



AN *IN-VITRO* STUDY ON ENDOGENOUS pH AND TITRATABLE ACIDITY OF APPLE CIDER VINEGAR-GARLIC COMBINATION MOUTHWASH

Yadav Chakravarth* and M.Subhashini

Department of Conservative Dentistry and Endodontics, Vinayaka Mission's Sankarachariyar Dental College, Salem, Tamilnadu, India.

ABSTRACT

The objective of this study was to determine whether this Custom mouthwash combination (Apple cider vinegar-Garlic extract combination) can be used at a near Neutral pH without causing any deleterious effect on the Tooth enamel. The study sample was composed of two mouthwashes based on different active ingredients. The experiments were performed in triplicate. The endogenous pH was evaluated by using a digital pH meter; titratable acidity was evaluated by the addition of 0.1N NaOH increments to the mouthwashes. pH value for Apple cider vinegar-Garlic extract combination mouthwash was evaluated as 4.50 and titratable acidity as 2.40. pH value for chlorhexidine mouthwash was evaluated as 3.27 and titratable acidity as 3.39 and thus chlorhexidine is classified as potentially erosive. On comparing the pH and titratable acidity of the two mouthwashes, mouthwash presented with low endogenous pH, even below the critical value for enamel dissolution ($\text{pH} < 5.5$), high titratable acidity may be concluded as potentially erosive to the dental tissues if not properly used.

Key words: Mouthwashes, Endogenous pH, Titratable acidity, Potentially erosive.

Corresponding Author **Yadav Chakravarth** Email: subachan98@gmail.com

INTRODUCTION

Mouthwashes has been used since centuries as chemical agents in daily oral hygiene which are beneficial in the prevention and treatment of a variety of oral or oropharyngeal diseases such as gingivitis, periodontitis and other inflammatory conditions. A cursory inspection of pharmacies, drugstores, supermarkets and other commercial establishments reveal that a large number of mouthwashes which are formulated for number of oral health benefits and usually do not require a prescription from a dentist thus making these products readily available to children and adults. However, relatively few mouthwash formulations to date have been proven to produce benefits to oral hygiene (Vivek S *et al.*, 2015).

Ideally, the pH of saliva lies within the range of 5.5–6.5; a pH of 5.5. This is generally accepted as the threshold level for the development of dental caries. While the oral cavity may recover when the pH within the oral cavity drops below this threshold, prolonged exposure to this pH or frequent cycling from the optimal (that is, neutral) pH to a value below the threshold can result in a more rapid demineralization of tooth enamel. Lowered salivary pH, often is a consequence of bacterial digestion of sucrose, fructose and similar carbohydrates, causing acidic byproducts to form in dental plaque. Tooth demineralization however may also occur due to dental erosion (Anthony J and Matthew M, 2004).

Dental erosion is the irreversible, usually painless, loss of dental hard tissue that occurs due to a chemical process, such as dissolution or chelation without the involvement of microorganisms (Anthony J and Matthew M, 2004).

The underlying acidity of mouthwashes is believed to be the primary factor in the development of dental erosion; this total acid level (known as *titratable*

Access this article online

Home page:
<http://onlineijp.com/>

DOI:
<http://dx.doi.org/10.21276/ijp.2018.9.1.2>

Quick Response code



Received:26.10.17

Revised:12.11.17

Accepted:06.12.17

acid), rather than the pH, is thought to be an important factor in erosion because it determines the actual hydrogen ion availability for interaction with the tooth surface. The measurement of a mouth washes's total acid content may be a more realistic and more accurate method for predicting erosive potential (Anthony J and Matthew M, 2004).

Various studies have demonstrated that acidic and low pH (less or equal to 5.5) mouthwashes can cause dental demineralization, erosion and significant loss of enamel within the first few minutes of contact with such acidic solution (Vivek S *et al.*, 2015). The erosive potential depends on low pH and buffering capacity of the mouth washes. It has been accepted that titratable acidity which is a measurement of the total acid content and pH value increase is seen as an important indicator in determining the erosive potential of mouth washes (Vivek S *et al.*, 2015).

Natural herbs like triphala, tulsipatra, jyestiamadh, neem, clove oil, pudina, ajwain and many more used either as whole single herb or in combination have been scientifically proven to be safe and effective medicine against various oral health problems like bleeding gums, halitosis, mouth ulcers and preventing tooth decay. The major strength of these natural herbs is that their use has not been reported with any side-effects till date (Nandhini T *et al.*, 2015).

A number of chemical agents are currently available in the market and are designed to assist individuals in their efforts to achieve and maintain oral health. A variety of products are available chemically for plaque control, which are divided into the first generation (e.g., phenols, quaternary ammonium compounds), second generation (e.g., bisbiguanides-chlorhexidine gluconate [CHXG]), and third generation (e.g., delmopinol).

Chlorhexidine mouth wash is considered as the golden standard for mouthwash and is a good example for chemical mouthwash. Chlorhexidine is an important ingredient for mouth washes to reduce plaques accumulation and bacterial growth (Renuka S and Muralidharan NP, 2007).

Draw backs of Chlorhexidine

Flotra et al reported various local side effects of Chlorhexidine mouthwash. The local side effects are: 1. Brown discoloration of the teeth, restorative materials and the tongue. 2. Alter taste sensation especially for salt taste. 3. Mucosal erosion with use of high concentration of Chlorhexidine rinse. 4. Parotid swelling in rare cases. 5. Increased rate of supragingival calculus formation (Amit P, 2015).

Mouthwashes contains fluoride in various forms as either sodium fluoride (NaF) or acidulated phosphate fluoride (APF). Though they promote remineralisation and are useful for the patient with high risk of dental caries, the drawback of fluoride containing mouthwash is found

to be contra-indicated in children less than six years of age because of risk of fluoride ingestion (Amit P, 2015).

In a study conducted to evaluate the efficacy of different brands of mouthwashes cetylpridinium-containing mouth rinses (e.g. Macleans) provide significantly greater oral microbial reductions than do rinses containing either phenolic compounds or glycerin/triclosan to which Listerine and Colgateplex belong and are currently used nowadays (Akande OO *et al.*, 2004)

Alcohol containing mouthwashes

Mouth is supposed to maintain accepted levels of moisture in order to ensure integrity of the oral health. Elimination of moisture below a certain level can lead to tooth decay, Oral Malodour, fungal infection risk and other oral health issues. Alcohol based mouthwashes should not be prescribed for patients with salivary problems (Rafey AJ *et al.*, 2004)

Ethanol is used as a preservative and solvent in a concentration range of 5 – 27 % in various commercially available mouthwashes. McCullogh and Farah stated that there may be a direct association between the alcohol content of mouthwashes and the risk of development of oral cancer. Alcohol containing mouthwashes have also shown to reduce the hardness of composite and hybrid resin restorations and may also alter the colour of composite restorations (Amit P, 2015).

Long-term use of alcohol based mouthwashes may lead to unwanted and harmful results like burning sensation and susceptibility to cancer. Alcohol itself is a contributing factor for mal odour and is an irritant to the oral epithelium. Leukoplakia also been reported due to prolong use of the mouth washes containing alcohol. Acetaldehyde, a potential carcinogen is also found intra orally in people using alcohol based mouth rinses. Thus, one can conclude that the long-term use of the alcohol mouthwashes should strictly be avoided (Rafey AJ *et al.*, 2004).

Povidone-iodine a broad spectrum antimicrobial agent also used as a mouthwash and it is found to reduce plaque formation and decreases the severity of gingivitis and radiation mucositis. This mouthwash is contraindicated in individuals having sensitivity to iodine and pre-existing thyroid disorders.

Apple cider vinegar

Apple cider vinegar is made by crushing apples and squeezing out the liquid. Bacteria and yeast are added to the liquid to start the alcoholic fermentation process, and the sugars are turned into alcohol. In a second fermentation process, the alcohol is converted into vinegar by acetic acid-forming bacteria (*Acetobacter*). The total acidity of vinegar is expressed as acetic acid which is the major organic acid in vinegar (Trial DA *et al.*, 2016).

According to Malicki, organic acids are considered weak acids with antimicrobial property caused by its undissociated forms. They passively diffuse through the bacteria cell wall and internalizing into neutral pH dissociating into anions and protons. Release of the protons causes the internal pH to decrease which exert inhibitory effects on the bacteria (Lingham T *et al.*, 2012).

The antimicrobial effects of vinegar have been previously confirmed. Ismael evaluated the effect of several types of vinegar on the biofilm of *Streptococcus pyogenes* isolated from patients and showed that vinegar eliminated a minimum of 90% of the biofilm (Narjis FI *et al.*, 2013). Komiyama *et al.*, in 2010 evaluated the antimicrobial and disinfecting effects of 0.12% chlorhexidine, 0.50% white vinegar and two other materials on *S. mutans*, *S. pyogenes*, *Staphylococcus aureus* and *Candida albicans* on toothbrush and showed that vinegar decreased the count of *S. mutans*, *S. pyogenes* and *S. aureus* (Fahiem MM *et al.*, 2016).

Researchers at the Food Biotechnology Department, Instituto de la Grasa (CSIC) in Seville, Spain conducted research on the antimicrobial activity of several food products including vinegar. The following microorganisms were used in the study: *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Escherichia coli* 0157:H7, *Shigella sonnei*, and *Yersinia sp.* Vinegar (5% acetic acid) showed bactericidal activity against all strains tested, which was attributed to its acidity. Various studies have compared the natural against the normal mouth rinses and reportable varied degrees of efficaciousness, particularly against *Streptococcus mutans*. However, there are very few studies done regarding the antimicrobial effect of natural mouth rinses (Fahiem MM *et al.*, 2016).

Scientific information about the biological effects of apple cider vinegar as a traditional medicine is inadequate. More valuable properties of apple cider vinegar, its ingredients, and also their therapeutic effects have been recently discovered.

In a study involving extensive analysis of possible risk factors on the pattern of erosion, it was concluded that the erosive tooth wear was induced by daily consumption of a glass of apple cider vinegar (Gambon DL *et al.*, 2012)

Garlic (*Allium sativum*)

Garlic (*Allium Sativum*) is a bulbous perennial medicinal plant which belongs to the family Liliaceae. The antimicrobial activity is attributed to thiosulphinates. Garlic can be used on microorganism that has particularly developed resistance to antibiotics. Garlic is a strong antibacterial agent against both Gram-positive and Gram-negative bacteria such as *E. coli*, *Salmonella spp.*, *Streptococcus spp.*, *Staphylococcus aureus*, *Klebsiella spp.*, *Proteus mirabilis*, *Shigella senteriae*, *Pseudomonas aeruginosa* and *Helicobacter pylori*, also it's effective even

against those strains that have become resistant to antibiotics (Atheer AK, 2014).

Grosso *et al* have observed a remarkable reduction of *Streptococcus mutans* after gargling with a 2.5% garlic mouthwash solution. Another study, reporting a reduction in levels of *S. mutans* after garlic mouthwash, mentioned that a 3% concentration was the minimum concentration at which a zone of inhibition was observed (Houshmand B *et al.*, 2013).

Studies have been done on the activities of garlic extract combination with apple vinegar (VGE) on the bacterial isolates. The results of garlic extract combination with vinegar (VGE) on bacterial isolates revealed that all bacterial isolates were highly sensitive to Vinegar Garlic Extract.

Qin *et al.*, pointed out that the bacterial effect of vinegar is strong on bacteria but weak on fungi. Both water garlic extract and apple vinegar pickled garlic extract had strong antimicrobial activity against both bacteria and fungi (Nadakhazalkadhim H, 2013).

Hence the objective of the present study was to determine whether this Custom mouthwash combination (Apple cider vinegar-Garlic extract combination) can be used at a Neutral pH without causing any deleterious effect on the tooth enamel.

Methodology of making Indigenous mouthwash

Fresh garlic bulbs (*Allium sativum* L) were cleaned, peeled then sun dried and cut in to small pieces: it was then ground using an electric blender and placed in clean container. 50 grams of Garlic powder dissolved in 100ml of Apple cider vinegar (weakly acidity) and sterilised by filtration (Nadakhazalkadhim H, 2013).

MATERIALS AND METHOD

- Apple vinegar-Garlic mouthwash
- Chlorhexidine (0.2%)(Hexidine mouthwash)
- Digital pH meter (Hanna Instrument)
- 0.1 N NaoH (Nice Laboratory Reagent)

Mouthwashes were selected based on the active ingredients and were coded. Endogenous pH of each mouthwash is measured immediately after package is opened at room temperature using a Digital pH meter. Titratable acidity is measured by titrating mouthwashes(100ml) adding increments of 0.1N sodium hydroxide and measuring the pH until it reached \geq 7 (neutral pH). Values are expressed as ml of NaOH.

STATISTICAL ANALYSIS

Mean and standard deviation of both the samples are tabulated. Data are analysed using SPSS version 17 software. Statistical significance are measured by using one-way ANOVA followed by Turkey's hoc test. P values <0.05 are considered statistically significant.

RESULTS

The experiments were performed in triplicate. The endogenous pH of Apple cider vinegar-Garlic extract combination mouthwash was measured immediately after preparation at room temperature using a digital pH meter and was found to be 4.37,4.62 and 4.50. The Acidic pH was buffered by adding 100ml of 0.1 N of NaOH (titratable acidity) of about 2.45,2.00,2.75 ml respectively and brought to Neutral pH (titratable acidity).

The endogenous pH of Chlorhexidine mouthwash was measured immediately after the package is opened at room temperature using a digital pH meter and was found to be 3.48,3.05 and 3.27. The Acidic pH of the mouthwash was buffered by adding 0.1 N of NaOH of about 3.69,2.94 and 3.53 ml of NaOH respectively and brought to Neutral pH (titratable acidity). The mean pH of Apple cider vinegar-Garlic extract combination mouthwash was found to be 4.50±0.13and

the mean pH of Chlorhexidine mouthwash was found to be 3.27±0.22. Distribution of the mouthwashes according to mean pH values and standard deviations is presented in Table 3 and Figure 1.

The mean titratable acidity of apple cider vinegar-garlic extract combination mouthwash was 2.40±0.38 and chlorhexidine was found to be 3.39±0.40. Distribution of the mouthwashes according to mean Titratable acidity values and standard deviations is presented in Table 4and Figure 2.

In this study Comparison of the results of pH of mouth washes (apple cider vinegar-garlic extract combination mouthwash, chlorhexidine) showed statistically highly significant difference at 1% with p value =0.001 and titratable acidity showed significant difference at 5% with p value=0.035.

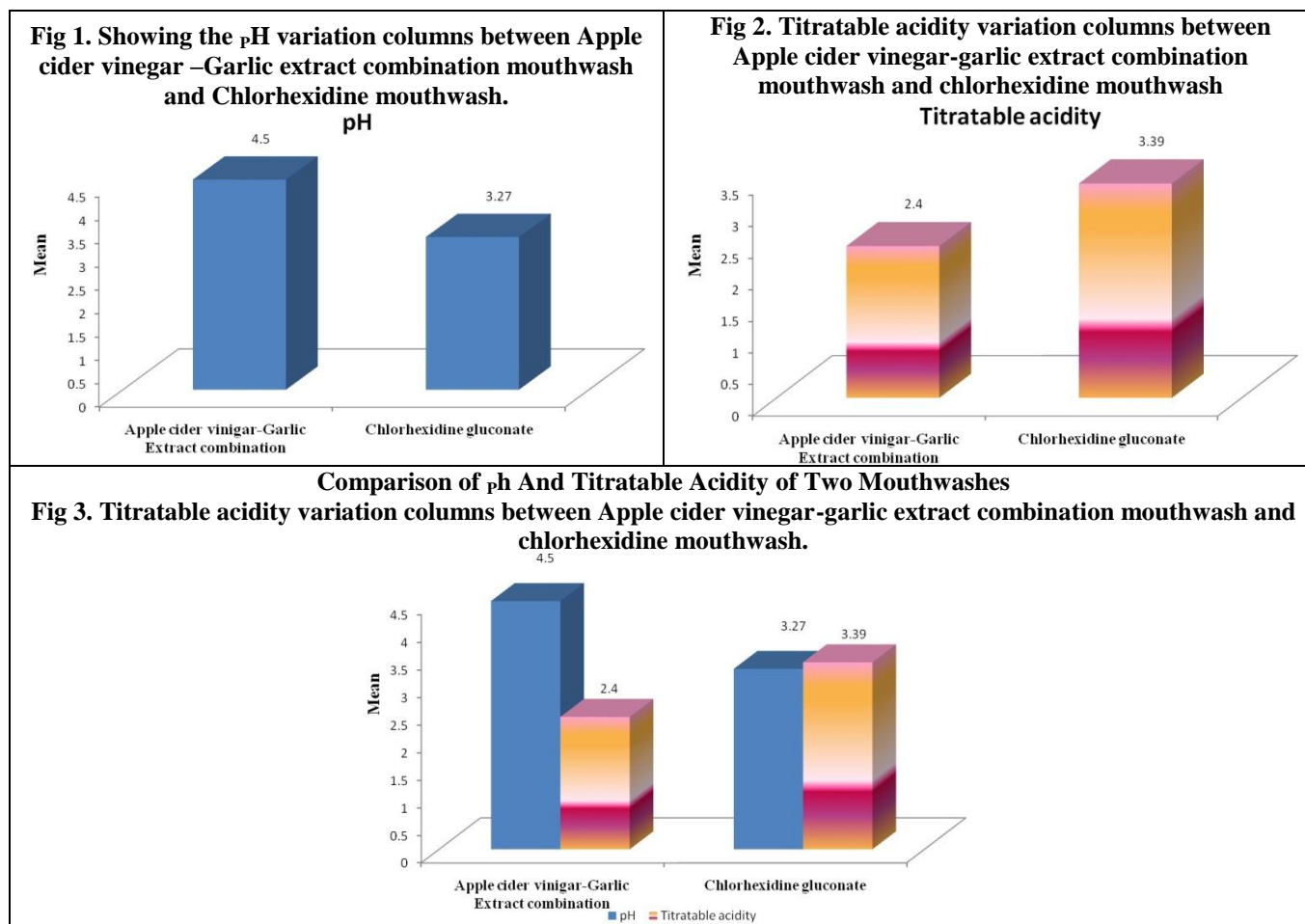


Table 1. Showing the Mean pH and standard deviation values of Apple -cider vinegar mouth wash on adding sodium hydroxide for its buffering capacity

S.No	pH value	MI of 0.1N NaOH	Mean+-SD
1	4.37	2.45	2.4±0.37
2	4.62	2.00	
3	4.50	2.75	

Table 2. Showing the Mean pH and standard deviation values of Chlorhexidine mouthwash on adding sodium hydroxide for its buffering capacity

S.NO	pH value	MI of 0.1 N NaOH	Mean+-SD
1	3.48	3.69	3.38±0.39
2	3.05	2.94	
3	3.27	3.53	

Table 3. Showing the difference in Mean pH values and statistical difference of Apple -cider vinegar and Chlorhexidine mouth wash

Mouth wash	N	pH			t	P
		Mean	Std. Deviation	Std. Error Mean		
Apple cider vinegar-Garlic Extract combination	3	4.50	0.13	0.07	8.57	0.001**
Chlorhexidinegluconate	3	3.27	0.22	0.12		

** Significant at 1 % (Highly Significant)

Table 4. Showing the difference in Titratable acidity values and statistical difference of Apple cider vinegar-Garlic extract combination mouthwash and Chlorhexidine mouth wash.

Mouth wash	N	Titratable acidity			t	p
		Mean	Std. Deviation	Std. Error Mean		
Apple cider vinegar-Garlic Extract combination	3	2.40	0.38	0.22	3.12	0.035*
Chlorhexidinegluconate	3	3.39	0.40	0.23		

* Significant at 5 %.

DISCUSSION

In 1970, Pindborg defined dental erosion as the irreversible loss of tooth structure due to chemical dissolution by acids and not of bacterial origin (Vivek S *et al.*, 2015)

Erosion depends on several intrinsic and extrinsic factors like acidic drinks, medications and foods lower the pH level of oral cavity hence their consumption causes the teeth to demineralise. Erosion is found initially in the enamel and if unchecked may proceed to the underlying dentin.¹Dental caries or cavities, more commonly known as tooth decay, are caused by a breakdown of the tooth enamel. This breakdown is the result of bacteria on teeth that breakdown foods and produce acid that destroys tooth enamel and results in tooth decay.

Mouthwashes have been used for centuries for medicinal and cosmetic purposes, but it is only in recent years that the rationale behind the use of chemical ingredients has been subject to scientific research and clinical trials. Drugs derived from plants are effective, easily available, and less expensive and rarely have side effects.

Herbal mouthwash contains a natural ingredients called phytochemical that contains desired anti-microbial and anti-inflammatory effect. Herbal mouthwash becomes more popular they work without alcohol, artificial preservatives, flavor, or colors.As it contains natural herbs

that have natural cleansing and healing property to teeth and gums, herbal mouth washes are safe alternative to pregnant women, people with dry mouth, diabetic and to children (Renuka S and Muralidharan NP, 2007).

Almost all chemical mouthwashes contain alcohol and fluoride which is toxic to our body in overdose. Due to the occurrence of unpleasant side effects and increasing resistance to the synthetic pharmaceuticals, there has been increasing interest in the quest of natural alternatives (Nadakhazalkadhim H, 2013).

Medicated oral rinses usually contains antimicrobial agents, such as chlorhexidine gluconate which is very potent chemoprophylactic agent, it has a broad spectrum action especially against Mutans Streptococci group. However it has many side effect like staining of teeth, altering the test of the mouth desquamation of oral mucosa.

Herbs are being widely explored to discover alternatives to synthetic antibacterial agents. Natural herbs like triphala, tulsipatra, jyestiamadh, neem, clove oil, pudina, ajwain and many more used either as whole single herb or in combination have been scientifically proven to be safe and effective medicine against various oral health problems like bleeding gums, halitosis, mouth ulcers and preventing tooth decay. The major strength of these natural herbs is that their use has not been reported with any side-effects till date. Apart from this, all herbal

mouthrinses do not contain alcohol, sugar, artificial colour artificial sweetner (such as saccharine),stannous fluoride (processed form of fluoride that can stain teeth)and cetylpyridinium chloride that can also cause staining (Renuka S and Muralidharan NP, 2007).

Apple cider vinegar is made by crushing apples and squeezing out the liquid. Bacteria and yeast are added to the liquid where the sugars are turned into alcohol in the fermentation procedure. In a second fermentation process, the alcohol is converted into vinegar by acetic acid-forming bacteria (*Acetobacter*) (Trial DA *et al.*, 2016). Among the natural products, vinegar, also known as acetic acid, contains sanitizing properties. The total acidity of vinegar is expressed as acetic acid which is the major organic acid in vinegar. Acetic acid, a monocarboxylic acid has a pungent odor and flavor. It is generally regarded as safe (GRAS) for general purpose and miscellaneous usage (Lingham T *et al.*, 2012).

According to Malicki, organic acids are considered weak acids meaning the antimicrobial effect of organic acids is mainly caused by its undissociated forms. They passively diffuse through the bacteria cell wall and internalizing into neutral pH dissociating into anions and protons. Release of the protons causes the internal pH to decrease which exert inhibitory effects on the bacteria (Lingham T *et al.*, 2012).

In this study Bragg Organic Raw Apple Cider Vinegar is used which is made from delicious, healthy, organically grown apples. Processed and bottled in accordance with USDA guidelines, It is Certified Organic by Organic Certifiers and Oregon Tilth; and is Kosher Certified. Its pH is 3.075.

Apple cider vinegar contains acetic acid, polyphenols, pectin, and carotenoids with antibacterial and prebiotic properties. Acetic acid is consumable at concentrations of 3–9% . Its antioxidant flavonoid content can reduce the harmful effects of high cholesterol diets (Trial DA *et al.*, 2016)

Historically, garlic has been used for centuries worldwide by various societies to combat infectious disease. Garlic can be provided in the form of capsules and powders, as dietary supplements and thus differ from conventional food or food ingredients. Louis Pasteur was the first to describe the antibacterial effect of onion and garlic juices.

The pharmacological effects and antimicrobial properties of garlic have been reviewed and found that the therapeutic effects of garlic is possible because of its oil and water-soluble organo sulfur compounds, which are responsible for the typical odour and flavor of garlic. Thiosulfinates play an important role in the antibiotic activity of garlic (Nadakhazalkadhim H, 2013).

Garlic (*Allium Sativum*) is a bulbous perennial medicinal plant which belongs to the family Liliaceae. The antimicrobial activity is attributed to thiosulphinates. Garlic can be used on microorganism that has particularly

developed resistance to antibiotics. Garlic is a strong antibacterial agent against both Gram-positive and Gram-negative bacteria such as *E. coli*, *Salmonella* spp., *Streptococcus* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Proteus mirabilis*, *Shigellasenteriae*, *Pseudomonas aeruginosa* and *Helicobacter pylori*, also it's effective even against those strains that have become resistant to antibiotics.

Garlic is also proven to have antioxidant, anti-atherogenic, hypolipidemic, antimicrobial, antithrombotic and antihypertensive activity. extracts of garlic may be used to break the chain of caries aetiology, by affecting the host factors and the microbial flora. pH value of garlic powder is 5.49. Garlic extracts stimulate the flow of saliva and facilitates the clearance of the substrates from the mouth. It reduces the *S. mutans* counts, their acid production and prevents the demineralisation of tooth structure (Shailesh K *et al.*, 2012; Mariam M and Usha Devi C, 2016)

Studies have shown that exposure to garlic increases acid production by *S. mutans*, but in the long term, inhibit its growth. In short term period, within 24 hours, this acid production is countered by salivation in response to the spicy flavour of garlic (Shailesh K *et al.*, 2012).

The spicy flavor of garlic stimulates salivary flow. Stimulated saliva contains greater concentration of bicarbonate ions in addition to other substances. This increases the buffering capacity of saliva and its ability to clear the acid and substrates from the plaque thus improving the pH of saliva and increasing resistance to caries Newbrun but There are reports of oral mucosal burn patients for the allergic reaction to garlic (Shailesh K *et al.*, 2012). Therefore, a thorough history has to be taken to rule out allergies. The characteristic flavour of garlic is not favoured by some patients.

Water is found to have an astounding effects in modifying the salivary pH, and hence can be used as daily routine regimen with the advantage of its feasibility and non side effects. It is noteworthy that G.V. Black (1914) was aware that the dental decay can be perfectly controlled by the use of tooth brush and plain water by the patient. The therapeutic modality of hydrotherapy considers disease processes to be pH mediated. The principle is to balance the pH of the body fluids like tissue fluids and saliva by regulating the intake of water (Shailesh K *et al.*, 2012).

In a study conducted to evaluate the effect of pH of garlic the combination mouthwash (garlic and chlorhexidine) was able to prevent the fall of the salivary pH significantly. The best results were obtained with the combination mouthwash of garlic and chlorhexidine, which probably produced a synergistic effect. Thus, the use of garlic incorporated combination mouthwashes or water rinsing may be a step forward in prevention of fall of salivary pH, thereby rendering more resistance against development of new carious lesions.

In a Traditional Chinese food product “Laba” garlic was carried out in the laboratory by soaking aged garlic in 5% (v/v, pH 2.33) acetic acid solution. Low pH (2.0–3.0) favors nonenzymatic reactions, while high pH (6.0 or above) is conducive to enzymatic reactions. Thus, the ideal pH for the entire process of garlic greening is between 4.0 and 5.0, which is a compromise of the optimal pH of both the enzymatic and nonenzymatic reactions (Bing *et al.*, 2015).

The results of vinegar garlic extract (VGE) of Gram positive bacteria (*E. faecalis*) were higher sensitive than Garlic Extract followed by, *S. pneumoniae* with inhibition zones were 50 mm and 40 mm respectively. Also, the results of Vinegar Garlic Extract (VGE) of Gram negative bacteria (*E. faecalis*, *P. aeruginosa*, and *S. typhi*) were higher sensitive than Garlic Extract followed by *P. fluorescens*, *P. Mirabilis*, *E. aerogenes*, *K. pneumoniae*, and *E. coli* with inhibition zones were 50 mm and 40 mm respectively (Nadakhazalkadhim H, 2013).

Statistical analysis showed significant differences between effect of garlic and garlic–apple vinegar extract combination on gram positive bacterial isolates with maximum inhibition concentration such as 40mm and 50mm and minimum inhibition concentration of 25mm and 30mm and on gram negative isolates with maximum inhibition concentration of 50mm and minimum inhibition concentration of 20mm and 25mm respectively. There were no significant differences between vinegar and garlic–vinegar extract combination on Gram positive bacteria with maximum inhibition zone of 15mm and 50mm and minimum inhibition concentration of 7mm and 30mm respectively and on gram negative bacteria it was found to have maximum inhibition zone values of 10mm and 50mm and minimum inhibition concentration values of 6mm and 25mm respectively. There were no significant differences between vinegar and garlic extract on bacterial isolates at level ($P \leq 0.05$) with maximum inhibition concentration of 15mm and 40mm on gram positive isolates and minimum inhibition concentration of 7mm and 25mm and on gram negative isolates with maximum inhibition concentration values of 10mm and 50mm and minimum inhibition concentration values of 6mm and 25mm respectively (Nadakhazalkadhim H, 2013).

Qin *et al.*, who pointed out that the bacterial effect of vinegar is strong on bacteria but weak on fungi. Both water garlic extract and apple vinegar pickled garlic extract had strong antimicrobial activity against both bacteria and fungi (Nadakhazalkadhim H, 2013).

Grosso *et al.* have observed a remarkable reduction of mutans streptococci after gargling with a 2.5% garlic mouthwash solution. Another study, reporting a reduction in levels of *S. mutans* after garlic mouthwash, mentioned that a 3% concentration was the minimum concentration at which a zone of inhibition was observed.

Studies were there on the activities of garlic extract combination with apple vinegar on the bacterial isolates. The results of garlic extract combination with vinegar on bacterial isolates revealed that all bacterial isolates were highly sensitive to Vinegar Garlic Extract (Nadakhazalkadhim H, 2013).

Based on studies published in the international dental literature, the present investigation evaluated two important physicochemical properties of mouthwashes i.e pH and titratable acidity (Vivek S *et al.*, 2015). The measurement of the pH is a practical method to assess the erosive potential by measuring acidity of a solution (Vivek S *et al.*, 2015).

Although pH value equal to or less than 5.5 is considered critical for enamel dissolution, mineral loss may begin even at higher pH; 6. pH is a logarithmic scale. Small changes in pH values equate with larger changes in the hydrogen ion concentration therefore, the prolonged use of oral rinses with pH below this value may be potentially harmful to dental (Vivek S *et al.*, 2015).

It is currently thought that titratable acidity is a more accurate measure of the total acid content of a solution and therefore, more realistic means of predicting erosive potential. In this study, titratable acidity determined the amount of acid present and the volume of NaOH necessary to buffer the test solution, a characteristic directly related to the buffering capacity of the saliva. Substances with low titratable acidity are readily neutralized by oral fluids, while those with high titratable acidity cause a prolonged drop in pH and greater demineralization of dental tissues (Vivek S *et al.*, 2015).

Although the erosive potential of various mouthwashes can be compared it is not possible to define the degree to which it will damage teeth. It mainly depends on protective effect of pellicle and the buffering capacity of saliva of individual (Vivek S *et al.*, 2015).

The low pH of oral care products increases the chemical stability of some fluoride compounds and favors the incorporation of fluoride ions into the lattice of hydroxyapatite and the precipitation of calcium fluoride onto the tooth surface (Vivek S *et al.*, 2015). Based on this statement, product labels were examined to identify mouthwashes containing fluoride.

Lack of fluoride and low pH may make these products harmful to dental tissues if not used carefully. Although mouthwashes have been formulated as pre- and post-brushing products for routine use, findings of a previous in situ study conducted by Pretty IA, have suggested that low pH mouthwashes should not be considered for long-term or continuous use and never as pre-brushing. Although baseline acidity is a major factor in determining erosive potential, baseline pH values give only a glimpse of the initial hydrogen ion concentration and therefore provide no indication as to the presence of undissociated acids (Vivek S *et al.*, 2015).

CONCLUSION

The results of the present study, shows that Apple cider vinegar-Garlic extract combination mouthwash was less acidic when compared to Chlorhexidine. Thus the use of garlic incorporated combination mouthwashes may be a step forward in prevention of fall of salivary pH, thereby rendering more resistance against development of new carious lesions. The findings of this in-vitro investigation cannot be directly extrapolated to the clinical situation. Modification by adding calcium and phosphate to the mouth rinses may be a helpful measure to reduce the erosive potential of these products.

There is clearly a great need for more in vivo studies to know the possible detrimental effect of this custom mouthwash preparation in order to balance formulations more advantageously for benefits provided by them. Oral hygiene products have to be regulated by regulatory bodies for their safety, efficacy, acceptability and quality control.

ACKNOWLEDGEMENT

Nil

CONFLICT OF INTEREST

No interest

REFERENCES

- Akande OO, Aladai ARA, Aderinokun GA and Ige AO. Efficacy of Different brands of mouth rinses on oral bacterial load count in healthy adults. *African Journal of Biomedical Research*, 7, 2004, 23.
- Amit P. Mouthwashes and Their Use in Different Oral Conditions - Review Article. *Sch. J. Dent. Sci.*, 2(2B), 2015, 186-191.
- Anthony J and Matthew M. Dissolution of dental enamel in soft drinks, Gateway, 2004, 29.
- Atheer AK. Antibacterial activity of Garlic extract (allium sativum) against *Staphylococcus aureus* In Vitro. *G.J.B.B.*, 3(4), 2014, 346-348.
- Bing B, Fang C, Zhengfu W, Xiaojun L, Guanghua Z and Xiaosong H. Mechanism of the Greening Color Formation of "Laba" Garlic, a Traditional Homemade Chinese Food Product. *J. Agric. Food Chem.*, 53(18), 2015, 7103-7107.
- Fahiem MM, Gurpreet S, Husham E, Iti G, Amr S. In Vitro Antimicrobial Effectiveness of Vinegar against Oral Microorganisms: Part I. *Journal of International Oral Health*, 8(11), 2016, 999-1002.
- Gambon DL, Brand HS, Veerman EC. Unhealthy weight loss. Erosion by apple cider vinegar. *Pubmed*, 119(12), 2012, 589-91.
- Houshmand B, Mahjour F, Dianat O. Antibacterial effect of different concentrations of garlic(*Allium sativum*) Extract on dental plaque bacteria. *Indian J Dent Res*, 24, 2013, 71-5.
- Lingham T, Besong S, Ozbay G, Lee JL. Antimicrobial Activity of Vinegar on Bacteria Species Isolated from Retail and Local Channel Catfish (*Ictalurus punctatus*). *J Food Process Technol*, 2012, 11-001.
- Mariam M and Usha Devi C. Chemical and Shelf life Analysis Of Dry Garlic Powder: A Golden Herb. *International Journal of Agriculture and Food Science Technology*, 7(1), 2016, 1-6.
- Nadakhazalkadhim H. In vitro Antibacterial activity of Aquatic Garlic extract, Apple vinegar and Apple vinegar- Extract combination. *American journal of phytomedicine and clinical therapeutic*, 2013,042-051
- Nandhini T, et al. Comparison of the Effectiveness of a Commercially Available Herbal Mouth Rinse with Chlorhexidine Gluconate at the Clinical and Patient Level. *J. Pharm. Sci. & Res*, 7(8), 2015, 595-597.
- Narjis FI. Vinegar" as Anti-bacterial Biofilm formed by *Streptococcus pyogenes* Isolated from Recurrent Tonsillitis Patients, In vitro. *Jordan Journal of Biological Sciences*, 6(3), 2013, 191 – 197.
- Rafey AJ, Shah SK, Mohammad FK, Zubaidah HR, Marina MB and Fathilah BA. Is Synthetic Mouthwash the Final Choice to Treat Oral Malodour? -A Review article. *Journal of the College of Physicians and Surgeons Pakistan*, 24(10), 2004, 757-762.
- Renuka S and Muralidharan NP. Comparison in benefits of herbal mouthwashes with chlorhexidine mouthwash-A review. *Asian j pharm clin res*, 10(2), 2007, 3-7
- Shailesh K, Shubha P, Vinitha P, Anand T. Effects of garlic extract on salivary pH: a clinical study: *Arch OrofacSci*, 7(1), 2012, 1-8.
- Trial DA, Cem A and Celalettin K. The Effect of External Apple Vinegar Application on Varicosity Symptoms, Pain, and Social Appearance Anxiety. *A Randomized Controlled- Research Article*, 2016, 6473678.
- Vivek S, Shwetha R. Endogenous pH titratable acidity of commercially available mouthwashes in Indian market-review article. *Int J Clin Trials*, 2(1), 2015, 20-24.

Cite this article:

Latha P, Sujatha D, and Chandra Sekhar KB. Protective Effect of *Cissus quadrangularis* in Streptozotocin Induced Type-2 Diabetes Rat Model and On SHSY5Y Neuronal Cells. *International Journal of Phytopharmacology*, 2018; 9(1): 9-16. DOI: <http://dx.doi.org/10.21276/ijp.2018.9.1.2>