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Comparative Evaluation of Antimicrobial Effect of - Ginger, Apple Cider Vinegar and Fruit Vinegar - An in Vitro UV Spectrophotometric Study

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Abstract: ***Aim: To evaluate the anti microbial and antifungal efficacy of ginger extract, commercially available apple cider vinegar and fruit vinegarand compare with that of 5.25% sodium hypochlorite. Materials and methods:The test materials used were commercially available forms of fruit vinegar (FV) and apple cider vinegar (ACV), and freshly made ginger extract (GE). The organisms tested were Enterococcus fecalis (E.fecalis) and Candida albicans (C.albicans). The antibacterial and anti fungal efficacy were evaluated with agar disc diffusion method and confirmation was done by UV spectrophotometry. Results: The study statistically proved that commercially available ACV and FV were effective against E. faecalis and C.albicans, however the GE was not effective against both the tested organisms.***

Keywords: Apple cider vinegar, Fruit vinegar, Ginger extract, Antibacterial, Antifungal

1. Introduction

Eradication of pathogenic microorganisms from the root canal system followed by sealing of the pulp space is inevitable to avoid possible sequela of endodontic infections. Compared to other systems in the human body, the complex anatomy of the root canal system makes it difficult and challenging to eliminate the microorganisms from an infected root canal. Microorganisms are able to survive in those shrouded complex anatomical sites and multiply by overcoming periods of starvation to produce inflammatory sequela and even life threatening complications. This mandates complete elimination of pathogenic microorganisms to avoid unwanted consequences.

Endodontic infections are associated with multispecies of bacteria and fungi, which can produceresistant infections when they are in planktonic state or as biofilm.[1] This necessitate us to acquire knowledge on the microbiota of oral cavity as well as the pathogenic microbes responsible for various pulpal and periapical diseases. Based on the microorganisms predominating in different endodontic infections and re-infections, the corresponding effective agent has to be selected to eradicate them. At the same time, the methods and agents used should not elicit any harmful reactions.

Enterococcus fecalis is one of the most common microorganisms involved in primary as well as secondary infections and in periapical lesions. Enterococcus fecalis form biofilms in medicated pulp spaces, endures prolonged periods of starvation and acquire gene coding resistance to antimicrobial agents.[2,3]

Fungi are also frequently isolated from oral sites, including the root canal systems. Studies have suggested a possible reaction between candida albicans and periodontal diseases, dentin and/or root caries. It also demonstrates quorum sensing with dentinophillic properties and affinity to smear layer.[4,5,6]

Various root canal irrigants and medicaments are used successfully to eliminate these pathogenic microorganisms. However, experiments are being conducted usingvarious naturally present herbs, fruits, plants etc.to avoid the toxic side effects of the synthetic chemical agents used in dentistry as well as to replace them with even more efficacious and less toxic or nontoxic natural extracts as root canal irrigants.

The aim of this study was to evaluate the anti-microbial and antifungal efficacy of ginger, apple cider vinegar and fruit vinegar compared with Sodium hypochlorite.

The tested hypothesis was that, the herbal extracts would show better antibacterial and antifungal properties.

2. Materials and Methods

Extraction of test materials

The test materials used in this study werepure ginger extract (GE), commercially available fruit vinegar (FV) and apple cider vinegar (ACV) without any dilutions.Rhizomes of Zingiber officinale Roscoe were obtained from the market. Approximately 50 g of fresh ginger rhizomes were washed thoroughly under running tap water and sliced into cross-sections at about 10 mm in thickness. The sliced ginger was subjected to blending, filtration and extraction. The crude extract was centrifuged at 5000rpm for 10 minutes and the supernatant was used as GE.

ACV and FV were procured from the local grocery shop and were experimented in the study without any dilution (100%).The positive and negative controls used were commercially available formulations of 5.25% sodium hypochlorite and 0.9% normal saline respectively.

Test organisms

Enterococcus fecalis (ATCC 29212)and Candida albicans (ATCC 18804) were obtained from Microbiology department of Dextrose Technologies. E.faecalis was grown in 10ml Brain heart infusion broth and incubated at 37ºC for 24 hours. Similarly Candida albicans was grown in Sabrous dextrose broth and incubated at room temperature for 24

hours.Gram staining was carried out to check the purity of the culture and confirmation of the sample.

Agar disc diffusion test

To carry out gel diffusion method, wells were punched on the agar plates.Brain heart infusion agar plates were used for inoculating E.faecalis whereas for C.albicans, Rose Bengal agar plates were used.50 µL of each sample was loaded into the wells and incubated at 37ºC for E.faecalis and at room temperature for C.albicans. After 24 hours of incubation,the zone of inhibition was measured using vernier caliper.

UV Spectrophotometric analysis

Similarly as mentioned above E.faecalis was grown in 10ml Brain heart infusion broth in a test tube and incubated at 37ºC for 24 hours and C.albicans was grown in Sabrous dextrose broth and incubated at room temperature for 24 hours.50µl of each samples were added to respective tubes and incubated at respective temperature.After 24 hours incubation optical density was measured using spectrophotometer at 660nm

3. Results

According to the results of the current study by agar disc diffusion method (table1),the mean value of zone of inhibition for ACV and FV against E fecalisare 2.2 and 2.5cm respectively. The inhibition zone shown by the positive control sodium hypochlorite wasonly 1.4cm. And the zone of inhibition exhibited by GE against E fecalis was nil.

With regards to C.albicans, the zones of inhibition (table2) exhibited by FV was maximum (2.05cm). Sodium hypochlorite (1.05cm) and ACV (1.1cm) exhibited only the half length of zone of inhibition when compared to FV. Again GE did not show any significant antifungal efficacy.

Coming to the spectrophotometric evaluation for the growth analysis of E.fecalis, ACV (0.455), FV (0.481) and sodium hypochlorite (0.475) showed minimal turbidityindicating minimal growth of organism (table 3).

Similarly, the growth analysis of C.albicans presented minimal turbidity of 0.484, for ACVfollowed by 0.564 byFV which were lesser when compared to that of sodium hypochlorite (0.678). However the turbidity or growth was maximum with the GE.

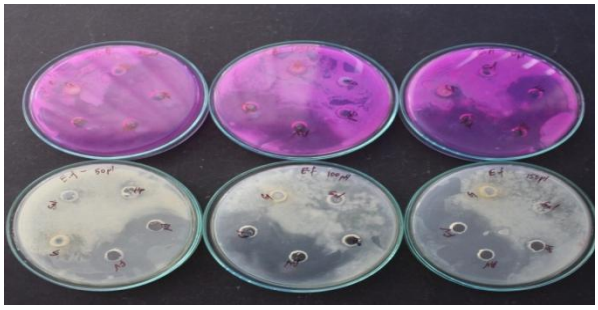
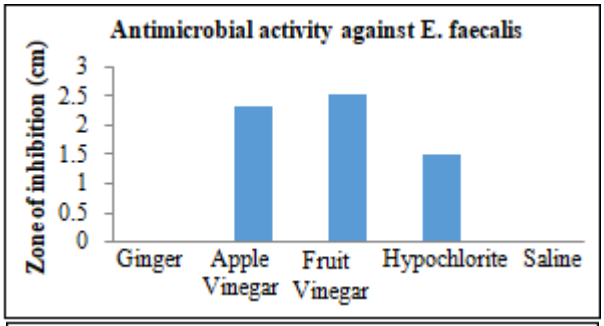


Figure: Zone of inhibition was analyzed for candida albicans and E. fecalis

Table 1: Zone of inhibition for E. faecalis and C.albicans

Sample	*Zone of inhibition [cm]*		
	Enterococcus faecalis		
	Trial 1	*Trial 2*	*mean*
GE	0	0	0
ACV	2.3	2.1	2.2
FV	2.5	2.5	2.5
S.Hypochlorite	1.5	1.3	1.4
Saline	0	0	0



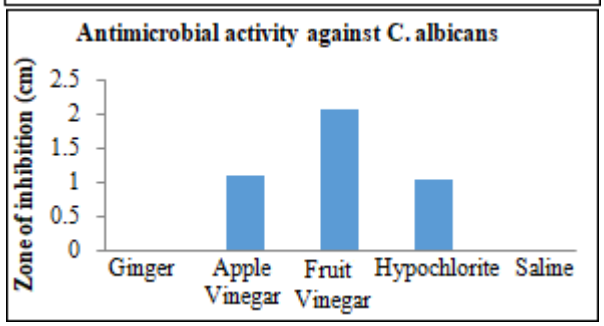
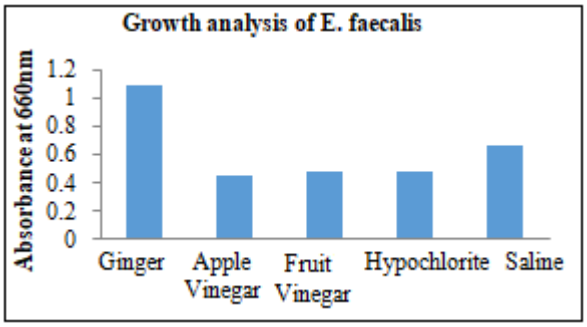


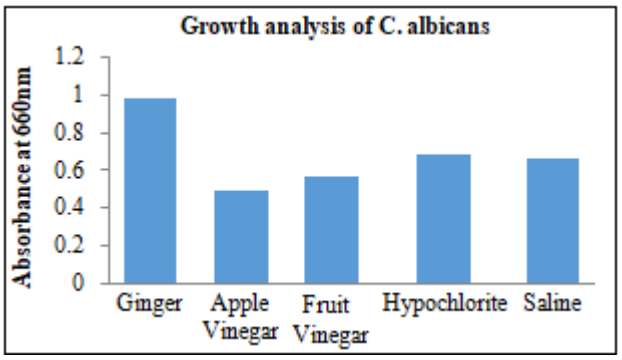
Table 2: Zone of inhibition for C.albicans

Sample	*Zone of inhibition [cm]*		
	Candida Albicans		
	Trial 1	*Trial 2*	*mean*
GE	0	0	0
ACV	1.0	1.2	1.1
FV	2	2.1	2.05
S.Hypochlorite	1.0	1.1	1.05
Saline	0	0	0

Table 3: Spectrophotometric analysis

Sample	Spectrophotometric analysis (absorbance or turbidity at 660nm)	
	E.faecalis	C.albicans
GE	1.088	0.980
ACV	0.455	0.484
FV	0.481	0.564
S.Hypochlorite	0.475	0.678
Saline	0.563	0.658





4. Discussion

The traditional system of medicine has been practiced successfully in countries such as India, Chinaetc., for almost 3000 years and witnessed an exponential growth in recent times. These systems deal with preparations that are derivatives of medicinal plants and are a rich source of phytonutrients.

Ginger, scientifically known as Zingiber officinaleRoscoe (family Zingiberaceae), is a vital plant with several remedial and nutritional principles. [7]Ginger has been used for its anti-inflammatory properties for centuries.Ginger is a rich source of antioxidants which act by scavenging superoxide anion, hydro peroxide, and hydroxyl free radicals. [7,8,9]Considering many of the already proven pharmaceutically beneficial aspects of ginger, it was decided to include in this studyas well.

The other 2 test materials used were fruit vinegar and apple cider vinegar. Vinegars are liquid products produced from the alcoholic and subsequent acetous fermentation of carbohydrate sources. While fruit vinegars are obtained from a mixture of many fruits and contain variety of biologically active components[10], apple cider vinegar which is extracted solely from apple juice was specifically attracted to be included in the current study, because of many of its proven therapeutic effects due to its component molecules.[10,11]

E. fecalis is one of the common microorganism involved in the endodontic pathologies with regard to primary as well as secondary infections. Fungi are also frequently isolated from root canal infections. Hence these twomicroorganisms were selected for this study.[12,13]

In this current study agar disc diffusion method was used as the primary method for testing the antimicrobial efficacy and then confirmation by UV spectrophotometric growth analysis by measuring the turbidity.

According to the results obtained from agar disc diffusion method for antibacterial efficacy,the zone of inhibition exhibited by FVand ACVwere almost the same whereas sodium hypochlorite showed only half of the above two test materials. However the turbidity measurement by UV spectrophotometry exhibited almost comparable values for ACV,FV and sodium hypochlorite. Thus it can be interpreted that both ACV and FV are equally or more effective against E.fecalis when compared to sodium hypochlorite.

The reason for the antibacterial activity could be the presence of various types of polyphenols micronutrients and other bioactive compounds found in vinegars contributing to their pharmacological effects (Madrera et al., 2010). [14,15]

The mode of antimicrobial action of the organic acids present in different types of vinegar was explained in 2011 by Zhang et al. These organic acids destroy the outer membrane of the organism's cell wall, inhibit the macromolecular synthesis, consume the microbial energy, as well as promote the production of the antimicrobial peptides in the host cells. [16]

Besides its antibacterial property, in many of the previous studies conducted, ACV was proved to be effective in removing the smear layer.[17,18,19,20]Estrela et al.assessed the smear layer removal capacity of apple vinegar used in isolation and/or associated with EDTA and they observed that the action of apple vinegar in removing the smear layer may be increased when EDTA is associated with the solution.

It is pointed that the antioxidative activities of vinegars derived mainly from its bioactive compounds including carotenoids and polysterols as well as phenolic compounds and vitamin C and E (Charoenkiatkul et al., 2016,Verzelloni et al.2007and Shahidi et al. 2008,Cheung et al. 2003)[10].Thusit appears that with regard to the antimicrobial/antioxidant activity of ACV, total phenolicshold a predominant role.[21,22]However small quantities of alcohol (acetic acid, organic acids, esters, ketones and aldehydes) resulting from the fermentation process which is responsible for reducing the surface tension of the solution, and strongly inhibits the growth of certain pathogens. [11, 14 ,15,23]

Thus the results of this study is in accordance with the results of the previous study done by Madrera et al 2010, Charoenkiatkul et al 2016,Verzelloni et al 2007, Shahidi et al 2008 and Cheung et al 2003.

Coming to the results of antifungal activity by agar disc diffusion method,FVshowed better antifungal activity when compared to other test materials and sodium hypochlorite. However, the fungal growth analysis by UV spectrophotometry presented the minimum growth by ACV followed by FV and then sodium hypochlorite.

This antifungal property is attributed to the organic acids which constitute the main ingredient in the vinegar.[24,25]

Also, the antifungal activityof ACV was explained in some studies through the fact that it inhibitsthe candidal adherence to the smooth surfaces and thus preventingits colonization and the ability to form an oral biofilm.[10] This could be the reason why both by agar disc diffusion method and UV spectrophotometry, ACV and FV exhibited activity against C.albicans.

Apart from these, the proteomic analyses of the microbes (E.coli, S. aureus and C. albicans.) in a study conducted demonstrated that ACV impaired cell integrity, organelles

and protein expression, proving its effectiveness against multiple organisms.[26]

The antibacterial and antifungal activities of GE with both the methodologies did not show comparable result with that of the other 2 test materials or the positive control.

But there are previous studies which have proved the positive effects of GE on endodontic pathogens.[27,28,29] A 10% ethanolic ginger extract showed good antimicrobial potential against S. mutans, E. faecalis and C. albicans in a study conducted in 2013.[30] It was verified that dehydrated rhizome of ginger was effective in eliminating the microorganisms,Candida albicans, Enterococcus faecalis, Escherichia coli and endotoxins in root canals.[31] Even though ginger possess many therapeutic benefits, the reason for its poor activity as per the results of the current study could be because of the type of extract (pure extract) used in this study. Because ethanolic and butanolic extracts of ginger has proven antibacterial efficacy against E.fecalis.[30,32]

5. Conclusion

Within the limitation of this in vitro study, both ACV and FV can be used as effective as sodium hypochlorite, which is one of the most effective intra canal irrigant used presently. However, the cytotoxic property of the sodium hypochlorite has to be kept in mind while using for canal disinfection. Hence herbal irrigants with equal or more antibacterial properties than sodium hypochlorite are becoming popular and can be used effectively. In this study, the commercially available form of vinegars are used without any dilutions. Further studies are needed to determine the minimum inhibitory concentrations and need to be tested in vivo for confirmatory results.

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