An *in vitro* evaluation of antimicrobial activity of aqueous *Curcuma longa* extract against endodontic pathogens

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**ABSTRACT**

The most commonly isolated bacteria from endodontically infected teeth are anaerobic, especially black-pigmented gram-negative organisms. However, facultative microorganisms such as *Enterococcus faecalis*, aerobes like *Staphylococcus aureus*, and yeasts like *Candida albicans* are considered by many to be the most resistant species, and possible causes of root canal treatment failure. Irrigating solutions and intracanal medicaments are required to eradicate microorganisms, and over a period, a variety of chemicals have been introduced. The alarming incidence of antibiotic resistance amongst the microbes, has led to the search of alternative antimicrobial drugs from medicinal plants to treat these infections. *Curcuma longa* (turmeric) belonging to the Zingiberaceae family, has been used for thousands of years as a flavouring agent, a medicinal herb, and a dyeing agent. Owing to its antimicrobial properties, it can prove to be useful in *Endodontics* as well. Therefore, in the present study, aqueous extract of the roots of *Curcuma longa* was prepared and solutions of different concentrations of the extract were made. Antimicrobial susceptibility tests were performed using the agar well diffusion method. The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) were calculated. The time-kill profile was also recorded. 2.3% percentage sodium hypochlorite solution was used as the positive control. The extract showed good antimicrobial properties against the endodontic pathogens. Hence, its future use as an endodontic irrigant or medicament should be considered and further evaluated.

**Keywords:** Aqueous extract; antimicrobial activity; *Curcuma longa*; endodontic pathogens

**INTRODUCTION**

One of the primary objectives of endodontic therapy is the microbial reduction or their elimination, to promote the normal healing and reestablishment of the health of the periapical tissues. Mechanical instrumentation cannot sufficiently disinfect root canals and hence irrigating solutions and intracanal medicaments are required to eradicate microorganisms. Over a period, a variety of chemicals has been introduced (Stephen Cohen, 9th Ed). An endodontic irrigant/medicament should ideally exhibit powerful antimicrobial activity, disinfect the root canal space, and have no cytotoxic effects on periradicular tissues.

Sodium hypochlorite, the commonly used endodontic irrigant, has many properties, but has a cytotoxic effect when injected into the periapical tissues. It is also known to produce allergic reactions; a foul smell and taste, tendency to bleach clothes and corrosive potential (Radcliffe, 2004). Studies have also shown the resistance of *Enterococcus faecalis* to it. Therefore, an equally effective and a safe irrigant/ intracanal medicament are desirable.

Currently, most bacteria isolated from endodontically infected teeth is anaerobic, especially black-pigmented gram-negative organisms. However, facultative microorganisms such as *Enterococcus faecalis*, aerobes like *Staphylococcus aureus*, and even *Candida albicans* are considered the most resistant species, and one of the possible causes of root canal treatment failure (Morgana Eli Vianna, 2004).

Antimicrobial agents are many a time associated with adverse effects on the host like, hypersensitivity, immune suppression, and allergic reactions. Also, the continuous evolution of bacterial resistance to currently available antimicrobial agents has necessitated the search for novel and effective antimicrobial compounds from alternative and natural sources like plants and herbs (Rana Pratap Singh, 2011).

According to World Health Organization (WHO), more than 80% of the world’s population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingre-
dients that can be used to treat chronic as well as infectious diseases (Diálo et al., 1999).

_Curcuma longa_ (tumeric) belonging to the Zingiberae-ceae family, has been used for thousands of years as a flavouring agent, a medicinal herb, and a dyeing agent. Ancient Indian medicine has touted _Curcuma longa_ as a herb with the ability to provide glow and luster to the skin as well as vigor and vitality to the entire body. Since it has antimicrobial, antioxidant, astringent, and other useful properties, it can be quite useful in Dentistry as well (Chaturvedi, 2009).

Various studies have shown the antimicrobial effects of extracts of roots of _Curcuma longa_ on various microorganisms like *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, *Candida albicans*, and *Candida kruseii* (Rambir Singh, 2002; Kang-Ju Kim, 2005; Park, 2005; Niamsa, 2009).

Considering the above-mentioned factors, the present study was conducted to evaluate the antimicrobial activity of _Curcuma longa_ extract on endodontic pathogens.

**MATERIALS AND METHODS**

**Collection and authentication of rhizomes**

Fresh rhizomes of _Curcuma longa_ of analytical grade, grown organically without the use of any pesticides were collected (Shobha Vana, Moodbidri, Karnataka). Authentication of the rhizomes was done at the NGSM Institute of Pharmaceutical sciences, Mangalore.

**Test organisms**

*Enterococcus faecalis* (ATCC 29212) [MicroBilogics, St. Cloud MN]. *Staphylococcus aureus* (ATCC 25923) [MicroBilogics, St. Cloud MN]. *Candida albicans* (NTCC 3736) [MicroBilogics, St. Cloud MN]

**Media used**

Nutrient Broth [HiMedia laboratories pvt ltd, Mumbai]. Mueller Hinton Agar [HiMedia laboratories pvt ltd, Mumbai]. Sabouraud’s Dextrose Agar [HiMedia laboratories pvt ltd, Mumbai]

**Extract preparation**

The rhizomes were washed with distilled water (B. K. Chemicals, Pune, India.) and dried. They were then cut into irregular large pieces and dried in an oven by tray drying process at a temperature of 45±5°C for a period of about 9-10 days till they were completely moisture free. The irregular large sized pieces were ground to form a coarse powder.

Maceration process of extraction was then performed on this coarse powder of the rhizomes. (Sukhdev Swami Handa, 2008) 500 gms of coarsely ground powder of the _Curcuma longa_ rhizomes was placed in a large glass chamber. 2500 ml of sterile distilled water was added into the glass chamber prepare the aqueous extract.

The glass chamber was then closed with a glass lid to prevent evaporation of the menstruum and this system was allowed to stand for 7 days with occasional stirring. The liquid i.e. the menstruum was then strained and the solid residue, called marc, was pressed to recover as much occluded solution as possible. The strained and expressed liquid thus obtained were mixed and clarified by filtration. The filtration was carried out in a beaker using a Whatman’s filter paper no 1 (Durga labs, Mangalore, India). 2000 ml of menstruum was obtained which was stored in a refrigerator at 4°C in two beakers.

China dishes (Rotek instruments, Kerala, India) were used for evaporation of the menstruum. These china dishes containing the menstruum were placed on a water bath (Rotek instruments, Kerala, India). After evaporation of the menstruum a thick dark brown colored sticky mass was obtained as the aqueous extract. The extract was stored in a dark colored pre-sterilized airtight container. The same procedure was performed for the remaining menstruum. It was then stored in a refrigerator at 4°C in a dark colored pre-sterilized airtight container until its further use. The total obtained extract, which was semi-solid weighed 19.5gms.

**Preparation of microbial inocula**

The density of selected organisms was adjusted equal to that of the 0.5 McFarland standards (1.5 x 10^8 CFU/ml) by adding them to nutrient broth for *Staphylococcus aureus and Enterococcus faecalis*; and Sabouraud’s dextrose broth for *Candida albicans*. A 24 hour old culture was used for the preparation of bacterial suspension. McFarland standards were used as a reference to adjust the turbidity of microbial suspension so that the number of microorganisms would be within a given range.

**Antimicrobial susceptibility test**

Agar well diffusion method was used to conduct the antimicrobial susceptibility test (Leonardo, 2000). Four different concentrations of the prepared extract were made using DMSO (Dimethyl Sulfoxide).The four concentrations were 1 g/ml, 0.75 g/ml, 0.5g/ml and 0.25 g/ml.

A sterile cotton swab was dipped into the respective microbial suspensions and surplus removed by rotation of the swab against the sides of the tube above the fluid level. The agar media plates were inoculated with the respective organisms by even streaking of the swab over the entire surface of the plate three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculums. Finally, it was swabbed all around the edge of the agar surface. Wells of 8 mm size were made with sterile borer into agar plates containing the bacterial inoculums. 100μl volume of each of the plant extract prepared in four concentrations was dispensed into the wells of inoculated plates.
Sterilized distilled water was used as a negative control which was introduced into one of the wells instead of plant extract. DMSO which was the solvent for preparation of different concentrations of the extract was used as a control for the solvent and was introduced into one well. 2.3% sodium hypochlorite was the positive control.

The plates thus prepared were refrigerated for 60 minutes allowing the diffusion of the extract into the agar. After incubation for 24 hrs at 37 °C, the plates were observed. If antibacterial activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the extract. The zone of inhibition was measured and expressed in millimeters. Antibacterial activity was recorded if the zone of inhibition was greater than 8 mm. The antibacterial activity results were expressed in terms of the diameter of zone of inhibition and <9 mm zone was considered as inactive; 9-12mm as partially active; while 13-18mm as active and >18mm as very active (Junior and Zanil, 2000). The tests were triplicated. The mean and standard deviation of the diameter of inhibition zones were calculated.

**Determination of Minimum inhibitory concentration (MIC)** (Vollekova et al., 2001; Usman, 2007)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacteriostatic concentration). The Vollekova et al., (2001) method modified by Usman et al., (2007) was employed. In this method, the broth dilution technique was utilized where the rhizome extract was prepared to the highest concentration of 750 mg/ml (stock concentration) in DMSO and serially diluted (two-fold) to a working concentration ranging from 0.08 mg/ml to 750 mg/ml using Nutrient broth for *Staphylococcus aureus* and *Enterococcus faecalis* and Sabouraud's dextrose broth for *Candida albicans*. These test tubes were then inoculated with 0.1 ml suspension of the test organism. After 24 hours of incubation at 37 °C, the test tubes were observed for turbidity. The least concentration where no turbidity is observed was determined and noted as the minimum inhibitory concentration (MIC) value.

**Determination of Minimum Bacterial Concentration (MBC)** (Vollekova et al., 2001; Usman, 2007)

The MBC is defined as the lowest concentration where no bacterial growth is observed (bactericidal concentration). This was determined from the broth dilution resulting from the MIC tubes by sub culturing to antimicrobial free agar.

In this technique, the contents of the test tubes resulting from MIC were streaked using a sterile wire loop on agar plates free of bacteria and incubated at 37 °C for 24 hours. The lowest concentration of each of the extract which showed no bacterial growth was noted and recorded as the MBC.

**Time-Kill Assay**

The same dilutions as prepared for MIC were used to determine the time-kill profile of the different concentrations of the aqueous extract.

The samples were immediately plated onto the agar plates soon after inoculation with organisms and this was noted as 0 hrs. The samples were again plated at time intervals of 15 mins, 30 mins, 45 mins, 60 mins, 120 mins, and finally at 24 hrs. These plates were immediately incubated and after 24 hrs of incubation, the colonies were counted. It was seen that the aqueous extract of *Curcuma longa* showed good inhibitory activity against *Staphylococcus aureus* and *Candida albicans*.

**RESULTS**

The results presented in Table 1 reveal that 75% concentration showed the greatest zone of inhibition of 13 mm against *Staphylococcus aureus* and the 50% concentration showed the best zone of inhibition of 15.66 mm against *Candida albicans*.

The 100%, 75% and 25% concentrations of the aqueous extract showed no inhibitory activity against *Enterococcus faecalis*; however 50% concentration showed mild activity with a zone of inhibition of 9 mm. The positive control ie 2.3% Sodium hypochlorite showed the best zones of inhibition (Table 1). The negative controls, distilled water and dimethyl sulfoxide (DMSO) did not show any inhibitory activity on the organisms.

(Figure 1) The MIC of aqueous extract against *Staphylococcus aureus* and *Enterococcus faecalis* was 37.5% (375 mg/ml) and 19% (190 mg/ml) for *Candida albicans*.

### Table 1: Antimicrobial activity of Aqueous *Curcuma longa* extract against endodontic pathogens. (Agar well diffusion method)

<table>
<thead>
<tr>
<th>S. NO</th>
<th>Different Concentrations of the extracts in %</th>
<th>CONTROL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>100%</td>
<td>75%</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (ATCC 25923)</td>
<td>10.66±1.15</td>
<td>13±1.00</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> (ATCC 29212)</td>
<td>--</td>
<td>8.33±0.577</td>
</tr>
<tr>
<td><em>Candida albicans</em> (NTCC 3736)</td>
<td>9.66±1.527</td>
<td>12.33±0.577</td>
</tr>
</tbody>
</table>

Diameters of zones of inhibition in mm. Values are means of three readings± SEM -- = No Activity
cans. (Figure 1) The MBC of aqueous extract for *Staphylococcus aureus* and *Enterococcus faecalis* was 75% (750 mg/ml) and 37.5% (375 mg/ml) for *Candida albicans*.

(Figure 2) Only 75% concentration of the extract showed complete inhibition at 24 hours, whereas bacteriostatic activity was seen from 45 mins to 120 mins. 37.5% concentration also showed bacteriostatic activity from 30 mins to 24 hrs. 2.3% NaOCl showed complete inhibition from 15 mins onwards.

(Figure 3) 75% concentration showed complete inhibition at 120 mins, 24 hours and bacteriostatic activity at 15 mins, 30 mins, 45 mins and 60 mins. 37.5% concentration showed complete inhibition from 45 mins-60 mins and bacteriostatic activity at 30 mins, 120 mins and 24 hours. 2.3% NaOCl showed complete inhibition from 30 mins onwards.

(Figure 4) 75% concentration showed complete inhibition from 30 mins to 24 hours and bacteriostatic activity from 0 mins to 15 mins. 37.5% concentration showed complete inhibition from 45 mins to 24 hours and also bacteriostatic activity was seen at 30 mins. 19%, 9.5% and 4.7% concentrations showed mild bacteriostatic activity from 15 mins to 60 mins. 2.3% NaOCl showed complete inhibition from 30 mins onwards.

DISCUSSION

An endodontic irrigant/medicament should ideally exhibit powerful antimicrobial activity, disinfect the root canal space, and have no cytotoxic effects on periapical tissues, among various other properties required. Therefore, an equally effective and a safe irrigant/medicament is desirable (Radcliffe, 2004).

Antimicrobial agents are many a time associated with adverse effects on the host like, hypersensitivity, immune suppression and allergic reactions. Also, the continuous evolution of bacterial resistance to currently available antimicrobial agents has necessitated the search for novel and effective antimicrobial compounds.

Various authors have shown the antimicrobial activity of *Curcuma longa* extracts against an array of pathogens (Rambir Singh, 2002; Kang-Ju Kim, 2005; Park, 2005; Niamsa, 2009). In the present study, it was seen that aqueous extract of *Curcuma longa* rhizome showed antimicrobial activity against the tested endodontic pathogens. Successive extraction and isolation
of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers used water as the primary solvent.

The results in the present study were in contrast to the results reported by various authors stating that alcoholic extracts showed better antimicrobial properties than aqueous extracts (Nadia Gul, 2004; Kang-Ju Kim, 2005; Ungphaiboon, 2005; Ong-and Lawhavinit, 2011).

The aqueous extract of Curcuma longa showed good inhibitory activity against Staphylococcus aureus and Candida albicans in accordance to a study by N Niamsa (2009). The greater concentrations did not show good inhibitory activity probably due to increased viscosity of the solution and hence lesser diffusion in the medium.

None of the concentrations matched the zone of inhibition of 2.3% sodium hypochlorite, which was the positive control; however 50% concentration of the extract showed equivalent zone of inhibition of 15.66 mm compared to 16 mm seen with that of control.

MIC and MBC were determined by the Volleкова et al. (2001) method; modified by Usman et al. (2007). The MIC (Minimum inhibitory concentration) of aqueous extract against Staphylococcus aureus, Enterococcus faecalis and Candida albicans were in the range of 19%-37.5% (190-375 mg/ml).

The MBC (Minimum bactericidal concentration) of aqueous extract for Staphylococcus aureus Enterococcus faecalis and Candida albicans were in the range of 37.5%-75% (375-750 mg/ml).

In time-kill assay against Staphylococcus aureus; only 75% concentration of aqueous extract showed complete inhibition at 24 hrs and bacteriostatic activity was seen by both 75% and 37.5%. Against Enterococcus faecalis; only 75% concentration of aqueous extract showed complete inhibition at 120 mins and 24 hrs and bacteriostatic activity was seen by both 75% and 37.5%. Candida albicans was completely killed by 75% and 37.5% of aqueous extract from 30 mins to 24 hours. Bacteriostatic activity was also seen by other concentrations.

The mechanism of antibacterial action of spices and derivatives is not clear. Hypothesis have been proposed different workers (Odhav et al., 2002; Lancelotti et al., 2004) which involve hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, followed by partition in the lipid bilayer; perturbation of membrane permeability consequent to its expansion and increased fluidity causing the inhibition of membrane embedded enzymes; membrane disruption; destruction of electrons transport systems and cell wall perturbation.

The fungicidal or fungicidal effect of spices is due to the inhibitory action of natural products and the mechanisms involved are cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular and extracellular enzymes. These biological events could take place separately or concomitantly culminating with mycelium germination inhibition (Cowan, 1999).

Various authors have stated that Curcuma longa extract showed better antifungal activity than antibacterial activity (Geoffrey et al., 1998; Dhingra, 2007; Abhijet Pandey et al., 2010). The present study also showed better antifungal activity against Candida albicans in accordance to the stated authors. But, in a study by Bhawan et al. (2011); it was reported that the antibacterial activity was better than the antifungal activity.

There can be several reasons for the lack of antimicrobial activity in the present study against the organisms. The plant part used or the type of extraction might have resulted in the nil activity, or the time of collection of the rhizomes and climate, might have affected the amount of active constituents in the plant material (Parekh and Chanda, 2007).

Two among the three tested organisms i.e. E. feacalis and C. albicans could be completely killed by the extract of Curcuma longa within 1 hour, the time usually spent for chemo mechanical preparation of the root canals (Morgan Eli Vianna, 2004). Hence, the Curcuma longa extract can be used as irrigating solution/medicament owing to their antimicrobial properties.

CONCLUSION

The aqueous extract showed good results against Staphylococcus aureus and Candida albicans and mild activity against Enterococcus faecalis. Also it was seen that the extract showed very good antifungal activity equivalent to that of the positive control. Thus; from the results obtained in the present study, it can be concluded that Curcuma longa extract can prove to be potent antimicrobial agent against endodontic pathogens. Hence, its future use as an endodontic irrigant/medicament needs to be studied further.

REFERENCES


