

Effect of different concentrations of garlic extract on dental plaque isolates obtained from patients attending Federal Medical Center Makurdi

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Abstract

Instead of the traditional microbiological pathogens, the microbes that make up the mouth's resident micro-flora are what cause oral diseases like dental caries/plaque and periodontal disorders. The aim of this study was determine the effect of different concentrations of garlic extract on dental plaque isolates obtained from patients attending Federal Medical Center Makurdi. Swap samples were collected from teeth of patients and were transported to the laboratory, analysis was done on different culture media and identification of isolates was done using the Bergy's manual of determinative bacteriology, biochemical characterization was carried following standard microbiological methods. The antibacterial activity was done using agar well diffusion method in which the garlic extract was introduced in concentrate. Concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL, 65.3 mg/mL and 35.5mg/mL were prepared and used for the susceptibility. The Isolates from results were *Streptococcus oralis*, *Streptococcus mutans*, *Enterococcus faecalis* and *Staphylococcus aureus*. The effect observed was concentration dependent as all the graphs obtained shows a linear series, the lowest inhibitory concentration shows that 250mg/ml was the lowest concentration that inhibits each of the isolates in ethanolic garlic extract while 500 mg/mL was the minimum inhibitory concentration for the aqueous garlic extract. Oral hygiene was recommended in order to help reduce the accumulation of dental plaque which usually harbours primary microbes that colonize the teeth surfaces resulting to caries and periodontal diseases. The use of mouth wash containing garlic and or toothpaste incorporated with alicin was recommended as this can help reduce the accumulation of dental plaque biofilm thereby minimising the incidence of dental caries and periodontal diseases.

Keywords: Antibacterial activity, Concentration, Dental plaque, Federal Medical Center, Garlic, Isolates

1. Introduction

Instead of the traditional microbiological pathogens, the microbes that make up the mouth's resident micro-flora are what cause oral diseases like dental caries/plaque and periodontal disorders (Hayho & Palombo, 2011). They primarily originate in the ecological discord in oral biofilms. Gram positive bacteria and cocci are prevalent in dental plaque, which accumulates on the top of teeth, due to high population of Gram positive microorganisms in the oral microbiome (Jonsdottir et al 2010).

The general quality of life is highly influenced by dental health whereas systemic disorders and chronic problems are linked to poor oral health. Lakshmi *et al.* (2011) discovered the link between oral illnesses and the oral microbiome. *Streptococcus mutans*, *S. sobrinus*, *S. oralis*, *S. intermedius*, *S. sanguis*, *S. salivarius*, *Lactobacillus acidophilus* and *S. mitis* are among the acid-producing bacteria that are significant initiators of caries infection (Pramod *et al.*, 2009). Caries, gingivitis, and periodontitis are just a few of the most prevalent diseases that impact people today. Dental plaque, the biofilm that accumulates on the surface of teeth, is one of the main causes of these conditions. Several systemic diseases manifest in the soft tissues of the oral mucosa of the mouth, and the oral cavity can be used to give hygiene to other parts of the body.

A deterioration in dental health has an impact on general health (Jonsdottir *et al.*, 2010, Lakshmi *et al.* 2011). Many different types of bacteria, viruses, protozoa, and fungi can be found in the oral cavity. These microorganisms live on and populate the tongue, cheeks, teeth, and palate, among other oral surfaces and this often happens when there is a gap in or lack of oral hygiene maintenance, the acid-producing bacteria may occasionally cause disorders of the oral cavity (Medeiros *et al.*, 2015). A lapse in oral hygiene can hasten the onset of diseases involving the oral cavity including halitosis, dental calculus, plaque, gingivitis, dental caries, and periodontitis. The loss of teeth and/or bone are serious consequences of inadequate oral hygiene (Bakri and Douglas, 2005).

Garlic cloves were shown to have sulfur-containing chemicals after a chemical examination (Jonsdottir *et al.*, 2010). These sulfur-based chemicals are what give garlic its antibacterial properties, with allicin and the breakdown products diallyl sulphide (DAS) and diallyl disulphide (DADS) being the most notable ones (Shams-Ghahfarokhi *et al.*, 2006). Because they bind with many of the nearby proteins, including the allinase enzyme, the reactive allicin molecules created in this way have a very short half-life and turn it into an almost suicide enzyme (Kulkarni, 2013). Allicin, which is derived from fresh garlic, is thought to have antioxidant properties, the ability to attach sulfur (SH) groups to enzymes, which inhibits sulfhydryl enzymes, and the ability to permeate cell membranes (Mikaili *et al.*, 2013).

2. Materials and Methods

Plant sample collection

The plant samples (garlic cloves) were acquired at the North Bank Market in Makurdi's capital city and delivered to the Joseph Sarwuan Tarka University's microbiology research facility. The paste from which the extract was made was made by peeling and blending fresh garlic cloves.

Swap sample collection

Swap samples were collected from the biofilm on teeth of patients attending Federal Medical Center Makurdi and were transported to Microbiology research laboratory, Joseph Sarwuan Tarka University Makurdi and analysis was done using standard procedures.

Sterilization and disinfection of materials

Sodium hypochlorite was used to properly disinfect work benches. During the bench work, all apparatus were cleaned with detergents, rinsed with clean water, and autoclaved at 121 °C for 15 minutes.

Garlic extract preparation

Fresh garlic paste that was homogenized was used in preparing the garlic extract.

Aqueous extract of garlic

Homogeneous mixture was made in a glass container by mixing 500 ml of distilled water with 250 grams of crushed garlic paste. The mixture was first periodically swirled for a while, and then it was filtered before being centrifuged once more at 10,000 rpm for about 20 minutes. The Whatman No. 1 filter paper grade 1 with 0.2 mm pore size was used to filter the supernatant to remove any contaminants.

Ethanollic garlic extract

Also, 250 grams of crushed garlic paste and ethyl alcohol (500 mL) were combined in a glass tube and stirred periodically for a while at 35°C to create a uniform mixed solution. The mixed solution was then centrifuged after filtering once more for about 20 minutes at 10,000 rpm. To get rid of any contaminants, the supernatant was filtered through grade 1 Wattman filter paper with a particle size of 0.2 mm. In order to produce the crude extract, ethyl alcohol was thus evaporated from the resulting alcoholic extract before it was heated to concentrate it.

Preparation of different concentrations of garlic extract

Double standard dilution method by (Fawole and Osho 2007) was used to obtain five different concentrations of the crude garlic extracts, these includes 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL 31.25 mg/mL, respectively. This was done by diluting appropriate milligrams of the extracts into corresponding volumes of solvents.

Media preparation

Preparation of nutrient agar

In order to fully dissolve the powder, the dehydrated medium was dissolved in the specified volume (i.e., 28 grams of dehydrated nutrient agar in 1000 ml of purified water). This then was heated with frequent stirring and brought to a boil for one minute. 15 pounds of pressure and 121°C were used during the autoclaving process. Before the inoculation, it was distributed into Petri dishes and given time to set (Hayhoe & Palombo, 2011).

Preparation of Brain Heart Infusion Agar (BHI)

Agar weighing 37.0g was added to a liter of distilled water and heated until it completely dissolved. The appropriate plates were filled with the required quantities, and sterilization took place at 121°C for 15 minutes (Dedua et al., 2014).

Preparation of Muller Hinton Agar

A liter of distilled water was used to suspend 38.0 g of the medium in the solution. To completely dissolve the medium, it was heated while being continuously stirred. A uniform depth was achieved by pouring the sterilized material into sterile Petri dishes at a low level on horizontal surfaces after 15 minutes of sterilization at 121°C.

Bacterial isolation

Inoculation of the swapped samples was done using spread plate method. Each swap stick was inoculated into agar plates by spreading it on the agar surfaces, and this was incubated for 24hours in an incubator. The colonies were sub-cultured onto blood agar and Brain heart infusion media and the morphological appearances and pigmentation were observed. Muller Hinton media was then applied in testing susceptibility of the isolated organisms.

Identification and characterization of bacteria isolates

The isolates' physical characteristics and the results of biochemical testing on the isolates were used to identify them. After 24 hours of growth, morphological characteristics such as colony appearance, colony surface, and pigmentation were observed. Characterization was carried out using the technique suggested by (Karuppiah & Rajaram, 2012).

Gram staining technique

On clean, grease-free slides, a smear involving the 24-hour-old cultures was made. They were fixed by passing them gently over a flame. After being stained for 60 seconds with 2 drops of crystal violet solution, they were washed with water. The smears were rinsed with distilled water after being saturated once more with an Iodine solution for about 30 seconds. They were then decolorized for 15 seconds with 70% alcohol. They were counter-stained for 60 seconds with two drops of safranin, this was rinsed and air-dried. They were put under the microscope after the application of immersion oil and were viewed. The result was read according to color appearance. Gram negative cells appeared pink/red while Gram positive organisms appeared purple (Karuppiah & Rajaram, 2012).

Test for Catalase

Using a sterile inoculating loop, a minute amount of the culture was placed on a drop of 3% hydrogen peroxide solution on a grease free slide. Based on gas's appearance as white froth, the result was interpreted (Kim et al., 2004).

Coagulase test

A loop of the isolates was formed into an emulsion on a microscope slide using regular saline solution. Five seconds were spent stirring the suspension after a drop of pure plasma was added. Colonies tend to group together when coagulase is present (Karuppiah & Rajaram, 2012; Kim et al., 2004).

Indole test

A loopful of the bacterial isolates was inoculated into each test tube after it had been filled with tryptone broth (5 mL), with one test tube remaining uninoculated as the control. The test tubes were then placed in an incubator for 48 hours at 37°C. Following the incubation, 0.5 mL of Kovacs reagent was added, gently mixed, and then let to stand for 20 minutes to allow the reagent to rise. Positive results are indicated by a red or red-violet hue on the tube's top surface, whereas negative results are indicated by yellow colouring (Karuppiah & Rajaram, 2012).

Citrate test

This test looks for an organism's capacity in utilizing citrate as its only energy and carbon source. 100ml of distilled water was used to dissolve about 2.4g of citrate agar. Each tube received roughly 10ml of citrate medium, was covered, sterilized, and cooled. By streaking the organisms once across the surface, the tubes were infected. Citrate use is indicated by a switch from green to blue (Hayhoe & Palombo, 2011).

Urease test

The surfaces of the urea agar slant were spotted with fragments of a well-isolated colony. The tubes were left with their caps loosely on and incubated at 35°-37°C in open air for 48 hours to 7 days. Throughout the course of seven days, the pink color's emergence was studied (Pramod et al., 2009).

Triple Sugar Iron (TSI) test

A sterile straight inoculation needle was used to contact the top of a well-isolated colony, and TSI agar was then inoculated by first stabbing through the medium's center to the tube's bottom, and then streaking across the agar's surface. The caps were left off when the tubes were incubated for 18 to 24 hours at 35°C in room air. (Karuppiyah & Rajaram, 2012).

Agar well diffusion procedure

Inoculum preparation using maccfaland standard

A straight nichrome wire that had been sterilized was used to transfer the colonies from the plates to the nutritional broth. Nutrient broth was used to visually adjust the turbidity to match a freshly made 0.5 MacFarland standard that was made by mixing barium chloride and sulfuric acid.

Inoculation of Agar Plate

The excess inoculum was removed by rotating a sterile cotton swab on the tube wall above the liquid after adjusting the inoculum to a 0.5 MacFarland unit turbidity standard. The Muller Hinton agar plate was streaked three times, rotating the plates between streaks by about 60 degrees, to ensure equal distribution. The inoculated plates were left to stand for no more than 15 minutes before drilling wells in the agar plate, but at least 3 minutes was required. An empty tube with a diameter of 5 mm was heated. When a well developed, it was taken from the inoculated agar plate and applied again. On each plate, six more wells were constructed. The following AGE and EGE concentrations were given to the respective wells on each plate: 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL, and 31 mg/mL. Metronidazole (flagyl) was used as a positive control. The plates were exposed to the chemical for 15 minutes and then incubated for 18 to 24 hours at 37 °C. It was possible to read the growth on confluent or almost confluent plates. The diameters of the inhibitory zone were measured using calipers to the nearest complete millimeter. (Shams-Ghahfarokhi et al., 2006).

Determination of the MIC of garlic

The minimum inhibitory concentration (MIC) of the aqueous garlic extracts (AGE) and ethanolic garlic extract was found using the macro broth dilution method. The concentrations used were as follows: 100 mg/mL, 50 mg/mL, 25 mg/mL, 125 mg/mL, 65 mg/mL, and 31.25 mg/mL. The appropriate broth media in each test tube, which contained 4ml of nutritional broth and 1ml of the extract, were added along with a loopful of the test organisms, which is equal to 0.5 McFarland standards. A set of tubes that contained just 1 ml of the broth medium and no diluted extract were grown as a control. The lowest dosage of garlic extract that completely stopped the growth of the organisms was known as the least inhibitory concentration.

3. Data analysis

The collected data was analyzed using Statistical Package for Social Sciences (SPSS).

4. Findings and discussion

The antibacterial properties of garlic have been shown to be effective against *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Streptococcus* spp., *Klebsiella* spp., *Proteus* spp., *Clostridium* spp., *Mycobacterium* spp., and *Helicobacter* species (Karuppiyah and Rajaram, 2012). Investigations in the past have demonstrated that there is a synergistic antibacterial effect when garlic extract and antibiotics are combined (Kim et al., 2004).

Garlic-infused mouthwash considerably reduced the number of bacteria in saliva, despite the fact that some oral streptococci have been shown to be resistant to the mouthwash's active ingredient (Mozaffari et al., 2014).

Fresh garlic extract has been shown to impede the growth of *Candida albicans* at all stages of the fungus' lifecycle, including the planktonic, adherent, and sessile forms. This raises the question of whether garlic possesses antifungal properties that can affect the growth of *Candida albicans* biofilm. (Kim et al., 2004).

In this study, the investigation on the effect of different concentrations of garlic extract against dental plaque biofilm isolates (*Streptococcus oralis*, *Streptococcus mutans*, *Enterococcus faecalis* and *Staphylococcus aureus*) obtained from patients attending Federal Medical Center (FMC) Makurdi, reveals a maximum inhibitory activity against *S. mutans* and *S. oralis* with a zone of inhibition of 23.33 ± 2.89 mm each, followed by *Staphylococcus aureus* with an inhibition zone of 18.00 ± 1.73 mm and lastly *Enterococcus faecalis* with an inhibition zone of 7.33 ± 6.43 mm for the ethanolic garlic extract both at a concentration of 500 mg/mL.

This is contrary to the report by Angle (2011), who recorded that garlic can increase bacteria biofilm attachment to orthodontic wire, as a result of non-antibacterial activity. Other concentrations also exhibited little inhibitory activities such as 250 mg/mL which showed an inhibitory activity with an inhibition zone of 17.00 ± 1.00 mm on *Streptococcus oralis*, 8.34 ± 7.43 mm, 8.33 ± 6.43 mm, and 6.67 ± 5.78 mm for concentrations of 125.5 mg/mL, 62.5 mg/mL and 31.3 mg/mL respectively. This agrees with the report of Behzad et al. (2013), who also reported antibacterial activity of garlic extract on oral *Streptococcus* at different concentrations.

The analysis also showed that the ethanolic garlic extract exhibited a higher inhibitory activity on the isolates than the aqueous garlic extract. The aqueous garlic extracts which showed an inhibitory activity with an inhibition zone of 19.00 ± 1.73 mm at concentrations of 500 mg/mL on *Enterococcus faecalis*, followed by *Streptococcus oralis* with an inhibition zone of 17.00 ± 1.00 mm, 13.33 ± 3.43 mm for *Streptococcus mutans*, and 13.33 ± 3.43 mm for *Staphylococcus aureus* respectively, This could result from the inhibitory activity which was dependent and varied with the extract concentrations, the species of organism and also dependent on the solvent used for the extraction, as the effect observed with ethanolic garlic extract was always higher in each case as compared to that observed with the aqueous garlic extract.

This result is also in agreement with that of Mohsenipour and Mehdi (2015), who reported a significant effect of garlic extract at each concentration on the dental plaque isolates and also stated that there exist a direct relationship between the effect exhibited by garlic extract and concentration of the extract, which implied that the higher the extract concentration the higher the antimicrobial activity and the lower the extract concentration the lower the antimicrobial activity.

The analysis of the minimum inhibitory concentration (MIC) of the ethanolic garlic on the test organisms revealed 250 mg/mL as the lowest concentration that inhibits each of the test organisms. However same analysis using aqueous garlic extract showed 250 mg/mL as the lowest concentration that inhibits *Streptococcus mutans* and *Streptococcus oralis* and 500 mg/mL as the lowest concentration that inhibited *Enterococcus faecalis* and *Staphylococcus aureus*. This therefore shows that ethanol as a solvent is more effective when used for extraction than aqueous. The minimum bactericidal concentration (MBC) analysis showed that both ethanolic garlic extract (EGE) and aqueous garlic extract (AGE) has little or no bactericidal activity as growth was observed on the MIC plates in each case. However little bactericidal activity was observed for the ethanolic garlic extract (EGE) at a concentration of 500 mg/mL on the test organisms as reduced number of colonies were recorded on the MIC plates bearing 500 mg/mL.

Table 1: Antibacterial Activity of Different Concentrations of Ethanolic Garlic Extract on Dental Plaque Isolates. Zones of Inhibitions Measured in Millimeter (mm)

Test Organisms	500 mg/mL	250 mg/mL	125 mg/mL	62.5 mg/mL	31.3 mg/mL	control
<i>Streptococcus mutans</i>	23.33±2.89	16.00±1.00	13.33±3.43	0.00±0.00	0.00±0.00	28.33±2.89
<i>S. oralis</i>	23.33±2.89	17.00±1.00	8.34±7.43	8.33±6.43	6.67±5.78	26.33±2.89
<i>Enterococcus faecalis</i>	7.33±6.43	6.67±11.55	3.33±5.78	0.00±0.00	0.00±0.00	27.33±2.45
<i>Staphylococcus aureus</i>	18.00±1.73	14.33±1.53	3.33±5.43	6.67±5.78	4.00±6.93	25.33±3.76

Result expressed in mean± standard deviation. P value at 0.05

Table 2. Antibacterial Activity of Different Concentrations of Aqueous Garlic Extract on Dental Plaque Isolates. Zones of Inhibitions Measured in Millimeter (mm)

Test Organisms	500 mg/mL	250 mg/mL	125 mg/mL	62.5 mg/mL	31.3 mg/mL	control
<i>Streptococcus mutans</i>	8.33±6.43	4.31±5.78	3.23±5.73	3.23±5.88	0.00±00	13.33±3.43
<i>S. oralis</i>	17.00±1.00	13.33±3.43	6.33±5.43	3.31±5.75	3.33±5.78	19.00±1.73
<i>Enterococcus faecalis</i>	19.00±1.73	13.33±3.43	7.33±5.78	3.33±5.78	0.00±00	26.33±1.53
<i>Staphylococcus aureus</i>	13.33±3.43	8.33±6.43	0.00±00	0.00±00	0.00±00	30.00±0.00

Result expressed in mean± standard deviation. P value at 0.05

Table 3. Minimum Inhibitory Concentrations (MIC) of Ethanolic Garlic Extracts on Dental Plaque Isolates

Test Organisms	500 mg/mL	250 mg/mL	125 mg/mL	62.5 mg/mL	31.3mg/mL
<i>Streptococcus mutans</i>	S	S	R	R	R
<i>S. oralis</i>	S	S	R	R	R
<i>Enterococcus faecalis</i>	S	S	R	R	R
<i>Staphylococcus aureus</i>	S	S	R	R	R

Key:

R= resistance

S= sensitive

Table 4. Minimum Inhibitory Concentrations (MIC) Of Aqueous Garlic Extracts on Dental Plaque Isolates

Test Organisms	500 mg/mL	250 mg/mL	125 mg/mL	62.5 mg/mL	31.3mg/mL
<i>Streptococcus mutans</i>	S	S	R	R	R
<i>S. oralis</i>	S	S	R	R	R
<i>Enterococcus faecalis</i>	S	R	R	R	R
<i>Staphylococcus aureus</i>	S	R	R	R	R

Key:

R= resistance

S= sensitive

Figure 1: Graphical representation of the effect of ethanolic garlic extract on dental plaque isolates

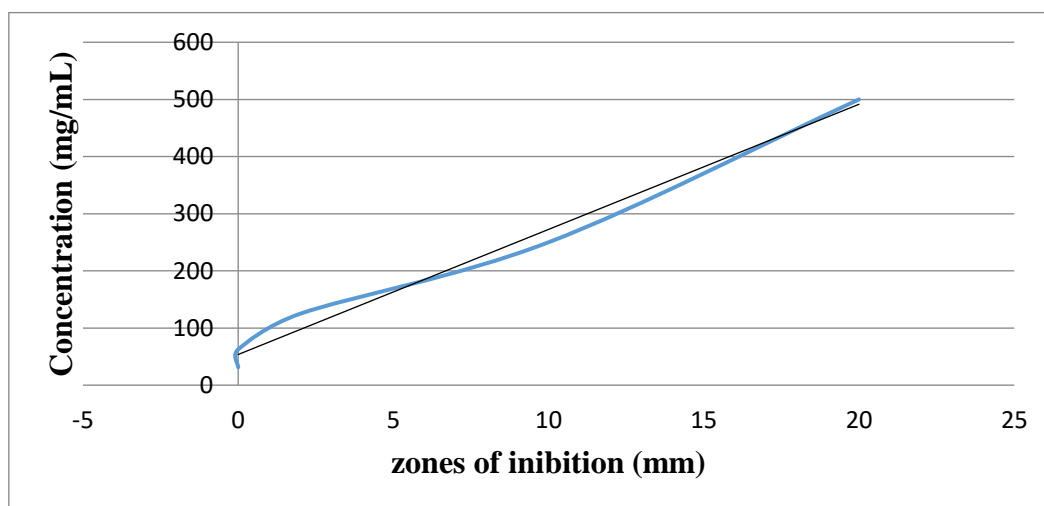
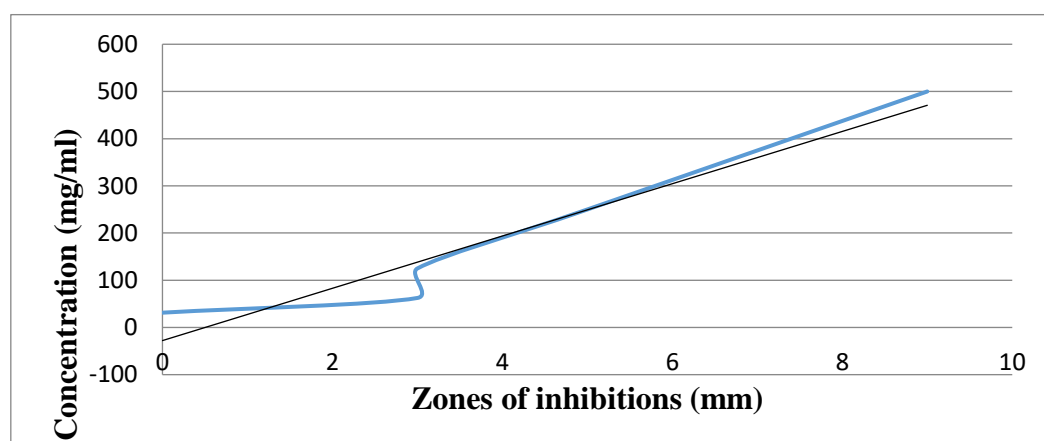


Figure 2: Graphical representation of the effect of aqueous garlic extract on dental plaque isolates



5. Contribution of the study to the body of knowledge

This study contributes to literature on the antibacterial effect of garlic on dental plaque isolates. It also proves

further the therapeutic effect of medicinal plants and how they can be used in the prevention and treatment of diseases.

6. Implications of the study

Garlic can therefore be used together with other substances to reduce the microbial biofilm on dental plaque. This can help to reduce the occurrence of oral diseases associated with those pathogens.

7. Recommendations and future study directions

Based on the result of this study the following recommendations are made;

- i. Oral hygiene is recommended in order to help reduce the accumulation of dental plaque which usually harbours primary microbes that colonize the teeth surfaces resulting to carries and periodontal diseases.
- ii. Agar well diffusion method is recommended for MIC instead of the microbroth dilution method in which the extract colour usually interferes with the test result.
- iii. The use of mouth wash containing garlic and or toothpaste incorporated with alicin is recommended as this can help reduce the accumulation of dental plaque biofilm thereby minimising the incidence of dental caries and periodontal diseases.

8. Conclusion

The crude ethanolic and aqueous extracts of garlic exhibited a minimal inhibitory effect at different concentrations on the tests organisms of dental plaque biofilm; *Streptococcus mutans*, *Streptococcus oralis*, *Enterococcus faecalis* and *staphylococcus aureus*, obtained from patients attending Federal Medical Center (FMC) Makurdi. The isolates were both sensitive to the extract at varying concentrations though no bactericidal activity was observed in any case on the test organisms at any concentration for the aqueous garlic extract while 500mg/ml was the Minimum Bactericidal Concentration for the ethanolic extract. Therefore extracts of Garlic can be used to manage oral problems caused by *Streptococcus* sp, *Enterococcus* sp and *Staphylococcus* sp.

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