

**Study of antimicrobial efficacy of some plant extracts against oral pathogens and comparative analysis of their efficiency against commercially available toothpastes and mouth rinses**

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**Abstract**

The present study was aimed to determine the antimicrobial efficiency of ethanol extracts of six medicinal plants and comparing their antimicrobial efficiency with commercially available toothpastes and mouth rinses against three oral pathogens, *Streptococcus mutans*, *Escherichia coli* and *Candida albicans*. Results from this study have shows *S. aromaticum* and *A. sativum* ethanol extracts were more effective in inhibiting the growth *S. mutans* and *C. albicans* and equally effective against *E. coli* when compared with marketed products.

**Keywords:** *S. aromaticum*, *A. sativum*, toothpastes and mouth rinses, zone of inhibition, phytochemical analysis.

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## 1.0 Introduction

Oral diseases are one of the major public health problems as they have a considerable impact on individuals and communities by causing pain, suffering, impairment of normal functions and reduced quality of life. Dental caries and related oral diseases like gingivitis and periodontitis are most common oral diseases in developed as well as developing countries affecting people from all ages of life. The frequency of these oral diseases is continuously increasing as a result of the changing food habit, tobacco use, inadequate exposure to fluorides, and lack of access to dental care (Petersen et al., 2005; Saini et al., 2003).

Dental plaque is the major cause of dental caries and periodontal disease. Plaque is a habitat for different microorganisms (Hardie, 1992). *Streptococcus mutans* is one of the main opportunistic pathogens of dental caries (Gamboa et al., 2004) which plays a central role in fermentation of carbohydrates resulting in acid production, and leading to the demineralization of the tooth enamel. In addition, other microflora like *Escherichia coli* and *Candida albicans* are also associated with active caries lesions. *C. albicans* is the most common yeast isolated from the oral cavity. It is by far the most commonly isolated fungal species from infected root canals, showing resistance to intercanal medication (Oztan et al., 2006).

Among various causes for oral diseases, poor oral hygiene is one of the major reason for accumulation of these microbes and their harmful activities. The oral diseases can be best avoided in most of the cases by the proper maintenance of oral hygiene. This is usually achieved by the regular brushing of teeth, which may be combined with the use of additional oral hygiene products such as mouth rinses and toothpastes. A recent trend has seen the inclusion of antibacterial agents in many oral hygiene products.

A number of chemical antibacterial agents, such as Cetylpyridinium chloride, Chlorhexidine, Triclosan, or antibiotics have been used in the prevention and management of oral diseases. Long term and irrational use of the antibiotics may lead to like toxicity, stain teeth and increased incidence of antibiotic resistance. Hence, many attempts have been made to emphasize the need for alternative

solutions with the aim of providing affordable, not toxic and effective alternatives to synthetic chemical agents. One among such effort is emphasis on investigation of natural phytochemicals or the compounds isolated from plants for management and treatment of oral diseases.

Since ancient times, medicinal plants are used for cosmetic, food, flavors, ornamental and medicinal purpose. (Syam et al., 2008). For centuries, plant products have been used for treatment for various diseases, and more recently in the development of new drugs (Hooper et al., 2011). Medicinal plants are part of complementary medicine practiced worldwide because of their potential health benefits (Kumar et al., 2014; Seydnejad et al., 2010). Previous studies have indicated that several phytochemicals are effective as natural antimicrobials in inhibiting the oral microflora (Parkar et al., 2013; Amin et al., 2012; Verkaik et al., 2011).

The literature survey of the folklore medicine reveals the use of *S. aromaticum* to maintain oral hygiene; it is used as natural analgesic, used as an anodyne for dental emergencies (Prashar et al., 2006) and applied to a oral cavity in a decayed tooth as it gives relief from toothache. It also helps to decrease infection in the teeth due to its antiseptic properties.

There is extensive literature available on antibacterial effects of fresh garlic extract. Garlic extract has been reported to inhibit growth of various gram-positive and gram-negative bacteria including: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Lactobacilli*, *Pseudomonas*, *Salmonella*, *Shigella* and *Proteus* (Nidadavolu et al., 2012; Eja et al., 2011; Rattanachaikunsopon and Phumkhachorn, 2009). Garlic extract is also active against multidrug-resistant organisms such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Candida albicans* (Palaksha et al., 2010; Iwalokun et al., 2004).

Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in-vitro to have antimicrobial properties (Adonu et al., 2013; Chaitra et al., 2012; Pathmanathan et al., 2010).

## Study of antimicrobial efficacy of some plant extracts against oral pathogens and comparative analysis of their efficiency against commercially available toothpastes and mouth rinses

By keeping in mind the antimicrobial significance of medicinal plants, the present study was designed to study the antimicrobial properties of ethanol extracts of six medicinal plants (*C. cassia*, *A. sativum*, *S. aromaticum*, *P. granatum*, *C. lemonium* and *H. sabdariffa*). An attempt has also been done to compare the antimicrobial efficiency of selected plant extracts with commercially available toothpastes and mouth rinses against three oral pathogens, and to identify the possible reasons for their activity by studying the phytochemical profile of the selected plants.

### 2.0 Materials and methods

#### Plant Materials

The selected dried samples (*C. cassia*, *S. aromaticum* and *H. sabdariffa*) were purchased from a well-known local retail markets for Egyptian herbal medicines at Sohag district, Egypt. Fresh plants (*A. sativum*, *P. granatum* and *C. lemonium*) were purchased from a local market at Sohag district, Egypt. Identification was done by botanists at the Botany Department, Faculty of Science, Sohag University.

#### Preparation of plant Extracts

Plant extracts were prepared according to the methods mentioned by Alade and Irobi (1993) with minor modification. A known weight (one hundred grams) of each powdered dry herbal plants were extracted separately by maceration method using

ethanol (70%). Maceration was continued for 48 h with frequent agitation and the resulting liquid is filtered using filter paper (Whatman No 1, Whatman Ltd., England). Extraction was repeated for five times and the all the filtrates were combined, solvent was removed under reduced pressure using Heidolph, VE-11 rotary evaporator (at 50°C) until the residue become completely dry. The dried plant extract was re-suspended in a small volume of the same solvent, transferred into pre-labelled and pre-weighed glass vials and allowed to dry. The total weight of the obtained extracts were measured and stored until use.

#### Microorganisms and Culture Media

Previously identified cultures of *S. mutans*, *E. coli* and *C. albicans* were obtained from patients with oral infections who attended the Dental Clinic, Department of Dentistry, Sohag University Hospital, Sohag District, for treatments for different oral diseases. *S. mutans* was cultured in brain heart infusion broth at 37°C for 24 h while *E. coli* was cultured in nutrient broth at 37°C for 24 h. *C. albicans* was cultured in sabouraud's dextrose broth at 37°C for 48 h.

#### Toothpastes and Mouthrinses

Five different brands of toothpastes and brands of mouthrinses were purchased from drug stores and their composition is depicted in Tables-1 and 2.

**Table-1: Ingredient details of toothpaste formulations tested for antimicrobial potential**

Brand name	Ingredients used
<b>Colgate</b>	Sodium monofluorophosphate 0.76%, sodium fluoride 0.1%, dicalcium phosphate dihydrate, aqua, sorbitol, glycerin, PEG-12, sodium lauryl sulfate, aroma, cellulose gum, tetrasodium pyrophosphate, sodium saccharin, limonene; contains no sugar
<b>Signal</b>	Sodium monofluorophosphate (1450 ppm fluoride), calcium glycerophosphate, calcium carbonate, sorbitol, water, hydrated silica, sodium lauryl sulfate, aroma, flavor, trisodium phosphate, potassium citrate, cellulose gum, sodium saccharin, phenyl carbinol, glycerin, limonene, CI74260
<b>Close up</b>	Sodium fluoride (1450 ppm fluoride), sorbitol, silica, water, PEG-32, mix of flavors, cellulose gum, sodium saccharin, limonene, CI 42090 , CI 47005
<b>Crest</b>	Sodium fluoride 0.321 % , sorbitol, hydrated silica, cellulose gum, aroma, sodium saccharin, trisodium phosphate, carbomer limonene, CI 74160, CI 77891
<b>Sensodyne</b>	Strontium chloride hexahydrate 10%

#### Antimicrobial Assay

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The antimicrobial activity of plant extracts, different toothpastes and mouth rinses was determined according to method described by Chrkraborty (1996). Agar media was poured into petri dish containing 0.1mL suspension of bacteria ( *S. mutans*, *E. coli* and *C. albicans* ). By using cork borer bores of 8 mm in diameter were made on media. After surface solidification, 100 µl of plant extracts (100 mg/ml) and different dentifrices at two different concentrations: 1:1 (50%) and full strength (100%) was added to each bore. Plates were incubated at 37°C for 18-24 h for bacteria and for 48 h for *C. albicans*. The antimicrobial activity of tested extracts and different dentifrices against the oral pathogens was determined by measuring the inhibition zones around each bore. Sterile DMSO served as the negative control and Ciprofloxacin (for bacteria), Amphotericin-B (for *Candida*) served as the positive controls.

### Effect of temperature and pH on stability of plant extracts

Thermostability of tested extracts was determined as following: Five milliliters of 100mg/ml of plant extracts were constituted in test tubes and exposed to 4, 30, 60, 90 and 120°C in a water bath for 30 minutes and tested for antimicrobial activity.

To determine the effect of pH, plant extracts were treated with various solutions of at varying pH ranges that is 5, 6, 7, 8 and 9 using 1N HCl and 1N NaOH solutions respectively for 30minutes. After 30 minutes of treatment, each of the treated extracts were neutralized (to pH 7) In vitro lethality assay of *Artemia salina* was used to check the toxicity of crude extracts and the extracts exposure to different temperature degrees and pH (Meyer et al., 1982). Brine shrimp eggs were placed in seawater (3.8% w/v sea salt in distilled water) and incubated at 24-28°C in front of a lamp. Eggs were hatched within 48h providing large number of larvae (nauplii). The hatched nauplii suspension was left to stand for 1 h without aeration, and then the nauplii were collected by pipetting from middle layer of solution, in which most of nauplii were swimming. A convenient number of nauplii were placed in vials containing 5ml of seawater and a different concentration of plant extracts (1, 10, 100 and 1000 µg/ml).

Control was made with the same volume of pure DMSO in seawater without addition of plant extracts. Live nauplii were counted

using 1N HCl and 1N NaOH as the case. may be, and then tested for antimicrobial activity by agar-well diffusion assay as described above.

Demonstration the cytotoxicity of plant extracts by Brine shrimp lethality assay Brine shrimp larvae (*Artemia salina*) are commonly used for cytotoxicity assays in pharmacology. These larvae are sensitive to toxic substances. The ratio between dead larvae (no motility) and living larvae (high motility) incomparison to a control without any toxic substances is used to estimate the toxicity of the test solutions. The test is not only used for predicting cytotoxicity, but is also used as a predictor of antitumor activity (Sanchez et al., 1993).

**Table-2: Mouthrinsers  
Ingredients as listed on packages**

Brand name	Ingredients used
<b>Ezafluor</b>	Cetylpyridinium chloride, Amine fluoride
<b>Orasan</b>	Triclosan, Citrimide, Glycerine
<b>Oracin</b>	Chlorhexidinegluconate, Methyl Salicylate, Methyl hydroxy benzoate, Butyl hydroxy toluene, Sodium benzoate, Tegosml, Tegosorb, Deionized water
<b>Fluocal</b>	Sodium fluoride, Potassium nitrate
<b>Betadine</b>	Povidone-iodine

after 24 h against a lighted background, and the average number of survived larvae in each triplicate was determined by using the following equation.

$$\% \text{ mortality} = \left( \frac{\text{No. of dead or stopped swimming brine shrimps}}{\text{total number of brine shrimps}} \right) \times 100$$

The results of the study is shown in table-3.

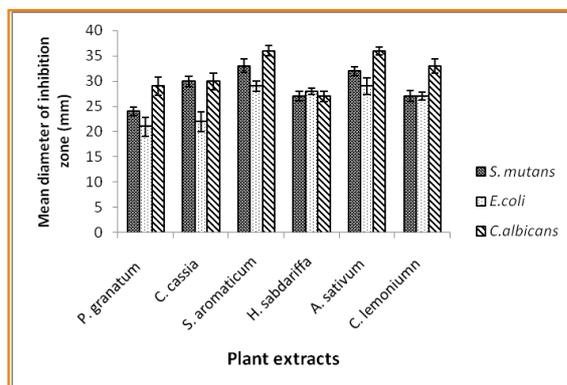
### Phytochemical Screening

Standard qualitative methods as described by (Tiwari et al., 2011) were adopted for phytochemical screening. The ethanol extracts of the tested plants were tested for phytochemical constituents using the following tests and reagents: reducing sugars with Fehling's test, anthraquinones with Borntrager's test, flavonoids with ammonia and sulphuric acid, saponins with foam test, tannins with Ferric Chloride test, alkaloids with Mayer's and

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Dragendorff's tests and cardiac glycosides with Keller- Killian's test. The results are

shown in table-4.



**Figure-1: Zones of inhibition produced by the most active plant extracts against the three tested microorganisms**

### 3.0 Results and discussion

The results obtained of the antimicrobial activity are summarized in Figure-1. It can be observed from the outcome of the study that the tested plant extracts have a good antimicrobial activity with varying

magnitudes against the tested pathogens as *C. albicans* was the most sensitive isolate followed by *S. mutans*, followed by *E. coli* for most of plant extracts.

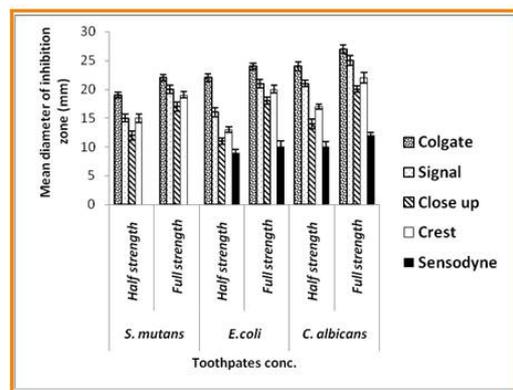
**Table-3: The number of shrimp nauplii that survived after treating with the six plant extracts and the percentage mortality**

Plant Extracts	Concentration (µg/mL)	Total Number of Survivors	% Mortality	LC <sub>50</sub> (µg/mL)
<i>P. granatum</i>	1	27	10	100
	10	21	30	
	100	15	50	
	1000	0	100	
<i>C. cassia</i>	1	29	3.33	130
	10	24	20	
	100	18	40	
	1000	0	100	
<i>S. aromaticum</i>	1	22	27	10
	10	15	50	
	100	0	100	
	1000	0	100	
<i>H. sabdariffa</i>	1	28	6.66	76
	10	19	36.7	
	100	13	56.6	
	1000	0	100	
<i>A. sativum</i>	1	24	20	10
	10	15	50	
	100	0	100	
	1000	0	100	
<i>C. lemoniumn</i>	1	23	23	54
	10	16	47	
	100	14	53	
	1000	0	100	

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\*No mortality was found in negative control (DMSO) group.

Moreover, ethanol extracts from *S. aromaticum* and *A. sativum* provided the highest antimicrobial efficacy against the tested species as the mean inhibition zone diameters of *S. aromaticum* extract was 36 mm for *C. albicans*, 28 mm for *E. coli* and 33 mm for *S. mutans*. As for *A. sativum* extract, the mean inhibition zone diameters were 36 mm for *C. albicans*, 29 mm for *E. coli* and 32 mm for *S. mutans*. It can be observed from the antimicrobial study that the ethanol extracts of most tested plants showed broader zones of inhibition than that of Ciprofloxacin toward bacteria and Amphotericin-B for *Candida*.



**Figure-2: Zones of inhibition produced by toothpaste formulations against the three tested microorganisms at two different dilutions**

Results of this investigation showed that different toothpaste brands exhibited wide range of inhibitory activity against the tested isolates (Figure-2). Among all the investigated toothpastes, Colgate toothpaste has emerged as the most effective compared formulation compared to all other toothpaste formulations (with inhibition zones ranged from 22 to 27 mm at full strength), followed by Signal, Crest and Close up toothpaste formulations respectively, whereas the lowest inhibitory effect were expressed by Sensodyne toothpaste with inhibition zones ranging from 0 to 12 mm.

The mean inhibition zone diameters of the five toothpaste brands at full strength ranged between 12 and 27 mm for *C. albicans*. However, the mean inhibition zone against *E. coli* ranged between 10 and 24 mm and between 0 and 22 mm for *S. mutans*.

Among the tested mouthrinses, the highest activity was detected from Oracin mouth rinse with 35 mm inhibition zone against *C. albicans*, 31 mm against *S. mutans* and 29 mm against *E. coli*, whereas the lowest activities was expressed by Betadine against all tested species with inhibition zones ranging from 0 to 12 mm. Antimicrobial activity of tested mouthrinses

is in decreasing order: Oracin > Orasan > Ezafluor > Fluocal > Betadine (Figure-3).

The results revealed that the inhibitory effect of different brands of dentifrice formulations against the tested pathogens were dose-dependent as the diameter of the growth inhibition zone was directly proportional to the concentration of the dentifrice. Moreover, in case of Colgate toothpaste and Oracin mouth rinse, their efficiency was high even at the lower concentration (1:1).

Results obtained here demonstrated that Oracin mouth rinse which considered the most effective chemical antiseptic product among all the tested toothpastes and mouth rinses provided higher antimicrobial efficacy than *P. granatum*, *C. cassia*, *H. sabdariffa* and *C. lemonium* extracts against all tested pathogens. While *S. aromaticum* and *A. sativum* extracts were more effective in inhibiting the growth of two target oral pathogens (*S. mutans* and *C. albicans*) and equally effective against *E. coli* when compared with Oracin mouth rinse.

### Effect of temperature and pH on antimicrobial activity of the extracts

It is clear from Figure-4 that various temperature ranges of 4, 30, 60 and 90°C had no effect on the antimicrobial efficiency of the extracts, but the extracts are losing

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their activity at 120°C. The experiment was repeated three times to avoid the errors and they produced similar results.

The stability of plant extracts at different pH levels was carried out at room temperature. The maximum zone of inhibition against all the tested pathogens

was observed at the original pH. A moderate level of activity was observed at the neutral pH (7) and at acidic pH (5, 6) the activity slightly increased, while at alkaline pH (8, 9) the activity of the plant extracts reduced as shown in Figure-5.

**Table-4: Phytochemical screening of ethanol extract of tested medicinal plants**

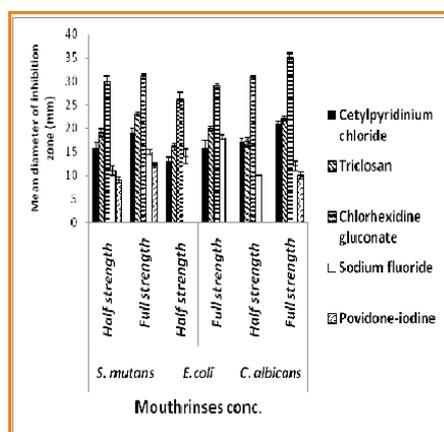
Plant Spp.	Alkaloids	Saponin	Tannins	Steroids	Flavonoids	Anthraquinone	Glycosides	Reducing sugar	Phenolic comp.
<i>C. lemonium</i>	-	-	+	++	+	-	++++	+	+
<i>A. sativum</i>	+	+++	-	++	+	-	++	+	+
<i>H. sabdariffa</i>	+	+	+	+	+	+	++	-	-
<i>S. aromaticum</i>	-	-	+	+++	+	+	-	-	++
<i>C. cassia</i>	-	-	-	+++	-	-	-	-	-
<i>P. granatum</i>	+	-	+	-	+	-	-	-	+

- = negative, + = positive; relative percentage (+, ++, +++, +++)

### Assessment of Bioactivity of the Most Promising Plants using Brine Shrimp (*Artemia salina*) Lethality Assay

Results from toxicity study revealed that maximum mortalities (100%) of shrimps were observed at a concentration of 1000µg/ml by all plant extracts whereas least mortalities were observed at 1µg/ml concentration. This clearly indicate that the

lethality of extracts was directly proportional to the concentration of the extract. Besides that, in case of both *S. aromaticum* and *A. sativum* extracts, maximum mortalities (100%) also were observed even at moderate concentration of 100 µg/ml –(Table 3).



**Figure-3: Zones of inhibition produced by mouth rinse formulations against the three tested microorganisms at two different dilutions**

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LC<sub>50</sub> values of the plant extracts were obtained by a plot of percentage of the shrimp nauplii killed against the concentrations of the extracts and the best-fit line was obtained from the data by means of regression analysis. The lethality concentration LC<sub>50</sub> of the *S. aromaticum* and *A. sativum* extracts were found to be 10µg/mL.

### Phytochemical Screening

Results of phytochemical study indicate that the Ethanolic extracts of *S. aromaticum* and *A. sativum* extracts contain saponins, steroids, cardiac glycosides, reducing sugar, alkaloids and phenolic compounds, whereas *S. aromaticum* contain steroids, phenolic compounds, flavonoids, tannins and anthraquinones.

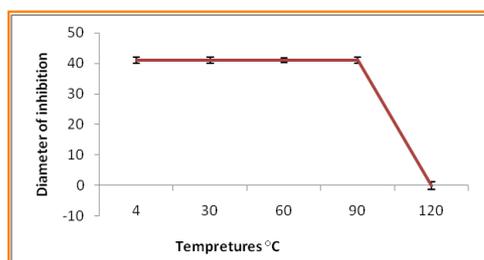


Figure-4: Thermostability of plant extracts

Maintenance of good oral hygiene is the key to the prevention of oral diseases. The importance of oral microflora being responsible for mouth odor and most oral diseases is well documented. (Ciancio, 2003).

Several studies have previously demonstrated the antibacterial potency of various plant extracts against oral pathogens (Kumar et al., 2014; Devi et al., 2012; Adebisi and Ojokoh, 2011).

The present study showed that all the investigated oral care products exhibited wide variations in their effectiveness against the three test pathogens, a feature that may have been largely attributed to the ingredients used in the toothpaste and mouth rinse formulations. Among all the investigated toothpastes, Colgate toothpaste emerged as the most effective against all the three organisms tested. This may be due to presence of sodium monofluorophosphate and sodium fluoride. Next to Colgate, Signal toothpaste has significant antimicrobial activity against the tested organisms. It contains sodium

monofluorophosphate and calcium glycerophosphate as active ingredients. This was followed by Close up toothpaste which contains only sodium fluoride.

Fluorides are popularly used in many oral health products as are reported to help in caries prevention (Marinho, 2009). The outcome of the present result was in agreement with the results obtained by Featherstone (2004) who reported that if the bacterial challenge is too high, it is not possible for fluoride to overcome the challenge completely. Lastly, Sensodyne toothpaste had the lowest inhibitory effect on tested pathogens which may be due to the presence of a single ingredient in its formulation (strontium chloride hexahydrate).

In case of mouth rinses, Oracin was found to be the most effective mouth rinse, which showed maximum antimicrobial efficacy against all the target oral pathogens. This may be due to the presence of Chlorhexidine gluconate as major ingredient in its formulation; this observation adds information to the earlier study carried out by (Prasanth et al 2011). Chlorhexidine formulations are considered to be the "gold standard" anti-plaque mouthrinses due to their prolonged broad spectrum antimicrobial activity and plaque inhibitory potential (Amornchat et al., 2006; Sheen et al., 2001).

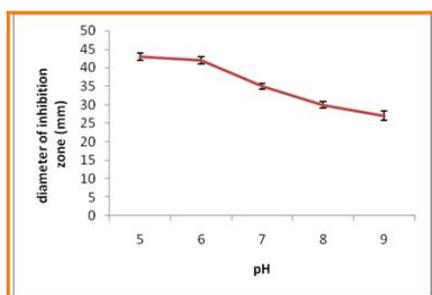
Activity of test-mouth rinses was in decreasing order based on their active ingredients: Chlorhexidine gluconate, Triclosan, Cetylpyridinium chloride, Sodium fluoride and lastly Povidone-iodine. When compared with plant extracts, the antimicrobial efficiency of the most active products (chlorhexidine mouth rinse) was lower than the efficiency of *S. aromaticum* and *A. sativum* extracts at the tested concentrations.

Previous studies have reported the efficacy of dentifrice containing herbal extracts in maintaining good oral health (Kothiwale et al., 2014; Hooper et al., 2011; Bradtke, 2008). Previous study also has shown that *A. sativum* juice was more effective against oral pathogens when compared with chlorhexidine mouthwash (Amin et al., 2012). There are reports in which it is documented that toothpowder consisting of herbal constituents was found to be as effective as the toothpastes marketed in the open markets (Vohra et al., 2012). Traditionally, the buds of *S. aromaticum* have been used to treat tooth

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ache due to its local anaesthetic activity (Cai and Wu, 1996).

Although the antimicrobial agents like Chlorhexidine, Cetylpyridinium chloride and fluorides are widely used; immediate hypersensitivity reactions, toxicity, tooth staining, changing of oral taste, desquamation of oral mucosa and other side effects have been reported (Mathur et al., 2011; Okpalugo et al., 2009). Moreover, it has been reported that Chlorhexidine is cytotoxic to human periodontal ligament cells, inhibit protein synthesis, and affect mitochondrial activity, thus having detrimental effects on vital tissues (Beaudouin et al., 2004; Chang et al., 2001).



**Figure-5: Stability of plant extracts against different pH values**

It is known fact that a balance exists in a person's oral microbial population. If this balance is lost, opportunistic microorganisms can proliferate, enabling the initiation of disease processes. Therefore, compounds possessing good antimicrobial properties even after dilution due to saliva in oral cavity may be useful in prevention and treatment of various oral diseases. In the present work, it has been found that extracts like *S. aromaticum* and *A. sativum* which possess good antimicrobial activity can be effective without loss the balance exists in a person's oral microbial population.

The activity of the most plant extracts tested was not affected when exposed to different temperature ranges (4, 30, 60 and 90°C), but the extracts lost their activity at 120°C. The temperature resistance studies are indication that the phytoconstituents are thermostable. This further supports the traditional usage of these plants where a very high temperature is used to boil them and for a longer period of time. It is a known fact that the loss of antimicrobial activity of natural products by heating to

120°C may be due to volatilization and/or the physical and chemical changes that take place during heating.

The antimicrobial activity of the extracts slightly increased at acidic pH, but reduced at alkaline pH. Increase in activity of phytoconstituents in the presence of acidic medium has earlier been reported (Salama and Marraiki, 2010; Doughari, 2006). The local application of these plants involves the addition of high doses of potash which is a strong basic salt, and for the fact that the activity of the extracts reduced at alkaline pH in this study, it may explain why the plant concoction is taken for longer period of time before any curative effect is noticed. This result agrees with the findings of other researchers on stability of plant extracts like Mehrotra et al (2010) who reported that bioactive components of ethanol extract of *S. aromaticum* buds were stable over a wide range of pH values and temperatures. Durairaj et al. (2009) concluded that *A. sativum* extract stored at room temperature showed inhibitory activity against the tested pathogens up to 7 days and when the extract was stored at 4°C, they exhibited moderate activity till 30 days and 60 days if the same extracts are stored at -20°C and also they have also reported that the antimicrobial activity of *A. sativum* extract decreased with the increase of the pH value.

In order to study the toxicity of the selected medicinal plants, brine shrimp lethality bioassay was employed which was based on the ability of tested samples to kill laboratory cultured brine shrimp (*Artemia salina* nauplii). The assay is considered a useful tool for preliminary assessment of toxicity since the brine shrimp is highly sensitive to a variety of chemical substances (Lachumy et al., 2010) and it represents a rapid, inexpensive, and simple bioassay for testing plant extracts bioactivity, which in most cases correlates reasonably well with cytotoxic and anti-tumor properties (Sam, 2010; Pisutthanan et al., 2004, Chanda and Baravalia, 2011). It is assumed that the medicinal properties of plant extracts may not be due to one of the component but rather due to a mixture of bioactive plant components present in the extracts.

The ethanol extracts of the six plants tested showed good brine shrimp larvicidal activity. Based on the results, the brine shrimp lethality of the extracts was found to be concentration-dependent. The ethanol

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extracts of *S. aromaticum* and *A. sativum* are very toxic to brine shrimps on exposure for 24 hours in a dose dependent manner in which the tested animals were killed at 100 and 1000 $\mu\text{g}/\text{mL}$  doses of the extract indicating the presence of potent cytotoxic components responsible for the observed toxicological activity. The result on the lethality of the medicinal plant extracts on brine shrimps is in agreement with other studies (Olowa and Nuñez, 2013; Gadir, 2012).

The lethality concentration LC<sub>50</sub> of the *S. aromaticum* and *A. sativum* extracts was found to be 10 $\mu\text{g}/\text{mL}$  which is within the range of 0-100 considered to be very toxic (Ogugu et al., 2012). Some brine shrimp results that are already available (Moshi et al., 2006; 2004) provide a circumstantial evidence that plant extracts with LC<sub>50</sub> values below 20 $\mu\text{g}/\text{mL}$  have a likelihood of yielding anticancer compounds.

According to the results of the phytochemical screening study, *S. aromaticum* and *A. sativum* extracts contain important phytochemicals viz phenols, flavonoids, alkaloids, flavonoids, saponins and tannins. It may be assumed that the presence of these phytochemicals may be responsible for the medicinal properties and the antimicrobial effect exhibited by these plants. These findings are comparable to the results from other workers (Amin et al., 2013; Rana et al., 2011; Olusanmi and Amadi, 2010, Musa, 2012).

We can conclude from the results of the present study that extracts of *S. aromaticum* and *A. sativum* which possess good antimicrobial activity may be used as natural mouth antiseptics in dental

preparations. Use of these plant extracts may be recommended as a supportive or alternative option to conventional formulations (toothpaste or mouth rinse) as these plant extracts may provide inexpensive, safe, effective and readily available alternative in maintaining oral hygiene.

### 4.0 Conclusion

The study concluded that *S. aromaticum* and *A. sativum* ethanol extracts have a significant antimicrobial effect against tested oral pathogens. Moreover, these natural antimicrobial agents were more effective in controlling the oral microflora compared to toothpastes and mouth rinses which contain synthetic antimicrobial agents like Chlorhexidine and Triclosan. These plant extracts can be used as a possible alternative or supportive alternative to different oral care products. However, further extensive research and development work should be undertaken on the active components of these plant extracts for their better economic and therapeutic utilization. In vivo clinical testing is also essential to conform in vitro results. More research on usefulness of the tested plants in dentistry can give us simple, effective solutions to oral diseases.

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