ORIGINAL ARTICLE

Decalepis hamiltonii (Swallow Root) as a Potential Antimicrobial Agent against Endodontic Pathogens: An *In Vitro* Study

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Abstract

Aim and objective: The aim of the article was to evaluate and compare the antimicrobial effect of alcoholic and hydroalcoholic extracts of *Decalepis hamiltonii* with those of *Curcuma longa*, *Azadirachta indica*, and *Zingiber officinale* against *Enterococcus faecalis*.

Materials and methods: Alcoholic and hydroalcoholic extracts of the herbs were prepared by cold maceration and filtration–decantation process. Minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) were determined for each extract; zone of inhibition (ZOI) was assessed separately for each extract and their different combinations on both laboratory strain and clinical isolates of *E. faecalis*.

Results: The extracts of *D. hamiltonii* showed a significant antimicrobial action, against endodontic pathogen *E. faecalis,* both independently and in combination preparations.

Conclusion: Extract of *D. hamiltonii* can be used as intracanal irrigant and medicament in endodontics after confirming its biocompatibility.

Keywords: Antimicrobial activity, *Decalepis hamiltonii*, Endodontic disinfection, *Enterococcus faecalis,* Herbal extracts, Natural products.

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Introduction

Natural products have played a significant role in eradicating infectious human diseases since time immemorial. There has been a renewed interest and exploration of natural remedies in modern medicine particularly in the last two decades.[1–4] The main goal of endodontics is to eradicate infectious material from canal space and ensure the healing of periapical tissues without reinfection. Chemical and mechanical methods have been popular in achieving this end. Irrigants form the mainstay of the chemical method of debridement and disinfection in endodontics.[5,6] The irrigants are required to possess antiseptic, bacteriostatic/bactericidal properties without periapical tissue irritation in the event of inadvertent extrusion. Conventional irrigants do not fulfill all of these requisites, besides structurally weakening dentin in the long term.[7] The other disadvantages of synthetic irrigants are host tissue toxicity, bacterial resistance, questionable and unwanted interactions, and caustic hazard. Hence, extracts of natural products are being tried as an alternative to conventional synthetic ones. The unique advantages of natural substances are ease of availability, long shelf life, and cost-effectiveness besides being safe and biocompatible.[3]

Turmeric (*Curcuma longa*), neem (*Azadirachta indica*), and ginger (*Zingiber officinale*) are edible and also commonly used in traditional medicine for their antimicrobial, anti-inflammatory, and anticarcinogenic properties.[3,7–10] These are also widely researched and reported in literature. Swallow root (*Decalepis hamiltonii*) is an edible root widely consumed in the southern parts of India, Sri Lanka, Malaysia, Singapore, and Indonesia. It contains many essential oils and aldehydes and is hence used in Ayurvedic medicine. However, it has been scarcely studied in modern medicine, especially dentistry.[11]

Hence, the aim of the present study was to evaluate the antibacterial effect of *D. hamiltonii* either separately or in combination with the extracts of *C. longa*, *A. indica*, and *Z. officinale* as compared to that of sodium hypochlorite on both standard laboratory strain and clinical isolates of *Enterococcus faecalis*.

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Materials and Methods

Preparation of Extracts

The coarse powder of the roots of *C. longa*, *Z. officinale*, and *D. hamiltonii* and leaves of *A. indica* were immersed in the respective solvents, ethanol (for alcoholic extract) or ethanol–water mixture (for hydroalcoholic extract), in individual stoppered containers and allowed to stand for 3 days at room temperature. Then, the mixture was strained and the damp solid material was pressed to yield the crude liquids. These liquids were clarified, filtered, and evaporated in a rotary vacuum evaporator and concentrated to get semisolid mass.

Microbiological Testing

The standard laboratory strain of *E. faecalis* ATCC 292120 and its clinical isolate (obtained from the Microbiology Department which had collected samples from the endodontic patients with symptomatic or asymptomatic irreversible pulpitis) were used in the microbiological assays. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and zone of inhibition (ZOI) were determined for the alcoholic and hydroalcoholic extracts of the selected four natural substances. ZOI was measured both individually for the extracts and in different combinations and compared to that of 2.5% sodium hypochlorite (NaOCl) which acted as a positive control.

E. faecalis ATCC 292120 and the clinical isolate were inoculated in 100 mL of Mueller Hinton Broth (MHB; HiMedia, India) separately and incubated at 37°C overnight. The turbidity was adjusted to 0.5 McFarland standards as recommended by the clinical and laboratory guidelines and the same was followed for preparing lawn culture for the assay.

Broth Microdilution Assay

Broth dilution is a technique, in which containers holding identical volumes of broth with an antimicrobial solution in incrementally increasing dilutions are inoculated with a known number of bacteria. Broth microdilution denotes the performance of the broth dilution test in a microtiter plate (500 µL capacity).

From the prepared test solutions, doubling dilutions were done from lower dilution to higher dilution in a series of microwell (33.2, 16.6, 8.3, 4.15, 2.07, 1.03, 0.51, 0.25, 0.12, and 0.06) plates (Table 1). The last well contained the negative control with the absence of test reagents. To all the microwell titer plates, 10 µL of *E. faecalis* suspension was added and incubated at 37°C overnight. The MIC was recorded as the lowest concentration in the series of dilutions that did not permit the growth (turbidity) of *E. faecalis*. To substantiate the MIC value, MBC assay was performed.

Minimum Bactericidal Concentration Assay

The broth microdilution MIC test was extended and confirmed by a subculture to measure the MBC. After incubation of the broth microdilution plate, 5 µL of the culture with herbal extracts and the negative control were inoculated on to Mueller Hinton agar plate and incubated overnight at 37°C. Bactericidal action is indicated by a reduction by at least 99% in the number of colonies.

Table 1: Dilution percentage and matching concentration of the extracts in mg/mL

Sl. No.	*%*	*Conc.*
1	100	33.2
2	50	16.6
3	25	8.3
4	12.5	4.15
5	6.25	2.07
6	3.125	1.03
7	1.0625	0.51
8	0.53125	0.25
9	0.265625	0.12
10	0.1328125	0.06

Agar Well Diffusion Assay

The Mueller Hinton agar plate surface was inoculated by *E. faecalis* culture adjusted to 0.5 McFarland standards to obtain a cell density of 1.5×10^8 cells/mL in MHB to obtain a lawn culture. Then, a well with a diameter of 8 mm was punched aseptically with a sterile cork borer and a paper disk saturated with a volume of 100 µL of the herbal extract solution at the desired concentration (MIC) was introduced into the well. Then, agar plates were incubated at 37°C for 24 hours. The diameter of ZOI was measured by a graduated scale.

Statistical Analysis

Students' *t*-test was used to evaluate the intergroup and intragroup differences in MIC, MBC, and ZOI with a confidence interval of 95% and *p* value at ≤0.05

Results

The method of extract preparation (hydroalcoholic/alcoholic) and the nature of the extract (plant source) had a significant effect on both MIC and MBC. MBC was generally higher than MIC.

When tested on ATCC strain, swallow root and turmeric did not show any difference between hydroalcoholic or alcoholic extracts for MIC and MBC values. But neem showed a lower value for hydroalcoholic than alcoholic extract while ginger showed the reverse.

All extracts showed higher MIC and MBC values on clinical isolates than ATCC strain. Neem, turmeric, and ginger showed lower values for hydroalcoholic extract than alcoholic extract. Swallow root showed the same values for both extracts.

Overall, neem had the lowest MIC and MBC of all the extracts, irrespective of the type of extract, or bacterial strain. The highest MIC and MBC values were found for alcoholic extracts of turmeric and ginger on clinical isolate and hydroalcoholic extract of ginger on ATCC strain. The hydroalcoholic extracts of swallow root, turmeric, and ginger showed the same values on clinical isolate (Fig. 1).

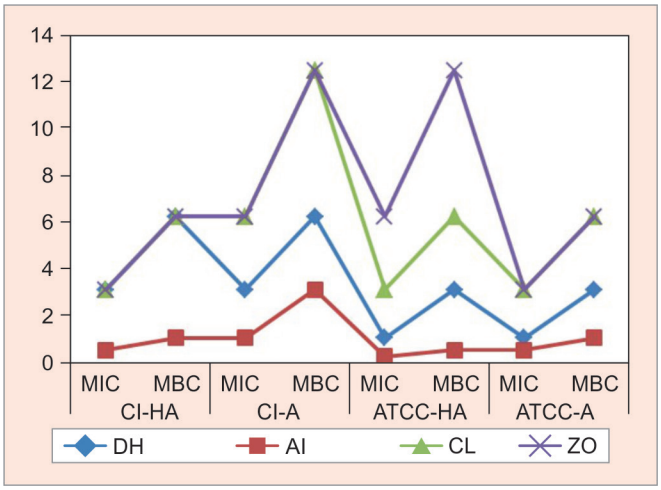

Fig. 1: Antibacterial activity of the test extracts on *Enterococcus faecalis*. *X*-axis, inhibitory/cidal activity of different types of extracts; *Y*-axis, inhibitory/cidal concentration in µg/mL; DH, *Decalepis hamiltonii*; AI, *Azadirachta indica*; CL, *Curcuma longa*; ZO, *Zingiber officinale*; CI, clinical isolate; HA, hydroalcoholic; A, alcoholic

When testing the extracts individually, in ATCC strain of *E. faecalis*, hydroalcoholic extract of turmeric showed the smallest ZOI; alcoholic extract of ginger was the largest; hydroalcoholic extract of neem was better than NaOCl while that of ginger was equivalent; others were inferior to the positive control NaOCl. In clinical isolate of *E. faecalis*, alcoholic extract of ginger was the largest followed by a hydroalcoholic extract of neem; swallow root was equivalent to the positive control NaOCl; others were inferior to the positive control NaOCl (Table 2). When the combination of extracts was tested, alcoholic extracts of neem + turmeric and neem + swallow root were the smallest; both types of extracts of neem + ginger was either equivalent or superior to the positive control NaOCl; hydroalcoholic extracts of neem + turmeric and neem + swallow root were equivalent to the positive control NaOCl; others were inferior to the positive control NaOCl (Table 3); however, ZOI for the cocktail of all four test extracts, both hydroalcoholic and alcoholic proved to be larger than the other combinations and the positive control NaOCl (Table 3).

Discussion

Endodontic infections are controlled by chemomechanical preparation, intracanal medicament placement, laser disinfection, photodynamic therapy, and ultrasonic agitation of irrigants. The long-term effect of certain conventional irrigants on dentin is far from acceptable and results in structural weakness. Moreover, the safety of some of these irrigants with caustic potential on host periradicular tissues is a cause for concern. Many naturally derived substances are being tried *in vitro* and *in vivo* for endodontic disinfection and debridement to overcome these disadvantages of conventional ones.

C. longa, a member of the ginger family contains curcuminoids, that includes mainly curcumin (diferuloyl methane), a yellow-colored pigment; demethoxycurcumin; bisdemethoxycurcumin; and various volatile oils, including turmerone, atlantone, and zingiberone.[1–3] It is used as a spice, food color, and preservative since time immemorial. It is also used in Ayurveda since 1900 BC for skin, lung, liver, and gastrointestinal (GI) disorders. Curcumin was first isolated in 1815 by Vogel and Pelletier and its chemical structure was determined in 1910 by J. Milobedzka and V. Lampe.[1] It possesses anti-inflammatory, antioxidant, hepatoprotective, antimicrobial, and anticancer activity.[3] Curcumin possesses antibacterial properties against a number of gram-positive and gram-negative bacteria. The molecule is pleiotropic and interacts with many molecules during inflammation. It regulates cytokines, transcription factors, protein kinases, adhesion molecules, redox agents, and enzymes. It inhibits bacterial cell division by interacting with FtsZ. A wide range of different concentrations have been found to be safe by Yeon et al.[12] Bulit et al. showed that it is not toxic to odontoblast-like cells, undifferentiated pulp cells, and human embryonic stem cells.[13] Mandroli et al. reported a MIC of 333.3 µg/mL against *Streptococcus mutans*, 125 against *Lacticaseibacillus casei*, 167 against *Actinomyces viscosus*, 208 against *Prevotella intermedia*, 125 against *Porphyromonas gingivalis*, and no activity against *E. faecalis*.[2] However, the results of another study by Neelakantan et al. were contradictory in that a significant antibacterial activity was seen compared to NaOCl.[14] Another study by Vinothkumar et al., using quantitative polymerase chain reaction (Q PCR), concurred with the superior efficacy of turmeric compared to *Aloe vera*.[15]

Curcumin was shown to prevent the biofilm formation by eliminating extracellular polymeric substances by Kishen et al.[16]

Table 2: Average ZOI (mean in mm and SD in parentheses) for individual extracts on *Enterococcus faecalis*

AT N A	*AT N HA*	*CI N A*	*CI N HA*	*AT SR A*	*AT SR HA*	*CI SR A*	*CI SR HA*	*AT T A*	*AT T HA*	*CI T A*	*CI T HA*	*AT G A*	*AT G HA*	*CI G A*	*CI G HA*	*AT SH*	*CI SH*
11.66^{kf} (0.57)	16.66^{b} (1.15)	10^{m} (0)	18.33^{a} (0.57)	13^{l} (0)	11.33^{ko} (0.57)	13.33^{l} (0.57)	12.33^{lfo} (0.57)	10.66^{mi} (0.57)	8.66^{d} (1.15)	11^{k}(0)	10^{m}(0)	22.33^{n} (1.15)	15^{e}(1)	21.33^{n} (0.57)	15^{e}(0)	14.66^{e} (0.57)	12.33^{f} (0.57)

ZOI, zone of inhibition; N, neem extract; SR, swallow root extract; T, turmeric extract; G, ginger extract; SH, sodium hypochlorite (2.5%); AT, ATCC strain; CI, clinical isolate; A, alcoholic extract; HA, hydroalcoholic extract. Values with the same superscript letter indicate statistically not significantly different

Table 3: Average ZOI (mean in mm and SD in parentheses) for the combination of extracts on *Enterococcus faecalis*

AT NT A	*CI NT A*	*AT NT HA*	*CI NT HA*	*AT NSR A*	*CI NSR A*	*AT NSR HA*	*CI NSR HA*	*AT NG A*	*CI NG A*	*AT NG HA*	*CI NG HA*	*AT 4 A*	*CI 4 A*	*AT 4 HA*	*CI 4 HA*
8^{d} (0)	7.5^{d} (1.5)	13.5^{ei} (1.5)	12^{fh} (1)	8^{d} (0)	7^{h} (0)	14.5^{e} (0.5)	13^{ei} (1)	14.5^{e} (0.5)	12^{f} (1)	16^{ebj} (1)	12.5^{fij} (2.5)	19.5^{ac} (0.5)	17.5^{b} (0.5)	20^{c} (0)	19^{a} (0)

ZOI, zone of inhibition; NT, neem + turmeric; NSR, neem + swallow root; NG, neem + ginger; 4, neem + turmeric + swallow root + ginger; AT, ATCC strain; CI, clinical isolate; A, alcoholic extract; HA, hydroalcoholic extract. Values with the same superscript letter indicate statistically not significantly different

Hemanshi Kumar showed that curcumin produced greater ZOI against anaerobes like *E. faecalis*.[9] Another study showed equivalent activity of turmeric and NaOCl superior to chlorhexidine (CHX) on *E. faecalis* biofilm in extracted teeth.[17] It was able to eliminate *E. faecalis* biofilm when used as an intracanal medicament (ICM), better than calcium hydroxide ($Ca(OH)_2$) but less than 2%CHX in a study by Yadav et al.[18] However, Pandey et al. showed that it is inferior to 5.25% NaOCl on *E. faecalis* biofilms.[19] In the present study also, turmeric had higher MIC and MBC when the alcoholic extract was used on clinical isolate. Its ZOI was also the smallest.

A. indica, known as the Indian neem/margosa tree, belongs to Meliaceae family. The chemical components present are nimbin, nimbinene, 6-desacetylnimbinene, nimbandiol, ascorbic acid, n-hexacosanol and amino acid, 7-desaacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol[20] which act as biocompatible antioxidant, antiviral, antifungal, antibacterial, and anticarcinogenic agents.[3] Subapriya et al. also reported similar properties.[21] In a study by Maina et al., it was found that the MIC of aqueous extract was 50 µg/mL and it inhibited 46% of *E. faecalis* at 24h and 85% at 48h. However, the ethanolic extract had a MIC of 0.1 µg/mL and inhibited 88% at 24h and 96% at 48h. The MBC was not detectable for aqueous extract while it was 6.25% for ethanolic extract.[7] This concurs with the findings of the current study.

Arati et al. found that the ethanolic extract of neem has significant antimicrobial activity against *E. faecalis*.[22] Hannah et al.[23] found that it is better than NaOCl in preventing bacterial adherence. Along with curcumin, it has better antimicrobial activity than other herbal extracts in a study by Vinothkumar et al.[15] However, in the present study, this combination was less effective than others. Bohora et al. found that both aqueous and alcoholic extracts were as effective as NaOCl[8] on a mixed culture of *E. faecalis* and *Candida albicans*. At a conc. of 25%, it was able to remove biofilm of *E. faecalis* to a limited extent only unlike 3% NaOCl which completely eliminated *E. faecalis* in a study.[24] In the present study, MIC and MBC were the lowest for neem when its hydroalcoholic extract was used, especially on ATCC. It also showed the largest ZOI for the hydroalcoholic extract on both ATCC and clinical isolate. Combining other natural extracts like ginger and swallow root with neem showed an additive effect.

Z. officinale, ginger, is used in traditional medicine for treating many diseases. Ginger's pungent components offer powerful anti-inflammatory and antioxidant activities, making it useful in arthritis, Alzheimer's disease, cancer, and cardiovascular disease. It contains zingibain, an enzyme that counteracts inflammation. The active compounds of ginger are divided into two groups: volatile essential oils and fragrant or harsh phenol compounds.[25] Among these, volatile essential components, which constitute zingerone and shagelol, were reported to be responsible for their antibacterial properties.[10] Its ethanolic extract was found to be effective against *C. albicans* and *E. faecalis* by Atai et al.[26] Similar results were reported by Gulve et al.[27] Gingerol in ginger was found to act on *P. gingivalis*, *Porphyromonas endodontalis, and P. intermedia* by Park et al.[28] It was found to act on bacterial endotoxin in a study by Maekawa et al.[29] In the present study, MIC of ginger was the highest when the hydroalcoholic extract was used against ATCC or when the alcoholic extract was used against clinical isolate of *E. faecalis*. However, its ZOI was the largest for its alcoholic extract when used on both ATCC and clinical isolate.

Roots of *D. hamiltonii* are edible and used for their health-promoting properties in traditional cuisine. They contain aldehydes, amyrins, lupeols, and volatile flavor compounds such as 2-hydroxy-4methoxybenzaldehyde; vanillin; and essential oils like methylresorcylaldehyde, atlantone, terpinene, and geraniol. A combinational molecule containing peptic polysaccharide with bound phenolics identified in the roots of *D. hamiltonii* and its breakdown products have been known to have health beneficial properties. It was found to have a maximum ZOI of 24mm against *E. faecalis* intermediate between those of ginger and 0.2% CHX. It showed a MIC of 2.5 mg/mL.[11] In the present study, swallow root had higher MIC and MBC when used on clinical isolate of *E. faecalis* which was similar to that of turmeric. Its ZOI was moderate and comparable to NaOCl. However, it should be noted that in the present study, ZOI was determined for MIC of the extracts, while many studies cited above have used maximum inhibitory concentration.

E. faecalis, a facultative anaerobe, is frequently used in biofilm models to test the potential antimicrobial actions of new medicaments, irrigants, irrigating protocols, instrumentation techniques, irrigant activation, sealers, and obturating materials in endodontics. The reason behind such practice is because it is one of the important organisms in endodontic infection and reinfection. It is commonly detected in asymptomatic, persistent endodontic infections and possesses virulence factors like lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid. It can survive by genetic polymorphism and it has the ability to bind to dentin, invade dentinal tubules, and survive starvation. It has been shown that it adheres to host cells, express proteins that allow it to compete with other bacterial cells, and alter host responses. Moreover, it suppresses the action of lymphocytes, potentially contributing to endodontic failure.[4] Several studies showed that *E. faecalis* was found more in the cases of failed endodontic treatment than in the cases with primary infections.[30] Thus, in the present study both standard laboratory strain and clinical isolate of *E. faecalis* were used as the microorganism to test the effect of plant extracts.

In this study, 2.5% NaOCl was used as the positive control as this concentration of the irrigant is widely used in endodontic practice than the other concentrations, and hence, the results simulate a routine clinical scenario. Further, this irrigant is considered as a gold standard for testing any new or potential ones, as it possesses a time-tested effect on a wide spectrum of microbes and tissue dissolution efficacy.

Phytochemicals and nutrients exert additive and synergistic effects when used in the right combination. Similarly, a wrong combination can exert an inhibitive or negative effect. Thus, in this study, the effects of combining four different plant extracts on endodontic pathogen were investigated and it appears that neem and turmeric may have a negating effect if used together, when other plant extracts are added, they can produce a significant positive effect. Exploring such combinations may be useful in choosing the best combination for the desired effect in clinical microbiology.

One limitation of this study is its design being *in vitro* and the fact that the biocompatibility of this extract of *D. hamiltonii* is not yet reported. However, since it is an edible root used in Asian cuisine for centuries, it may be probable that it is also safe like the ginger and turmeric family which has many similarities. Future studies can focus on this aspect and test the efficacy in clinical studies before it can be recommended as an alternative to conventional irrigants.

Conclusion

The potential for using the extract of swallow root for antimicrobial action was tested in this study. This edible root, similar to ginger, was found to be having satisfactory antimicrobial action against both standard strain and clinical isolate of *E. faecalis*. Hence, it has the potential to be used as an alternative to conventional irrigants and medicaments in endodontics. The combination cocktail of the four natural extracts proved to be more effective than the positive control of 2.5% NaOCl.

Highlights

- Extracts of natural substances have the potential for being safer and effective alternatives to synthetic ones in endodontic disinfection and cleaning
- *D. hamiltonii* (swallow root) has similar potential as *C. longa* in inhibiting *E. faecalis*
- Swallow root extract can be tried as an alternative to sodium hypochlorite in endodontic irrigation.

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