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 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 hypochlorite, chlorhexidine, and ethylenediaminetetraacetic acid. 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. Uses of herbal preparations, such as extracts of triphala, green tea extract, and Morinda citrifolia have been explored. Herbal preparations can be nontoxic and more biocompatible than other

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. This bacteria can survive even in the absence of adequate nutrients and in low pH. It can be isolated from the root canals several days after the completion of biomechanical procedures. 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. 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 hypochlorite, chlorhexidine, and ethylenediaminetetraacetic acid. 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. Uses of herbal preparations, such as extracts of triphala, green tea extract, and Morinda citrifolia have been explored. Herbal preparations can be nontoxic and more biocompatible than other
Antibacterial Activity of Leaf Extract of Annona muricata and Simarouba glauca on Enterococcus faecalis

The Journal of Contemporary Dental Practice, August 2016;17(8):650-653

AIMS AND OBJECTIVES

The aim of this article is to determine the antimicrobial effect of water extracts of leaves of Annona muricata and Simarouba 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 Annona muricata and Simarouba 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 Annona muricata was 0.6 gm and that of Simarouba 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.

RESULTS

The results of experiment with Annona 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.

<table>
<thead>
<tr>
<th>Sl. no</th>
<th>1% NaOCl (cm)</th>
<th>2% Annona extract (cm)</th>
<th>1% Annona extract (cm)</th>
<th>0.5% Annona extract (cm)</th>
<th>Distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2</td>
<td>1.7</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.4</td>
<td>1.5</td>
<td>1.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>1.6</td>
<td>1.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1.4</td>
<td>1.5</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1.3</td>
<td>1.3</td>
<td>1.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1.6</td>
<td>1.4</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Mean 1.4 ± 0.14 1.5 ± 0.41 1.38 ± 0.15 0 0

Mean and Cultural Conditions

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

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

<table>
<thead>
<tr>
<th>Sl. no</th>
<th>1% NaOCl (cm)</th>
<th>2% Annona extract (cm)</th>
<th>1% Annona extract (cm)</th>
<th>0.5% Annona extract</th>
<th>Distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1</td>
<td>1.4</td>
<td>1.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.3</td>
<td>1.7</td>
<td>1.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
<td>1.6</td>
<td>1.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1.2</td>
<td>1.2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1.3</td>
<td>1.7</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1.5</td>
<td>1.3</td>
<td>1.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>1.3 ± 0.14</td>
<td>1.48 ± 0.21</td>
<td>1.35 ± 0.22</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sl. no</th>
<th>1% NaOCl (cm)</th>
<th>2% Annona extract (cm)</th>
<th>1% Annona extract (cm)</th>
<th>0.5% Annona extract</th>
<th>Distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5</td>
<td>1.6</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.7</td>
<td>1.5</td>
<td>1.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
<td>1.5</td>
<td>1.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1.3</td>
<td>1.4</td>
<td>1.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1.3</td>
<td>1.7</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>1.47 ± 0.16</td>
<td>1.55 ± 0.10</td>
<td>1.45 ± 0.10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

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

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

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. 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.

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*.

**ACKNOWLEDGMENT**

Authors would like to thank Dr. CKK Nair, Director, Research Center, St. Gregorios Dental College, Kerala, India, for his invaluable help.

**REFERENCES**