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ORIGINAL RESEARCH

Antibacterial Activity of Leaf Extract of *Annona muricata* and *Simarouba glauca* on *Enterococcus faecalis*

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ABSTRACT

Aim: To determine the antimicrobial effect of water extracts of leaves of *Annona muricata* and *Simarouba glauca* on *Enterococcus faecalis* using agar diffusion method.

Materials and methods: Dried leaves of *A. muricata* and *S. glauca* were powdered and extracted in a soxhlet apparatus. *Enterococcus faecalis* was grown overnight in Trypticase soy agar plates. About 10 µL of each extract was placed on agar plates and incubated overnight. The zone of inhibition was measured after 24 hours. About 1% sodium hypochlorite and distilled water were used as positive and negative controls.

Results: The leaf extract of *A. muricata* showed similar effectiveness as that of sodium hypochlorite, whereas the leaf extract of *S. glauca* showed only a slight reduction in growth of *E. faecalis*.

Conclusion: Leaf extract of *A. muricata* can be developed as an alternative to sodium hypochlorite for root canal irrigants.

Clinical significance: Success of endodontic treatment depends on complete disinfection of the root canals. Root canal irrigants have a major role in complete disinfection of the root canals. Chemical root canal irrigants are more or less toxic to the oral environment. In this study, naturally derived leaf extracts of *A. muricata* and *S. glauca* are compared with sodium hypochlorite for its effectiveness against *E. faecalis* – the most common pathogen found in the root canals.

Keywords: *Annona muricata*, *Enterococcus faecalis*, Root canal irrigants, *Simarouba glauca*, Sodium hypochlorite.

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INTRODUCTION

Complete disinfection of the root canal is still a big concern for endodontists. The success of root canal treatment lies completely on proper disinfection of root canals. Among the several microorganisms present in the root canals, *Enterococcus faecalis* is the most common.[1] This bacteria can survive even in the absence of adequate nutrients and in low pH.[1,2] It can be isolated from the root canals several days after the completion of biomechanical procedures.[1,3,4] A study by Siren et al has demonstrated a high failure rate for those root canals from which *E. faecalis* has been isolated compared with those root canals having only other nonenteric bacteria.[5] Thus, developing an irrigant solution, i.e., highly effective against *E. faecalis* is of great importance.

Root canal irrigants have a major role in the success of root canal treatments. The irrigant should not only be antibacterial but also be nontoxic. It should not cause any untoward reactions if spilled to the oral cavity as well as to the periapical tissue. The most commonly used irrigants are sodium hypochloride, chlorhexidine, and ethylenediaminetetraacetic acid.[6] Use of other irrigants, such as MTAD (a mixture of 3% doxycycline, 4.25% citric acid, and detergent), HEBP (1-hydroxyethylidene-1, 1-bisphosphonate), chlorine dioxide, silver diamine fluoride, tetraclean, triclosan, and Gantrez has also been suggested by several workers.[6] Uses of herbal preparations, such as extracts of triphala, green tea extract, and *Morinda citrifolia* have been explored.[6] Herbal preparations can be nontoxic and more biocompatible than other chemical preparations. This study aims to examine the antibacterial activity of the extracts of *Annona muricata* and *Simarouba glauca* against *E. faecalis* in comparison with 1% sodium hypochlorite.

Annona muricata is an edible fruit-bearing evergreen tree with large glossy dark green leaves. It is cultivated throughout India. The extracts from its leaf, stem roots, and seeds have demonstrated the antibacterial activity against a plethora of microorganisms.[7-9] However, its antibacterial effect against *E. faecalis* has not been reported so far.

Simarouba glauca is another plant of medicinal importance. The extracts of its leaves have demonstrated antibacterial, antioxidant, hemolytic, and thrombocytic activities.[8,10,11]

Sodium hypochlorite is a commonly used root canal disinfectant. It acts as a solvent when it comes into contact with organic tissues.[6] It releases chlorine that combines with amino acids to form chloramines. The chloramines interfere with cell metabolism.[6] Chlorine that is a strong oxidant has an antimicrobial action.[6] Because it exhibits an excellent antimicrobial action against root canal microorganisms, it is used as a negative control to compare the effectiveness of leaf extracts of *A. muricata* and *S. glauca*.

AIMS AND OBJECTIVES

The aim of this article is to determine the antimicrobial effect of water extracts of leaves of *A. muricata* and *S. glauca* on *E. faecalis* using agar diffusion method. This preliminary study was conducted to collect data for further studies to develop some herbal root canal irrigants.

MATERIALS AND METHODS

Bacterial Strain

Enterococcus faecalis (MTCC number 439) was purchased.

Culture Media and Chemicals

Trypticase soy broth and agar were purchased. All chemicals used in this study were of analytical grade procured locally from reputed manufacturers.

Plant Materials

Healthy leaves of *A. muricata* and *S. glauca* were collected from locally grown trees in December 2015. These leaves were dried in shade and stored.

Preparation of Extract

The dried leaves were powdered and 30 gm of each powder was extracted in a soxhlet apparatus with 300 mL of water for 16 hours. The extract was evaporated in a water bath to obtain the powdered extracts. The total yield for *A. muricata* was 0.6 gm and that of *S. glauca* was 1.2 gm. Solutions of 2, 1 and 0.5% concentration were obtained by mixing 2, 1, and 0.5 mg of powders with 100 mL of distilled water.

Media and Cultural Conditions

The bacterial strains of *E. faecalis* were grown overnight at 37°C in Trypticase soy broth (3%) without aeration. For the solid medium, the broth containing 2% agar was used.

Determination of Minimum Inhibitory Concentration

To determine the minimum inhibitory concentration (MIC) of the extracts of the bacterial strains of *E. faecalis*, 100 µL of overnight cultures of the bacterial strains were spread over Trypticase soy agar plates. Disks of Whatman No. 3 paper of 6 mm diameter holding 10 µL solutions of the extracts of *Annona* and *Simarouba* at different concentrations were placed on the agar plates. As positive controls, disks carrying 10 µL of 1% solution of NaOCl and as negative control, disks carrying 10 µL of distilled water were also kept on each of the agar plates. After 24 hours of incubation at 37°C, the plates were examined and the zone of inhibition of bacteria around each of the disks was measured. The results were recorded and the data were expressed as the diameter of the zone of inhibition d standard deviation in the table. The experiment was repeated three times.

RESULTS

The results of experiment with *A. muricata* extract were tabulated in Tables 1 to 3 and in Figures 1A to F. Inhibition zones were not obtained for *Simarouba* extract and hence, were not included in the table.

Mean Zone of Inhibition

1% sodium hypochlorite – 1.39 ± 0.06
2% *Annona* extract – 1.51 ± 0.04
1% *Annona* extract – 1.39 ± 0.05.

Table 1: Experiment 1 – Zone of inhibition of growth of *E. faecalis* and disks with *A. muricata* extract, 1% sodium hypochlorite, and distilled water

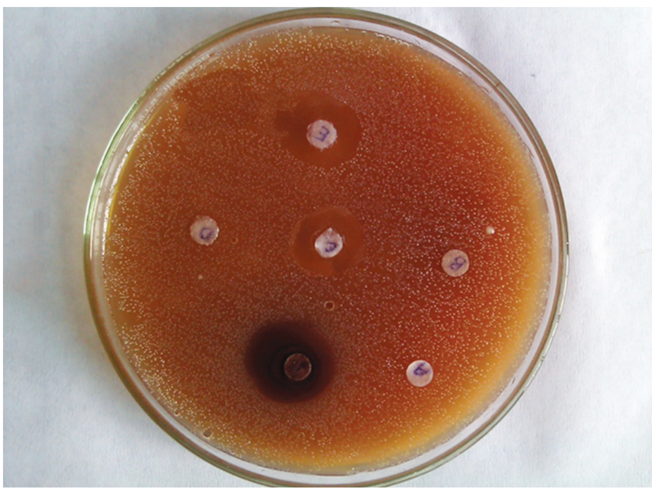
Sl. no	*1% NaOCl (cm)*	*2% Annona extract (cm)*	*1% Annona extract (cm)*	*0.5% Annona extract*	*Distilled water*
1	1.2	1.7	1.3	0	0
2	1.4	1.5	1.4	0	0
3	1.5	1.6	1.2	0	0
4	1.4	1.5	1.3	0	0
5	1.3	1.3	1.5	0	0
6	1.6	1.4	1.6	0	0
Mean	1.4 ± 0.14	1.5 ± 0.41	1.38 ± 0.15	0	0

Table 2: Experiment 2 – Zone of inhibition of growth of *E. faecalis* and disks with *A. muricata* extract, 1% sodium hypochlorite, and distilled water

Sl. no	*1% NaOCl (cm)*	*2% Annona extract (cm)*	*1% Annona extract (cm)*	*0.5% Annona extract*	*Distilled water*
1	1.1	1.4	1.2	0	0
2	1.3	1.7	1.4	0	0
3	1.4	1.6	1.5	0	0
4	1.2	1.2	1	0	0
5	1.3	1.7	1.6	0	0
6	1.5	1.3	1.4	0	0
Mean	1.3 ± 0.14	1.48 ± 0.21	1.35 ± 0.22	0	0

Table 3: Experiment 3 – Zone of inhibition of growth of *E. faecalis* and disks with *A. muricata* extract, 1% sodium hypochlorite, and distilled water

Sl. no	*1% NaOCl (cm)*	*2% Annona extract (cm)*	*1% Annona extract (cm)*	*0.5% Annona extract*	*Distilled water*
1	1.5	1.6	1.3	0	0
2	1.7	1.5	1.5	0	0
3	1.4	1.5	1.4	0	0
4	1.3	1.4	1.4	0	0
5	1.3	1.7	1.6	0	0
6	1.6	1.6	1.5	0	0
Mean	1.47 ± 0.16	1.55 ± 0.10	1.45 ± 0.10	0	0



Figs 1A to F: Inhibition zones of *A. muricata*, *S. glauca*, sodium hypochlorite, and distilled water: (A) Distilled water; (B) *S. glauca* leaf extract; (C) 1% *A. muricata* leaf extract; (D) 0.5% *A. muricata* leaf extract; (E) 1% sodium hypochlorite; and (F) 2% *A. muricata* leaf extract

STATISTICAL ANALYSIS

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 19. The t-test was used to test the difference between 1% sodium hypochlorite and 2% leaf extract of *A. muricata* and that of 1% sodium hypochlorite and 1% leaf extract of the plant extract. High p values are obtained, showing that there is no statistically significant difference between the 1% sodium hypochlorite and the plant extract.

This shows 1% leaf extract of *A. muricata* is equally effective as 1% sodium hypochlorite against *E. faecalis*.

DISCUSSION

This study shows the antibacterial activity of *A. muricata* leaf extract against *E. faecalis*, a facultative anaerobic Gram-positive coccus, which is the most common *Enterococcus* species found in nonhealing endodontic cases. There are about 23 species of *Enterococcus*.[12] They are divided into 5 groups based on their interaction with mannitol, sorbose, and arginine. *Enterococcus faecalis* comes under group II. It forms acid in mannitol broth and hydrolyzes arginine but does not form acid in sorbose broth.[12] *Enterococcus* species grows as a commensal in human intestine and oral cavities. They can resist extreme alkaline pH, salt concentrations, ethanol, bile salts, detergents, heavy metals, azide, and desiccation.[12] They can survive a temperature of 60°C for 30 minutes.[12] They can survive in root canals for prolonged periods without any nutritional supplies and can recover when adequate nutritional supplies are made available.[12] *Enterococcus faecalis* can survive calcium hydroxide – a commonly used intracanal medicament if high pH is not maintained.[12] It has been estimated that there is nine to one chance of detecting *E. faecalis* in failed endodontic treatments compared with primary root canal infection.[13]

The antibacterial activity of the plant extract was compared with 1% sodium hypochlorite, a commonly used root canal irrigant. Sodium hypochlorite is a powerful oxidizing agent with unpleasant taste, high toxicity, and its inability to remove the smear layer. Furthermore, hypochlorite is a very caustic, nonspecific agent whose action is not limited to necrotic tissue.[14] It has a deleterious effect on the dentin. It may cause reduction of elastic modulus and flexural strength of the dentin.[15] Accidental spillage of sodium hypochlorite to clothing causes rapid, irreparable bleaching, and spillage to the eyes, skin, and oral cavity causes severe tissue damage.[16] Extrusion of sodium hypochlorite into the periapical tissue results in sudden onset of swelling both intraorally and extraorally. There will be rapid tissue necrosis and rapid onset of pain.[16] In spite of these disadvantages, hypochlorite is still used as an irrigant in the absence of a suitable and effective alternative.

The constant increase in antibiotic-resistant strains and the side effects of inorganic and synthetic drugs have prompted researchers to look for herbal alternatives. This study had demonstrated the effectiveness of 1% solution of *A. muricata* leaf extracts as an antibacterial agent against *E. faecalis*. This suggests its effectiveness as a root canal irrigant comparable with 1% sodium hypochlorite.

Annona muricata belongs to the Annonaceae family. It is commonly known as graviola or soursop.[17] It is grown in the tropical regions of Central and South America, Western Africa, and Southeast Asia.[18] It bears an edible fruit. Extracts from its bark, root, seed, or leaves are used for varied medicinal purposes throughout the world.[18]

Annonaceous acetogenins are some powerful phytochemicals found in *A. muricata*.[7] They are a series of polyethers that have antitumor, antiparasitic, antimalarial, insecticidal, and antibacterial activities.[19]

The leaf extract of *A. muricata* is a naturally derived solution that has been used to treat various bacterial diseases, such as pneumonia, diarrhea, urinary tract infections, and some skin diseases.[19] It has not demonstrated any toxic effect so far. The synergism of flavonoids, steroids, and alkaloids found in the extracts of *A. muricata* is attributed to its antibacterial activity.[7,18,20]

This study of *in vitro* antimicrobial evaluation of the leaf extract of *A. muricata* provides a primary platform for further phytochemical and pharmacological evaluation of the extract as an alternative to sodium hypochlorite for root canal irrigation.

CONCLUSION

A nontoxic but effective root canal irrigant is the need of the hour. The leaf extract of *A. muricata* offers a good choice. This is a preliminary study where the whole of the leaf extracts is used as such. Further studies are needed to isolate the active component in the leaf extract and to develop a root canal irrigant from the leaf extract of *A. muricata*.

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