

## Antimicrobial Efficacy of Herbal Extracts

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### ABSTRACT:

**Background and objectives:** The aim of the study was to evaluate and compare extracts of ginger, garlic, aloe vera, neem, turmeric and sodium hypochlorite as root canal irrigants.

**Methods:** Freshly prepared extracts of ginger, garlic, aloe vera, turmeric, neem and 2% sodium hypochlorite were used and compared against *Enterococcus faecalis* using the agar diffusion test. Agar plates were incubated at 37° C for 24 hours in an incubator. The diameter of bacterial inhibition zones around each well was recorded to the nearest size in mm.

**Results and interpretation:** Higher mean zone of inhibition was recorded in Sodium Hypochlorite followed by Garlic Extract, Neem Extract, Ginger Extract and Turmeric Extract respectively. The lowest mean zone of inhibition was found in Aloe Vera Extract.

**Conclusion:** There was no significant difference between the antimicrobial property of garlic extract and 2% sodium hypochlorite.

**Keywords:** Antimicrobial, Herbal extracts, Irrigants

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### INTRODUCTION

*Enterococcus faecalis* is the most commonly isolated species from the canals of teeth presenting post-treatment diseases. *Enterococcus faecalis* accounts for up to 77% of therapeutic failures<sup>1</sup>.

Among the procedures involved in the control of endodontic infection, irrigation is an important step in eliminating microorganisms from the root canal system. Sodium hypochlorite is the most widely used root canal irrigating solution. The advantages of sodium hypochlorite are its ability to dissolve necrotic tissues and its antibacterial properties against most microorganisms. However, it has several undesirable characteristics such as tissue toxicity, risk of emphysema on overfilling, allergic potential, and disagreeable smell and taste<sup>2</sup>.

As the irrigants currently used in the field of endodontics have their share of limitations, the search for an ideal root canal irrigant continues<sup>3</sup>. The use of herbal extracts as endodontic irrigants is today gaining popularity. In ginger rhizome there are several components which have antibacterial and antifungal effect. The gingerol and shagelol were identified as more active agents<sup>4</sup>. The main active component of garlic is allicin. It is antibacterial and has immune regulatory functions. Allicin destroys cell wall and cell membrane of root canal bacteria<sup>5</sup>. Aloe vera contains aloins and barbadoins as main chemical constituents. Aloe Vera has gel has inhibitory effects on *S.pyogens* and *E.faecalis* because of the presence of anthraquinone as antimicrobial<sup>6</sup>. Neem contains Nimbidin which demonstrated antiinflammatory, antiarthritic,

antipyretic, hypoglycaemic, antigastric ulcer, spermicidal, antifungal, antibacterial and diuretic properties<sup>7</sup>. Curcumin [diferuloylmethane], the main yellow bioactive component of turmeric has shown to have a wide spectrum of biological actions, including antimicrobial, anti-inflammatory and antioxidant activities<sup>8</sup>.

The aim of the study was to evaluate and compare extracts of ginger, garlic, aloe vera, neem and turmeric with sodium hypochlorite as a root canal irrigant.

### MATERIALS AND METHODS

Freshly prepared extracts of ginger, garlic, aloe vera, turmeric, neem and 2% sodium hypochlorite were used and compared against *Enterococcus faecalis*.

**Preparation of ginger extract:** Outer covering of ginger was peeled off. 20 g of ginger was kept in closed containers after being chopped into small pieces. 50 ml of diethyl ether was added to it and the mixture was left for 6 hours. The mixture was periodically agitated during this period every 15 minutes. It was then filtered and the ether was vaporized in an evaporator (60° C)<sup>9</sup>. The extract obtained at the end of this process was used in a non-diluted form for analysis on the same day.

**Preparation of garlic extract:** Fresh peeled garlic (80gm) was chopped and homogenized in 100ml sterile distilled water, centrifuged, filtered through

Whatman No 1 filter paper and kept at  $-70^{\circ}\text{C}$  until use<sup>10</sup>.

**Preparation of aloe vera extract:** The pulp was removed from 100 gm of aloe vera leaves and converted into liquid form in a mixer. The liquid was mixed well with distilled water in 1:5 ratio and the mixed solution was placed in a crucible on a water bath for dehydration. Precipitate of extract was dissolved in methanol and used<sup>11</sup>.

**Preparation of neem extract:** 100 gms of neem leaves were obtained and tied in muslin cloth which was soaked in 800 ml of distilled water in a beaker. The beaker was boiled over low flame till the extract reduced to 400 ml to obtain a 25% concentration of aqueous neem extract. After the extract cooled down, it was filtered in Whatman filter paper and the extract was stored in amber bottle for further usage<sup>12</sup>.

**Preparation of turmeric extract:** The extract was prepared by elemental hydro-distillation method. 100g dried turmeric and 500 ml distilled water were mixed in a 1,000 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 into a dark bottle and stored at  $4^{\circ}\text{C}$  until use<sup>13</sup>.

**Agar-diffusion test:** The bacterial stock culture *Enterococcus faecalis* was obtained and culture was grown overnight in brain heart infusion (BHI) broth and inoculated in Mueller- Hinton agar plates. Inoculation was performed by using sterile swab brushed across the media. One round well, 4 mm deep and 8 mm diameter was punched in each agar plate using sterile cork-borer and the prepared irrigants were added to the wells. Agar plates were incubated at  $37^{\circ}\text{C}$  for 24 hours in an incubator. The diameter of bacterial inhibition zones around each well was recorded to the nearest size in mm. The results were tabulated and statistically analyzed using analysis of variance (ANOVA) (Table 1).

## RESULTS

Higher mean zone of inhibition was recorded in Sodium Hypochlorite followed by Garlic extract, Neem extract, Ginger extract and Turmeric extract respectively. The lowest mean zone of inhibition was found in Aloe Vera extract.

There was no statistically significant difference between sodium hypochlorite and garlic extracts. In order to find out among which pair of groups there existed a significant difference, multiple comparisons were carried out using Tukey's test (Table 2).



**Fig 1: Aloe vera extract- least zone of inhibition**



**Fig 2: Garlic extract- highest zone of inhibition**

**Table 1: Mean zone of inhibition of different herbal extracts**

| <b>Irrigant</b>     | <b>Mean zone of inhibition</b> |
|---------------------|--------------------------------|
| Sodium Hypochlorite | 23.00                          |
| Turmeric Extract    | 8.00                           |
| Ginger Extract      | 11.00                          |
| Garlic Extract      | 22.00                          |
| Neem Extract        | 16.00                          |
| Aloe Vera Extract   | 6.40                           |

Table 2

| (I) Irrigant        | (J) Irrigant      | Mean Difference (I-J) | Std. Error | 95% CI for mean difference |             | P-Value |
|---------------------|-------------------|-----------------------|------------|----------------------------|-------------|---------|
|                     |                   |                       |            | Lower Bound                | Upper Bound |         |
| Sodium Hypochlorite | Turmeric Extract  | 15.000                | 0.787      | 12.565                     | 17.435      | <0.001* |
|                     | Ginger Extract    | 12.000                | 0.787      | 9.565                      | 14.435      | <0.001* |
|                     | Garlic Extract    | 1.000                 | 0.787      | -1.435                     | 3.435       | 0.798   |
|                     | Neem Extract      | 7.000                 | 0.787      | 4.565                      | 9.435       | <0.001* |
|                     | Aloe Vera Extract | 16.600                | 0.787      | 14.165                     | 19.035      | <0.001* |
| Turmeric Extract    | Ginger Extract    | -3.000                | 0.787      | -5.435                     | -0.565      | 0.010*  |
|                     | Garlic Extract    | -14.000               | 0.787      | -16.435                    | -11.565     | <0.001* |
|                     | Neem Extract      | -8.000                | 0.787      | -10.435                    | -5.565      | <0.001* |
|                     | Aloe Vera Extract | 1.600                 | 0.787      | -0.835                     | 4.035       | 0.355   |
| Ginger Extract      | Garlic Extract    | -11.000               | 0.787      | -13.435                    | -8.565      | <0.001* |
|                     | Neem Extract      | -5.000                | 0.787      | -7.435                     | -2.565      | <0.001* |
|                     | Aloe Vera Extract | 4.600                 | 0.787      | 2.165                      | 7.035       | <0.001* |
| Garlic Extract      | Neem Extract      | 6.000                 | 0.787      | 3.565                      | 8.435       | <0.001* |
|                     | Aloe Vera Extract | 15.600                | 0.787      | 13.165                     | 18.035      | <0.001* |
| Neem Extract        | Aloe Vera Extract | 9.600                 | 0.787      | 7.165                      | 12.035      | <0.001* |

\*denotes significant difference

The difference in mean zone of inhibition was found to be statistically significant between Sodium Hypochlorite & Turmeric Extract (P<0.001), Sodium Hypochlorite & Ginger Extract (P<0.001), Sodium Hypochlorite & Neem Extract (P<0.001), Sodium Hypochlorite & Aloe Vera Extract (P<0.001), Turmeric Extract & Ginger Extract (P<0.05), Turmeric Extract & Garlic Extract (P<0.001), Turmeric Extract & Neem Extract (P<0.001), Ginger Extract & Garlic Extract (P<0.001), Ginger Extract & Neem Extract (P<0.001), Ginger Extract & Aloe Vera Extract (P<0.001), Garlic Extract & Neem Extract (P<0.001), Garlic Extract & Aloe Vera Extract (P<0.001) as well as between Neem Extract & Aloe Vera Extract (P<0.001).

## DISCUSSION

Healing potential of plants is an ancient idea, but in recent times it has gained renewed interest and importance. The major advantages of using herbal alternatives are easy availability, cost-effectiveness, increased shelf life, low toxicity, and lack of reported microbial resistance.<sup>14</sup>

The present study showed that garlic extract had good inhibitory efficacy against *Enterococcus faecalis*. The antimicrobial action of garlic is primarily attributed to allicin whose mechanism of action partially inhibits DNA and protein synthesis, and entirely inhibits RNA synthesis. DNA transcription and other DNA activities are also influenced by allicin<sup>15</sup>.

In a study antibacterial activities of two natural plants (freshly minced garlic extract and fresh lemon solution) were compared with that of three traditional intracanal medications (10% citric acid, 5.25%naocl, and CPCP) on mixed root canal flora on blood agar plates as well as inside prepared sterile root canals. The results showed that both freshly minced garlic and CPCP represented the statistically significant largest inhibitory zones when compared with other three medications used<sup>16</sup>.

Various parts of the neem tree have been used as traditional ayurvedic medicine in India from time immemorial. Its use for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer has also been evident. Nimbidin, a major crude

bitter principle extracted from the oil of seed kernels of *A. indica* demonstrated antiinflammatory, antiarthritic, antipyretic, hypoglycaemic, antigastric ulcer, spermicidal, antifungal, antibacterial and diuretic properties<sup>7</sup>. Neem also has an anti-adherence activity by altering bacterial adhesion and ability of organism to colonize<sup>17</sup>.

Use of neem as an endodontic irrigant might be advantageous because it is a biocompatible antioxidant and thus not likely to cause any adverse reactions. Bitter taste associated with this plant can be altered by modifying the formulations with the addition of sweeteners and flavors thereby increasing the patient compliance and acceptability. A study using neem leaf extract has shown that it is a viable medicament against *C. albicans*, *E. faecalis* and even mixed culture when compared to sodium hypochlorite<sup>18</sup>.

In the present study neem extract showed significant zone of inhibition compared to the other extracts, but the zone of inhibition was less compared to garlic extract.

Aloe vera has various therapeutic uses in arthritis, asthma, digestive and bowel disorders, skin problems (eg: eczema, psoriasis, acne, burns), ulcers etc in general. In dentistry aloe vera is used in cases of aphthous ulcers, lichen planus, alveolar osteitis<sup>6</sup>. Aloe vera leaves contain aloins and barbadoin as main chemical constituents.

In a study aloe vera extract has shown significant antibacterial activity against *E. faecalis* and *C. albicans*<sup>11</sup>. But in the present study aloe vera extract showed the least zone of inhibition against *E. faecalis*.

A study has shown that ginger extract had good inhibitory efficacy against *Enterococcus faecalis*<sup>19</sup>. Another study has reported significant antifungal activity of ginger extract on oral species of *Candida albicans*<sup>20</sup>. Studies have shown that the extract of ginger can inhibit the growth of oral bacteria<sup>21</sup>. However in the present study ginger extract showed lesser zone of inhibition when compared to the previous studies.

Traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis<sup>8</sup>.

Curcumin [diferuloylmethane], the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions, including antimicrobial, anti-inflammatory and antioxidant activities<sup>8</sup>.

The lower antimicrobial efficacy of turmeric, aloe vera, ginger, neem in the present study as compared to the earlier studies could be attributed to the constituents of the herbs depending on the area where these were grown, the method of extract preparation or the use of these preparations. However, several disadvantages of herbal irrigants

like fresh solutions have to be prepared each time, the unacceptable odour and taste, short shelf life have to be overcome. The smell and taste of the herbal irrigants has to be modified by adding flavouring agents to make it palatable and acceptable by the patient. More research for prolonging the shelf life of these irrigants has to be done so that these irrigants are more widely accepted. There is not much evidence about the overdose reactions of these herbal irrigants. More research about the use of particular concentrations of these irrigants to achieve maximum therapeutic effects have to be done.

## CONCLUSION

Garlic extract showed to be a potent antimicrobial against *E. faecalis* when compared to the other herbal extracts used in this study. There existed no statistical difference between antimicrobial activity of sodium hypochlorite and garlic extract. However, further research has to be done in the field of herbal extracts as endodontic irrigants to identify the concentration to be used, the method of extract preparation and differences between the efficacy of the herbal extracts and traditional root canal irrigants.

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