WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 3, 92-114 Research Article ISSN 2278 – 4357

ANTIMICROBIAL ACTIVITY OF *ARTOCARPUS ALTILIS*, (BREADFRUIT), *AVERRHOA BILIMBI* (SOURIE) AND *CORDIA CURASSAVICA* (BLACKSTAGE) STEM EXTRACT AGAINST *STREPTOCOCCOUS MUTANS*, IN PURSUIT OF NATURAL ANTICARIES AGENTS

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Article Received on
02 Jan. 2019,

Revised on 18 Jan. 2019,
Accepted on 08 Feb. 2019,

DOI: 10.20959/wjpps20193-13258

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ABSTRACT

The antibacterial activity of three plant extracts: *Artocarpus altilis* fruit and leaves (Breadfruit), *Averrhoa bilimbi* (Sourie) fruit and leaves and *Cordia Curassavica* (Blackstage) stem and leaf extracts were evaluated against *Streptococcous mutans* with the aim of discovering new natural anticaries agents. The disc diffusion assay was used in the antimicrobial evaluation process. The extracts were evaluated at three different concentration of 100%, 90% and at 80%. At the 100% of extract concentration, the highest AZOI of 124.41 mm^2 was induced by the sourie leaf extract. At the same concentration, the lowest AZOI of 52.4 mm^2 was induced by the breadfruit extract. At the 90% extract concentration, the highest AZOI of 157.69 mm^2 was induced by the sourie leaf extract. The lowest AZOI of 66.02 mm^2 was induced by the breadfruit leaf extract. At the lowest 80% concentration of the extract, the highest AZOI of 131.09 mm^2 was induced by the black stage stem extract. There seems to be a variation in the antimicrobial potency of each fruit and leaf extract at the different concentrations. *Streptococcus mutans* showed antimicrobial susceptibility to all plant extracts investigated and this varied with the concentration of the plant extracts. The results were statistically analysed. The plant extracts also studied are not only antibacterial in nature, but also well within the pH range of the typical dentrifices.

KEYWORDS: *Artocarpus altilis, Averrhoa bilimbi* (Sourie), *Cordia Curassavica* (Blackstage), *Streptococcous mutans, disc diffusion assay*

INTRODUCTION

Guyana has a rich biodiversified flora, whose organic and aqueous extracts have been shown to possess potent and selective antimicrobial activity, compared with standard antibiotics: penicillin, nystatin and ampicillin[1-8] etc. Research in the design and synthesis of antimicrobials will continue to be problematic on our planet, considering bacteria and fungal resistance to antimicrobials over a period of time.[6-13] This is primarily due to indiscriminate use of commercial antimicrobial drugs used for the treatment of infectious diseases and antibiotic resistance.[6-13] The latter is of immediate global concern. Many synthetic antimicrobial drugs have several adverse side effects which are usually irreversible when administered and the cost of synthesizing drugs in most cases is an expensive endeavor.[7-9] Fruits are non toxic to humans and thus would be expected to show little or no side effect as compared to synthetic drugs. Clinical trials will not be expected either. Extracts from fruits can also be incorporated in soaps, detergents and cough syrups to boost their antimicrobial potency, a significant impetus to Pharmaceutical companies locally and internationally. We have reported the antimicrobial activity of the stems and leaves of selected plants against pathogenic organisms such as *E. coli*, *S. aureus*, *K. pneumoniae*, *B. subtilis* and *C. albicans*. However, there is no report by us on the antimicrobial activity of various plant parts against *Streptococcus mutans*, a bacteria that is responsible for dental caries.[1-8]

Dental caries has been studied for a long time, with great emphasis being placed on the carious process itself.[14] After this process was outlined, scientist then focused on ways this process can be inhibited or controlled.[15] Fluoride supplementation was identified as a preventive means of avoiding dental caries development.[16] However, the use of fluoride supplementation and fortification is now being questioned by the public. This gave rise to movement of developing herbal/organic means of fighting dental caries.[17]

There are several reports on the use of plant extracts as antimicrobial agents. The antimicrobial activity of different concentrations of aqueous extracts of cinnamon and ginger on *Streptococcus mutans* and *Lactobacillus* bacteria has been reported.[18] In high concentrations, antimicrobial activity of the cinnamon extracts were more against *L. bacillus* than its activity against the *S. mutans.* With the ginger extracts, it was found that the antimicrobial properties against *L. bacillus* were increased with higher concentrations generally, than against activity against *S. mutans.*[18]

Studies were also done using essential oils.[19] It was found that *Achillea ligustica, Baccharis dracunculifolia, Croton cajucara, Cryptomeria japonica, Coriandrum sativum, Eugenia caryophyllata, Lippia sidoides, Ocimum americanum, and Rosmarinus officinalis* were the effective species with antibacterial potential against cariogenic bacteria. It was also noted that in some cases, the major phytochemical compounds determined the biological properties of these essential oils. Menthol and eugenol were considered outstanding compounds demonstrating an antibacterial potential.[19]

Some Plant extracts were tested on common oral pathogens. These extracts include that of ten plants, six juices and propolis and a combination of these extracts on the *in vitro* growth of two oral pathogens; *Streptococcus mutans* and *Candida albicans*. Combinations that involved three and four extracts were tested with the most active extracts. The results demonstrated that all of the tested 70% ethanol extracts inhibited the growth of both organisms. The most active inhibitors were cloves, cinnamon. propolis, lavender and sage. Little activity was noted in apple, black chokeberry, black elderberry, cranberry, Japanese quince, and lemon juice. When mixed in double 1:1 combinations, eight extract combinations showed a synergistic action and eleven combinations resulted in an antagonistic action to the inhibition of the growth of *C. albicans.*[20]

Natural phytochemicals that were extracted from plants that were medicinal in nature were used to combat the growth of the *S. mutans.* Extracts from different parts of the *Psidium guajava* plant were tested for their antimicrobial activity against the oral plaque forming bacteria *Streptococcus mutans.* The three solvents (acetone, ethanol and methanol) based extracts of *Psidium guajava* showed good activity. Both the acetone and ethanol leaf extract (*Psidium guajava)* is highly active against *Streptococcus mutans* . However, the flowers showed to have the largest zone of inhibition. (Investigation of Biofilm Inhibition Activity and Antibacterial Activity of Psidium guajava Plant Extracts against *Streptococcus mutans* Causing Dental Plaque.[21]

The antibacterial activity of herbal extracts of *Cudrania tricuspidata, Sophora flavescens, Ginkgo biloba,* and *Betula Schmidtii* against normal oral streptococci, planktonic and biofilm of *S. mutans, Streptococcus gordonii, Streptococcus oralis, Streptococcus salivarius, Streptococcus sanguinis,* and *S. mutans* has been reported.[22]

Antibacterial potency of aqueous plant extracts against *S. mutans* has been reported[23]. In this study the inhibition effect of aqueous extracts of nine plants (Coriander, Black Tea, Bitter Fennel, Cubeb, Dry Black Lime, Ginger, Nutmeg, Turmeric and Senna) was tested against *Streptococcus mutans*. The results showed that the antibacterial effect of these extracts was better in case of adding the extract to the medium than the case of evaporating the extract and then adding it to the medium except in case of Black tea and Nutmeg (was better in case two than in case one.[23]

The effect of aqueous extracts of a number of known medicinal plants against the *S. mutans* has been investigated.[24] The results highlighted that *Syzygium aromaticum* showed significantly higher zone of inhibition on the *Mutans-Sanguis* agar plates in agar well diffusion assay. Using the broth culture, *Syzygium aromaticum* and *Psidium guajava* showed higher inhibition of biofilm formation in anti-biofilm activity.

Nineteen plant extracts obtained from plants from the Brazilian Amazon showed activity against planktonic *Streptococcus mutans*, an important bacterium involved in the first steps of biofilm formation and the subsequent initiation of several oral diseases.[2]

Abelmoschus esculentus (okra) extracts were tested against *S. mutans*.[26] The largest zone of inhibition was recorded (4mm) when the combination of the skin (peel) and seed were used. All the combinations of the plant extract produced antibacterial activity that was at least equal to or higher than the activity of the positive control (Ofloxacin).[26]

Various extracts of *Stevia rebaudiana* leaves were tested against *S. mutans.* These include aqueous, methanol, ethanol and acetone extracts. Acetone and ethanol extracts of the leaf gave the highest zone of inhibition against *S. mutans*. Aqueous extract of this plant was not effective on S. *mutans.*[27]

Several studies were done on *Artocarpus altilis* leaf extracts and a few fruit extracts of breadfruit, testing its antimicrobial activities on *S. mutans* [28-30]. However, there is no report of for *Averrhoa bilimb* extract as an antimicrobial agent against *S. mutans*. However, the effects of *Averrho bilimbi* leaf extract on some other bacteria which indicates that it has antibacterial potential has been reported.[31-33]

The antibacterial activities of *Averrhoa bilimbi* leaf extract 6.25%, 12.5%, 25%, 50%, and 100% were used, with chlorhexidine gluconate (0.2%) as the control. The smallest inhibitory

zone was noted in the 6.25% 10.08 mm, the biggest inhibitory zone in 100% was 23.07 mm.[31-32]

Internationally and locally using black sage (*C. curassavica*) to brush teeth is deemed a substitute to conventional brushing. *Salvadora persica* (miswak) chewing twig is widely used by Muslims around the world to help care their teeth.[33] However, there is no published article that document the use of black sage (*C. curassavica*) as having antimicrobial activities against *S. mutans*. In Guyana, blacksage is used by some inhabitants to clean and brush teeth.

This paper reports the antimicrobial activity of the aqueous extract of leaves and fruits of *Averrhoa bilimbi, Artocarpus altilis* and leaves and stem of *Cordia curassavica* against *S. mutans.* To the best of knowledge, no research on the antimicrobial activity of aqueous extract of *Artocarpus altis* against *S. mutans* has been reported. So, too, the antimicrobial activity of the organic and aqueous extract of *Averrhoa bilimbi* and *Cordia curassavica* against *S. mutans*.

2.0. MATERIALS AND METHODS

2.1. Specimen Collection

Leaves and fruits of *Averrhoa bilimbi, Artocarpus altilis and leaves and stem of Cordia curassavica* were obtained from areas along the East Coast of Demerara. Fig. 1.0. shows the approximate location the plants were obtained. Leaves weren't collected from diseased plants or plants that exhibit any form of notable deficiencies. Original samples of the species being studied were authenticated at the Biodiversity centre of the University of Guyana. The leaves, fruits and stem were then stored according to species and taken to the lab. Fresh leaves and fruits of the *Averrhoa bilimbi and Artocarpus altilis* were obtained and gently rinse with distilled water to remove any debris. Excess water was drained and a certain weight of leaves was added to a clean blender. A specified volume of deionized distilled water was added to the contents of the blender and the contents were blended. Upon completion of the blending process, the contents were then filtered using a glass funnel and Whatman's #1 filter paper to obtain the aqueous extracts. The aqueous extracts was then stored in large glass bottles and refrigerated until antimicrobial susceptibility test was done. The same procedure was done for the leaves and stem of the *Cordia curassavica* contents. Once these extracts were obtained and purified they were then tested on cultured *Streptococcus mutans* to determine any level of inhibition.

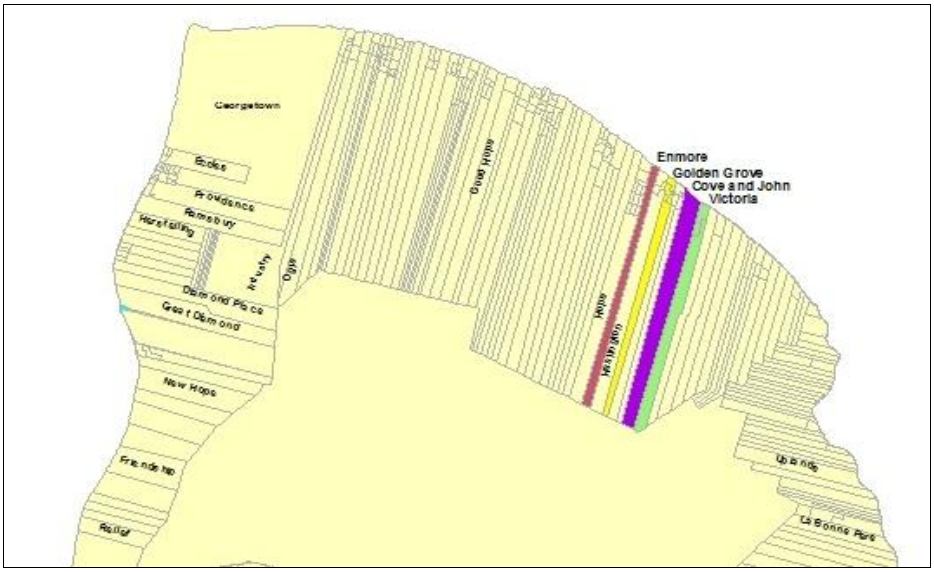


Fig. 1.0. Identifying the approximate location that plants were obtained

Each plant extract was tested at three different concentrations (100%, 90% and 80%) against the bacteria, *S. mutans*. Additional susceptibility test was also carried out on the solvent used, which is water and this was the negative control. Susceptibility test was also carried out on chlorhexidine gluconate, the reference compound at varying concentrations. Once these extracts were obtained and purified they were then tested on cultured *S . mutans* to determine any level of inhibition.

2.2. Microscopic analysis

The *S. mutans* bacteria were obtained from the University of Guyana, Department of Biology laboratory. However, additional assessment of the bacteria was done to confirm the identity of the said bacteria. The identification assessment includes: Gram staining, microscopic observation of the bacteria.

2.3. Gram Staining[34]

The prepared slides were heated and fixed using the Bunsen burner, gently passing over two to three times (couple seconds). The slide was then flooded with crystal violet and timed for one minute. After one minute, it was rinsed with water and then flooded again, this time with iodine for one minute. After one minute, it was rinsed with water and then decolourised with alcohol for five to ten seconds. The slide was then finally flooded a final time with safranin for one minute, rinsed and viewed under the microscope to observe any changes. Bacteria can generally be classified either gram-positive (stains dark purple) or gram-negative (stains pink). *S. mutans* is expected to stain dark purple (Gram positive).

2.4. Microscopic observation[35]

The microscope light was switched on. Using rheostat, the brightness of the light was adjusted. The prepared microscope slide was placed onto the stage and clipped in position. Once in position, the nosepiece was rotated to the lowest-power objective which is 4 x objective lens, this is usually colour coded with red band. The iris diaphragm was adjusted to the largest diameter, which allows the greatest amount of light to pass through. Using the coarse adjustment, knob adjustments were made until the specimen was within view. This particularly helps with positioning the objective lens correctly. Detail focus was achieved by using the fine adjustment knob. The iris diaphragm was adjusted to get a clearer image. By using a lower power lens initially, the researcher was able to view a general overview of the prepared slide. The nosepiece was then rotated to the 10 x objective that has a yellow band. The iris diaphragm was closed to 1/4 of the diameter, which allowed a greater amount of light to pass through. Once again, the coarse adjustment knob was used to adjust the specimen until it is in focus. This was followed by making fine adjustment via the fine adjustment knob and iris diaphragm for clearer image. The nosepiece was once again rotated to the 40 x objective, which is blue in colour. The iris diaphragm was closed to 1/2 of the diameter, thereby restricting the amount of light entering again. Once again, the coarse adjustment knob was used to adjust the specimen until it is in focus. This was followed by making fine adjustment via the fine adjustment knob and iris diaphragm for clearer image. The nose piece was rotated such that the specimen was between 40 X and 100 X objective. A small drop of oil was placed on the centre of the slide. The nose piece was rotated so that the oil immersion objective touches the oil. No coarse adjustment was made, only the fine adjustment knob was adjusted for a clearer image. At that point, morphological observations were made and documented carefully in table provided (see appendix 4). Once all observation was completed steps for caring the microscope was followed. The oil immersion (100X) objective was wiped with lens paper to remove all oil. The glass slide was removed, the microscope was unplug and then cleaned.

2.5. Antimicrobial assay: Disc Diffusion Assay Method[36-38]

Molten agar was poured in 90 mm diameter sterile dishes to a depth of 4mm (about 30ml per plate). The plates were poured on a level surface so that the depth of the medium was uniform. Using Stokes Disc diffusion sensitivity testing technique, an innoculum containing bacterial cells was applied on to the nutrient agar plates. On each plate, four discs were applied. The disc were punched out from filter paper using a perforator (5 mm in diameter)

The discs were then placed in a vial of 10ml of respective concentration of the various extracts for a few minutes (5 minutes), so as to absorb the liquid. Four disc of the same extract and concentration were then placed on the bacteria inoculated plate equal distance apart.

This step was repeated in triplicates for each concentration of the various plant samples, and in duplicates for the positive control (Chlorhexidine gluconate) and negative control (water). The antimicrobial compound was expected to diffuse from the discs into the medium. Following an overnight incubation, the cultures were examined for area of no growth around the discs (area of inhibition) and the widest diameter of the zone of inhibition was measured. (The end point of inhibition is considered where growth starts. Larger the inhibition zone, greater is the antibacterial activity. The inhibition zones produced by the different antimicrobials against the same organism vary in size due to differences in antimicrobial molecular structures and nature. Those that diffuse rapidly generally have larger inhibition zones. It was anticipated that a no growth area will be induced around the disc inoculated with the plant extracts and the positive control (chlorhexidine gluconate). However, test done for the negative control (water) should not exhibit any area of inhibition. Zones of inhibition were then compared to that achieved in the reference susceptibility tests. The results were then tabulated and graphs were constructed. Appropriate analysis was done using Mean, Standard Deviation, ANOVA and Tukey comparison tests to determine the significance of the values obtained.

3.0. RESULTS

Table 1.0: Characteristic features of the bacteria as observed under the microscope.

Bacteria, *S. mutans*	**Findings**
Gram Test	+ ve (purple stain retain)
Shape	Round

Table 2.0: Average diameter and Average Area of Zone of Inhibition induced by Breadfruit extract against *S. mutans* at varying concentration.

Sample	Concentration (%)	Diameter of Zone of Inhibition, DZOI (mm)	Average diameter of Zone of Inhibition (mm)	Average Area of Inhibition (mm^2)
Breadfruit	100	8.0	8.17 ± 0.288675	52.4
Breadfruit	100	8.5		
Breadfruit	100	8.0		
Breadfruit	90	8.75	9.42 ± 0.57735	69.67
Breadfruit	90	9.75		
Breadfruit	90	9.75		
Breadfruit	80	9.0	9.25 ± 0.433013	67.23
Breadfruit	80	9.0		
Breadfruit	80	9.75		

Table 3.0: Average diameter and Average Area of Zone of Inhibition induced by Breadfruit leaf extract against *S. mutans* at varying concentration.

Plant	Concentration (%)	Diameter of Zone of Inhibition (mm)	Average Diameter of Zone of Inhibition (mm)	Average Area of Zone of Inhibition (mm^2)
Breadfruit leaf	100	10.25	9.42 ± 1.233221	69.67
Breadfruit leaf	100	8.0		
Breadfruit leaf	100	10.0		
Breadfruit leaf	90	8.50		
Breadfruit leaf	90	10.50	9.16 ± 1.154	68.02
Breadfruit leaf	90	8.50		
Breadfruit leaf	80	8.25		
Breadfruit leaf	80	10.25	9.50 ± 1.089725	70.91
Breadfruit leaf	80	10.00		

Table 4.0: Average diameter and Average Area of Zone of Inhibition induced by Sourie Fruit Extract extract against *S. mutans* at varying concentration.

Plant	Concentration (%)	Diameter of Zone of Inhibition (mm)	Average Diameter of Zone of Inhibition (mm)	Average Area of Zone of Inhibition (mm^2)
Sourie Fruit	100	11.0	10.25 ±1.089725	
Sourie Fruit	100	9.0		82.55
Sourie Fruit	100	10.75		
Sourie Fruit	90	9.75		
Sourie Fruit	90	9.50	9.75 ± 0.25	74.69

Sourie Fruit	90	10.0		
Sourie Fruit	80	12.0		
Sourie Fruit	80	9.75	10.67 ± 1.18	89.4
Sourie Fruit	80	10.25		

Table 5.0: Average diameter and Average Area of Zone of Inhibition induced by Sourie leaf Extract extract against *S. mutans* at varying concentration

Plant	Concentration	Diameter of Zone of Inhibition	Average Diameter of Zone of Inhibition (mm)	Average Area of Zone of Inhibition, AZOI
Sourie leaf	100	12.0		
Sourie leaf	100	12.25	12.58 ± 0.803638	124.41
Sourie leaf	100	13.50		
Sourie leaf	90	15.25		
Sourie leaf	90	12.25	14.17 ± 1.664582	157.69
Sourie leaf	90	15.00		
Sourie leaf	80	12.75		
Sourie leaf	80	11.50	12.33 ± 0.721688	119.52
Sourie leaf	80	12.75		

Table 6.0: Average diameter and Average Area of Zone of Inhibition induced by Black Stage Stem Extract extract against *S. mutans* at varying concentration.

Plant	Concentration (%)	Diameter of Zone of Inhibition (mm)	Average Diameter of Zone of Inhibition	Average Area of Zone of Inhibition (mm)
Black stage stem	100	10.00		
Black stage stem	100	10.0	9.67 ± 0.577	73.42
Black stage stem	100	9.0		
Black stage stem	90	10.25		
Black stage stem	90	9.0	9.25 ± 1.12	67.23
Black stage stem	90	8.0		
Black stage stem	80	17.75		
Black stage stem	80	10.0	12.92 ± 4.22	131.09
Black stage stem	80	11.00		

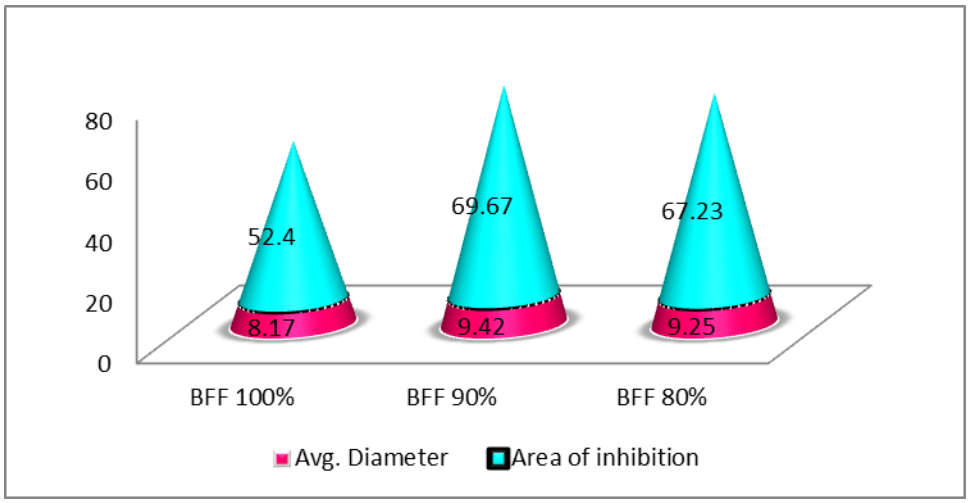
Table 7.0. Average diameter and Average Area of Zone of Inhibition induced by Black Stage leaf Extract extract against *S. mutans* at varying concentration.

Plant	Concentration	Zone of Inhibition (mm)	Average Diameter of Zone of Inhibition	Average Area of Zone of Inhibition
Black Stage leaf	100	10.50		
Black Stage leaf	100	9.0	9.5 ± 0.866	73.42
Black Stage leaf	100	9.0		
Black Stage leaf	90	10.50		
Black Stage leaf	90	10.75	10.58 ± 0.144	67.23
Black Stage leaf	90	10.50		
Black Stage leaf	80	12.50		
Black Stage leaf	80	9.25	11± 1.64	131.09
Black Stage leaf	80	11.25		

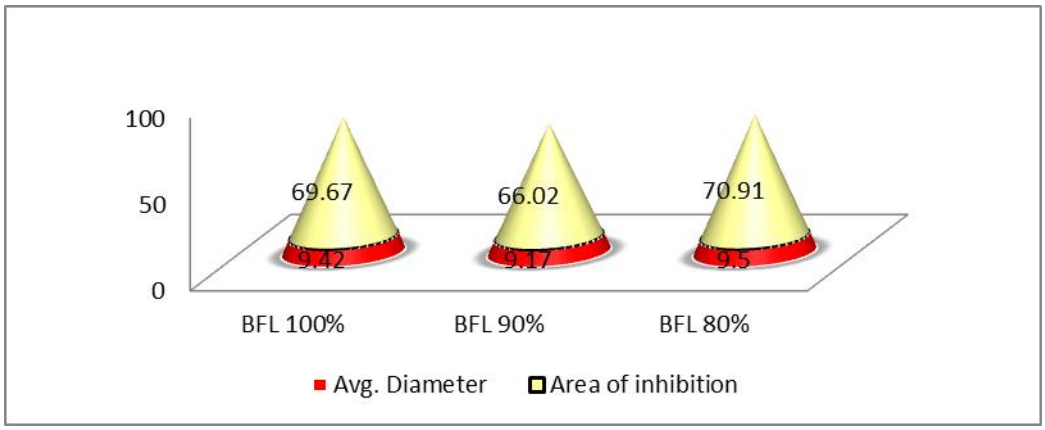
Table 8.0: Average diameter and Average Area of Zone of Inhibition induced by Reference compound against *S. mutans* at varying concentration.

Reference	Concentration (%)	Plate Number	Diameter of Zone of Inhibition (mm)	Average Diameter of Zone of Inhibition	Area of Zone of Inhibition (mm^2)
Chlorohexidine gluconate	0.20	Plate 1	7.5	6.75 ± 0.75	36.24
Chlorohexidine gluconate	0.20	Plate 2	6		
Chlorohexidine gluconate	0.18	Plate 1	6.5	6.5 ± 0.0	33.2
Chlorohexidine gluconate	0.18	Plate 2	6.5		
Chlorohexidine gluconate	0.16	Plate 1	5.5	5.5 ± 0.00	23.77
Chlorohexidine gluconate	0.16	Plate 2	5.5		
Chlorohexidine gluconate	0.15	Plate 1	5.5	5.63 ± 0.18	24.87
Chlorohexidine gluconate	0.15	Plate 2	5.75		

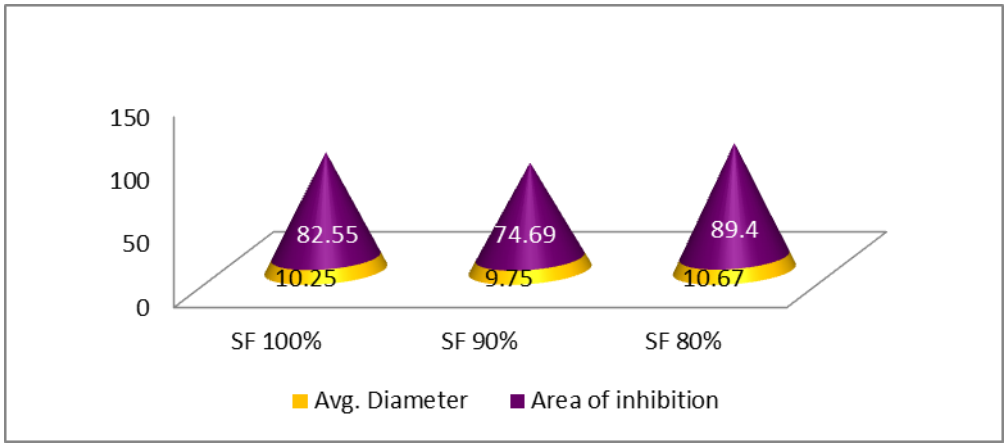
Graphs



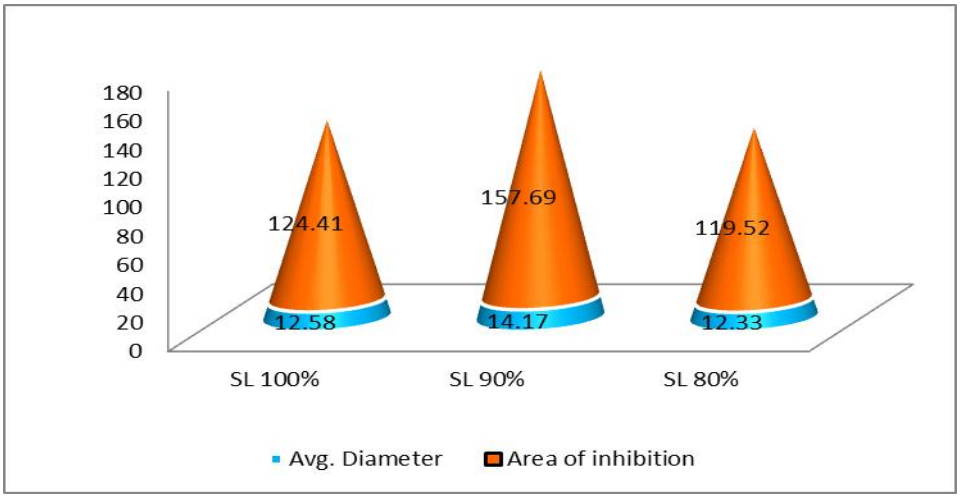
Graph 1: Pyramid chart comparing the average diameter, ADZOI and average area of inhibition AAZOI across concentrations of bread fruit extracts.



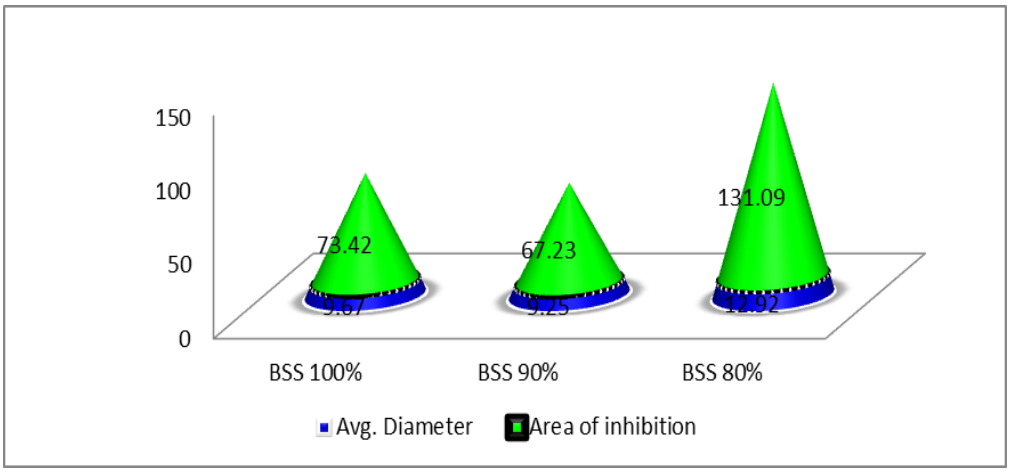
Graph 2: Pyramid chart comparing the average diameter, ADZOI and average area of inhibition AAZOI across concentrations of bread fruit leaf extracts.



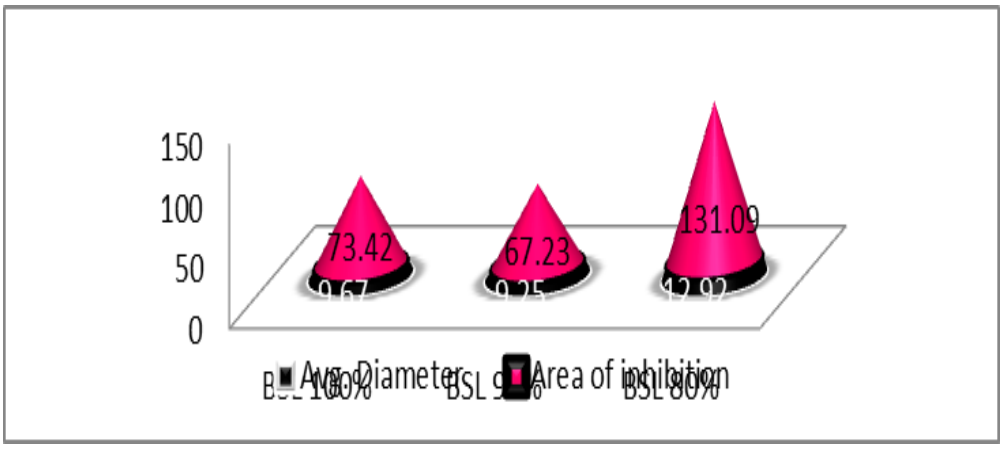
Graph 3: Pyramid chart comparing the average diameter, ADZOI and average area of inhibition AAZOI across concentrations of bread fruit leaf extracts.



Graph 4: Pyramid chart comparing the average diameter, ADZOI and average area of inhibition AAZOI across concentrations of Sourie leaf extracts.



Graph 5: Pyramid chart comparing the average diameter, ADZOI and average area of inhibition AAZOI across concentrations of black stage stem extracts.

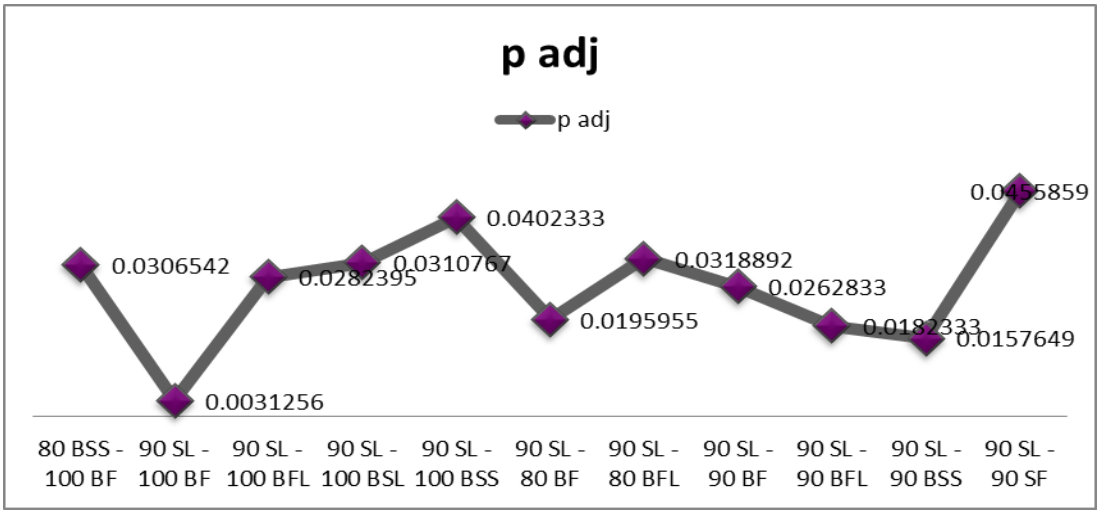


Graph 6: Pyramid chart comparing the average diameter, ADZOI and average area of inhibition AAZOI across concentrations of black stage leaf extracts.

ANOVA RESULTS

Table 9.0: Results Obtained For Anova Test Sample Conc. And Areas of Inhibition.

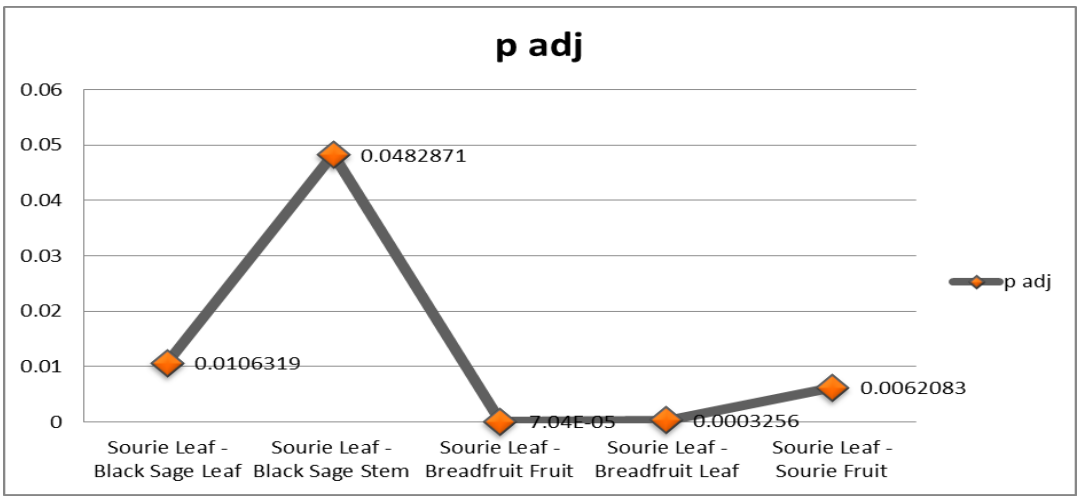
	Df	Sum Sq.	Mean Sq.	F. Value	Pr(>F)
Concentration	17	43441	2555.3	3.432	0.000911
Residuals	36	26801	744.5		



Graph 7.0: Line chart illustrating the sample concentrations with significant difference between areas of inhibition.

Table 10.00: Depict the Results Obtained for Anova Test: Area of Inhibition and Plant Extract.

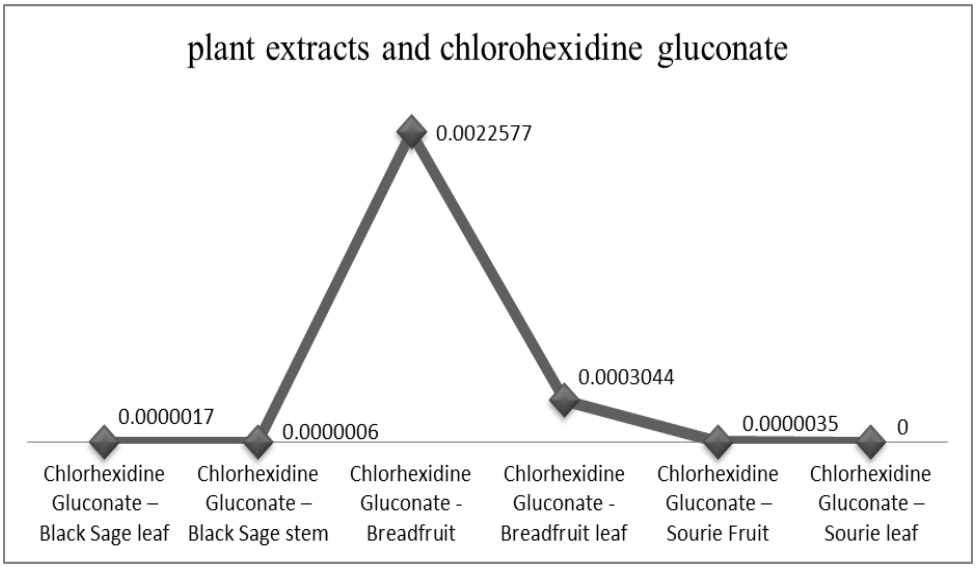
	Df	Sum Sq.	Mean Sq.	F. Value	Pr(>F)
Plant extract	5	28644	5729	6.61	9.38E-05
Residuals	48	41598	867		



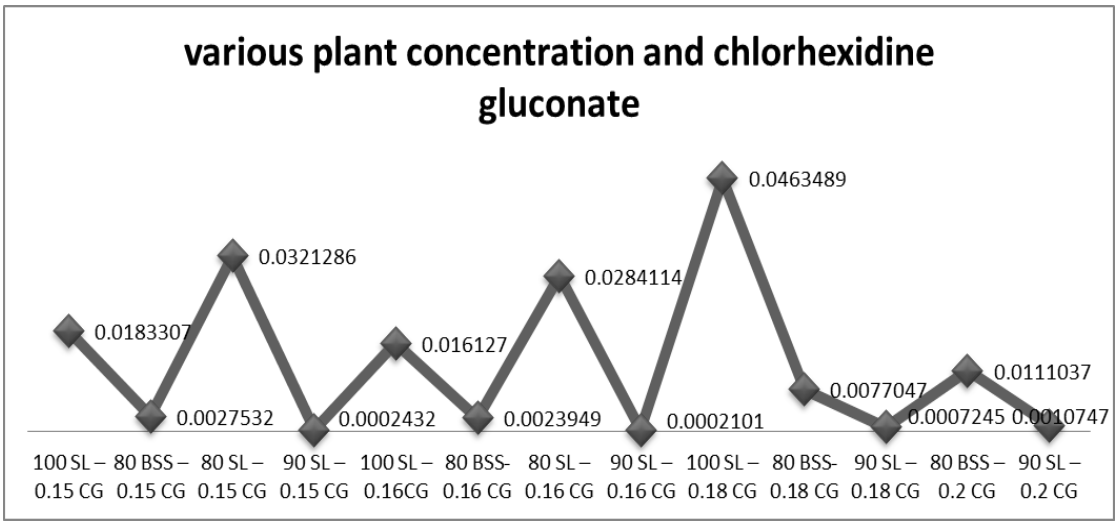
Graph 8.0: Line chart illustrating the plant extracts with significant difference between area of inhibition.

Table 11.0: *Anova Test:* Area Of Inhibition And Samples Including Chlorhexidine Gluconate.

	Df	Sum Sq.	Mean Sq.	F. Value	Pr(>F)
Plant extract	6	52718	8786	11.66	2.21e-08
Residuals	55	41458	754		



Graph 9.0: Chart depicting the results obtained from Tukey multiple comparisons of means identifying the extracts with significant difference to Chlorhexidine gluconate area of inhibition).



Graph 10.0. depicts the results obtained from Tukey multiple comparisons of means identifying the various concentration of extracts with significant difference to Chlorhexidine gluconate(Zone of inhibition)

DISCUSSION

The plant extracts were investigated at three different concentrations of 100%, 90% and 80%. The zone of Inhibition, ZOI and Area of Zone of Inhibition, AZOI were used as indicators of plant extracts antimicrobial activity. The results are presented in Tables 2.0 to Table 7.0. The greater the AZOI, greater is the extract's antimicrobial activity. At 100% of extract concentration, the highest AZOI of 124.41 mm^2 was induced by the sourie leaf extract. At 100% extract concentration, the lowest AZOI of 52.4 mm^2 was induced by the breadfruit extract. At the 90% extract concentration, the higest AZOI of 157.69 mm^2 was induced by the sourie leaf extract. The lowest AZOI of 67.23 mm^2 was induced by the blackstage stem extract. At the lowest concentration of extract, 80%, the highest AZOI of 131.09 mm^2 was induced by the black stage stem and black stage leaf extract.

There seems to be a variation in the antimicrobial potency of each fruit and leaf extract at the three different concentrations. For the breadfruit and sourie leaf extract, there was an increase in antimicrobial activity at the 90% concentration and a decrease at the 80% concentrations. As an example, AZOI of 124.41 mm^2, 157.69 mm^2 and 119.52 mm^2 was noted for the sourie leaf extract at 100%, 90% and 80% concentration. For the breadfruit leaf, sourie fruit, black stage stem and black stage stage leaf, there was a general decrease in antimicrobial activity at the 90% concentration and an increase at the 80% concentration. For example, for the blackstage stem, the AZOI of 73.42 mm^2, 67.23 mm^2 and 131.09 mm^2 was recorded at the 100%, 90 % and 80% concentration of plant extracts.

Streptococcus mutans showed susceptibility to all plant extracts investigated. The AZOI range from 52.4 mm^2 to 157.69 mm^2. Of these extracts, the sourie leaf exhibited the best antimicrobial activity with AZOI ranging from 119.52 mm^2 to 157.69 mm^2..

Thus, the order of antimicrobial potency of plant extracts toward *streptococcus mutans* at specified concentration is.

100%: Sourie leaf > Sourie fruit > Black stage stem = Black stage leaf > Breadfruit leaf > Breadfruit.

90%: Sourie leaf > sourie fruit > breadfruit > blackstage stem = Blackstage leaf.

80%: Black stage leaf = Black stage stem > Sourie leaf > Sourie fruit > breadfruit leaf > Bread fruit.

Thus, at the 100% and 90% plant extract concentration, the sourie leaf and sourie fruit were more potent antimicrobially against *S. mutans*. However, at the 80% concentration, the blackstage stem and blackstage leaf were more potent antimicrobially.

Table 8.0. shows the Average diameter of Zone of Inhibition, AZOI and Average Area of Zone of Inhibition, AZOI induced by the reference compound, Chlorohexidine gluconate against *S. mutans* at varying concentration of 0.15% to 0.20 %. Chlorohexidine gluconate showed an average AZOI $(mm)^2$ that range from 23.77 mm^2 to 36.24 mm^2. It is clearly evident that as the concentration of chlorohexidine gluconate increases, the AZOI increases.

Pyramids graphs, comparing the average diameter, ADZOI and Average area of zone of inhibition, AZOI, across all three concentrations of plant extracts were constructed. These are shown in Graphs 1.0 to Graph 6.0.

The Results, Table (2)-Table (8) were statistically analysed.[39-43] The ANOVA test addresses the significance of the results which is usually followed by a Tukey means of comparison[39-43] ANOVA tests were carried out to determine if there was statistical significance difference between Sample concentration, and areas of zones of inhibition, Area of Zone of inhibition and plant extract (sample). These results are presented in Table 9.0, Table 10.0 and Table 11.0. Also, in graphs 7.0, 8.0, 9.0 and 10.0.

From Table 9.0, the P value = 0.000911 < 0.05, the alpha level, indicating significance difference in Sample concentration, and areas of inhibition. A Tukey multiple comparisons of means were done to identify the samples with significance and these values are plotted in Graph, 7.0 to Graph 10.0. Graph 7.0. shows all eleven (11) values are less than, p = 0.05, indicating that there is indeed significance difference between the sample concentration and areas of Zone of Inhibition. The minimum significance occurs at 90% SL-100% BF extract (Tukey Test), P = 0.0031256. The maximum significance occurs at 90SL-90SF extract (Tukey Test, P = 0.0455859.

Table 10.00, shows that $P = 9.38 \times 10^{-5} < 0.05$, indicating that there is statistically significance difference between the Area of Zone of Inhibition, AZOI and type of plant extracts. The corresponding Graph 8.0 shows that a minimum significance occurs at Sourie Leaf-Breadfruit (Tukey Test, $P = 7.04 \times 10^{-5}$). A maximum significance occurs at Sourie Leaf-Black Sage Stem (Tukey Test, P = 0.0482871).

Table 11.0 shows the ANOVA test for the Area of Zone of Inhibition, AZOI and Samples including Chlorhexidine gluconate. Graph 9.0 depicts the results obtained from Tukey Multiple comparisons of means identifying the extracts with significant difference to chlorhexidine gluconate area of inhibition. The graph shows that all P values are less than 0.05, suggesting that there is no significance difference between chlorohexidine gluconate and the type of plant extracts. A maximum significant value of 0.0022577 occurs for Gluconate and breadfruit extract. A minimum value of zero occurs for chlorhexidine gluconate and sourie leaf extract. Graph 10.00 depicts the results obtained from Tukey Multiple comparisons of means, identifying the various concentration of extracts with significant difference to chlorhexidine gluconate. All values are less than 0.05, suggesting significance difference between the different Chlohexidine gluconate concentration and the various plant extracts concentration. The highest value of 0.0463489 occurs at 100 SL vs 0.18 CG. The lowest value of 0.0002432 occurs at 90 SL-0.15 CG.

Questions might be asked about the effects of the plant extracts and their ability to cause demineralization of tooth structure. This is addressed in the radar chart shown in Fig. 2.0. As is seen from this chart, the pH of the samples being studied were within the range of 5.78-5.9. These values are well beyond the critical pH of the enamel (pH 5.5). It has been noted that the pH of most toothpaste was within the range of 4.22-8.35. This confirms that the plant extracts studied are not only antibacterial in nature, but also well within the pH range of the typical dentrifices.[40]

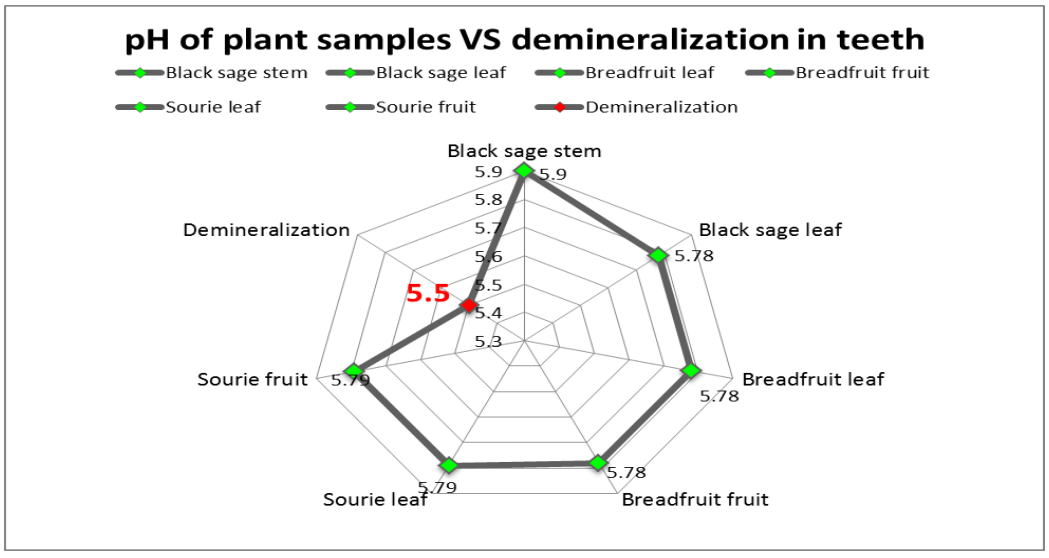


Fig. 2.0. Radar chart illustrating the pH of the samples being studied at 100% concentrations versus the pH at which tooth structure begins to demineralise.

CONCLUSION

The antibacterial activity of three plant extracts: *Artocarpus altilis* fruit and leaves (Breadfruit), *Averrhoa bilimbi* (Sourie) fruit and leaves and *Cordia Curassavica* (Blackstage) stem and leaf extracts were evaluated against *Streptococcous mutans* with the aim of discovering new natural anticaries agents. The disc diffusion assay was used in the antimicrobial evaluation process. The extracts were evaluated at three different concentration of 100%, 90% and at 80%. At the 100% of extract concentration, the highest AZOI of 124.41 mm^2 was induced by the sourie leaf extract. At the same concentration, the lowest AZOI of 52.4 mm^2 was induced by the breadfruit extract. At the 90% extract concentration, the highest AZOI of 157.69 mm^2 was induced by the sourie leaf extract. The lowest AZOI of 66.02 mm^2 was induced by the breadfruit leaf extract. At the lowest 80% concentration of the extract, the highest AZOI of 131.09 mm^2 was induced by the black stage stem extract. The results were statistically analysed. There seems to be a variation in the antimicrobial potency of each fruit and leaf extract at the different concentrations. *Streptococcus mutans* showed antimicrobial susceptibility to all plant extracts investigated. The plant extracts also studied are not only antibacterial in nature, but also well within the pH range of the typical dentrifices.

ACKNOWLEDGEMENT

The author thanks his research student, M. Monize for his enthusiasm and hardwork exhibited.

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