Short communication

Antibacterial efficacy of *Lawsonia inermis* (*Henna*) against the predominant endodontic pathogen *(Enterococcus faecalis*)-An *in vitro* antimicrobial assay

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ABSTRACT

Introduction and Aim: The prospect of a successful root canal therapy relies on three main factors which includes proper instrumentation, disinfection and obturation of root canal. The goal of the present study is to analyse the anti-microbial efficacy of *henna* (*Lawsonia inermis*) against the major endodontic pathogen, *Enterococcus faecalis.*

Materials and Methods: In this study, the antibacterial efficacy of *Lawsonia inermis* is investigated against *Enterococcs feacalis* using the method of agar disc diffusion. Extracts of *L. inermis* were prepared by using methanol as an extraction solvent, whereas DMSO (Dimethyl sulfoxide) and water were used as dissolution solvents. The diluted henna sample was used as the test sample, while positive control used was chlorhexidine gluconate solution and the negative control used was saline. The bacteria were cultured, and the samples were placed to measure the zone of inhibition.

Results: Extracts of *L. inermis* displayed noteworthy antimicrobial activity against *E. fecalis*. The zone of inhibition of henna against *E. fecalis* was found to be 11mm whereas the positive control had 14mm.

Conclusion: In conclusion, the henna extracts had shown acceptable antimicrobial efficacy and thus this study provides the scientific reason for the use of *L. inermis* in dentistry.

Keywords: *Lawsonia inermis;* irrigants; antimicrobial activity; zone of inhibition; *Enterococcus faecalis*.

INTRODUCTION

The primary dentition must be retained until the normal exfoliation of the tooth to maintain and preserve the arch integrity and to aid in proper function. The pulpally infected primary tooth has to be treated with pulp therapy aiming complete elimination of pulp and periapical pathogens within the root canals (1). The success of an endodontic treatment in deciduous teeth strongly depends on achieving an adequate level of disinfection within their root canals (2). Evidence has revealed that instrumentation done using hand / rotary files leave a considerable portions of infected root canal walls uncleaned or untouched; therefore, it is necessary to reduce the bacterial load within the root canal and to eradicate the microorganisms along with their by-products, to the most possible extent, by utilizing clinically effective and biocompatible irrigants (3).

Generally, the necessity for irrigation is: a) removal of the remains of infected pulp tissue, dentin debris (smear layer), blood, exudates, food, and medications; (b) providing detergent, antiseptic, and bleaching action; and (c) hydration and lubrication of the canal walls during instrumentation (4). In clinical practice, different intracanal irrigants have been proposed for primary teeth, such as physiological saline solution (NaCl), Sodium hypochlorite (NaOCl), chlorhexidine gluconate (CHX), ethylenediaminetetraacetic acid (EDTA), citric acid, MTAD, hydrogen peroxide, and others (5). Although several in vivo; in-vitro and ex vivo approaches have been applied in efforts to establish the effectiveness of diverse disinfecting substances, the search for an ideal irrigating solution in paediatric dentistry remains an issue.

Chlorhexidine (CHX) solution is one among the commonly used and potent antiseptic irrigant possessing low surface tension. It is commonly used in root canal therapy of primary teeth because of its superior ability to penetrate accessory canals and dentin tubules to a depth of 100 µm without exhibiting toxic effects (6). Chlorhexidine has shown to have bacteriostatic action when used in low concentrations and bactericidal action when used in high concentrations for 48–72 hours against a wide range of aerobic and anaerobic bacteria and is sought mostly due its substantivity (7). However, the activity of Chlorhexidine is pH dependent which is substantially reduced in the presence of organic debris. Although the microorganisms are killed by CHX, the biofilm and other organic debris present in the root canal are not removed by it. Additionally, rare cases of

inflammatory responses were also reported when chlorhexidine was injected accidently beyond the apex of the root.

Normal Saline as 0.9%W/V is commonly used irrigant in paediatric endodontics because of its isotonicity to the body fluids. It provides gross debridement and lubrication of root canals by its flushing action. It is found to have zero side effects, even if injected beyond the root apex and into the periapical tissues because the osmotic pressure of normal saline is same as that of the human blood (8). Normal saline lacks the antibacterial activity when used alone, and they do not dissolve tissue either. Moreover, water and saline solutions bear the risk of contamination if used from containers that have been opened more than once to the external environment.In the discipline of traditional herbal medicine, plants are in use for several years. Therefore, plants are considered as a natural source of efficient and potent antimicrobial agents (9).

Lawsonia inermis (*L. inermis*) is a scientific name of a tall shrub plant commonly known as Henna or Mehndi that belongs to: Kingdom: Plantae, Division: Angiospermae, Class: Dicotyledoneae, Order: Myrtales, Family: Lythraceae, Genus: Lawsonia, Species: *L. inermis*.

The principal constituents of henna are Lawsone (2-hydroxynaphthoquinone), mucilage, mannite, and tannic acid (10). In Ayurveda, henna and its extracts are used to treat many diseases as it possesses properties such as anti-inflammatory, antioxidant, antihemorrhagic, immunostimulant and antimicrobial properties (11). The present study aims to assess the antimicrobial efficacy from the leave extracts of *L. inermis* against *E. faecalis.*

MATERIALS AND METHODS

This *in vitro* study was conducted in Department of Pediatric and Preventive Dentistry and in collaboration with Department of Microbiology.

Preparation of broth and bacterial growth

The species of microorganism used in this experiment was Enterococcus faecalis (*E. faecalis*) ATCC 29212. The standard bacterial strain of E. faecalis was obtained from the King George Medical University and Hospital, Lucknow, Uttar Pradesh, India. The purity of test strain was evaluated and confirmed. Brain Heart infusion (BHI) broth was prepared, and Quality Control was checked for sterility and growth potential.

Preparation of the culture medium

For the preparation of culture medium, Mueller Hinton agar was slowly added to distilled water and was gently mixed by shaking the container. The resultant mixture was then sterilized by autoclaving at 121°C with 15 lb pressure for 15 minutes. The liquid was then cooled down to room temperature after 15 minutes. This liquid medium was poured in the petri dishes of size 90mm and allowed to set in a laminar flow chamber. In the petri dish three wells were cut to test the test material in addition to a positive and a negative control.

Obtaining plant material

In this study, the dried leaves of *L. inermis* (*Henna)* used were purchased from the traditional medical shop in Chennai, India

Preparation of plant extract

To evaluate the antimicrobial efficacy of *L. inermis*, methanol extract was prepared. For the preparation of methanol extract, initially the dried leaves of Henna were ground to fine smooth powder mechanically in an electric grinder. The fine powdered leaves weighing about 10 g were added with in the flasks of 100 ml volume and 50ml of methanol solvent was added to the flask separately. Then the flasks were kept in incubator at 37°C overnight for incubation. The contents present within the flask were initially filtered through the uniform layers of muslin cloth and then with the Whatman's filter paper. The resultant filtrate was evaporated in rotary evaporator and the weight of residues was also recorded. To dissolve the residues of methanol, DMSO (Dimethyl sulfoxide) was used.

Placement of the test materials and incubation

Each freshly mixed experimental material was placed into the wells of petri dishes. The material was allowed to diffuse into the Mueller Hinton agar and incubated for 24 hours at 37°C.

The quantity of materials dispensed is as follows: Henna extract- 500 µl; Positive control (0.12% chlorhexidine digluconate) - 20 µl; Negative control (Normal physiologic saline) – 500 µl

Inoculation of bacterial strain onto culture media

Each agar plate with Mueller Hinton agar was inoculated with 0.1mL microbial suspension matching McFarland turbidity standard of 0.5 using sterile swab and was allowed overnight at 37°C.

Measuring zone of inhibition

The diameter of zones of inhibition formed was measured in mm by measuring the shortest distance between the outer margin of the well and initial bacterial growth after 24 hours of incubation**.** (Fig. 1)

RESULTS

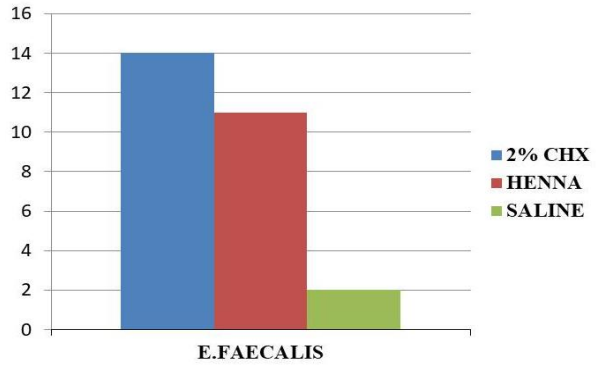
Chlorhexidine gluconate (positive control) had shown 14 mm of zone of inhibition whereas saline (negative control) had shown only 2 mm of zone of inhibition. The extracts prepared from *Lawsonia inermis* showed 11 mm zone of inhibition against the bacterial strain of *Enterococcus faecalis* (Table 1; Fig. 1).

Table 1: Table showing the inhibition zones of the test groups.

Test groups	Zone of inhibition
Henna extract	11 mm
Chlorhexidine gluconate	14 mm
Normal saline	2 mm

Figure 1: Zone of inhibition (mm) exhibited by Henna, chlorhexidine gluconate and saline against E. faecalis.

Graph 1 depicts the zone of inhibition between the three groups. Chlorhexidine as a positive control and as a standard irrigating agent has shown a marked and highest zone of inhibition. On the other hand, the extract from *L. inermis* has shown a relatively distinct zone of inhibition of about 11 mm. The antimicrobial activity of these plants could be attributed to the presence of secondary metabolites and the active constituent of these secondary metabolites which include phenolic compounds and tannins. In henna the major chemical components present are lawsone (2-hydroxynaphthoquinone), mannite, mucilage gallic acid and tannic acid which aid in its antibacterial property (10).



Graph 1: Graph showing the inhibition zones of the test groups

DISCUSSION

Pulpectomy is indicated in deciduous teeth when pulp has become irreversibly denatured, infected or necrotic due to caries, trauma, or other causes. For the better success of pulp therapy, substances with antimicrobial/ antibacterial properties are advocated as intracanal irrigants in deciduous teeth. *E. faecalis* was chosen as a test strain in this study, as it is a primary endodontic pathogen which causes persistent endodontic infection. Its prevalence in recurrent infection ranges from 24% to 77%. It can survive and grow within dentinal tubule and reinfect an obturated root canal. *E. faecalis* was reported to be the predominant human enterococcus flora causing oral diseases, such as dental caries, endodontic infections, periodontitis, and peri-implantitis. Most importantly, *E. faecalis* rapidly colonizes dentinal tubules compared to other species and therefore is particularly difficult to eradicate. It has been often noticed in failure of endodontic treatment, due to high resistance to endodontic medicaments, and its ability to form biofilms both in treated and untreated root canals (12).It represents a classic standard against which the antimicrobial action of a medicament should be tested.

In the present study, to assess the antimicrobial activity of Henna, agar diffusion method was used because it is one among the commonly used authentic methods. The agar disc diffusion test works on the principle where the material to be tested will diffuse through the paper disk or small well into an agar medium containing the test organisms. However, the results are often influenced by the degree of diffusion of material across the medium (13). Till date, there is no single irrigating agent in the market that sufficiently covers all functions of an ideal irrigant. A wide range of synthetic antimicrobial agents were used over the years as endodontic irrigants. Due to the incidence of increased antibiotic resistance to these antimicrobial agents, there is a demand for the search of alternative agents which are affordable, non-toxic, and effective.

There are several studies that have highlighted the effects of various herbal irrigants. Neelakantan *et al.*,

conducted an *in vitro* study where they showed that curcumin has eminent anti-bacterial activity against *E. faecalis* and could be used as an adjunct to sodium hypochlorite for root canal irrigation in primary teeth. (14). In another study by Al-Qathami and Al-Madi, assessed the anti-microbial efficacy of propolis, sodium hypochlorite and saline as endodontic irrigants and concluded Propolis showed an anti-microbial activity on par to that of sodium hypochlorite (15). Some authors have observed that ethanolic extract of neem had significant anti-microbial activity against E. *faecalis*.

In the present study, the commercially available dried leaves of *L. inermis* were obtained and were used to prepare the *henna* extracts. According to the existing literature it has been proven that preparation from the dried leaves have more concentrated and active phytochemical and biological compounds than fresh plant material (16). The findings of Papageorgiou *et al.,* revealed that the phytochemical constituents of *L. inermis* exhibited antimicrobial activity against gram positive bacteria, which is in accordance with the results obtained in this study. The study results of Bhuwaneshwari *et al.,* Habbal *et al.,* and Hussain *et al.,* firmly suggests the antibacterial activity of *L.inermis* against different bacterial strains which support the findings of the present study (17).

Limitations

It is known that about 25% of all medicines available in the market have been derived directly or indirectly from plants. Herbal medicines are generally believed as safe. Besides, it is mandatory to evaluate their biological safety to avoid fatal consequences. Also, the staining property of *henna* due to its 'lawsone molecule' must also be studied. Evidence shows that *L. inermis* (Henna) has immense pharmacological properties, but its toxicological assessment is also indispensable. Hence, further *in vivo* studies with larger sample size are needed to evaluate the antimicrobial efficacy of *henna,* in clinical settings.

CONCLUSION

In conclusion, the present study emphasizes the scientific rational of *L. inermis* for its medicinal and therapeutic use. The zone of inhibition of henna against *E. fecalis* was found to be 11mm whereas the positive control had 14mm. Therefore, the herbal extracts of *L. inermis* can be of great significance as an alternative antimicrobial agent in therapeutics and in dentistry. Further clinical trials are required for *henna* to be considered an efficient root canal irrigants.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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