

**ANTIBACTERIAL PROPERTIES OF METHANOL AND AQUEOUS EXTRACTS OF
ANOGEISSUS LEIOCARPUS AND TERMINALIA MICROPTERA AGAINST SELECTED
ORAL PATHOGENS**Amuda Oladunni*¹, Samuel Alimi Garba¹, Galadima Musa¹, and Jigam Ali Audu²¹Department of Microbiology, Federal University of Technology, Minna, Niger State, Nigeria.²Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria.***Corresponding Author: Amuda Oladunni**

Department of Microbiology, Federal University of Technology, Minna, Niger State, Nigeria.

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ABSTRACT

In Africa, chewing sticks are commonly used for maintaining oral hygiene. Phytochemical contents and antibacterial activity of aqueous and methanol extracts of *Terminalia microptera* and *Anogeissus leiocarpus* were investigated using standard methods. Qualitative phytochemical screening of the two extracts of *T. microptera* and *A. leiocarpus* revealed the presence of all the tested phytochemicals, except anthraquinones, steroids and terpenoids which are absent in aqueous extract of *A. leiocarpus*. Quantitative phytochemical analysis (mg/100g) indicates the presence of phenols, flavonoids, tannins, saponins and alkaloids at concentration ranging from 805.231±10.83-880.393±6.05, 295.661±100.32-337.556±3.67, 142.321±12.62-253.25±8.56.63, 4.254±0.10-7.338±0.84 and 85.762±10.21-134.231±8.63 respectively. The antibacterial activity of the aqueous and methanol extract of the plants at 20, 30 and 40mg/mL revealed a spectrum of activity which is significant at p<0.05 with the standard drug (Ampiclox and Amoxicilin). Minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the methanol and aqueous extract of the plants ranged from 0.625-10mg/mL and 1.25 – 40mg/mL respectively in all the test organisms. The results revealed the potency of these extracts as a good source of treating orodental and other diseases cause by these pathogens.

KEYWORDS: *Anogeissus leiocarpus*, *Terminalia microptera*, Antibacterial, Ampiclox, Amoxicilin, Pathogens, Oro dental.

1.0 INTRODUCTION

In Africa, chewing sticks are commonly used for maintaining oral hygiene, and roots, stems and twigs of numerous plants are employed for this purpose. Chewing sticks are recommended for oral hygiene by the World Health Organization, and some of them, or their extracts, are also used in the ethnomedical treatment of oral infections by the local herbal practitioners.^[1] Preliminary screening of these plants or their extract have shown their antimicrobial activity against a broad spectrum of microorganisms, including those commonly implicated in orofacial infections.^[1]

Almost the entire rural population of Nigeria uses chewing sticks for orodental hygiene. Previous studies have demonstrated the antiplaque and antibacterial actions of extracts of these Nigerian chewing sticks (NCS) against oral bacteria, such as *Streptococcus mutans* and *Micrococcus luteus*, *Streptococcus mitis* and oral anaerobes,^[1] which are the organisms commonly implicated in dental caries and orodental infections. Most of the studies on orodental infections stress the importance of oral anaerobes, particularly black

pigmented bacteroides, in the etiology of periodontal diseases.^[1]

The choice of chewing sticks to be used in most cases depends on its cleansing action of the teeth; the therapeutic value, or preferred taste or flavour. The sticks (which may be stem or root with bark removed or retained) are cut to convenient lengths and washed thoroughly with fresh water to get rid of the earth or any dirt. The diameter should afford good grip, say between 0.5-1.30cm.^[2] reported that some of the chewing sticks being used are obtained from the following plants: *Garcinia mannii*, *Masularia acuminita*, *Terminalia glaucescens*, *Anogeissus leiocarpus*, *Pseudocedrela kotschyi*, *Xanthoxylum gilletti* and *Azadiracta indica*. Investigations carried out on some of these chewing sticks showed that they possess antimicrobial activity against oral microbial flora such as *Staphylococcus aureus* and *S. auricularis*,^[2] *Candida albicans*, *Aspergillus flavus*, *Microsporium gypseum* and *Trichophyton metagrophytes*.^[3]

Terminalia macroptera Guill. & Perr. (Combretaceae) is a tree, up to 20 m high, which occurs widely in West Africa. In Mali, *T. macroptera* is used against a variety of ailments, and more than 30 different indications have been mentioned by the traditional healers in ethnopharmacological studies. The stem bark and leaves are most commonly used against sores and wounds, pain, cough, tuberculosis and hepatitis.^[4] The roots are used against hepatitis, gonorrhoea and various infectious diseases, including *H. pylori*-associated diseases.^[4-8] Flavonoids,^[5-7] triterpenoids,^[5,6] ellagitannins^[7] and related phenolics,^[5,9] have been identified from different parts of *T. macroptera*. Ellagitannins are known antimicrobial compounds which may be related to the use of the leaves against wounds and infections.^[9] Water decoctions of *T. macroptera*, administered orally, are the most common preparations used by the traditional healers in Mali.^[4]

Anogeissus leiocarpus is also another plant species used in traditional medicine as a remedy for many ailments of livestock and man, which include helminthiasis, schistosomiasis, leprosy, diarrhea and psoriasis.^[11] In addition to these applications, Hollist^[12] reported that *A. leiocarpus* is one of the major plants commonly used as chewing stick in Nigeria. *Fagara zanthoxyloides* is also widely distributed in African countries. The root-bark extract is used in treating elephantiasis, toothache, sexual impotence, gonorrhoea, malaria, dysmenorrhoea and abdominal pain.^[11] Many studies have demonstrated the antimicrobial, anticaries, anti-periodontic and antifungal properties of both aqueous and ethanol extracts of various chewing sticks.^[13] There are documented reports on the antimicrobial activity of *A. leiocarpus* on oral microflora.^[14] reported the antimicrobial effect of its root extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa*.^[11] documented the antibacterial activity of its bark extract on *Bacteriodes gingivalis* and *Bacteriodes malaninogenicus*. Workers in West Africa have also reported the anti-sickling and antimicrobial activity of the extracts of *Fagara zanthoxyloides*.^[11] Water extracts from the plant showed activities against bacteria significant to periodontal disease.^[11] The anthelmintic activity of the methanol extract of the root-bark of *F. zanthoxyloides* was also reported,^[11] and it is a very popular anthelmintic amongst the various tribes in Uganda. It has also been found that the alcoholic extracts of the root-bark possess considerable antibacterial activity.^[11] An anti-sickling agent^[11] and an anti-inflammatory amide were isolated from the plant.^[11]

Finally, it must be stressed that the development of virile herbal toothpaste is consequent upon the bioactivity of the constituent chewing sticks against a wide range of oral pathogens. Hence the aim of this paper is to report the antimicrobial activity of the methanol and aqueous extracts of two Nigerian chewing sticks; *Terminalia macroptera* and *Anogeissus leiocarpus* on oral bacteria pathogens such as *Micrococcus luteus*, *Streptococcus*

mutans, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection and Identification of plant materials.

Fresh stem of *Terminalia macroptera* and *Anogeissus leiocarpus* were both collected from Kaba, in Kogi State, Nigeria in October, 2016. The plants were identified at National Institute of Pharmaceutical Research and Development, Idu-Abuja, Nigeria and assigned a voucher number NIPRD/H/6797 and NIPRD/H/6799 for *T. macroptera* and *A. leiocarpus* respectively.

2.1.2 Sources and Characterization of Bacteria Pathogens

Samples were collected from orodental patients attending General Hospital Minna, using sterile swab stick soaked with sterile normal saline. The swab stick sample was inoculated into prepared nutrient broth and incubated for 6 h, so as to activate the bacterial. After activation on the nutrient broth, the nutrient broth culture was subculture into nutrient Agar, Blood Agar, MacConkey Agar and Manitol salt Agar so as to isolate the bacteria. This was characterized after isolation and compared with known existing taxa of.^[15] The bacteria pathogens identified includes: *Micrococcus luteus*, *streptococcus mutans*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*.

2.1.3 Ethical Consideration

Ethical clearance to conduct this research was sought from the research ethics and publication committee of the hospital. Informed consent was obtained for each respondent before physical examination. Subjects less than 18 years had their consent sought from their relatives or guidance.

2.2 Methods

2.2.1 Plant processing and extraction

The stem of *A. leiocarpus* and *T. macroptera* were collected washed and air-dried at room temperature at the Centre for Genetic Engineering and Biotechnology, Federal University of technology, Minna, Niger State. The dried stem were blended using blending machine to obtain a fine powder.^[16] Fifty grams (50 g) of the plant powder was extracted with 400mL of methanol and distilled water using reflux method at a temperature of 45°C for 2 hours and the extract was filtered using muslin cloth followed by further filtration using whatman No 1 filter paper with pore size of 0.7µm to obtain a fine filtrate. The filtrate was then concentrated using RE-6000 rotary evaporator at 50°C and further concentrated using the water bath at 45°C to ensure the extract is totally free from the solvent used in the extraction.

The percentage yield of the extracts was calculated as follow:

2.2.2 Qualitative Phytochemical Screening of Extracts

Preliminary qualitative phytochemical screening which involved performing simple chemical tests to detect the presence of secondary metabolites such as tannins, flavonoids, phenols, phenolic compounds, saponins, and glycosides, was carried out according to Trease and Evans and Sofowora.^{[9],[17]}

2.3 Quantitative determination of Phytochemicals

Quantitative estimation of phytochemicals such as alkaloids and saponins was carried out according to,^[26] total phenolic content^[27] and flavonoids using Aluminum Chloride colorimetric method.^[28]

2.4 Determination of Antibacterial Activity of the Extract

2.4.1 Assay for Antibacterial Activity

Agar well diffusion method was used to evaluate the antibacterial activity of the Crude extracts.^[36] Eighteen-hour culture of bacteria adjusted to 0.5 McFarland standard was used as inoculum on sterile Mueller Hinton agar. The plate was kept on flat bench for 30 minutes to solidify. Five wells (4mm) deep were made in the agar using a sterile 6mm diameter cork borer. Then 0.5 mL of the reconstituted extract at a concentration of 20, 30 and 40 mg/mL was pipetted into the wells using micro pipette. Zero point five milliliter (0.5mL) each of 5mg/mL of Ampiclox and Amoxiline solution were used as positive controls and 0.5mL of Di-Methyl Sulphoxide (DMSO) as a negative control. The plates were allowed to stand on a flat bench for 30 min to allow diffusion of the extract into the agar before incubation at 37°C for 24 h. Each test was carried out in triplicates and mean zone diameter of inhibition was recorded.

2.4.1 Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extract was determined using the double fold dilution. An aliquot of 1mL of the reconstituted extract with 50% DMSO at a concentration of 40 mg/mL was diluted serially to give concentrations of 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg/mL in eight test tubes. One milliliter (1mL) of 18h culture of bacteria previously adjusted to 0.5 McFarland standard (1.0×10^6 cfu/mL) was added to each of the test tubes and the content was mixed thoroughly. The tubes were incubated at 37°C for 4 h. The 9th test tube contained 1mL of 50% DMSO with no extract served as negative control. The 10th and 11th test tubes containing a solution of 5 mg/mL of Ampiclox and Amoxiline served as positive control. The procedure was repeated for the test and the control. The test tube with the lowest concentration of the extract without visible turbidity of growth was taken as the MIC.^[18]

2.4.2 Determination of the Minimum Bactericidal Concentration (MBC)

From each of the test tubes without any visible growth, a loopfull of the broth was aseptically inoculated on a sterile Mueller Hinton agar. The inoculated plates were incubated for 24hr at a temperature of 37°C. After incubation, the MBC was determined as the lowest concentration with no visible growth on the plate.^[19]

2.5 Statistical Analysis

Data obtained in this study were analysed using the IBM Statistical Package for Social Science (SPSS) 20.0, 2011 version (SPSS Inc., Chicago, Illinois, USA). Numerical data were presented as mean \pm standard error of mean (SEM) of the triplicate.

3.0 RESULTS

Table 1: Percentage yield of the extracts of *A. leiocarpus* and *T. microptera*.

Samples	WDP (g)	Methanol g (%)	Aqueous g (%)
<i>Anogeissus leiocarpus</i>	500	88.45 (17.69)	39.15 (7.83)
<i>Terminalia microptera</i>	500	35.20 (7.04)	40.80 (8.16)

Key: WDP = Weight of dry powder plant.

Table 2: Results of Qualitative Phytochemical Screening of methanol and aqueous extracts of *A. leiocarpus* and *T. microptera*.

Plants	Extract	Alkaloids	Phenols	Tannins	Saponins	Cardiac glycosides	Phlobatannins	Anthraquinones	Steroids	Terpenes	flavonoids
<i>T. microptera</i>	Methanol	+	+	+	+	+	+	+	+	+	+
	Aqueous	+	+	+	+	+	+	+	+	+	+
<i>A. leiocarpus</i>	Methanol	+	+	+	+	+	+	+	-	+	+
	Aqueous	+	+	+	+	+	+	-	-	-	+

Keys: + = Present, - = Absent

Table 3: Results of Quantitative phytochemical analysis of methanol and aqueous extracts of *A. leiocarpus* and *T. microptera* (mg/100g).

Plants extract	Total phenols	Total flavonoids	Alkaloids	Tannins	Saponins
ALA	826.661±67.35	337.556±3.67	134.231±8.63	253.25±8.56.63	4.254±0.10
ALM	850.535±10.92	320.412±35.36	122.209±16.92	191.292±3.63	4.292±0.03
TMM	880.393±6.05	305.667±222.94	133.863±8.50	145.009±1.74	7.338±0.84
TMA	805.231±10.83	295.661±100.32	85.762±10.21	142.321±12.62	6.881±0.53

Values are expressed in mean ± standard error of mean of duplicate determination.

Keys:ALA: Aqueous extract of *A. leiocarpus*, ALM: Methanol extract of *A. leiocarpus*, TMM: Methanol extract of *T. microptera*, TMA: Aqueous extract of *T. microptera*

Table 4: Mean zones of inhibition (mm) of methanol and aqueous extracts of *Terminalia microptera*.

Test organisms	TMM 20mg/mL	TMM 30mg/mL	TMM 40mg/mL	TMA 20mg/mL	TMA 30mg/mL	TMA 40mg/mL	Amoxicilin* 5mg/mL	Ampiclox* 5mg/mL
<i>M. luteus</i>	0.00±0.00 ^a	14.67±0.67 ^b	20.33±0.67 ^c	22.33±0.33 ^{cd}	23.00±1.00 ^d	23.00±1.53 ^d	26.33±0.33 ^e	22.33±0.33 ^{cd}
<i>P. aeruginosa</i>	17.33±0.88 ^{bc}	16.00±0.5 ^b	23.67±1.86 ^d	25.00±1.00 ^e	23.33±0.67 ^d	27.33±0.33 ^f	13.67±0.33 ^a	20.67±0.33 ^c
<i>S. mutans</i>	20.67±1.20 ^{ab}	20.33±0.67 ^{ab}	23.67±0.33 ^{bc}	19.00±0.58 ^a	22.00±1.00 ^b	22.00±1.00 ^b	25.67±0.33 ^c	28.00±1.00 ^d
<i>K. pneumoniae</i>	18.67±0.33 ^a	20.33±0.33 ^b	25.33±0.33 ^{cd}	18.67±0.33 ^a	25.00±1.00 ^{cd}	23.67±0.33 ^c	19.00±1.00 ^{ab}	24.33±0.33 ^c
<i>S. pneumoniae</i>	19.00±0.58 ^d	19.67±0.33 ^d	21.33±0.6 ^{dc}	0.00±0.00 ^a	12.00±1.00 ^b	16.33±0.67 ^{cd}	15.670.33 ^c	24.33±0.67 ^e
<i>S. pyogenes</i>	17.00±1.00 ^b	13.33±0.33 ^a	17.00±0.67 ^b	18.33±0.33 ^{bc}	21.33±0.88 ^c	22.67±0.88 ^d	26.00±0.58 ^e	27.67±0.33 ^f

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significant difference (p>0.05), n=3

TMM: *Terminalia microptera* methanol extract, TMA: *Terminalia microptera* aqueous extract.

* Specification for Amoxicillin and Ampiclox are: ≤19mm (resistance) and ≥20mm (susceptible) (CLSI, 2012).

Table 5: Mean zones of inhibition (mm) of Methanol Extracts of *Anogeissus leiocarpus* stem.

Test organisms	ALM 20mg/mL	ALM 30mg/mL	ALM 40mg/mL	ALA 20mg/mL	ALA 30mg/mL	ALA 40mg/mL	Amoxicilin* 5mg/mL	Ampiclox* 5mg/mL
<i>M. luteus</i>	22.33±0.67 ^{bc}	21.00±1.00 ^b	24.33±1.20 ^{cd}	19.33±0.88 ^a	19.33±0.88 ^a	23.33±0.67 ^c	26.33±0.33 ^d	22.33±0.33 ^{bc}
<i>P. aeruginosa</i>	17.67±0.67 ^b	22.67±0.33 ^{cd}	22.67±0.33 ^{cd}	15.00±0.58 ^{ab}	21.00±0.00 ^c	25.67±1.20 ^d	13.67±0.33 ^a	20.67±0.33 ^c
<i>S. mutans</i>	21.67±0.88 ^b	24.00±1.00 ^c	23.33±1.20 ^{bc}	18.33±0.33 ^a	21.00±1.00 ^b	21.67±0.33 ^b	25.67±0.33 ^{cd}	28.00±1.00 ^d
<i>K. pneumoniae</i>	18.33±0.33 ^b	23.33±0.33 ^c	23.33±0.33 ^c	15.00±0.58 ^a	19.33±1.33 ^{bc}	22.67±0.33 ^c	19.00±1.00 ^b	24.33±0.33 ^d
<i>S. pneumoniae</i>	20.67±0.67 ^b	21.00±0.58 ^b	25.00±0.58 ^d	15.33±0.33 ^a	22.00±1.00 ^{bc}	24.33±0.67 ^c	15.670.33 ^a	24.33±0.67 ^c
<i>S. pyogenes</i>	20.33±0.88 ^a	20.33±0.88 ^a	28.33±0.67 ^d	21.00±1.15 ^a	20.67±1.67 ^a	20.67±0.33 ^a	26.00±0.58 ^c	27.67±0.33 ^d

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significant difference (p>0.05), n=3

ALM: *Anogeissus leiocarpus* methanol extract, ALS: *Anogeissus leiocarpus* aqueous extracts

* Specification for Amoxicillin and Ampiclox are: ≤19mm (resistance) and ≥20mm (susceptible) (CLSI, 2012).

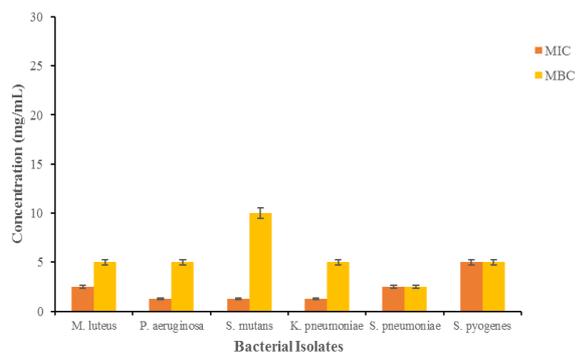


Figure 1: MIC and MBC of methanol extract of *T. microptera*.

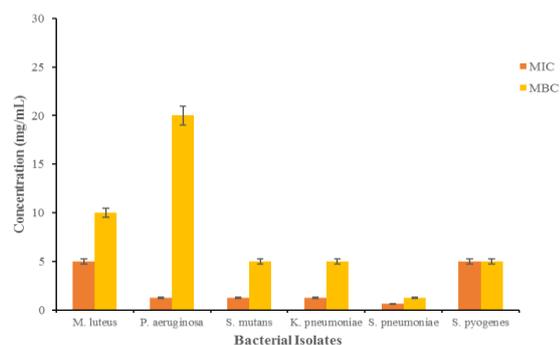


Figure 2: MIC and MBC of aqueous extract of *T. microptera*.

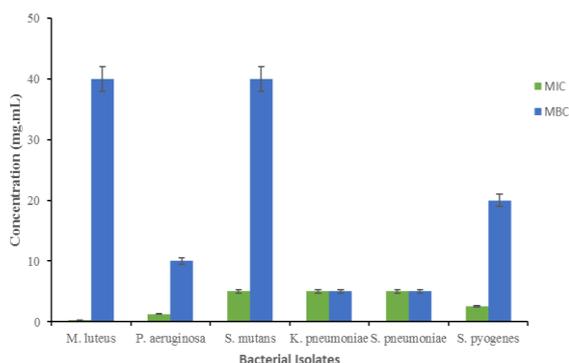


Figure 3: MIC and MBC of methanol extract of *A. leiocarpus*.

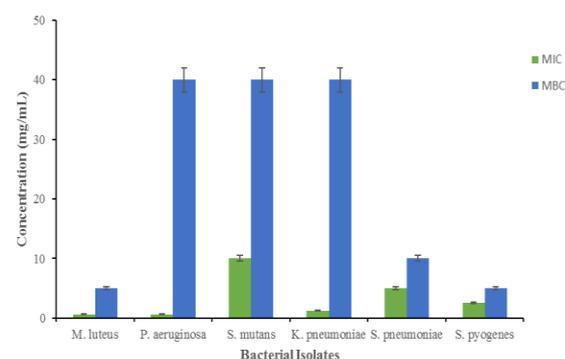


Figure 4: MIC and MBC of aqueous extract of *A. leiocarpus*.

4.0 DISCUSSIONS

About 88% of people in the developing world depend on plant-based traditional medicines for their primary health care.^[20] According to the World Health Organization, a medicinal plant is any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemopharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. These non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals ('phyto-'from Greek - *phyto* meaning 'plant') or phytoconstituents and are responsible for protecting the plant against microbial infections or infestations by pests.^[21,22,23]

Higher yield of the extract was obtained in aqueous extract of *A. leiocarpus* (17.67%) followed by aqueous extract of *T. microptera* (8.16%). Methanol and aqueous extract of *T. microptera* and *A. leiocarpus* 7.04 and 7.86% respectively (Table 1). The yield of plants depends on the solvent used for extraction, the method of extraction and the part of the plant used for the extraction. Extraction from the plant is an empirical exercise in which different solvents are utilized under a variety of conditions such as time and temperature of extraction. The success or failure of the extraction process depends on the most appropriate assay.^[20]

Preliminary phytochemical screening of the aqueous and methanol extract of *A. leiocarpus* and *T. microptera* revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, cardiac glycosides, anthraquinones, steroids and terpenes and saponins in all the extracts while anthraquinones, steroids and terpenes were absent in the aqueous extract of *A. leiocarpus* (Table 2). This report agreed with the findings of^[24] and^[25] who reported the presence of all these phytochemicals in 70% methanol extract of *T. microptera* and aqueous extract of *A. leiocarpus* respectively. Quantitative phytochemical analysis (mg/100g) indicates the presence of phenols, flavonoids, tannins, saponins and alkaloids at concentration ranging from 805.231±10.83 - 880.393±6.05, 295.661±100.32 - 337.556±3.67, 142.321±12.62-253.25±8.56.63, 4.254±0.10-7.338±0.84 and 85.762±10.21 - 134.231±8.63 respectively (Table 3). A large number of phytochemicals have been reported to have antibacterial activity. Well known examples include phenols, unsaturated lactones, saponins, cyanogenic glycosides and glucosinolates.^[25] The presence of these phytochemical in the investigated plant parts of *A. leiocarpus* and *T. microptera* may be responsible for the demonstrated antibacterial activity of the extracts (Table 4 and 5). In this regard, the higher concentration of these phytochemicals in the extract may have been responsible for a relatively higher antimicrobial activity demonstrated by the extract on the tested oral pathogens.

Previous reports have indicated that the root of *A. leiocarpus* is often used as chewing stick^[2] and^[14] while *T. microptera* have been reported to possess various medicinal values.^[4]

The determination of the minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) extracts was to provide a minimum concentration of the extracts that would selectively inhibit or kill the pathogens. The minimum inhibitory concentration (MIC) of the methanol and aqueous extracts of *A. leiocarpus* and *T. microptera* ranged between 0.625 - 10mg/mL in aqueous and methanol extracts of the plants while the MBC ranged between 1.25 - 40mg/mL as shown in Figure 1-4.

4.1 CONCLUSIONS

From the results of this study, aqueous and methanol extract of *T. microptera* and *A. leiocarpus* possesses active phytochemical constituents and antibacterial activities against the selected oral pathogens. It can therefore be inferred that methanol and aqueous extract of these plants may be used as chewing sticks since it contains bioactive components that can inhibit the growth and activities of the microorganisms in the oral cavity. Also, the plants can also be used in the manufacture of herbal paste and drugs which can be used in the treatment of ailments caused by these pathogens.

4.2 CONFLICT OF INTEREST

The author has declared there is no conflict of interest.

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