicans* using agar diffusion test. Sodium hypochlorite (NaOCl) served as a standard control for comparisons.

**Materials and Methods:** The bacterial strains of *C. albicans* and *S. aureus* culture were grown overnight (18–20 h) in the brain heart infusion broth at 37°C and inoculated in Mueller–Hinton agar plates. Antibacterial inhibition was assessed using agar well-diffusion method using the methanolic extracts of the three plants to be tested and NaOCl. Bacterial inhibition zone around each well was recorded. The results were tabulated and analyzed statistically for significance.

**Results:** The novel *A. paniculata* showed significantly higher zone of inhibition against *C. albicans* ( $P < 0.0001$ ) compared to the experimental irrigants. Against *S. aureus*, it exhibited similar results as that shown by NaOCl ( $P > 0.05$ ).

**Conclusion:** Zones of inhibition exhibited by novel herbal agent *A. paniculata* were higher against *C. albicans* and similar against *S. aureus*, when compared to NaOCl.

**Keywords:** *Andrographis paniculata*; antimicrobial efficacy; *azadirachta indica*; *Candida albicans*; *Curcuma longa*; *Staphylococcus aureus*

## INTRODUCTION

The prime objective of endodontic therapy is thorough debridement and cleaning of the entire root canal system (RCS), resulting in removal of infected and necrotic pulp tissue, to receive an inert filling, thus minimizing the possibility of reinfection.<sup>[1]</sup> Microorganisms colonizing in the necrotic pulp tissue cause primary endodontic

infection. They are polymicrobial in nature, dominated by obligate anaerobic bacteria. The success of endodontic treatment depends on complete eradication of these microorganisms. This is not always achieved completely because of anatomical complexity and the limitation in accessing the canal system by instruments and irrigants.<sup>[2,3]</sup>

Anatomically complex RCSs containing lateral wall ramifications, cul de sac, and other canal irregularities, result in incomplete eradication of microorganisms

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following mechanical instrumentation. The only way of impacting such areas is with the help of irrigation.<sup>[4,5]</sup> Irrigation not only has a germicidal role but it also keeps the canal lubricated during instrumentation and removes smear layer, tissue remnants, and dentin chips. The effectiveness of irrigating solutions depends on multiple factors such as microbial susceptibility, concentration of the solution, penetration in infected areas, and its toxic effects on host cells.<sup>[4]</sup>

Facultative microorganisms such as *Enterococcus faecalis*, *Candida albicans*, and *Staphylococcus aureus* have been considered to be the most resistant species in the oral cavity and possible cause of failure of root canal treatment.<sup>[6]</sup> *E. faecalis* is associated with persistent periradicular lesions after root canal treatment. *C. albicans* and *S. aureus* are part of microbiota with failed endodontic therapy and may be considered a dentinophilic microorganism.<sup>[7]</sup> *C. albicans* seen in 18% of the retreatment infections,<sup>[8]</sup> whereas *S. aureus* is seen in 0.7%–15% of the cases.<sup>[9]</sup>

Sodium hypochlorite (NaOCl) has remained as gold standard for root canal irrigation because of its antimicrobial potential and its ability to dissolve organic matter. Nevertheless, it is not only irritant to the periapical tissues but also possesses disadvantages such of instruments, burning of surrounding tissues, unpleasant taste, high toxicity, corrosive to instruments, inability to remove the smear layer, reduction in elastic modulus, and flexural strength of dentin.<sup>[3]</sup>

*Andrographis paniculata*, an annual herbaceous plant, popularly known as king of bitters in English, belongs to the *Acanthaceae* family. It is also infamously called as Bhui-neem, which means “neem of the ground” as it has a similar strong bitter taste as that of the large neem plant. It is commonly cultivated in southern Asia, in China, and in some parts of southeast Asia.<sup>[10]</sup> Being a potent stimulator of the immune system, it is effective against a wide array of infections and oncogenic agents. It is effective against several viruses and bacteria and is also a powerful antimalarial and antidiarrheal.<sup>[11]</sup>

The present study is aimed to explore novel herbal irrigant (*A. paniculata*), as potential antimicrobial agents in the inhibition of *C. albicans* and *S. aureus* in comparison to *Azadirachta indica* (neem), *Curcuma Longa* (curcumin), and NaOCl.

## MATERIALS AND METHODS

### Preparation of test herbal irrigation solutions

The plants of *A. paniculata* and *A. indica* were washed with distilled water and dried for 10–12 days. They were then ground to fine powder in the grinder for further extract procurement. Alcoholic extract of both was prepared. The

powder was added to 50 ml of absolute ethanol (Sterling Chemicals and Alcohols Pvt. Ltd., Mumbai). The mixture was macerated for 1–2 min to form an extract. Then, this extract was filtered through muslin cloth for coarse residue and then through Whatman filter paper no. 41 for finer residue. The extract for curcumin was prepared by the elemental hydrodistillation method. Hundred gram dried turmeric and 500 ml of distilled water were mixed in a 1000 mL round-bottomed flask. Distillation was conducted continuously for 60 h. After turning on the valve to remove the distilled water, the essential oil was collected as stock solutions and kept in a dark bottle and stored at 4°C until use.

### Test for antimicrobial assay

#### *Staphylococcus aureus*

A loopful of culture was inoculated in nutrient broth and was kept at 37°C at shaker condition 150 rpm, for 18–24 h. Optical density was checked at 620 nm after 18 h and was adjusted to 0.2. Following which, Mueller–Hinton Agar butts were prepared and cooled slightly. 1 ml of culture was subsequently added to the agar butt and was poured into the petri dish. This was then allowed to solidify. The procedure was repeated two more times. Separate MH agar plates were used to compare the antimicrobial efficacy of the herbal irrigants (*A. paniculata*, neem, and curcumin) against *S. aureus*. Using cork borer, four wells were made on each plate, and 50  $\mu$ L of sample was added to these wells. Petri dishes were then refrigerated at 4°C for 20 min. Following which, they were incubated at 37°C for 24 h. Zone of inhibition was observed and measured in millimeters.

#### *Candida albicans*

The protocol was the same, with the only difference being in the culture media used. Sabouraud Dextrose Agar was used instead of Muller–Hinton Agar to grow these organisms.

## RESULTS

The mean zones of inhibition by the irrigating agents used are summarized in Tables 1 (*C. albicans*) and 2 (*S. aureus*). The observations exhibited a significant difference between the groups tested on application of one-way ANOVA ( $P < 0.01$ ) for both microorganisms. *A. paniculata* exhibited larger zones of inhibition against *C. albicans* compared to the other groups tested ( $P < 0.01$ ), whereas similar to NaOCl when used against *S. aureus* ( $P > 0.05$ ). The mean wise distribution of the mean zones of inhibition is plotted in Graph 1.

## DISCUSSION

The healing potential of herbal plants is an ancient belief; however, it has gained interest and importance in the

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**Table 1: Mean, standard deviation, and statistical analysis of antibacterial efficacy against *Candida albicans* by different irrigants**

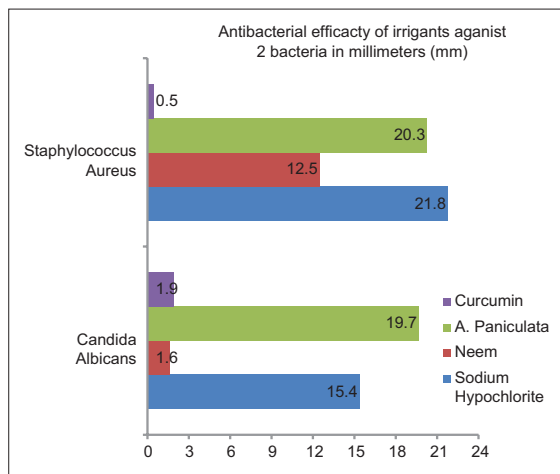
Antibacterial efficacy against <i>Candida albicans</i> by different irrigants					
Groups	Irrigants	Mean zone of inhibition in mm with SD	P (one-way ANOVA)	Post hoc test for multiple comparisons	
1	Sodium hypochlorite	15.4±1.4	<0.0001	>2, 4; <3	
2	Neem	1.6±1.1		<1, 3; ≅4	
3	<i>Andrographis Paniculata</i>	19.7±1.2		>1, 2, 4	
4	Curcumin	1.9±0.8		<1, 3; ≅2	

SD: Standard deviation

**Table 2: Mean, standard deviation, and statistical analysis of antibacterial efficacy against *Staphylococcus aureus* by different irrigants**

Antibacterial efficacy against <i>Staphylococcus aureus</i> by different irrigants					
Groups	Irrigants	Mean zone of inhibition in mm with SD	P (one-way ANOVA)	Post hoc test for multiple comparisons	
1	Sodium hypochlorite	21.8±2.0	<0.0001	>2, 3, 4	
2	Neem	12.5±1.8		<1, 3; >4	
3	<i>Andrographis Paniculata</i>	20.3±1.7		≅1; >2, 4	
4	Curcumin	0±0		<1, 2, 3	

SD: Standard deviation



**Graph 1: Mean wise distribution of zone of inhibition against *Candida albicans* and *Staphylococcus aureus* by different irrigants in millimeters (mm)**

recent times. It can be attributed to their easy availability, cost-effectiveness, increased shelf life, low toxicity, and lack of reported microbial resistance. The present study compared the antibacterial activity of novel herbal agent *A. paniculata* against *C. albicans* and *S. aureus*, which are commonly encountered in recalcitrant endodontic infections.<sup>[12]</sup>

The current study appears to be the first report on the endodontic applications of *A. paniculata*. This herbal agent exhibited antibacterial, antifungal, and antiviral effectiveness in previous reports.<sup>[13-15]</sup> It has proved antibacterial activity against Gram-positive (+) and Gram-negative (-) bacteria including avirulent *Mycobacterium smegmatis* and *M. tuberculi*. A previous study on crude methanol and chloroform extract of *A. paniculata* has shown significant antimicrobial activity only against *Bacillus subtilis* and *C. albicans*. It also showed specific

antibacterial activity against only *S. aureus*, *Escherichia coli*, *B. subtilis*, and *Pseudomonas aeruginosa*.<sup>[16]</sup> The minimum inhibitory concentration (MIC) against *C. albicans* 100 µg/ml and *S. aureus* was 250 µg/ml, which are in accordance with previously reported studies.<sup>[14,16]</sup> It also has anti-inflammatory, immunostimulant and antipyretic effects,<sup>[17]</sup> which helps in the treatment of acute upper respiratory tract infections. It is known to have anticancer and immunomodulatory effects on human cells. It also shows significant antimicrobial and antioxidant properties, which was the basis of considering this extract as a prospective endodontic irrigant in our study.<sup>[10,17]</sup> In the present study, *A. paniculata* exhibited higher antimicrobial activity against the most common endodontic pathogens associated with reinfected RCSs, which are *C. albicans* (19.7 ± 1.2) and *S. aureus* (20.3 ± 1.7).

Different parts of the neem tree have been used for its medicinal properties, in Ayurvedic science.<sup>[18]</sup> Nimbidin a product of the seed kernel of *A. indica* demonstrates anti-inflammatory, antibacterial, antifungal, and anti-pyretic properties. Furthermore, according to Botelho,<sup>[19]</sup> neem exhibited substantial efficacy against periodontal pathogens and is biocompatible with PDL fibroblasts. Hence, its use as a biocompatible irrigant might be beneficial in endodontic therapy. Mistry et al.<sup>[20]</sup> concluded in their study that neem extract showed significant activity against *S. aureus* with a MIC of 125 µg. This was in synchrony with our results, which showed the considerable antimicrobial activity of neem (*A. indica*) extract against *S. aureus*. Whereas, Bohora et al. and<sup>[21]</sup> Tyagi et al.<sup>[3]</sup> reported neem to be an effective root canal medicament against *E. faecalis* and *C. albicans*. However, the results of the present study were not in accordance with them and neem exhibited no activity against *C. albicans*.

Curcumin has been proved to be a highly pleiotropic molecule, which can interact with various molecular

targets associated with inflammation. Various clinical trials show that curcumin can be used in a wide range of concentration, without causing toxicity.<sup>[22]</sup> Accounting to its antibacterial and anti-inflammatory properties, it can have several possible applications in endodontics, one of them being a potentially effective endodontic irrigant. Aqueous extract of *C. longa* showed good inhibitory activity against *C. albicans*.<sup>[23,24]</sup> Both of these are not in accordance with our results, which showed no antimicrobial activity against both *S. aureus* and *C. albicans*. The discrepancy in results can probably be due to the difference in concentration of extracts used in the study and the method of procuring the extracts.

## CONCLUSION

*A. paniculata* is known for its anticancer and immunomodulatory action and has conventionally been used as a medicine for treatment of various diseases. The high antimicrobial activity against *S. aureus* and *C. albicans* shown by *A. paniculata* compels us to think of its probable applications in the field of dentistry as a propitious endodontic irrigant. Its medicinal properties can be harnessed to prevent and treat secondary endodontic infections. However, these results are based on an *in vitro* aerobic culture technique which may not immediately reflect clinical efficacy *in vivo*. The results have to be corroborated on a biofilm model to better reflect clinical efficacy.

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## Conflicts of interest

There are no conflicts of interest.

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