

A Jamaican Study: Invitro Comparison of the Effects of *Lantana camara*, *Gouania lupuloides* and Commercial Mouthwashes on Oral Microorganisms

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Abstract Objectives: The study was to compare the efficacy of selected commercial mouthwashes and natural products against oral microorganisms. **Method:** Red sage (*Lantana camara*) and Chew stick (*Gouania lupuloides*) plant material were collected from Northern Caribbean University farm. Ethanolic and aqueous extracts of the plants were extracted and concentrated with a rotor vapor. The extracts were utilized for the preparation of different concentrations for antimicrobial sensitivity. Commercial mouthwashes: Crest, ACT, Ultra Care, Cari-Med and Listerine were tested against the isolates: *Streptococcus mutans*, *Escherichia coli*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* from oral cavity. Phytochemical screening was done on the plant extracts to determine their active ingredients. **Results:** The results showed that Red sage and Chew stick extracts had the highest zone of inhibition against the oral microorganisms compared to the commercial mouthwashes. The microorganisms were weakly sensitive to the mouthwashes. Aqueous plant extracts tested positive for phytochemical screening. **Conclusion:** It is evident that the plant extracts had antimicrobial properties. The oral microorganisms were sensitive to the ethanolic and aqueous extracts. Red sage aqueous extracts was the most potent against all tested microorganisms. Thus, further work is in progress to consider Red sage and chew stick natural products as mouthwashes for treatment of oral cavity infections.

Keywords: oral microorganisms, commercial mouthwashes, plants extracts and antimicrobial activity

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1. Introduction

Normal flora is described as the variation of organisms that are regularly found at any anatomical site of the body [1]; they colonize the human body during birth or shortly thereafter, remaining through life. Normal flora can be found in many sites of the human body including the skin, respiratory tract, urinary tract and the digestive tract. On the other hand, areas of the body, such as the brain, the circulatory system and the lungs are intended to remain sterile. The human body provides many unique environments for different bacterial communities [2].

1.1. Oral Cavity and Microorganisms

The oral cavity is comprised of many surfaces, each coated with a plethora of bacteria. Some of these bacteria have been implicated in oral diseases such as caries and periodontitis, which are among the most common bacterial infections in humans [3] Oral bacteria can be classified primarily as Gram positive and Gram negative microorganisms, and secondarily as either anaerobic or facultative anaerobic according to their oxygen requirements. An

opportunistic pathogen is an infectious microorganism that is normally a commensal or does not harm its host but can cause disease when the host's resistance is low [4] When the immune system is not adequately functioning, normal flora can overpopulate or move into areas of the body where they do not normally occur [4] Opportunistic bacteria include *Pseudomonas*, *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus pneumoniae* and *Escherichia coli*. *Streptococcus mutans* is naturally found in the oral cavity.

Pseudomonas aeruginosa is a Gram-negative, aerobic rod belonging to the *Pseudomonadaceae* family [4,5] This blue-green pus forming bacterium acts as normal flora in the lower gastrointestinal tract but opportunistically infects individuals and has become an important cause of infection in patients who are immuno-compromised. *Pseudomonas* rarely causes infection in healthy persons but it is a major cause of nosocomial infections. *Staphylococcus aureus* belongs to the family of *Staphylococcaceae* and is a Gram-positive, non-motile coccus It is commonly found residing on the skin and within the nose, pharynx and mouth [6].

Streptococcus pneumoniae belongs to the *Streptococcaeae* family and is a Gram-positive coccus [5] It is present in

the upper respiratory tract (pharynx and mouth), but if it invades the lower respiratory tract it can cause pneumonia. This bacterium causes 95% of all bacterial pneumonia [1]. *Escherichia coli* is a Gram-negative rod-shaped bacterium commonly found in the lower intestine of warm-blooded organisms [5]. *E.coli* is the most prevalent infecting organism in the family of *Enterobacteriaceae* and is the most studied free-living organism [7].

Streptococcus mutans is the primary bacterium involved in plaque formation and initiation of dental caries. *S. mutans* has been found to not only be a potential pathogen but also serves a role in causing cavities in the teeth (Mac, 2011). The *Streptococcus mutans* is a potent initiator of caries because there is a variety of virulence factors unique to the bacterium that have been isolated that play an important role in caries formation. *S mutans* is an anaerobic bacterium known to produce lactic acid as part of its metabolism.

Mouthwashes are used for a variety of reasons: to freshen breath, to help prevent or control tooth decay, to reduce plaque, to reduce the speed that tartar forms on the teeth or a combination of these effects (Mouthrinses, 2015). Basic ingredients include water, alcohol, cleansing agents, flavouring ingredients and colouring agents (Mouthrinses, 2015).

1.2. Plants with Medicinal Value

Lantana camara also known as the Red Sage plant has several uses, mainly as a herbal medicine and in some areas as firewood and mulch. The leaves are used to relieve itching. Other uses are against flu, colds, coughs, fevers, yellow fever, dysentery and jaundice. The roots are used for gonorrhoea. The use of lantana extracts as potential biocides have been suggested. Plant extracts are used as medicine for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atony of abdominal viscera. (Lantana camara, 2008).

Gouania lupuloides also known as Chew stick is a plant or herb of the family *Rhamnaceae* that is found in the West Indies. It is popularly grown in the woodlands of Jamaica and is preferably used by many people to naturally clean the teeth. It contains antibacterial as well as anti-cariogenic properties. It is not popularly used today, but it still retains its importance in dental and oral hygiene. Chew stick is herbal remedy for gum ailments and preventing tooth decay. Therefore, utilizing it is not only for whitening of the teeth but it may be used for health benefits as well. It can also help to keep away tartar and plaques build up. This is due to the fact that the foam from this herb will circulate even between closely knit teeth and will work as a dental floss. (Chew stick medicinal herb, 2015).

1.3. Objectives

The aim for this study was to determine the efficacy of natural products and antiseptic mouthwashes against oral microorganisms. Phytochemical compounds in *Lantana camara* and *Gouania lupuloides* extracts were analyzed to determine their antimicrobial properties.

2. Methodology

2.1. Sample Collection of Antiseptic Mouthwashes

Mouthwashes in the study were selected according to their contents and active ingredients. Five commercial brands were purchased and utilized: Listerine, Cari-Med, Crest (Prohealth), UltraCare, Corsidine

2.1.1. Plant Materials

The plants were collected randomly from Northern Caribbean University (NCU) Farm and identified taxonomically by a botanist. These plants (2) included: *Lantana camara* (Chewstick) and *Gouania lupuloides* (Redsage).

2.1.2. Sample Preparation and Treatment

For each plant material different parts were separated: flowers, leaves, stems and roots, air-dried and blended to give a coarse powder. Extracts were obtained using distilled water and ethanol and were decanted, filtered and the solvent was evaporated on a rotary evaporator and oven at 55°C.

2.2. Subculture Strains of Oral Bacteria

A standard strain of oral bacteria isolates such as: *Streptococcus mutans*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were sub-cultured in blood and MacConkey agar.

2.2.1. Agar Diffusion (Disk/Well Plate)

Selected antiseptic mouthwashes and different concentrations of plant extracts (10mg, 20mg, 30mg, 40mg, 50 mg) were used for antimicrobial sensitivity testing. 10 µl of suspension was transferred to the Muller Hinton agar to determine zones of inhibition of the organisms.

2.3. Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Detection of the following compounds was done: alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, terpenes, proteins and amino acids.

3. Results

Table 1. Zones of Inhibition (mm) of Commercial Mouthwashes Against The Oral Pathogen Isolates

Organisms	Crest	ACT Restoring	Ultra Care	Cari Med	Listerine
<i>S. aureus</i>	30 (S)	26 (S)	0 (R)	0 (R)	0 (R)
<i>E.coli</i>	18 (S)	0 (R)	0 (R)	8 (R)	7 (R)
<i>P. aeruginosa</i>	0 (R)	7 (R)	7(R)	8(R)	7(R)
<i>S.pneumoniae</i>	24 (S)	18 (S)	0(R)	0(R)	0(R)
<i>S. mutans</i>	30 (S)	20 (S)	0(R)	0(R)	0(R)

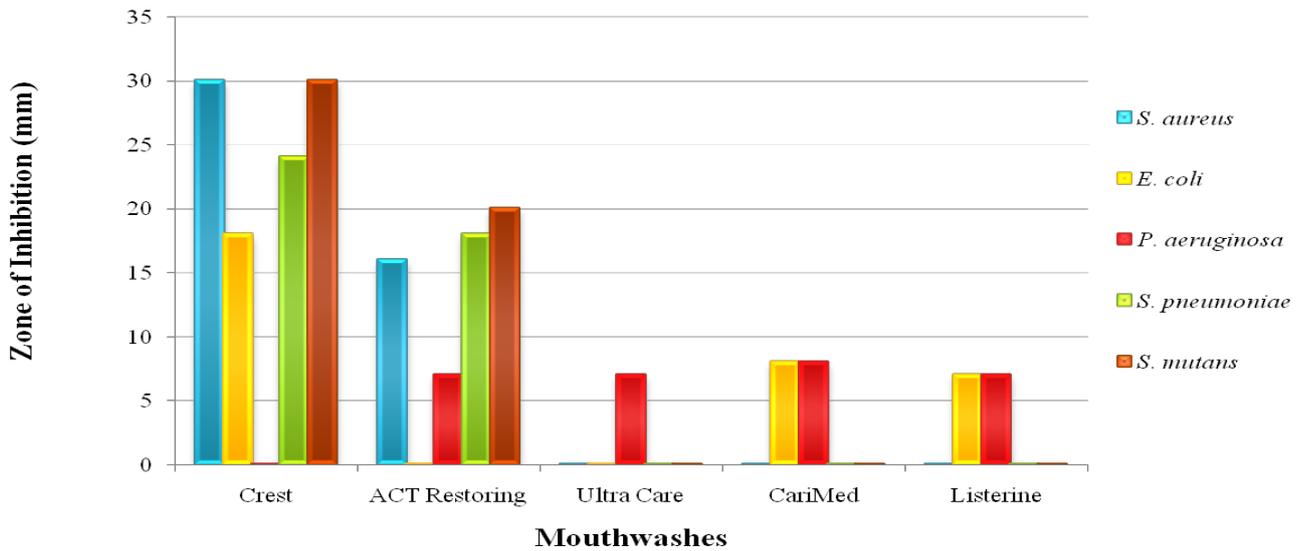


Figure 1. Zones of Inhibition (mm) of The Isolates Against Mouthwashes

Table 2. Zones of Inhibition (mm) of Plant Extracts Against The Isolates

Organisms	Extract	Conc. 10mg	Conc. 20mg	Conc. 30mg	Conc. 40mg	Conc. 50mg
<i>S. aureus</i>	PPE	14(S)	11(S)	13(S)	17(S)	20 (S)
	PFW	0(R)	0(R)	0(R)	0 (R)	0 (R)
<i>E.coli</i>	RFW	0 (R)	0 (R)	10(S)	14(S)	19 (S)
	PFE	0(R)	0(R)	0(R)	0 (R)	0 (R)
<i>P. aeruginosa</i>	PFE	10(S)	18(S)	14(S)	15(S)	19 (S)
	PFW	0(R)	0(R)	0(R)	0(R)	0(R)

Key: PFW- Pink flower water, PFE- Pink flower ethanol, RFW- Red flower ethanol

Table 3. Zones of Inhibition (mm) of Plant Extracts Against The Isolates

Organisms	Extract	Conc. 10mg	Conc. 20mg	Conc. 30mg	Conc. 40mg	Conc. 50mg
<i>S. pneumoniae</i>	RFW	14(S)	18(S)	17(S)	15(S)	15 (S)
	PFW	0(R)	0(R)	0(R)	0 (R)	0 (R)
<i>S.mutans</i>	RFW	10 (S)	11 (S)	11(S)	14(S)	14 (S)
	PFW	0(R)	0(R)	0(R)	0 (R)	0 (R)

Key: PFW- Pink flower water, PFE- Pink flower ethanol, RFW- Red flower ethanol

S. aureus

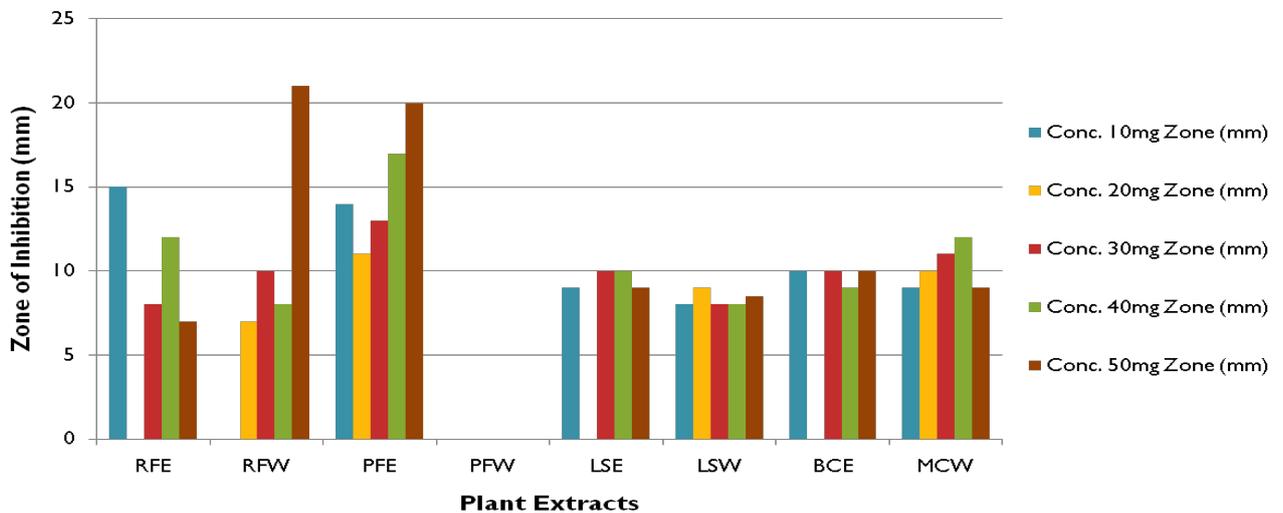


Figure 2. Comparison Among Eight Plants Extracts Concentrations versus The Zones of Inhibition (mm) *Staphylococcus aureus*

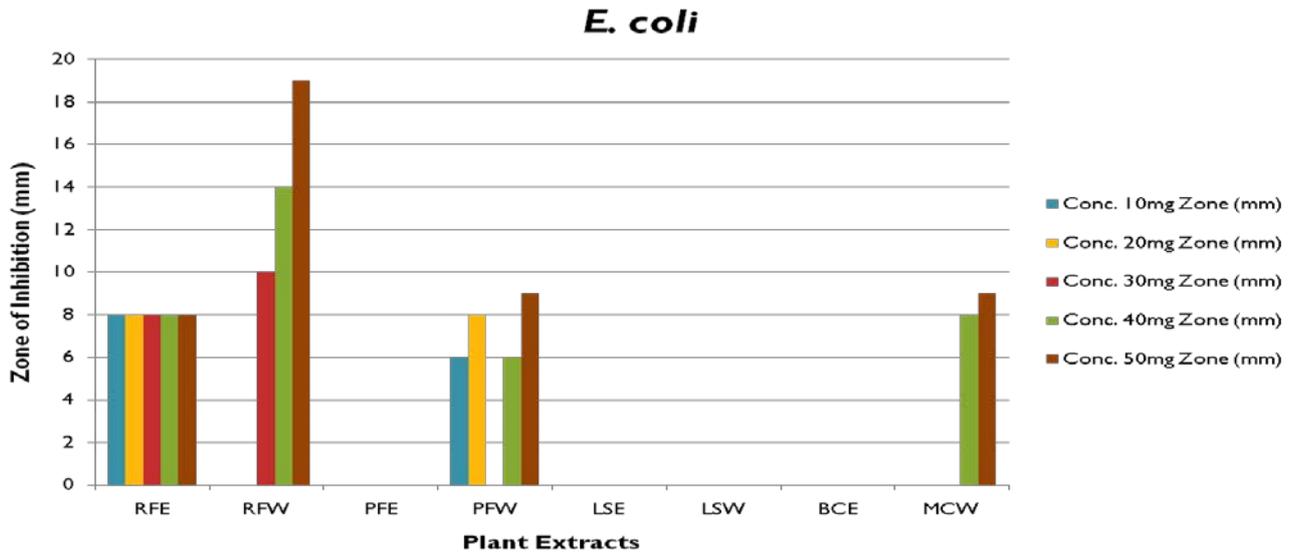


Figure 3. Comparison Among Eight Plants Extracts Concentrations versus The Zones of Inhibition of *Escherichia coli*

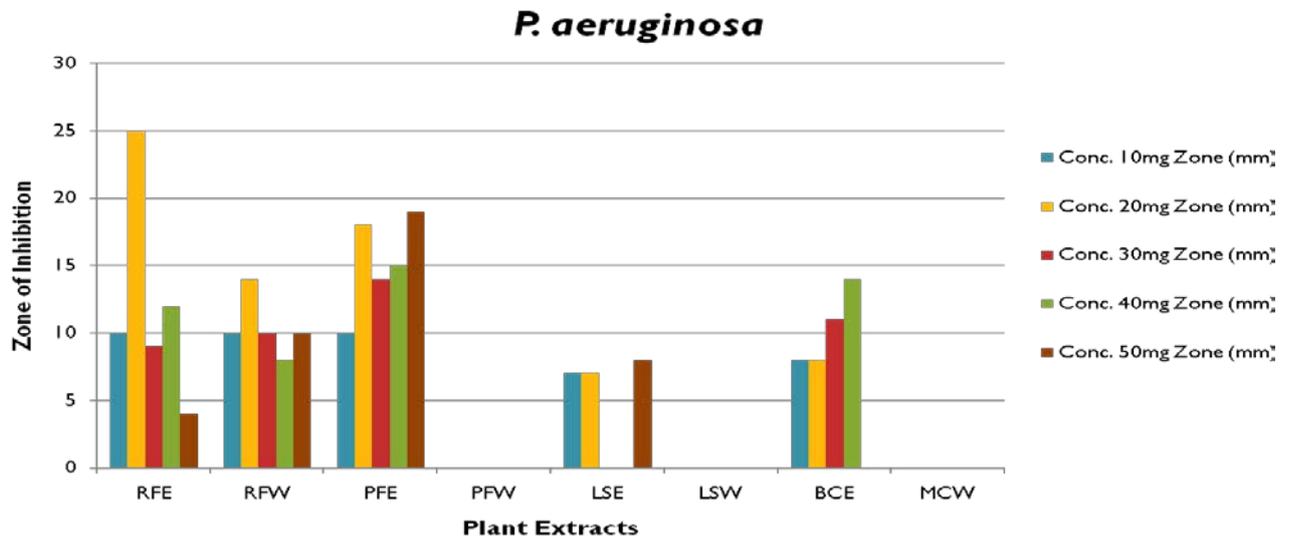


Figure 4. Comparison Among Eight Plant Extracts Concentrations versus The Zones of Inhibition of *Pseudomonas aeruginosa*

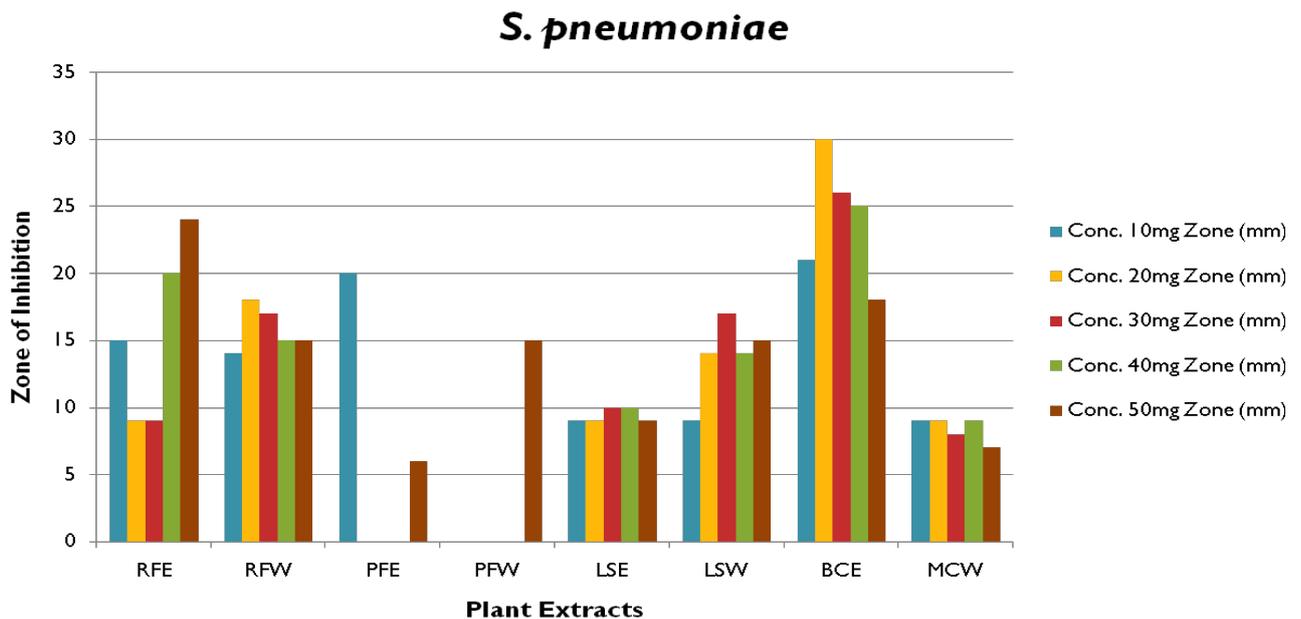


Figure 5. Comparison Among Eight Plants Extracts Concentrations versus The Zones of Inhibition *Streptococcus pneumoniae*.

S. mutans

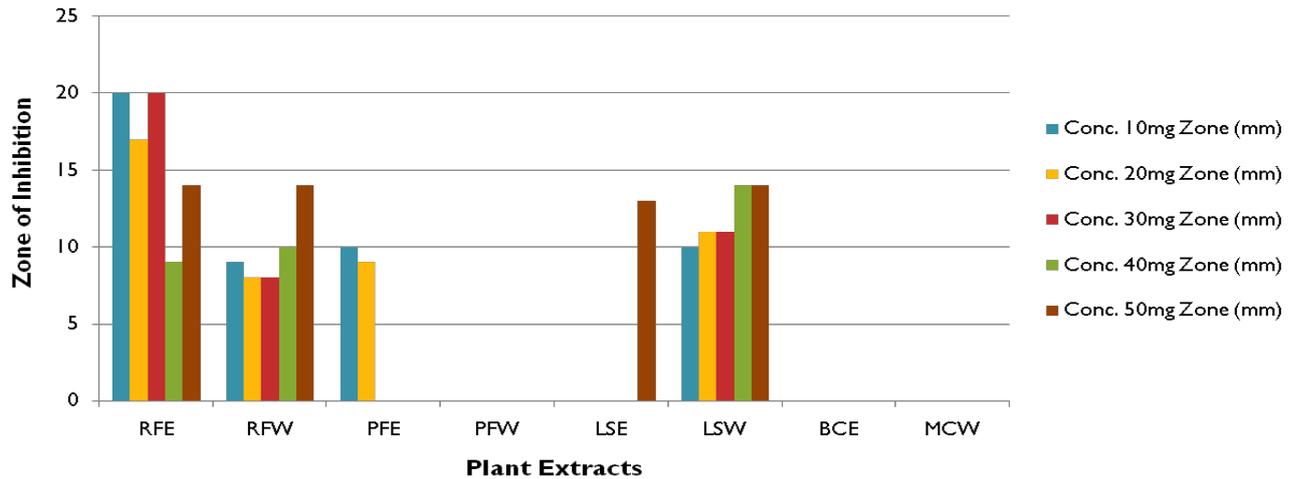


Figure 6. Comparison Among Eight Plant Extracts Concentrations versus The Zones of Inhibition (mm) Streptococcus mutans

Table 4. Phytochemical Tests For Water Extracts

Test	Red Sage Flower	Pink Sage Flower	Stem and Leaf	Chew Stick
Reducing sugar	+	+++	-	+++
Saponins	+++	++	++	+++
Tannin	++	++	++	-

Key: + - Low, ++ - Medium, +++ - High, (-) - Absent

Table 5. Phytochemical Tests For Ethanol Extracts

Test	Red Sage Flower	Pink Sage Flower	Stem and Leaf	Chew Stick
Terpenoids	+++	+++	+	++
Flavanoids	++	++	+	-
Cardiac glycosides	++	+	+	-

Key: + - Low, ++ - Medium, +++ - High, (-) - Absent.

4. Discussion and Conclusion

Based on the findings Crest Mouthwash was the most effective mouthwash (Table 1). Most of the isolates were highly sensitivity to this mouthwash in comparison to the other mouthwashes utilized. Ultra Care mouthwash was the least effective mouthwash (Table 1). All the isolates were resistant to this mouthwash showing small zones of inhibition which was below the acceptable standard and in some cases there were no zones. The high antimicrobial activity of Crest mouthwash could be attributed to the presence of Sodium Fluoride which is the active ingredient. This compound inhibited the growth of the isolates as it was seen (Figure 1). There was a high peak showing the large zones of inhibition in comparison to other mouthwashes.

It was evident that the plants used in this study had antimicrobial properties. This is because the oral pathogens were highly sensitive to different concentrations of plant extracts tested (Figure 2 – Figure 6). Redsage extracts were the most effective natural product against the isolates. The extracts showed high zones of inhibition (Figure 5, Figure 6). Table 3 showed S. pneumoniae being the most susceptible organism to Redsage ethanolic extracts (red flower) in all concentrations. The growth of S. mutans bacteria was inhibited by Redsage plant extracts but showed resistance to Chew Stick extracts (Figure 6). S. aureus was the most sensitive organism to ethanolic

extracts of pink flower Redsage (Figure 2). E. coli was susceptible to Redsage in aqueous extracts at high concentrations and resistant at low concentrations (Figure 3). The growth of P. aeruginosa was strongly inhibited by Redsage (pink flower) in ethanolic extracts (Figure 4).

The antimicrobial activity of Redsage and Chew Stick could be attributed to the active ingredients in phytochemical screening. Both plants had a high amount of terpenoids and saponins present in their extracts (Table 4, Table 5). Reducing sugars were high in pink flower Redsage and Chew Stick extracts (Table 4, Table 5). Tannins compounds were moderately present in Redsage but absent in Chew Stick (Table 4, Table 5). Flavonoids and cardiac glycosides compounds were also present in moderation in Redsage extracts and absent in Chew Stick plant extracts (Table 4, Table 5).

This study concluded that these compounds might have inhibited the growth of the oral pathogens at different concentrations. The efficacy of the plant extracts was concentration dependent based on (Figure 2 - Figure 6), the sizes of zone of inhibitions determined the effectiveness of the extract. The larger the zone the more sensitive was the organism. Most of the extracts from Redsage had larger zones than Chew Stick extracts. These extracts proved to be successful in inhibiting growth of oral cavity microorganisms. Therefore, preventive measures should be utilized in oral hygiene to prevent invasion of these microorganisms tested. Knowledge of mouthwashes and natural products that inhibit oral

microorganisms is important in predicting and preventing not only dental diseases but also associated systemic complications caused by them.

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