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Evaluation of Antimicrobial Efficacy of Apple Cider Vinegar on Periodontal Pathogens –An Invitro Study

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Abstract

Introduction:*Apple cider vinegar is an ancient folk remedy which has various health benefit*. ***Aim:****To evaluate the antimicrobial efficacy of Apple cider vinegar on periodontal pathogens.* ***Methodology:****The study was conducted on 5 different strains of organisms and 3 groups were made:Group A: Apple cider vinegar, Group B: chlorhexidine (gold standard) GROUP C: normal saline.Apple cider vinegar was used at a concentration of 0.5%, 2.5% and 5% concentration. Reference strains ofA.a, P. gingivalis, P. intermedia, S. mutans and Candida albicans were selected as colonizers in oral microbial flora. Organisms were grown on prefabricated blood agar plates and were incubated in a CO2 jar for 24 hours at 37°C. Inhibition zone diameters were measured.* ***Results****: For A.a, ACV at 5% concentration presented a zone of inhibition of 30 mm while chlorhexidine presented 25 mm.At 2.5% concentration of ACV, the zone of inhibition seen was in comparable range with CHX. However, at 0.5% concentration of ACV, Aa* was found to be resistant. For candida albicans, 5% concentrations of ACV presented a zone of inhibition of 18mm while chlorhexidine 15mm. *P.gingivalis, P.intermedia and S.mutans were more susceptible to chlorhexidine when compared to ACV.For A.a* and candida albicans, ACV was significantly more effective than CHX at 5%. Significant difference was seen between all 3 groups at 2.5% and 0.5% concentrations. ***Conclusion:****Apple cider vinegar demonstrated better antimicrobial activity at 5% than chlorhexidine and can therefore become a substitute for chlorhexidine.*

Keywords: Apple Cider Vinegar; Chlorhexidine;Aggregatibacteractinomycetemcomitans; Steptococcusmutans; Candida Albicans; Porphyromonasgingivalis; Prevotella Intermedia.

Introduction

It is deep rooted in the field of periodontology that plaque is an initiating factor for the development of gingivitis when it comes in contact with periodontal tissue.[1]Gingivitis is an inflammation of gingiva caused by accumulation of supra gingival plaque characterized by edema and light bleeding which is a moderate stage of periodontal disease. Periodontitis, a more severe stage of periodontal disease in which the alveolar bone around the teeth is slowly and progressively lost and the periodontal ligament supporting the tooth is detached resulting in the formation of periodontal pockets. This suggests that the prevention of periodontal disease must rely on measures directed at plaque control, this preventsgingivitis and is a primary requisite for good oral hygiene.[2] Various mechanical plaque control measures like toothbrushes, floss, interdental brushes are used for removal of plaque.[3] But the inadequacy of these mechanical measures in complete removal of plaque has fuelled up a search for chemical agents.[4]

A large number of chemical plaque control agents are commercially made available in market these days. Chlorhexidine is one such agent which is considered as a gold standard and is the most commonly prescribed agent as an adjunct with mechanical measures for effective plaque control. But chlorhexidine cannot be prescribed for extended periods as it may cause tooth staining, altered taste distribution and in rare cases it can also cause painful desquamation of the oral mucosa.[5]Besides chlorhexidine rinses, certain essential oils have been extensively used.

However, the most common shortcoming of these products is its unpleasant taste and its alcohol content. These days patients are more concerned about their oral health and the side effects of artificial chemical products[6].Thus naturally occurring substances can be used which will meet a new way of restoration of health in the most valuable and least nocuous way.

Researchers all around the globe are using a large number of herbal products as a remedy for treating periodontal diseases. Apple cider vinegar is one such

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popular yet under rationalized remedy. Apple cider vinegar is recently one of the most discussed topics amidst the home remedy seekers. The influence of Apple cider vinegar has been documented to have investigated over a period of hundreds of years. It is documented to have been used 5000 years ago in 400 BC by Hippocrates, the father of modern medicine. He used a mixture of Apple cider vinegar and honey for the treatment of various diseases. Apple cider vinegar had been used during the American civil war for disinfecting the wounds of soldiers. It is produced from cider that has undergone acetous bioconversion and has relatively low acidity (5% acetic acid). It also contains organic acids, flavonoids, polyphenols, vitamins and minerals[7]. ACV has been used as a supplement aiding weight loss, hyperlipidemia, hypercholesterliaemia, nutritional support, antioxidant defence and lowering blood pressure[7,8]. Recently Apple cider vinegar is gaining popularity and has been the object of investigations so the researchers can introduce it in various areas of health field. Studies so far have not documented the antibacterial efficacy of Apple cider vinegar in periodontal pathogens.Therefore, the current study was designed to evaluate the antimicrobial efficacy of apple cider vinegar on strains of Porphyromonasgingivalis, Prevotellaintermedia, Streptococusmutans, Aggregatibacter *actinomycetemcomitans* and Candida albicans by determination of minimum inhibitory concentration (MIC).

Materials and Methods

The study was conducted on 5 different strains of organisms keeping apple cider vinegar in the test group, chlorhexidine (gold standard) as a positive control and normal saline as a negative control. The following three groups were made

1) GROUP A: NORMAL SALINE
2) GROUP B: CHLORHEXIDINE 0.12% GOLD STANDARD
3) GROUP C: APPLE CIDER VINEGAR
   - 3a) 0.5%
   - 3b) 2.5%
   - 3c) 5%

Apple cider vinegar was used at a concentration of 0.5%, 2.5% and 5% concentration. Reference strains of Aggregatibacter *actinomycetemcomitans*, Porphyromonasgingivalis, Prevotella intermedia, Streptococusmutans and Candida albicans were selected as colonizers in oral microbial flora. Organisms were grown on prefabricated blood agar plates. Pure growth suspensions of the respective organisms were prepared at Maratha Mandal Dental College, Belgaum, India. Blood agar plates were prepared for diffusion and the bacteria were lawned on the plates. Plates were dried and 3 wells of approximately 6mm in diameter were cut with the help of cork. Apple cider vinegar was added in 5 plates in 3 different concentrations. Plates were incubated in a CO2 jar for 24 hours at 37°C. Inhibition zone diameters were measured. Similar procedure was performed using chlorhexidine and normal saline.

Results

Data was thus obtained using SPSS version 23.Descriptive statistics, One Way ANOVA Test was done to check the dilution of all the three groups for different strains of pathogen. For Aggregatibacter *actinomycetemcomitans*, apple cider vinegar at 5% concentration presented a zone of inhibition of 30mm while chlorhexidine presented a zone of inhibition of 25mm which shows that 5% Apple cider vinegar has antimicrobial efficacy greater than that of standard concentration of chlorhexidine(0.12%). Similarly, at 2.5% concentration of apple cider vinegar the zone of inhibition seenwas in comparable range with chlorhexidine. However, at 0.5% concentration of Apple Cider Vinegar, Aggregatibacter *actinomycetemcomitans was found to be resistant. When tested for candida albicans, 5% concentrations of apple cider vinegarpresented a zone of inhibition of 18mm while chlorhexidine presented a zone of inhibition of 15mm thus stating that the 5 % Apple Cider Vinegar is more effective than Chlorhexidine.* Porphyromonasgingivalis, Prevotella intermedia and Streptococusmutans were more susceptible to chlorhexidine when compared to the Apple cider Vinegar At different Concentrations. For Aggregatibacter *actinomycetemcomitans and candida albicans, Group C was significantly more effective than Group B at higher concentration of 5%. Significant difference was seen between all 3 groups at 2.5% and 0.5% concentrations. For obtaining uniform results, the present study was also performed using normal saline as negative control. The results for the Group A revealed that all the potential pathogens of the present study were resistant against Normal saline and were grown effectively by disc diffusion method.*

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Table1/Fig 1. *Zone of Inhibitions for apple cider vinegar, chlorhexidine and normal saline*

	Apple cider vinegar		CHX	Normal saline	
	5%	2.5%	0. 5%		
Streptococusmutans	19mm	15mm	13mm	20mm	R
Porphyromonasgingivalis	25mm	23mm	20mm	26mm	R
Prevotellaintermedia	26mm	22mm	18mm	28mm	R
Aggregatibacter *actinomycetemcomitans*	30mm	28mm	R	25mm	R
Candida albicans	18mm	10mm	08mm	15mm	R

Table 2/Fig 2. *Intergroup comparison of disc*

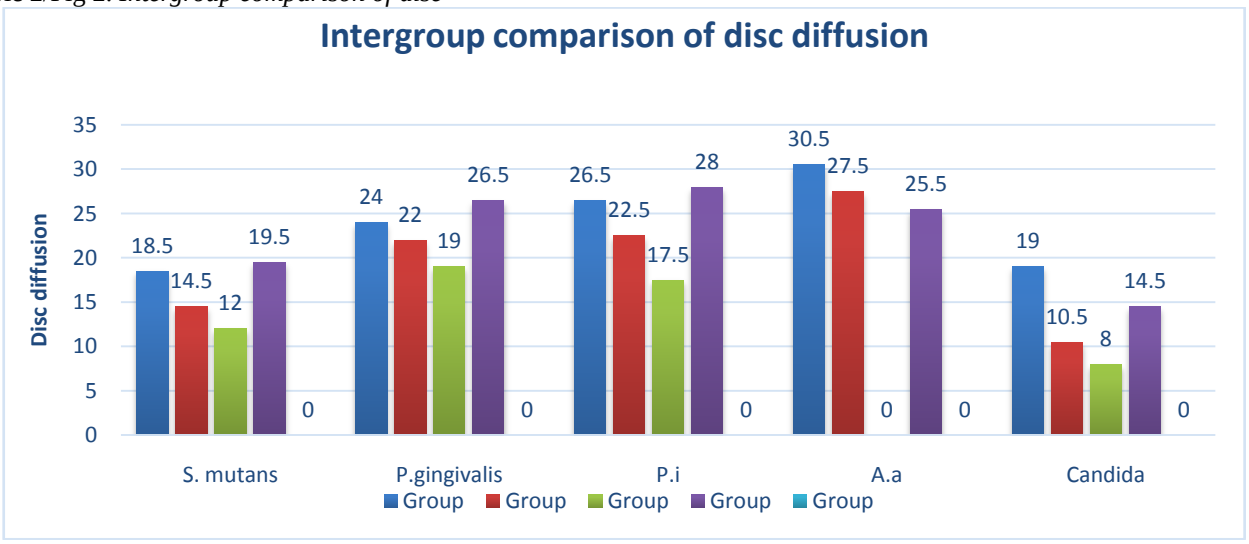
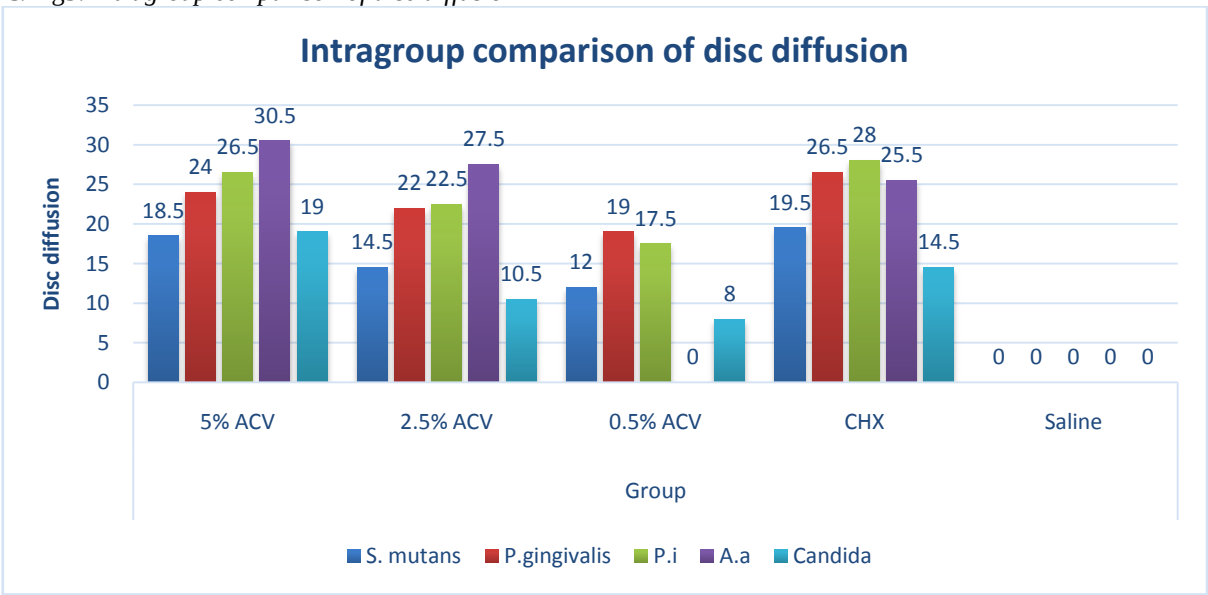


Table 3/Fig3. *Intragroup comparison of disc diffusion*



The above tables (table 2 and 3) show the comparison of mean values of disc diffusion of various bacteria in various solutions. There is statistically significant difference present in the mean disc diffusion values within groups and between groups ($p<0.001$).

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Figure 4. Bar diagram showing difference between apple cider vinegar and chlorhexidine at 5% 2.5% and 5% concentration for Aggregatibacteractinomycetemcomitans

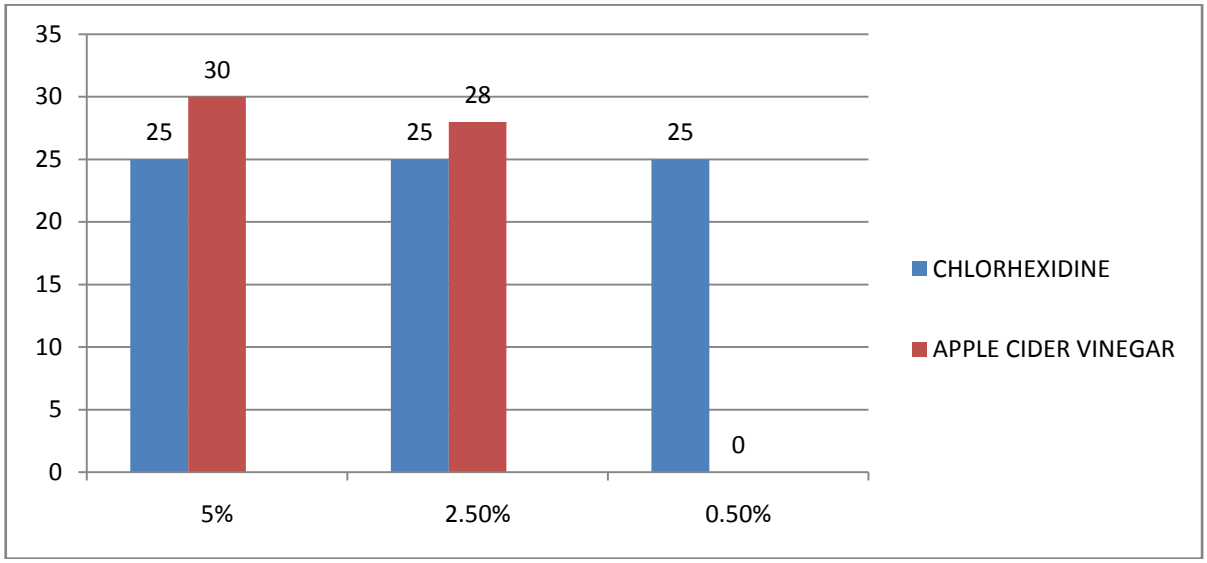

Figure 5. Bar diagram showing difference between apple cider vinegar and chlorhexidine at 5% 2.5% and 5% concentration for Candida Albicans

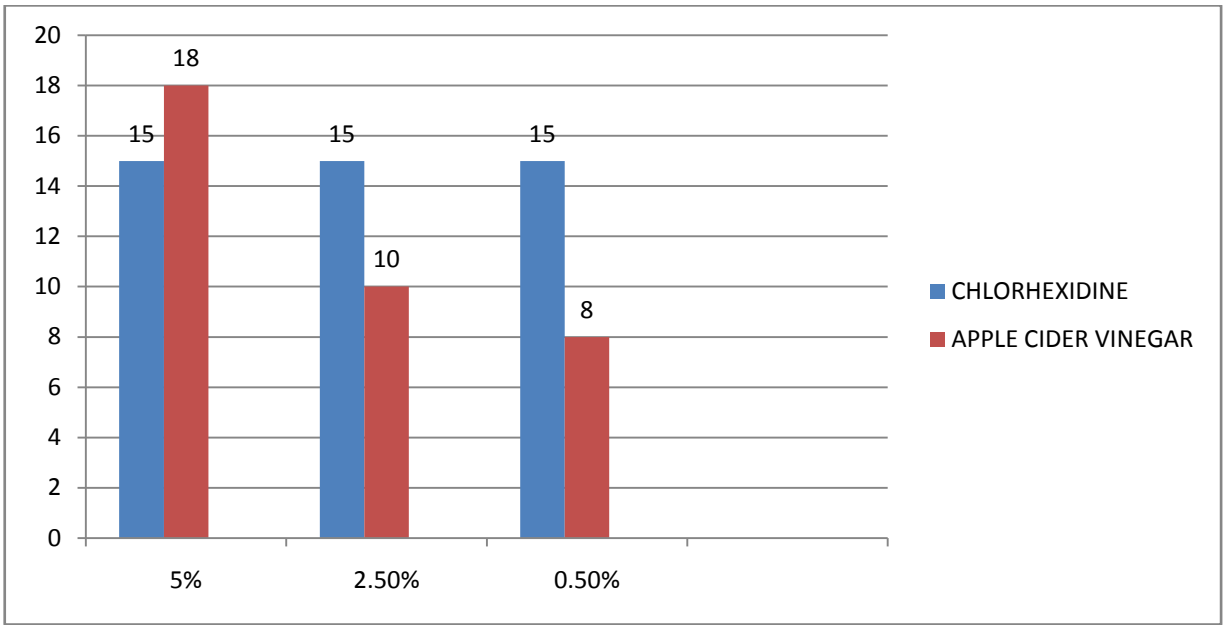

Figure 6. Bar diagram showing difference between apple cider vinegar and chlorhexidine at 5%, 2.5% and 0.5% concentration for Prevotella intermedia

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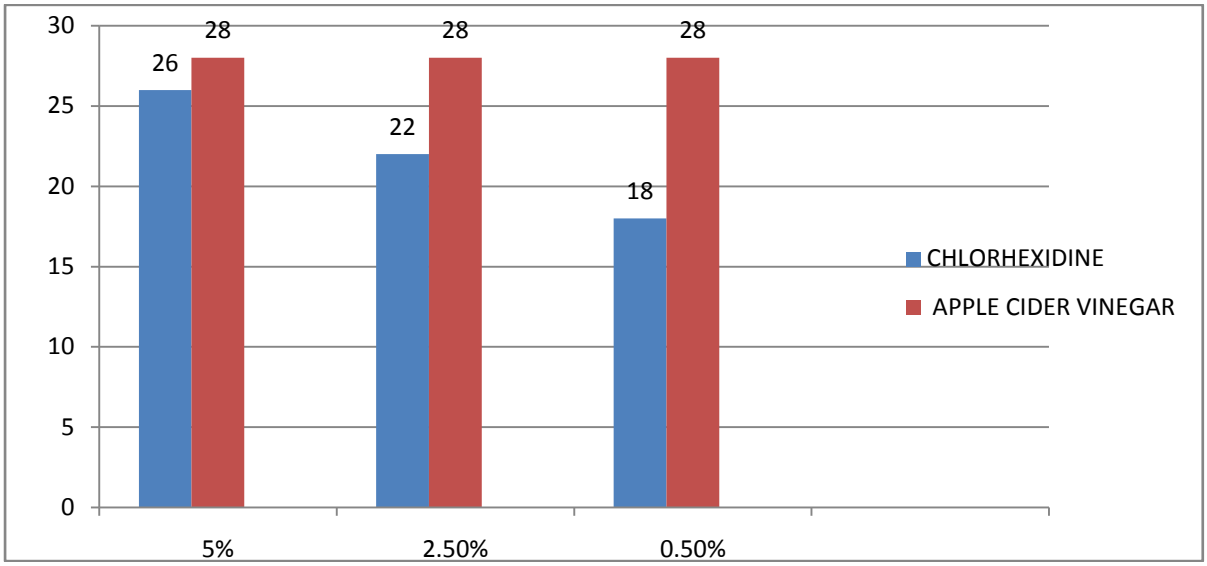

Figure 7. Bar diagram showing difference between apple cider vinegar and chlorhexidine at 5%, 2.5% and 0.5% concentration for Porphyromonasgingivalis.

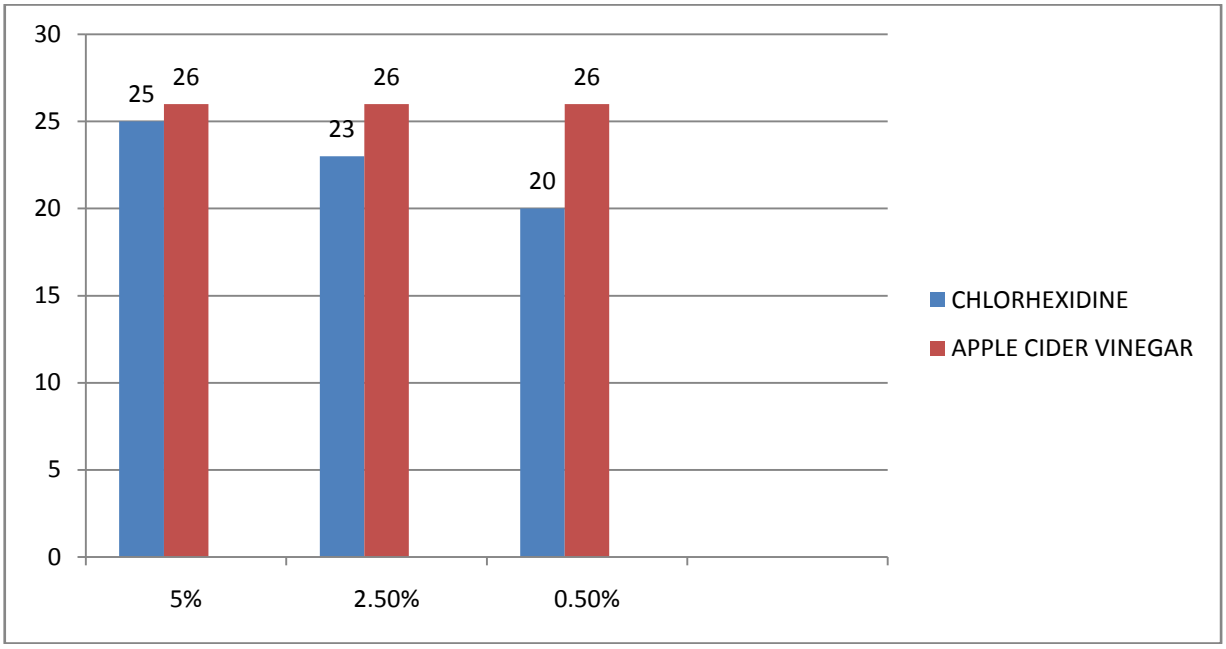

Figure 8. Bar diagram showing difference between apple cider vinegar and chlorhexidine at 5%, 2.5% and 0.5% concentration for Streptococusmutans.

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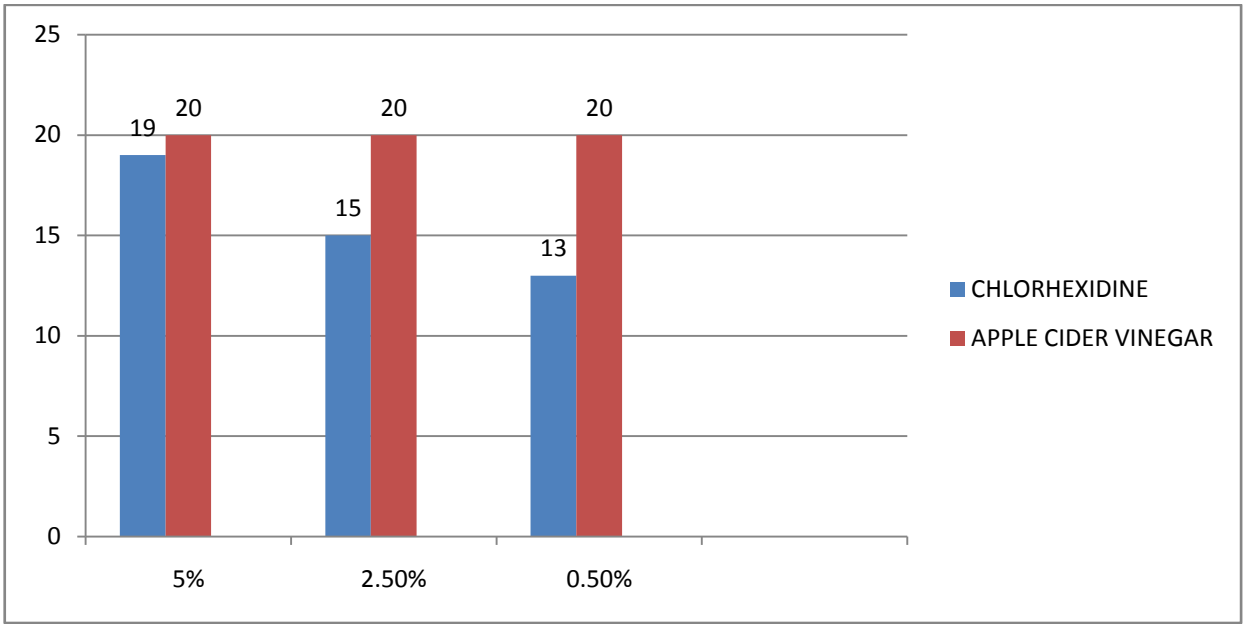

Discussion

These days the search for a reliable agent for plaque control has intensified and since naturally occurring substances have been used successfully in treating various ailments they have emerged as an alternative for chemical plaque control agents. Studies so farhave not documented the antimicrobial efficacy of apple cider vinegar for periodontal pathogens. Therefore, the current study was designed to evaluate the antimicrobial efficacy of apple cider vinegar on different strains of Porphyromonasgingivalis, Prevotella intermedia, Aggregatibacter *actinomycetemcomitans*, Streptococusmutans and Candida albicans by determination of minimum inhibitory concentration.

Periodontitis is a common, progressive disease that eventually affects the majority of the population[8]. The local destruction of periodontitis is believed to result from a bacterial infection of the gingival sulcus, and several clinical studies proved that the red complex bacteria Porphyromonasgingivalis, is implicated in severe forms of periodontal diseases like P gingivalis studies have found that *Prevotella intermedia and Aggregatibacteractinomycetemcomitans* are also associated with the etiology of different periodontal conditions[9].

Aggregatibacteractinomycetemcomitans being a second colonizer does not initially colonize tooth surface but adheres to the bacteria already in the plaque mass and is implicated in destruction of periodontal disease and thus can be considered as a key pathogen[10].

Recently certain studies have reported high traces of Smutans in patients with periodontitis. S mutans have been isolated from root caries and is believed to participate in periodontal destruction.[11,12,13]

Periodontal alterations are believed to be the result of an exacerbated immune response against the host tissue. Changes in cellular and humoral immune response may allow different species like candida to colonize the subgingival environment. It has been reported that the proportion of yeastin periodontal pocket is similar to that of some bacterial periodontal pathogens which suggests the role of candida albicans in the pathogenesis of periodontal diseaseCandidal species are found in more numbers in patients suffering from diabetes[14].

The current study demonstrates the inhibition action against Porphyromonasgingivalis, Prevotellaintermedia, Aggregatibacter *actinomycetemcomitans*, Streptococusmutans and Candida albicans.The minimum inhibitory concentration (MIC) is the lowest concentration of a chemical, usually a drug, which prevents visible growth of bacterium.It is the lowest concentration of an antibacterial agent which is required to inhibit visible growth.The MIC is determined by preparing solutions of the chemical in vitro at increasing concentrations, incubating the solutions with the separate batches of cultured bacteria, and measuring the results using agar dilution or broth microdilution[15].In the present study chlorhexidine was used as a control group for comparison of inhibition zones.*Chlorehexidine was effective against all the mentioned periodontal pathogens even at 0.12% concentration. Apple cider vinegar on the other hand at 5% concentration was found to be more effective than chlorhexidine against aggregatibacteractinomycetemcomitans and candida albicans. However at 5% concentration minimal difference in the inhibition zone was observed between chlorhexidine and apple cider vinegar for strains of porphyromonasgingivalis(CHX*26mm;ACV25mm), Prevotellaintermedia(CHX28mm;ACV26mm) andStreptococusmutans (CHX19;ACV20*).For obtaining uniform results, the present study was also performed using normal saline as negative control. The results*

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revealed that all the potential pathogens of the present study were resistant against Normal saline and were grown effectively by disc diffusion method.

The acetic acid in ACV is permeable to intact cell membranes, facilitating its access to the molecular target and causing an increase of its conc within the cell so that it becomes toxic. This situation leads to activation of H + ATPase and there is loss of cell integrity after exposure to high concentrations of acetic acid.A study done by Yaganik Darshana showed that ACV causes alteration of the microbial protein physiology destroying structural pathogenic proteins and metabolic enzymes.[16]Thus this might be the reason for the effectiveness of apple cider vinegar against the strains of periodontal pathogens.

Chlorhexidine is considered as the most effective and least harmful chemical plaque control agent and so the current study is designed in complete accordance with other similar literatures with respect to the antimicrobial efficacy of chlorhexidine.

Since apple cider vinegar was found to be more effective against candida albicans and agregatibacteractinomycetemcomitans further studies should be carried out testing its efficacy in diabetic patients and in patients having aggressive periodontitis.

*The limitations of the present study are the use of a short duration of incubation i.e.24 hours. Studies with longer duration of incubation period should be carried out. The present study is an invitro study and so the results of the study cannot be directly extended in invivo studies and therefore further research should be carried out. The overall results of the present study show that apple cider vinegar can be used as a substitutefor chlorhexidine owing to its better antimicrobial activity and higher nutritional content.*Apple cider vinegar demonstrated better antimicrobial activity at 5% concentration. Although chlorhexidine still continues to be the gold standard, Apple cider vinegar can become a substitute for avoiding the side effects of chlorhexidine. Further in vivo studies should be conducted to test the efficacy of apple cider vinegar on periodontal pathogens.

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