ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 10 | October - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

EVALUATION OF ANTIBACTERIAL EFFICACY OF NEEM,TURMERIC AND WHEAT GRASS EXTRACT AGAINST ENTEROCOCCUS FAECALIS - AN INVITRO STUDY

Dentistry

Dr Sravani Nirmala — Assistant Professor, Department of Conservative Dentistry and Endodontics SVS Institute of Dental sciences Mahabubnagar.

Dr Naresh Kumar* — Reader, Department of conservative Dentistry and Endodontics SVS Institute of Dental sciences Mahabubnagar. *Corresponding Author

Dr M.Narender Reddy — Professor, Department of Conservative Dentistry and Endodontics SVS Institute of Dental sciences Mahabubnagar.

Dr C.Rakesh Reddy — Associate, Department of Conservative Dentistry and Endodontics SVS Institute of Dental sciences Mahabubnagar.

Dr D.Sainath — Professor, Department of Conservative Dentistry and Endodontics SVS Institute of Dental sciences Mahabubnagar.

Dr Sneha Pallepagu — Assistant Professor, Department of Conservative Dentistry and Endodontics Malla reddy Institute of Dental Sciences Hyderabad

ABSTRACT

Aim: The aim of the study is to evaluate the antimicrobial efficacy of Neem, Turmeric and Wheat grass extract against Enterococcus faecalis using Agar well diffusion method. **Materials and Methods:** E. faecalis American Type Culture Collection 21292 was inoculated onto brain heart infusion agar plate. Ethanolic extract of Neem , Turmeric and Wheat grass were prepared by adding 20grams of powders in 20ml of ethanol , incubated for about 48hrs.Absolute ethanol was taken as controls.50 µl of the inoculum of E faecalis was spread over petri plates with the help of L-arm loop. Wells of 5 mm diameter were punched using a sterile cork borer and 50 µl of the prepared herbal medicaments were added to the wells incubated at 37°C aerobically for 48h and growth inhibition zones were measured. Statistical analysis was done using ANOVA test complimented by Post hoc tukeys test. **Results:** Neem has shown highest zone of inhibition followed by turmeric. Wheat grass has shown limited zone of inhibition. **Conclusion:** wheat grass has limited antibacterial properties against Enterococcus faecalis using lesser concentration of the prepared solution. Further studies should be carried out for the better antibacterial properties with increased concentrations

KEYWORDS

Enterococcus faecalis, Wheat grass juice, Neem, Turmeric, Ethanol.

INTRODUCTION

Majority of bacterial species play a crucial role in the causation of pulp and periapical diseases. The penetrating ability of these bacteria and the anatomical variations inside the root canal system increased there survival beyond the host defense mechanism.

Enterococcus faecalis a gram positive facultative anaerobe is associated with persistent periradicular infections.its prevalence is nine times more in failed endodontic treatments and studies have investigated its occurrence to be 24-77% in root filled teeth(1-2).

The most commonly used conventional endodontic irrigant, sodium hypochlorite (NaOCl) is highly efficient in eliminating biofilms of E. faecalis.But, the major disadvantages of NaOCl is its toxicity, unpleasant taste and odor, corrosion of instruments, inability to remove the smear layer, reduction in the elastic modulus, and flexural strength of dentin(3).

The other most commonly used irrigant is a cationic bisbiguanoid that's Chlorhexidine (CHX) which has a broad spectrum of antimicrobial activity and demonstrates substantivity. It overcomes the disadvantages of NaOCl, but its inefficiency in dissolving the organic content and also discoloration of the teeth are its main drawbacks(4).

Pharmacological studies confess the importance of medicinal plants as probable source of bioactive compounds. The constant increase in antibiotic resistant strains and side effects caused by synthetic drugs enlight the researchers for herbal alternatives. These herbal plant extracts has antibacterial and therapeutic effects suggesting its potential to be used as an endodontic irrigant(3).

Azadiracta indica (Neem) is the most popularly used traditional medicinal plant in India. There are nearly 140 biologically active compounds in Neem. The presence of alkaloids,glycosides, saponins, flavonoids, steroids, anthraquinone,tannic acid and active constituents such as nimbidin, nimbin, nimbolide etc aid for its antibacterial properties (3,5).

Curcuma longa (commonly called as turmeric) extract had considerable antimicrobial properties which is attributed to its biologicial active compound Curcumin. . It has a potent antibacterial activity against a number of pathogenic bacteria including Enterococci(6).

Triticum aestivum is a young grass of wheat plant which grows rich in vitamins and minerals, A,E,C,K, and, calcium, phosphorous, selenium, magnesium,iron,sodium,potassium,zinc,sulphur and with full spectrum of B vitamins. It has a complete source of protein, consisting of all the essential amino acids and is chemically active and contains over 80 enzymes such as protease, amylase, lipase, cytochrome oxidase, transhydrogenase, superoxide dismutase. Wheatgrass juice is the KING OF LIVING JUICES because of its antibacterial, anti-inflammatory, anti-hypertensive, anti-carcinogenic, detoxifier, immune boosting, and anti-aging properties. It is particularly high in 70% chlorophyll and considered as green blood, which cleanses and builds the blood. It is the first product of light and therefore contains more healing properties than any other element.It builds RBC which carry oxygen to every cell and maintains cellular function at an optimal level in highly oxygenated environment. It has the ability to repair and heal the damaged cells(7-9).

The major advantages of herbal irrigants are safety, ease of availability, prolonged shelf-life, economical, and lack of microbial resistance so far(10). The aim of the study is to evaluate the antimicrobial efficacy of Neem, Turmeric and Wheat grass extract against Enterococcus faecalis using Agar well diffusion method.

MATERIALS AND METHODS

Procurement Of The Microorganism

A pure culture of E. faecalis (ATCC 29212)was inoculated on BHI agar plates, incubated at 37°C overnight for 24 hrs

Preparation Of Extracts

Ethanolic extract of Neem , Turmeric and Wheat grass were prepared by adding 20gram of powders in 25ml of ethanol and the extracts were placed in airtight container for 48hrs. Absolute ethanol was taken as control. 50 µl of the inoculum of E faecalis was spread over petri plates

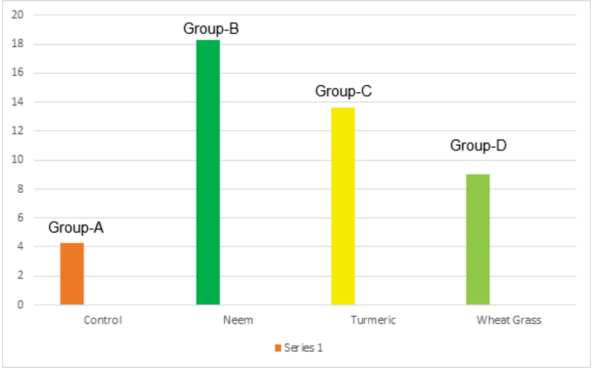
with the help of L-arm loop.

Antibacterial Assay
Wells of 5 mm diameter were punched using a sterile cork borer and 50 µl of the prepared solutions were added to the wells with the help of a micropipette as follows:
Group A- Ethanol (control)
Group B - Neem
Group C- Turmeric
Group D- Wheat grass

The plates were then incubated at 37^{0}C for 24hrs and the diameter of bacterial inhibition zones were recorded to the nearest size in millimetres using vernier callipers.

STATISTICALANALYSIS
The data was statistically analysed using ANOVA test complimented by Post hoc tukeys test.

RESULTS
Neem has shown highest zone of inhibition followed by turmeric. Wheat grass has shown limited zone of inhibition. There is no statistical difference among different groups.



Graph-1 Zone of inhibition (in millimeter) of herbal extracts and ethanol against Enterococcus faecalis.

DISCUSSION
E.faecalis was selected as the test organism because it is a facultative anaerobe that is nonfastidious, easy to grow resist high environmental conditions and efficiently and rapidly colonizes into the dentinal tubules. It has been choosen as test species because of its high prevalence of 63% in post endodontic treatment diseases and due to the high level of resistance to a wide range of antimicrobial agents. ATCC 29212 strains of E.faecalis which has been the standard strain used in the previous studies was selected for the present study(11).

The agar diffusion method permits direct evaluation of the materials beside the organisms, representing the potential of the test materials to remove microorganisms in the local microenvironment of the root canal system. The results may differ based upon the capacity of the material to diffuse across the medium and not only on the toxicity of the material for the specific organism(2).

In the present study neem has shown the highest zone of inhibition when compared to the other groups and the results are in accordance with the study done by vinothkumar et al. Similar results are seen in a study done by Archana et al and Arindam et al(3,5,12).The neem extracts has gone through extensive pharmacological screening and found to have several medicinal properties due to the presence ofseveral active constituents like nimbidin, nimbin, nimbolide,gedunin, azadirachtin, mahmoodin, margolone andcyclictrisulphide responsible for its antibacterial action. It has an anti-adherence activity by altering bacterial adhesion and the ability of organism to colonize has resulted in maximum reduction in adherence of E. faecalis to dentin(3).

Curcumin, has shown good zone of inhibition when compared to that of Group-A and Group-D but it was less when compared to that of Group-B and similar results were seen in a study done by vinothkumar et al and Sharmila et al(3,13). Similar results were seen in a study done by Neelakantan et al who concluded that curcumin in combination with NaOCl had synergistic effect on E.feacalis formed biofilms. Curcumin is a yellow bioactive pigment,is a polyphenolic compound, which strongly inhibits bacterial cell proliferation by inhibiting the assembly dynamics of FtsZ in the Z-ring responsible for bacterial cell division such as anti-inflammatory, antioxidant, antifungal and antibacterial activities[6,3].

In the present study a noval ingrediant wheat grass juice exhibited limited zone of inhibition against E faecalis when compared to that of other groups. But the zone of inhibition was higher when compared to that of control group.In a study they have concluded that fresh and undiluted wheatgrass juice exhibited mild antibacterial activity against Staphylococcus aureus, Bacillus cereus, Salmonella typhimurium and Kleibsella pneumoneae(7). Sharma et al concluded that wheat-grass has antibacterial activity against many common human pathogens. Its antimicrobial properties are attributed to its high content of Bioflavonoids which include Apigenin, Quercitin, Luteonin (8).

However, the major drawbacks of herbal irrigants is that, the fresh solutions has to be prepared each time, the unacceptable odour and taste, short shelf life have to be overcome . Flavouring agents need to be added to make it palatable and acceptable by the patient. More research has to be done to prolong the shelf life of these irrigants so that these irrigants are more widely accepted[11].

CONCLUSION
Within the limitations of the present study it can be concluded that wheat grass has limited antibacterial properties against Enterococcus faecalis using lesser concentration of the prepared solution. Further studies should be carried out for the high antibacterial properties with increased concentration.

REFERENCES
1. Comparative evaluation of antimicrobial efficacy of three herbal irrigants in reducing intracanal E. faecalis populations: An in vitro study. Alpa Gupta-Wadhwa , Jitesh Wadhwa , Jigyasa Duhan J Clin Exp Dent. 2016;8(3):e230-5.
2. Antibacterial Efficacy of Neem, Tulsi Extract, Aleo Vera, Turmeric and 5% Sodium Hypochlorite Against Enterococcus faecalis: An in-vitro Study.Ravikumar K1, Paul U2, Nanda SS3, Selvan AS4, Revankar VD4, Mallikarjunan DY1, Ranjith G1. Int J Cur Res Rev | Vol 13 Issue 05 March 2021.
3. In vitro evaluation of five different herbal extracts as an antimicrobial endodontic irrigant using real time quantitative polymerase chain reaction. Thilla S Vinothkumar, Mohamed I Rubin, Lakshmi Balaji, Deivanayagam Kandaswamy. Journal of Conservative Dentistry | Mar-Apr 2013 | Vol 16 | Issue 2.
4. Antibacterial Effect of Azadirachta indica (Neem) or Curcuma longa (Turmeric) against Enterococcus faecalis Compared with That of 5% Sodium Hypochlorite 2% Chlorhexidine in vitro. Dakshita Joy Sinha, Kanwar D.S.Nandha,Natasha Jaiswal et al.Bull Tokyo Dent Coll(2017)58(2):103-109.
5. Azadirachta indica A. juss, Morinda citrifolia L and Triphala as herbal endodontic irrigants. Archna Agnihotri, Swaty Jhamb,Urvashi Shrama, and Sumidha Rohtagi: A scoping review 2020 Jul-Sep; 41(3): 148–158.
6. Effectiveness Of Curcumin Against Enterococcus Faecalis Biofilm.Prasanna Neelakantan , Chandana Subbarao , Subash Sharma , February 2013,Acta Odontologica Scandinavica 71(6).
7. Wheat Grass- A Perfect Food And Its Anti-Microbial Properties From The Different Solvent Extract.Dr. Amit Kumar Dutta And Dr. Wasim Raja. World Journal Of Pharmacy And Pharmaceutical Sciences Vol 5, Issue 9, 2016.
8. Evaluation of Anthocyanin Content, Antioxidant Potential and Antimicrobial Activity of Black, Purple and Blue Colored Wheat Flour and Wheat-Grass Juice Against Common Human Pathogens. Natasha Sharma, Vandita Tiwari, Shreya Vats. Molecules 2020, 25, 5785.
9. Nutritional Quality and Antioxidant Activity of Wheatgrass (Triticum aestivum) Unwrap by Proteome Profiling and DPPH and FRAP assays Santosh B. Parit, Vishal V. Dawkar , Rahul S. Tanpure et al. Journal of Food Science Vol. 0, Iss. 0, 2018.
10. An in vitro evaluation of antimicrobial activity of five herbal extracts and comparison of their activity with 2.5% sodium hypochlorite against Enterococcus faecalis Divya Saxena, Suparna Ganguly Saha, Mainak Kanti Saha et al. Indian J Dent Res 2015;26:524-7.
11. A Comparative Evaluation And Effectiveness Of Different Antimicrobial Herbal Extracts As Endodontic Irrigants Against Enterococcus Faecalis And Candida Albicans- An In-Vitro Study. Suresh Pandey, Rhitu Shekhar, Rohit Paul, et al.University J Dent Scie 2018; Vol. 4, Issue 2.
12. Comparative anti-microbial efficacy of Azadirachta indica irrigant with standard endodontic irrigants: A preliminary study Arindam Dutta, Mala Kundabala1Journal of Conservative Dentistry | Mar-Apr 2014 | Vol 17 | Issue 2.
13. Antibiofilm efficacy of photoactivated curcumin, triple and double antibiotic paste, 2% chlorhexidine and calcium hydroxide against Enterococcus fecalis in vitro Sharmila Devaraj, Nithya Jagannathan & Prasanna Neelakantan.Scientific Reports volume 6, Article number: 24797 (2016).