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Evaluation of antimicrobial efficacy of four different natural extracts against persistent root canal pathogens: E. faecalis and Candida albicans

[1]Arun Teja S, Post Graduate student, Department of conservative dentistry and endodontics, Krishnadevaraya college of dental sciences, Bangalore, India

[2]Manje Gowda, Reader, Department of conservative dentistry and endodontics, Krishnadevaraya college of dental sciences, Bangalore, India

[3]Jayalakshmi K.B, Professor and HOD, Department of conservative dentistry and endodontics, Krishnadevaraya college of dental sciences, Bangalore, India

[4]Arul Selvan, Professor and HOD, Department of microbiology, Krishnadevaraya college of dental sciences, Bangalore, India

[5]Prasanna Latha Nadig, Professor, Department of conservative dentistry and endodontics, Krishnadevaraya college of dental sciences, Bangalore, India

Corresponding Author: Arun Teja S, Post Graduate student, Department of conservative dentistry and endodontics, Krishnadevaraya college of dental sciences, Bangalore, India

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Abstract

Plants produce a diverse range of bioactive molecules, making them rich source of different type of medicines. Most of the drugs today are obtained from natural sources or semisynthetic derivatives of natural products and are being used in traditional system of medicine. Thus, it is a logical approach to screen traditional natural products in endodontic treatments. Root canal infections are multi-microbial. E. faecalis and candida albicans are the most commonly isolated microbes from root canals of failed endodontic treatment cases. The aim of the study is to compare the antimicrobial efficacy of natural extracts of bark of Cinnamomum, seeds of Nutmeg, seeds of Bitter melon, Guava leaf extract and 5.2% Sodium hypochlorite against E. faecalis and Candida albicans

Keywords: Antimicrobials, Agar diffusion, E. faecalis, Herbal extracts.

Introduction

The success of root canal treatment depends on complete elimination of microorganisms in the root canal environment followed by three dimensional obturation providing an appropriate environment for tissue healing.[1,2]

However complete elimination of microorganisms from infected root canals is a complicated task which can be effectively achieved by combining mechanical instrumentation with chemical irrigants.[3,4] Numerous measures have been described to reduce the number of microorganisms in the root canal system, including the use of various instrumentation techniques, irrigation regimens, and intracanal medicaments.[5] Irrigation dynamics plays an important role and complementary to instrumentation in facilitating the removal of pulp tissue and/or microorganisms.[4] Primary root canal infections are polymicrobial, typically dominated by obligatory anaerobic bacteria. On the other hand, facultative bacteria such as non mutans Streptococci, Enterococci and Lactobacilli, once established, are more likely to survive chemo mechanical instrumentation and root canal medication.[3,4] Enterococcus faecalis, a facultative anaerobic gram-positive coccus and Candida albicans are the most commonly isolated species in persistent root canal infections. The most widely used endodontic irrigant is 0.5% to 6.0% sodium hypochlorite (NaOCl), because of its bactericidal activity. However, adverse effects of NaOCl have been reported including unpleasant odor and taste, toxicity, possible paresthesia of the mandibular nerve, allergy, and an increase in coronal microleakage of adhesive restorations.[4,5,6] Chlorhexidine when mixed with sodium hypochlorite produces a carcinogenic product, i.e., parachloroanaline.[5]

Because of the cytotoxic reactions of most of the commercial irrigants used and their inability to totally eliminate bacteria from root canals, recent medicine attempts to use biologic irrigants extracted from natural plants. In dentistry, phytomedicines have been used as anti-inflammatory, antibiotic, analgesic, and sedative agents.[1,2] Hence, the present study is aimed to compare the antimicrobial efficacy of natural extracts of bark of cinnamomum, seeds of nutmeg, seeds of bitter melon, guava leaf extract and 5.2% sodium hypochlorite against E.faecalis and candida albicans.

Methodology

Preparation of cinnamomum bark extract: Fresh cinnamomum bark sticks were cleaned and reduced in size by cutting into small parts and then dried under the shade. The bark sticks were coarsely powdered with the help of a blender. Coarse powder of sticks was then exhaustively extracted in the soxhlet apparatus. In this apparatus 250g of dried powder was extracted using 500ml of ethyl alcohol and distilled water. The extracts were concentrated by distilling the solvents, and preserved under refrigeration for further studies.

Preparation of Nutmeg seed extract

The seeds were cleaned and reduced in size by cutting into small parts and then dried under the shade. The seeds were coarsely powdered with the help of a blender. This powder was then exhaustively extracted in the Soxhlet apparatus. In this apparatus 250g of dried powder was extracted using 500ml of ethyl alcohol and distilled water. The extracts were concentrated by distilling the solvents and preserved under refrigeration for further studies.

Preparation of Guava leaf extract

Fresh guava leaves were collected, properly washed under tap water and rinsed in sterile distilled water. Leaves were dried in fresh open air, protecting from direct exposure to sunlight. The leaves were coarsely powdered with the help of a blender.50g of powdered leaves was taken into a beaker containing 500ml of distilled water. Prepare the hot water extract till the menstrum was reduced to about 125ml. After complete evaporation the resultant liquid was filtered with filter paper.

Preparation of Bitter melon seed extract

The seeds were cleaned and reduced in size by cutting into small parts and then dried under the shade. The seeds were

coarsely powdered with the help of a blender. This powder was then exhaustively extracted in the Soxhlet apparatus. In this apparatus 250g of dried powder was extracted using 500ml of ethyl alcohol and distilled water. The extracts were concentrated by distilling the solvents and preserved under refrigeration for further studies.

E.faecalis and candida albicans strains were obtained and inoculated in culture plates with set layers of Mueller-Hinton agar and Sabouraud dextrose agar respectively. E.faecalis and candida albicans strains were subcultured in Butylated hydroxyanisole agar broth and sarbouraud dextrose broth respectively and incubated at 37oc for 4hrs. The turbidity of the culture medium was adjusted to McFarland opacity standard,0.5 for E.faecalis and 1 for C.albicans. 30ml of Mueller-Hinton agar and sabouraud dextrose agar was freshly prepared and autoclaved. The media was cooled to 50oc and 25microlitres of the adjusted microbial cultures were added to medium and poured into sterile petriplates of 90mm diameter. After the media has set completely, wells of 6mm diameter were made using sterile templates. The wells were inoculated with 50microlitres of experimental irrigants.

After incubation period, plates was checked for zones of inhibition of microbial growth and diameter of zones achieved by each group against E.faecalis and Candida albicans, which was recorded in mm and each experiment was repeated thrice.

The solutions were divided into five different groups:

GROUP-1: Cinnamomum bark extract.

GROUP-2: Nutmeg seed extract.

GROUP-3: Guava leaf extract.

GROUP-4: Bitter melon seed extract.

GROUP-5: 5.2% sodium hypochlorite.

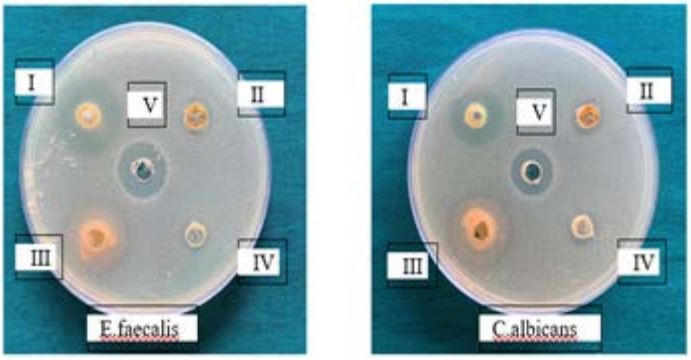


Figure 1: Zone of inhibition

Statistical Analysis

One-way ANOVA test followed by Tukey's post hoc analysis was used to compare the mean Zone of Inhibition (in mm) for E. Faecalis and C. Albicans between different study groups.

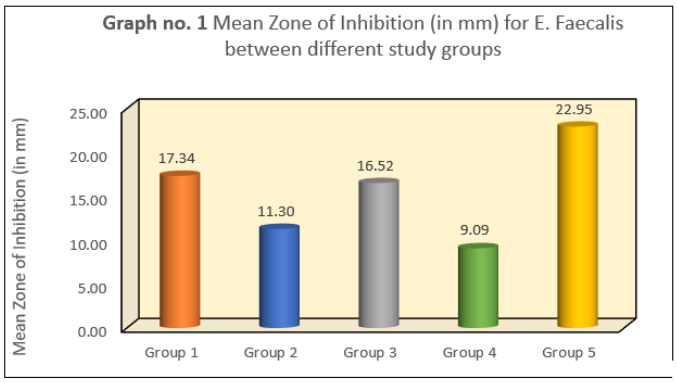
The level of significance was set at P<0.05.

Result

The mean Zone of Inhibition (in mm) for E. Faecalis between different study groups demonstrates that Group 5 shows significantly highest mean zone of Inhibition compared to other study groups at P<0.001, this followed by Group 1 showing significantly higher mean Zone of Inhibition (in mm) compared to other study groups at P<0.001 except for Group 3 [P=0.17], and this was followed with Group 3 and Group 2 showing significantly higher mean zone of Inhibition compared to Group 4 at P<0.001(Refer Table & Graph 1)

Table no. 1 Comparison of mean Zone of Inhibition (in mm) for E. Faecalis between different study groups using One-way ANOVA test

Groups	N	Mean	SD	Min	Max	P-Value
Group 1	3	17.34	0.49	16.9	17.9	<0.001*
Group 2	3	11.30	0.15	11.2	11.5	
Group 3	3	16.52	0.54	15.9	16.8	
Group 4	3	9.09	0.46	8.6	9.5	
Group 5	3	22.95	0.27	22.8	23.3	



* - Statistically Significant

Table & Graph no. 1: compares the mean Zone of Inhibition (in mm) for E. Faecalis between different study groups.

The mean Zone of Inhibition (in mm) for C. Albicans between different study groups demonstrates that Group 5 shows significantly highest mean zone of Inhibition compared to other study groups at P<0.001, this followed by Group 3 showing significantly higher mean Zone of Inhibition (in mm) compared to other study groups at P<0.001 and this was followed with Group 1 showing significantly higher mean zone of Inhibition compared to Group 2 and Group 4 at P<0.001.

However, no significant difference was observed between Group 2 and Group 4 [P=0.66] [Refer Table & Graph 2]

Table no.2 Comparison of mean Zone of Inhibition (in mm) for E. Faecalis between different study groups using One-way ANOVA test

Groups	N	Mean	SD	Min	Max	P-Value
Group 1	3	16.58	0.29	16.2	16.8	<0.001*
Group 2	3	8.77	0.48	8.4	9.3	
Group 3	3	18.20	0.27	17.9	18.4	
Group 4	3	8.37	0.43	8.0	8.9	
Group 5	3	20.53	0.31	20.2	20.8	

*-Statistically Significant

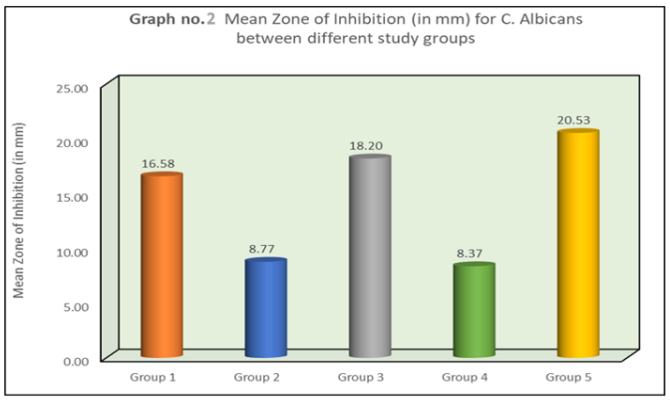


Table & Graph no. 2: compares the mean Zone of Inhibition (in mm) for C. Albicans between different study groups.

Discussion

The success of the root canal treatment depends up on the effectiveness of endodontic files, rotary instrumentation, irrigating solutions and chelating agents to clean, shape, and disinfect root canals which underpins the success, longevity, and reliability of modern endodontic treatments.[5] Elimination of microorganisms from infected root canals is a complicated task and failure to eliminate these microorganisms may result in root canal treatment failure. [5,6,7]

The root canal system is complex, and accessory features such as fins, cul de sacs, and intercanal communications are colonized by microorganisms once the tooth becomes infected. Self-aggregates of monobacterial morphotypes and coaggregates of different bacterial morphotypes are also found adhering to teeth. [2,5,7,8] The interbacterial spaces are occupied by an amorphous material, spirochetes, and hyphal-like structures that are suggestive of fungi. [9,10]

Enterococcus faecalis is a microorganism commonly detected in asymptomatic, persistent endodontic infections. Its prevalence in such infections ranges from 24% to 77%.[2,3,4] Enterococcus gram positive cocci can

occur singly, in pairs, or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen. Enterococci survive very harsh environment including extreme alkaline pH (9.6) and salt concentrations. They resist bile salts, detergents, heavy metals, ethanol, azide, and desiccation. They can grow in the range of 10oC to 45°C and survive a temperature of 60°C for 30min.[7,8]

It possesses serine protease, gelatinase, and collagen-binding protein (Ace), which helps it bind to dentin. It is small enough to proficiently invade and live within the dentinal tubules. It has the capacity to endure prolonged periods of starvation until an adequate nutritional supply becomes available. Once available, the starved cells are able to recover by utilizing serum as a nutritional source. Serum, which originates from alveolar bone and the periodontal ligament, also helps E.faecalis bind to type I collagen. E.faecalis is able to form a biofilm that helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than non- biofilm producing organisms.[11]

Waltimo et al. revealed the presence of fungi in 5% - 20% of infected root canals concomitant with apical periodontitis.[9] Candida albicans (CA) has a major role in endodontic treatment failure as the most important fungus isolated from the root canal system.

Egan et al. carried out a study to determine the presence of C.albicans in teeth requiring root canal treatment, with and without periapical lesion. The study revealed that the prevelance of C.albicans was 13.8 times higher in patients with periapical lesion.[10]

C.albicans adapt to an extreme range of pH, low oxygen and nutritional environment. Candida is polymorphic fungus that exists in blastophores, germ tubes, true hyphae, pseudohyphae and chlamydospores depending on environmental conditions. This provides the ability to penetrate dentinal tubules via hyphal adherence and able to bind to collegen type I and IV. Candida species has ability to produce secreted aspartyl proteases, collegenases, hyaluronidases, acid and alkaline phosphatases which helps to degrade variety of host dentinal collagen and other extracellular proteins.[12]

Among various chemical root canal irrigants available sodium hypochlorite (NaOCl) and 2% chlorhexidine (CHX) are successfully used in endodontics. NaOCl is the gold standard antimicrobial agent with tissue dissolving properties and is widely used as root canal irrigating solution.[13,14,15] Ideal root canal irrigants should be biocompatible, nontoxic with a desirable taste and smell. Chemical irrigants, even though effective in root canal irrigation, are associated with several disadvantages. NaOCl causes allergic reaction, tissue toxicity, staining of instruments, irritation to periapical tissue, inability to remove smear layer, and has an undesirable taste and smell.[13] Constant increase in antibiotic resistant strains and side effects of chemical irrigants has led to the search for alternative herbal medicaments. Antimicrobial properties of herbal extracts, such as Cinnamomum bark extract, Nutmeg seed extract, Guava leaf extract, Bittermelon seed extract were compared in this current study. Among the study groups compared, cinnamomum bark extract and guava leaf extract showed the highest antimicrobial acitivity against both E.faecalis and C.albicans where as nutmeg seed extract and bittermelon seed extract has shown the least antimicrobial activity among all other groups. Sodium hypochlorite is used as a control group which has shown the highest zone of inhibition compared to all the other experimental groups.

Based on the results obtained, the high antibacterial activity observed in cinnamomum extract may be due to the action of trans cinnamaldehyde, considered as its single major compound. It has been reported that trans

cinnamaldehyde possesses the highest antimicrobial action in comparison with other constituents of cinnamomum.[16,17] Usually, Gram-negative bacteria are more resistant to plant extracts and their constituents than Gram-positive bacteria, because the cell wall of Gram-negative bacteria is more complex. Porin proteins serve as hydrophilic transmembrane channels for small hydrophilic solutes, which easily pass through the outer membrane of Gram-negative bacteria; however, it is hard for hydrophobic antibiotics to penetrate the cell and this is one of the reasons that makes Gram negative bacteria more resistant. Trans cinnamomum- a commonly studied phenylpropene molecule, rich in phenolics is able to pass through the phospholipid bilayer of bacterial cell wall and bind to proteins, to prevent them from performing their normal functions.[17] The antimicrobial efficacy of cinnamomum extract against candida albicans is also studied in this study where cinnamomum extract was more effective against E.faecalis compared to candida albicans, which is in contrast to the study done by zinnat et al where cinnamomum bark extract exhibited potent inhibitory activity against Candida albicans. The antifungal activity might be because cinnamomum bark extract has been shown to alter cell membrane permeability, fluidity, and inhibit biofilm formation. Cinnamaldehyde, the major constituent of cinnamomum bark extract, targets the membrane and causes increased cell wall thickness in C.albicans, this can be attributed to β-1- 3-glucan synthase inhibition as observed in Saccharomyces cerevisiae. The increase in bud scar formation upon cinnamaldehyde exposure also suggests an impact on cell division, resulting in decreased viability. Other constituents like Benzyl benzoate and Linalool affect membrane fluidity and induce cell cycle arrest at the G2-M and G1 phases, respectively at concentrations greater than the minimum inhibitory concentration (MIC).[16,18] The other experimental group which showed greater antibacterial activity was Guava leaf extract which has showed the mean zone of inhibition of 16.52 ± 0.54 for E.faecalis and 18.20 ± 0.27 for Candida albicans which is almost similar to the cinnamomum bark extract . The anti-bacterial activity of guava leaf extract against E.faecalis might be due to the flavonoids such as mosin glycosides, quercetin, and quercetin glycosides. The aqueous extracts of guava leaf can cause a marked reduction in the adhesion of the early organisms of plaque biofilm formation.

One of the main virulence factors of Candida is the cell dimorphism, which depends on the environmental conditions in which microorganisms are found growing. A study done by Maria et al has proved that based on the results of the MIC, the tests were performed to verify that the Guava extracts influenced the morphological transition of yeast. Guava leaves extract has affected the phenotypic plasticity of C.albicans and C.tropicalis reducing hyphae and pseudohyphae formation process in so far as their concentrations were increased. [19,20,21]

Other herbal extracts which was used in this study was nutmeg seed extract. The antimicrobial activity of these nutmeg seed extracts showed least anti-microbial activity when compared to cinnamomum and guava leaves extract. The mean zone of inhibition for nutmeg seed extract was 11.30 ± 0.15 for E.faecalis and 8.77 ± 0.48 for candida albicans. This might be due to poor diffusion of the extract into the surrounding agar from the wells owing to least zone of inhibition against tested microrganisms.

Bitter melon seed extract also exhibited least antimicrobial activity in this study. The mean zone of inhibition observed for both E.faecalis and Candida albicans found to be 9.09 ± 0.46 and 8.37 ± 0.43 respectively. Bitter melon contains glycosides, terpenoids, and momordicin-1 which could be responsible for its “medicinal properties”. Momordicin inhibits the production of ribosomal proteins.

It is postulated that this inhibition of protein may make bitter melon bacteriostatic. However, the reason for the poor antimicrobical activity of bitter melon is still unknown.[22]

The findings of this study concluded that herbal extracts like cinnamomum bark extract and guava leaf extract exhibited highest antimicrobial activity against both E.faecalis and Candida albicans. Hence cinnamomum and guava leaf extracts can be effectively used as a root canal irrigant. However further studies will be required to evaluate the reason for the least antimicrobial activity of nutmeg and bittermelon seed extract.

Conclusion

Within the limitation of this study, the following conclusions can be made

i. The Ethanol extract of Cinnamomum bark has a significant antimicrobial efficacy against both E.faecalis and C.albicans.

ii. The aqueous extract of Guava leaves has a significant antimicrobial efficacy against both E.faecalis and C.albicans.

iii. The Ethanol extracts of Nutmeg seeds and Bittermelon seeds were not effective against both E.faecalis and C.albicans.

References

1. Ahmed F, Thosar N, Baliga MS and Rathi N. Single Visit Endodontic Therapy: A Review. Austin J Dent. 2016; 3(2): 1035.
2. Narayanan LL, Vaishnavi C. Endodontic microbiology. J Conserv Dent. 2010 Oct;13(4):233-9. doi: 10.4103/0972-0707.73386. PMID: 21217951; PMCID: PMC3010028.
3. Gomes BPFA, Herrera DR. Etiologic role of root canal infection in apical periodontitis and its relationship with clinical symptomatology. Braz. Oral Res. 2018;32(suppl):e69
4. Muliyar S, Shameem KA, Thankachan RP, Francis PG, Jayapalan CS, Hafiz KA. Microleakage in endodontics. J Int Oral Health. 2014 Nov-Dec;6(6):99-104. PMID: 25628496; PMCID: PMC4295468.
5. Jaju S, Jaju PP. Newer root canal irrigants in horizon: a review. Int J Dent. 2011;2011:851359. doi: 10.1155/2011/851359. Epub 2011 Nov 30. PMID: 22190936; PMCID: PMC3235459.
6. Zahed Mohammadi Yazd, Sodium hypochlorite in endodontics: an update review. International Dental Journal (2008) 58, 329-341
7. Portenier et al. Enterococcus faecalis – the root canal survivor and ‘star’ in posttreatment disease. Endodontic Topics 2003, 6, 135–159
8. Charles H. Stuart, DDS, Scott A. Schwartz, DDS, Thomas J. Beeson, DDS, and Christopher B. Owatz, DMD. Enterococcus faecalis: Its Role in Root Canal Treatment Failure and Current Concepts in Retreatment
9. Hengameh Ashraf, Mohammad Samiee, Gita Eslami. Presence of Candida Albicans in Root Canal System of Teeth Requiring Endodontic Retreatment with and without Periapical Lesions. IEJ -Volume 2, Number 1, Spring 2007
10. Persoon IF, Crielaard W, Ozok AR. Prevalence and nature of fungi in root canal infections: a systematic review and meta-analysis. International Endodontic Journal, 50, 1055– 1066, 2017
11. Mohammadi Z. Sodium hypochlorite in endodontics: an update review. Int Dent J. 2008 Dec;58(6):329-41. doi: 10.1111/j.1875-595x.2008.tb00354.x. PMID: 19145794.
12. Mukaremera L, Lee KK, Mora-Montes HM, Gow NAR. Candida albicans Yeast, Pseudohyphal, and Hyphal Morphogenesis Differentially Affects Immune

Recognition. Front Immunol. 2017 Jun 7;8:629. doi: 10.3389/fimmu.2017.00629. PMID: 28638380; PMCID: PMC5461353.

13. Verma N, Sangwan P, Tewari S, Duhan J. Effect of different concentrations of sodium hypochlorite on outcome of primary root canal treatment: a randomized controlled trial. Journal of endodontics. 2019 Apr 1;45(4):357-63.
14. Vinothkumar, et al.: Antimicrobial effi cacy of herbal extracts. Journal of Conservative Dentistry | Mar-Apr 2013 | Vol 16 | Issue 2
15. Divya Sundaram et al., Honey, Neem Leaf Extract, Sodium Hypochlorite as Intracanal Irrigant. Journal of Clinical and Diagnostic Research. 2016 Aug, Vol-10(8): ZC88-ZC91
16. Gupta A, Duhan J, Tewari S, Sangwan P, Yadav A, Singh G, Juneja R, Saini H. Comparative evaluation of antimicrobial efficacy of Syzygium aromaticum, Ocimum sanctum and Cinnamomum zeylanicum plant extracts against Enterococcus faecalis: a preliminary study. International Endodontic Journal, 46, 775–783, 2013.
17. Ala Mahdi et al . A comparative evaluation of antimicrobial activity of the ethanolic extract of Cinnamomum zeylanicum and NaOCl against oral pathogens and against swabs taken from nonvital teeth - An in vitro study. International Journal of ChemTech Research, 2017,10(4): 39-47.
18. Revati S, Bipin C, Chitra PB, Minakshi B. In vitro antibacterial activity of seven Indian spices against high level gentamicin resistant strains of enterococci. Arch Med Sci. 2015 Aug 12;11(4):863-8. doi: 10.5114/aoms.2015.53307. Epub 2015 Aug 11. PMID: 26322099; PMCID: PMC4548039.
19. Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li HB. Antibacterial and Antifungal Activities of Spices. Int J Mol Sci. 2017 Jun 16;18(6):1283. doi: 10.3390/ijms18061283. PMID: 28621716; PMCID: PMC5486105.
20. Biswas B, Rogers K, McLaughlin F, Daniels D, Yadav A. Antimicrobial activities of leaf extracts of guava (Psidium guajava L.) on two gram-negative and gram-positive bacteria. International journal of microbiology. 2013 Oct 20;2013.
21. Jose J, Krishnamma S, Peedikayil F, Aman S, Tomy N, Mariodan JP. Comparative evaluation of antimicrobial activity of QMiX, 2.5% Sodium Hypochlorite, 2% Chlorhexidine, Guava Leaf extract and Aloevera extract against Enterococcus faecalis and Candida albicans–An in-vitro Study. Journal of clinical and diagnostic research: JCDR. 2016 May;10(5):ZC20.
22. Joseph B, Jini D. Antidiabetic effects of Momordica charantia (bitter melon) and its medicinal potency. Asian Pac J Trop Dis. 2013 Apr;3(2):93–102. doi: 10.1016/S2222-1808(13)60052-3. PMCID: PMC4027280.