GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Evaluation of the antibacterial activities of methanol stem extract of *Massularia acuminata* and methanol twig extract of *Garcinia kola* on oral Bacteria and their effect in combination

Dan John [1], Lambert Okwubie [2,*] and Efe J. Oyakhire [1]

[1] *Department of Pharmaceutical Microbiology and Biotechnology, University of Port Harcourt.*
[2] *Department of Pharmacognosy and Phytotherapy University of Port Harcourt.*

GSC Biological and Pharmaceutical Sciences, 2022, 18(01), 118–128

Publication history: Received on 19 December 2021; revised on 19 January 2022; accepted on 21 January 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.18.1.0037

Abstract

Massularia acuminata and *Garcinia* kola are chewing sticks widely used in Nigeria rural population for oral and dental care. This research investigates the individual antibacterial effects of methanol extracts of stem and twigs of these plants respectively and the combined effect of both extracts, against isolates of the oral cavity. The isolates were obtained from the oral cavities of healthy undergraduate students. The bacteria isolates obtained were Staphylococcus aureus, Klebsiella pneumonia, *Streptococcus spp., Pseudomonas aeruginosa* and *Escherichia coli*. Screening for antibacterial effect of each of the extracts and the combined extract on the standardized isolates was done by the Agar well diffusion method. On S. aureus, the methanol extract of M. acuminate and G. kola had minimum inhibitory concentration (MIC) of 12.5mg/ml and 12.5mg/ml with mean inhibitory zone diameter (IZD) of 3mm and 10mm respectively. The combination of both plant extracts showed MIC of 25mg/ml and a mean IZD of 4mm. On *E. coli, M. acuminate* and *G. kola* had MIC of 25mg/ml and 12.5mg/ml with mean IZD of 2mm and 3mm respectively. The combination of both extracts gave MIC of 25mg/ml and a mean IZD of 1mm. Both extracts didn't exhibit any effect on the other 3 isolates. Statistical analysis using ANOVA showed the values were significant at $p<0.05$ for antibacterial activity of *G. kola, M. acuminata* and the combined extracts against *S. aureus*, also significant for *G. kola* ($p<0.05$) against *E. coli* but insignificant for *M. acuminata* and the combined extracts ($p>0.05$). This study proved that *M. acuminata* and *G. kola* extracts can be used for oral hygiene, but not in combination

Keywords: *Massularia acuminate; Garcinia kola*; Oral cavity; Methanol

1. Introduction

Dental carries are known to be one of the most rampant and chronic infections of the tooth, globally affecting all ages and populations resulting in severe socioeconomic burden. [1]. This multifactorial disease is characterized as a progressive dissolution and demineralization of tooth resulting from complex interactions between tooth structures and oral microbial flora, dental film formation, dietary remnant accumulation, salivary dysfunction and genetic disturbances. [2]. The most popular and commonly used oral hygiene kits remain the regular toothbrushes and a plethora of toothpastes. [3]. However, Sharda and Sharda, [4], stated that individuals either by medical advice or by personal preference have adapted to other oral hygiene kits that includes traditional chewing sticks. In A comparative study carried out by Tedwins *et al,* [3] to determine the effect of *Massularia acuminata* and mouthwash against isolates from the oral cavity, the aqueous and ethanolic extract of *Massularia acuminata* showed a high inhibitory activity against the bacteria species tested, since it exhibited the highest antibacterial inhibition with a minimum inhibitory

* Corresponding author: Lambert Okwubie; Email: okwubie1@yahoo.com
Department of Pharmaconosy and Phytotherapy University of Port Harcourt

concentration of 25mg/ml against all the test isolates. Therefore, the aim of thhis study was to evaluate the antibacterial effect of methanol extracts of Massularia *acuminata* and *Garcinia kola* on oral bacteria and their effect in combination

2. Material and methods

2.1. Culture Media Used

The media used include; Nutrient agar (Titan biotech limited India), Mannitol salt agar (Titan Biotech Limited, India), Mac-Conkey agar (Titan Biotech Limited, India), Mueller hinton agar (Titan Biotech Limited, India), Peptone water (Lab M Limited, England). They were prepared according to the manufacturer's specifications and sterilized by autoclaving at 121°c for 15 minutes.

2.2. Equipment/ Apparatus and Reagents

The various apparatus used include Sterile swab sticks, Oven, incubator, autoclave, refrigerator, Bunsen burner, wire loop, measuring cylinders, beakers, bent glass rod, sterile disposable petri dishes, sterile Cork borer, sterile pipettes, sterile forceps, infusion bottle, conical flask, hand gloves, cotton wool, universal bottles, Mccartney bottles, sterile syringes (1ml, 2ml, 5ml), extraction jar, crucibles, filter paper, Antibiotic discs (Rapid Labs, Uk, no: QF10/P).

The reagents used in this study include; Safranin Red, Crystal Violet, Lugol's Iodine, 95% alcohol, Kovac's reagent, 3% Hydrogen peroxide, Dimethyl sulfoxide (DMSO), Distilled water, methanol, (Kermel limited), hydrogen peroxide, Dragendoff's Reagent, Mayer's reagent, Hager's reagent, 5% Ferric chloride, catalase reagent, Safranin red, 1% Hydrochloric acid. COLLECTION OF SAMPLES

2.3. Collection of Plant Samples

The twigs (branches) of *Garcinia kola* and stem wood of *Massularia acuminata* were obtained from a village called Echie situated in Rivers State, Nigeria. The specimens were identified in the Faculty of Agriculture, University of Port Harcourt, and Rivers state. Voucher specimen of the plants were deposited in the herbarium of the Faculty of Pharmaceutical sciences in the same university where the plants were assigned voucher numbers UPHG301 and UPHR302 respectively. Plant samples were prepared for extraction by scraping the bark, mincing it into small pieces and shade drying for about two weeks. Once drying was complete, further disintegration was achieved by grinding it in a Wiley mill. The powdered samples were kept separately in sterile, dried, screw capped bottles and stored in a cool dry place for subsequent use.

2.4. Sources of Bacterial Isolates

Bacterial isolates were obtained from mouth swabs collected from 120 undergraduate students of the University of Port Harcourt consisting of 60 females and 60 males using sterile swab sticks and these isolates were used for the study.

2.5. Preparation of Extracts

About 600g of each sample was weighed into an extraction jar and macerated with 1000ml of 80% methanol and extraction was allowed for three days with occasional agitation. The solvents were then filtered using Whatman No. 1 filter paper and the filtrate obtained was concentrated in a rotary evaporator after which it was transferred to an evaporating dish and concentrated to dryness in a water bath at 40°c. A 100mg/ml stock solution of each of the extracts was prepared by dissolving 1g of the extract in 10ml of DMSO in universal bottles. 5ml was then taken from each of the bottles to prepare the combined extracts of the same concentration after which all the bottles were properly labeled and stored in a sterile refrigerator at 4°c prior to use.

2.6. Cultivation and Preservation of Bacterial Isolates

The cultivation and preservation of bacteria isolate were done by standard method according to Koneman *et al.* [5]

2.7. Microbial Identification and Characterization

This was carried out in order to determine the true identity of the organisms through the following methods:

2.8. Morphological Identification

The morphological characteristics of the isolates were determined such as their shape (spherical, rod, shaped, comma shaped, spiral shaped, filamentous), size, arrangement and strain of the organism.

2.9. Gram Staining

The gram staining was carried out according to method by Koneman *et al.* [5]

2.10. Cultural Identification

The microorganisms isolated were identified based on how they appear on different selective media using; pigmentation, size, odour and shape of microbial colonies as they grow on agar plates.

2.11. Biochemical Tests

Biochemical tests were used in the identification of the bacteria species based on the differences in the biochemical activities of the different bacteria. The organisms from the Agar slants were streaked onto various media (Blood Agar, Macconkey Agar, Mueller Hinton Agar, Mannitol Salt Agar), incubated at 37°c for 24 hours and the slants were kept in a refrigerator. The different biochemical tests carried out on the isolates include:

2.12. Catalase Test

2.12.1. Procedure

A drop of 3% hydrogen peroxide was placed on a clean non greasy microscope slide. A flamed and cooled wire loop was used to emulsify a colony of each isolated microorganism on the drop of reagent aseptically. Presence or absence of gas bubbles was observed. Presence of gas bubbles indicates positive result and the absence of gas bubbles indicates a negative result.

2.13. Oxidase Test

2.13.1. Procedure

A colony of the organism was smeared on a piece of Whatman (No. 1) filter paper that has been previously soaked with oxidase reagent (containing 1% solution of N, N, N', N' - tetramethyl-p-phenylenediamine dihydrochloride in purified water) with the aid of clean grease-free glass slide which had been previously flamed and cooled. It was monitored and observed for the presence or absence of a colour change from violet to purple within 10-30 seconds.

2.14. Citrate Test

2.14.1. Procedure

This test was carried out according to the method by (Koneman *et al.* [5]

2.15. Indole Test

2.15.1. Procedure

Sterile Peptone water measuring 15ml volume each in a McCartney bottles was inoculated with a loopful of 24 hours Broth culture of the bacteria isolates and incubated at 44°c for 48 hours. After the incubation period 2 – 3 drops of Kovac's reagent was added to the mixture with a sterile pasteur pipette after which it was observed for formation of a red ring at the top layer which indicates a positive result.

2.16. Coagulase Test

2.16.1. Procedure

A 1 in 10 dilution of plasma was collected and 0.5ml of the aliquot was placed into two bijou bottles. A 0.5ml of 24 hours' broth culture was added into one of the bottles and both bottles were incubated at 37°C. The bottles were examined after 1 hour and after 24 hours of incubation for the occurrence of clumping.

2.17. Triple Sugar Iron Test

2.17.1. Procedure

A well isolated colony was aseptically inoculated into the sterile Triple Sugar

Iron agar slant in a test tube by first stabbing through the center of the medium to the bottom and then streaking the surface of the slant. The test tube was then incubated at 37c for 24 hours after which they were observed for results.

2.17.2. Standardization of Culture

The isolated organisms were inoculated into 0.1% peptone water with the aid of a sterile wire loop and matched with 0.5 Mc-Farland turbidity standard (1×10^{8} cfu/mL) and incubated at 37°C for 24 hours. [6]

2.17.3. Antibiotic Susceptibility Testing for the Isolates

The antibiotic tested are Ceftazidime (30ug), Cefuroxime(30ug), Gentamicin (10ug), Ciprofloxacin (5ug), Ofloxacin (5ug), Amoxicillin/clavulanate (30ug), Nitrofurantion (300ug) and Ampicillin (10ug Erythromycin, Cloxacillin, and Ceftriaxone. After incubation 0.1ml each of the standardized Gram positive cultures were inoculated into sterile, cooled molten Muller Hinton agar of 20ml in universal bottles respectively. The content of each bottle was mixed thoroughly by rotating the bottle in an anticlockwise direction on the palm. The mixtures were poured aseptically into sterile petri dishes respectively and allowed to solidify. A sterile forceps was used to place commercially prepared antibiotic discs/Rapid Labs® on the surface of the agar. The antimicrobial discs were placed on a plate at equidistance to each other according to modified Kirby-Bauer disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI). The procedure was carried out in duplicates. The plates were incubated at 37° C for 24 hours after which zones of inhibition were measured and recorded to the nearest whole millimeter. Using the interpretative chart derived from inhibitory zone diameter of standard organisms according to Cheesbrough. [7], the zone of inhibition size of each antimicrobial agent was interpreted and the isolates were reported as being "resistant", "intermediate" or "susceptible"

2.17.4. Antibacterial Assay of the Plant Extracts

Agar well diffusion method

About 0.1ml of the standardized 24 hour cultures were inoculated into sterile, cooled, molten Muller Hinton agar of 20ml volume in universal bottles. The content of each bottle was mixed thoroughly by rotating the bottle gently on the work bench. The content of each bottle was poured aseptically into sterile Petri dishes respectively and allowed to solidify. With the aid of a sterile Cork borer, seven wells of 6mm diameter were made into the previously seeded Muller hinton agar plates and using a sterile micropipette, two wells labeled 'A' were filled with 2ml of the *Massularia acuminata* extract at a concentration of 100mg/ml. Two wells labeled 'B' were also filled with the *Garcinia kola* extract after which another two wells labeled 'C' were filled with the 2ml of a combination of both extracts. The same 2ml quantity of DMSO (Dimethyl sulfoxide) served as negative control and was poured into the last well labeled 'D'. The plates were left to stand for 30 minutes at room temperature to allow the extracts and the drug diffuse across the agar. This was done in duplicates after which the plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for inhibition zones around the wells. The diameters were measured and the mean inhibition zone diameter was recorded to the nearest whole millimeter.

Determination 0f Minimum Inhibitory Concentrations of the Extracts

Using the agar well diffusion method, about 0.1ml of the standardized (1×10^{8}cfu/ml) 24 hour cultures were inoculated into sterile, cooled, molten Mueller Hinton agar of 20ml volume in universal bottles respectively. The content of each bottle was mixed thoroughly by rotating the bottle gently on the work bench. The content of each bottle was then poured aseptically into sterile Petri dishes respectively and allowed to solidify. A sterile Cork borer was used to create 14 wells of 6mm diameter equidistant from each other in each of the sterile petri plates, after which the wells were labeled. Using a sterile Pasteur pipette 2ml of various concentrations of each of the extracts; 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml respectively were used to fill their corresponding wells, 2ml of Dimethyl sulfoxide was introduced into one of the wells as Negative control while equal volume of Streptomycin(5mg/ml) prepared by dissolving 50mg Streptomycin tablet in 10ml of Sterile distilled water was used as positive control in another well. Tests were performed in duplicates. The plates were left to stand for 30 minutes at room temperature to allow the extracts and the drug to diffuse into the agar. This was done in duplicates for each of the extracts, after which the plates were incubated at 37°C for 24hours. After incubation, the plates were observed for inhibition zones around the wells and the lowest dilution that inhibited growth of the organism was taken as the Minimum Inhibitory concentration. The diameters were measured and the mean inhibition zone diameter was recorded to the nearest whole millimeter.

2.18. Phytochemical Screening

This was carried out by the method described by sofowora, [8], Ajayi *et al*. [9] and Edeoga, *et al.* [10]. The phytochemicals screened are: Alkaloids, flavonoids, Phlobatannins, Anthraquinones, Cardiac glycosides, saponins and Tannins.

3. Results

3.1. Bacteria Isolation

The result of the isolation of microorganisms from the oral cavities of 60 males and 60 females in which microbial isolates recovered were 70 in number is shown in the table one below.

Table 1 Isolation of Isolates

Organism	Number of isolates	Occurrence (%)
Staphylococcus aureus	27	38.6%
Streptococcus spp.	34	48.6%
Klebsiella pneumonia	2	2.9%
Escherichia coli	4	5.7%
Pseudomonas aeruginosa	3	4.2%
Total number of isolates	70	100

Examination of the occurrence of the Isolates as shown on table 1, showed that *Streptococcus spp*. has the highest occurrence of 48.6%, while *Klebsiella pneumonia* has the lowest occurrence of 2.9%.

3.2. Result for Phytochemical Screening

The result for the phytochemical screening of the methanol extracts of *Massularia acuminata* stem include Alkaloids, Tannins, Flavonoids, Carbohydrates, Saponins, Phlobatanins and Cardiac Glycosides with the absence of Anthraquinones while *Garcinia kola* methanol extracts showed the presence of alkaloids, tannins, flavonoids, anthraquinones, phlobatanins, carbohydrates and cardiac glycosides with the absence of saponins.

3.3. Result of Biochemical Characterization

The results of biochemical Identification tests and morphological characterization carried out on the isolates is presented in table two below.

Table 2 Gram Staining and Biochemical Test Results

Test Organism	Gram Staining	Catalase Test	Coagulase Test	Indole Test	Oxidase Test	Citrate Test	Triple Sugar Iron Test	Urease Test
Escherichia coli	Negative rod	-	-	+	-	-	Slope=Yellow Butt= Yellow Gas = + H2S = -	-
Klebsiella pneumonia	Negative rod	-	-	-	-	+	Slope=Yellow Butt =Yellow Gas = + H2S = +	+
Staphylococcus aureus	Positive cocci	+	+	-	-	-	Slope= Red Butt= Red Gas = -	-

							H2S = -	
Streptococcus spp.	Positive cocci	-	-	-	-	-	Slope=Yellow Butt=Yellow Gas = - H2S = -	-
Pseudomonas aeruginosa	Negative rods	-	-	-	+	-	Slope=Yellow Butt =Yellow Gas = - H2S = -	-

Key: (+) = Present, (-) = Absent

3.4. Result of the Antibacterial activities of *Garcinia kola* and *Massularia acuminata* and their activity in combination

Table 3 Antibacterial Activities of methanol extracts of *Massularia acuminata* and *Garcinia kola* and combination of both plant extracts at various concentrations against the test bacteria

Mean Inhibitory Zone Diameter (mm)				
Test organisms	**Concentrations of extract (mg/ml**	**Garcina kola**	**Massularia acuminate**	**Combination**
Staphylococcus aureus	100	24	9	12
	50	22	6.5	10
	25	14	4	4
	12.5	10	3	2.5
	6.25	Nil	Nil	Nil
	3.125	Nil	Nil	Nil
Escherichia coli	100	6	4	3
	50	5	3	2
	25	4	2	1
	12.5	3	Nil	Nil
	6.25	Nil	Nil	Nil
	3.125	Nil	Nil	Nil
Streptococcus spp	100	Nil	Nil	Nil
	50	Nil	Nil	Nil
	25	Nil	Nil	Nil
	12.5	Nil	Nil	Nil
	6.5	Nil	Nil	Nil
	3.125	Nil	Nil	Nil
Klebsiella pneumonia	100	Nil	Nil	Nil
	50	Nil	Nil	Nil
	25	Nil	Nil	Nil
	12.5	Nil	Nil	Nil
	6.25	Nil	Nil	Nil

	3.125	Nil	Nil	Nil
Pseudomonas aeruginosa	100	Nil	Nil	Nil
	50	Nil	Nil	Nil
	25	Nil	Nil	Nil
	12.5	Nil	Nil	Nil
	6.25	Nil	Nil	Nil
	3.125	Nil	Nil	Nil

Examination of the results from table 3 showed that the extracts of both plant are active against *Staphylococcus aureus* and *Escherichia coli* but not active or zero activities against *Streptococcus spp*., *Klebsiella pneumonia* and *Psudomonas aeruginosa.*

Table 4 Showing the results for minimum inhibitory concentrations (MIC) of methanol Extracts of *Massularia acuminata, Garcinia kola* and combination of both

Minimum inhibitory concentration (MIC) (mg/ml)			
Test Organism	**Garcina kola**	**Massularia acuminate**	**Combination**
Staphylococcus aureus	12.5	12.5	25
Escherichia coli	12.5	25	25
Streptococcus spp	Nil	Nil	Nil
Klebsiella pneumonia	Nil	Nil	Nil
Pseudomonas aeruginosa	Nil	Nil	Nil

From table 4, *Garcina kola* has the least minimum inhibitory concentration 12.5mg/ml for *E¸coli* and the same MIC of 12.5mg/ml with *Massularia acuminate*, while the combined extracts gave the highest minimum inhibitory concentrations of 25mg/ml for both the two sensitive bacteria.

Table 5 Comparison of Antibacterial activities of Standard Antibiotics to that of methanolic extracts of *Massularia acuminata* and *Garcinia kola* as well as their combination against the test organisms

Mean Inhibitory Zone Diameter (mm)						
Organisms	**Standard Antibiotics**	**Mean inhibitory zone diameter (mm)**	**Concentrations of extracts (mg/ml)**	***Garcinia kola***	***Massularia acuminata***	**Combination**
Escherichia coli	Ceftazidime	Nil	100	6	4	3
	Cefuroxime	10	50	5	3	2
	Gentamicin	13	25	4	2	1
	Ciprofloxacin	10	12.5	3	Nil	Nil
	Ofloxacin	11	6.25	Nil	Nil	Nil
	Augmentin	20	3.125	Nil	Nil	Nil
	Nitrofurantoin	16				
	Ampicillin	Nil				
Staphylococcus aureus	Erythromycin	16	100	24	9	12

	Cloxacillin	Nil	50	22	7	10
	Ofloxacin	16	25	14	4	4
	Amoxicillin/Clavulanate	11	12.5	10	3	Nil
	Ceftazidime	12	6.25	Nil	Nil	Nil
	Cefuroxime	Nil	3.125	Nil	Nil	Nil
	Gentamicin	16				
	Ceftriaxone	16				
Klebsiella pneumoniae	Ceftazidime	20	100	Nil	Nil	Nil
	Cefuroxime	16	50	Nil	Nil	Nil
	Gentamicin	18	25	Nil	Nil	Nil
	Ciprofloxacin	19	12.5	Nil	Nil	Nil
	Ofloxacin	12	6.25	Nil	Nil	Nil
	Augmentin	17	3.125	Nil	Nil	Nil
	Nitrofurantoin	20				
	Ampicillin	17				
Pseudomonas aeruginosa	Ceftazidime	Nil	100	Nil	Nil	Nil
	Cefuroxime	10	50	Nil	Nil	Nil
	Gentamicin	13	25	Nil	Nil	Nil
	Ciprofloxacin	20	12.5	Nil	Nil	Nil
	Ofloxacin	Nil	6.25	Nil	Nil	Nil
	Augmentin	Nil	3.125	Nil	Nil	Nil
	Nitrofurantoin	Nil				
	Ampicillin	Nil				
Streptococcus spp	Erythromycin	20	100	Nil	Nil	Nil
	Cloxacillin	14	50	Nil	Nil	Nil
	Ofloxacin	15	25	Nil	Nil	Nil
	Amoxicillin/Clavulanate	20	12.5	Nil	Nil	Nil
	Ceftazidime	Nil	6.25	Nil	Nil	Nil
	Cefuroxime	10	3.125	Nil	Nil	Nil
	Gentamicin	18				
	Ceftriaxone	17				

Key: Nil= No Activity

In table 5, comparison with standard antibiotic showed that Garcina kola is more active with inhibition zone diameter of 24mm on *Staphylococcus aureus* than the standard antibiotic (Erythromycin, Gentamicin, and Ceftriaxone) that are more sensitive to the bacteria among the sensitive antibiotics. However, the standard antibiotics are more active against the *E. coli,* than the two individual plant extracts and their combination.

4. Discussion

4.1. Bacterial Isolation

From table 1 of the bacteria isolates obtained, it was not in line with the results of the study reported by Hassan *et al* [11], who isolated about 45.6% *Streptococcus spp*. 13.2% *Staphylococcus aureus*, *Pseudomonas aeruginosa* 6% and

Klebsiella pneumoniae 20.0%, *Lactobacillus spp.* 15.2% from 80 samples collected from the Dental Clinic of Obafemi Awolowo University Teaching Hospital complex. Similarly, Kolapo *et al*. [12] isolated about 38% *Streptococcus spp.,* 13% Candida albicans and 12% Lactobacillus spp. as the predominant species in plaque of 20 students at Ile – Ife, except that in this work, *Lactobacillus spp.* was not isolated. However, odontho pathogens include hundreds of microorganisms of which *Streptococcus spp.* is by far the most dominant as shown by these studies.

4.2. Phytochemical Screening

The result of phytochemical Screening of two medicinal plants; *Massularia acuminata* and *Garcinia kola*, conforms with the study reported by Dowe *et al*, [13] and Florence *et al* al, [14], except that she reported absence of Alkaloids and presence of Anthraquinones in ethanolic extracts of *Massularia acuminata*. This variance in phytochemical constituents could be attributed to the difference in the solvents used. It could also be due to seasonal variations and stage of maturity of the plant as proposed by Buenz *et al* [15].

The antimicrobial effects of these plant extracts could be attributed to the presence of some of these secondary metabolites [8]. These phytochemical compounds exert antimicrobial activities through various mechanisms [16].

4.3. Antibacterial activity

The evaluation of the antibacterial activities of methanolic extracts of *Massularia acuminata* and Garcinia kola against the test organisms were carried out and the activities of the plant extracts varied with the test organisms. It was observed that the methanolic extracts of *Massularia acuminata* showed antibacterial activity against *Staphylococcus aureus* at a minimum inhibitory concentration of 12.5mg/ml with a mean inhibitory zone diameter of 3mm, while *Garcinia kola* showed antibacterial activity against *Staphylococcus aureus* at a minimum inhibitory concentration of 12.5mg /ml with a mean inhibitory zone diameter of 10mm. The combination of both plant extracts showed antibacterial activity against *Staphylococcus aureus* with a minimum inhibitory concentration of 25mg/ml and a mean inhibitory zone diameter of 4mm.

Furthermore, *Massularia acuminata* showed antibacterial activity against *Escherichia coli* with a minimum inhibitory concentration of 25mg/ml with a mean inhibitory zone diameter of 2mm, while *Garcinia kola* showed antibacterial activity against *Escherichia coli* with a minimum inhibitory concentration of 12.5mg/ml and a mean inhibitory zone diameter of 3mm. The combination of both plant extracts showed antibacterial activity against *Escherichia coli* with a minimum inhibitory concentration of 25mg/ml and a mean inhibitory zone diameter of 1mm. It was also observed that *Streptococcus spp., Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were all resistant to both extracts and their combination. This was indicated by the absence of visible zones of inhibition at all the concentrations used. This was however, not in line with the results obtained by Dowe *et al* [13], who reported a minimum inhibitory concentration of 50mg/ml when concentrations of 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml respectively of *Massularia acuminata* were used against *Staphylococcus aureus.* Another report by Ndukwe *et al*. [17] showed that aqueous extracts of *Massularia acuminata* was inactive against *Staphylococcus aureus, Streptococcus spp.* And *Escherichia coli* at concentrations of 40mg/ml, 20mg/ml, 10mg/ml, 5mg/ml and 2.5 mg/ml respectively while *Garcinia kola* showed antibacterial activity against *Escherichia coli* with a minimum inhibitory concentration of 10mg/ml, *Staphylococcus aureus* and *Escherichia coli* with a minimum inhibitory concentration of 2.5mg/ml each.

It was observed in this research work that the antibacterial effect of the plant extracts in combination was an antagonistic effect on *Staphylococcus aureus* and *Escherichia coli.* This was observed from the mean inhibitory zone diameter of the combined plant extracts which was 1mm at a minimum inhibitory concentration of 25mg/ml when used against *Escherichia coli* while *Garcinia kola* and *Massularia acuminata* gave mean inhibitory zone diameters of 4mm and 2mm respectively at the same concentration against *Escherichia coli.* The combination of both plant extracts also showed antibacterial activity against *Staphylococcus aureus* with a mean inhibitory zone diameter of 4mm at a minimum inhibitory concentration of 25mg/ml while *Garcinia kola* and *Massularia acuminata* showed mean inhibitory zone diameters of 14mm and 4mm respectively at the same concentration against *Staphylococcus aureus.* An antagonistic effect implies that the effect of the two plants in combination is less than the effect of the most effective component of the combination; AB < A (AB is less than A), where A is the most active individual plant extract. There was however no antibacterial effect of the plant extracts in combination against *Streptococcus spp., Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at all the concentrations used in this research.

4.4. Comparison of Antibacterial activities of aqueous methanol extracts of *Massularia acuminata* and *Garcinia kola* with that of Standard antibiotics

Comparing the antibacterial activities of methanol extracts of *Massularia acuminata* and *Garcinia kola, Garcinia kola* plant extract showed a better antibacterial effect against *Staphylococcus aureus* with mean inhibitory zone diameters of 24mm and 22mm at 100mg/ml and 50mg/ml concentrations respectively while the Standard Antibiotics Erythromycin, Ofloxacin, Gentamicin and Ceftriaxone all gave a mean inhibitory zone diameter of 16mm each when tested against the same organism. When tested against standardized culture of *Escherichia coli, Garcinia kola* showed less antibacterial activity with mean inhibitory zone diameter ranging from 6mm to 3mm at concentrations ranging from 100mg/ml to 12.5mg/ml respectively compared to that of the Standard antibiotics; Augmentin, Nitrofurantoin and Gentamicin which gave mean inhibitory zone diameters of 20mm,16mm and 13mm respectively when tested against the same organism. *Escherichia coli* was however resistant to Ampicillin and Ceftazidime. The *Massularia acuminata* plant extract when tested against *Staphylococcus aureus* the mean inhibitory zone diameters observed at concentrations ranging from 100mg/ml to 12.5mg/ml were 9mm, 7mm, 4mm and 3mm respectively which was less compared with that of Standard antibiotics; Erythromycin, Ofloxacin, Gentamicin and Ceftriaxone which all showed mean inhibitory zone diameters of 16mm respectively. This was also the case with standardized culture of *Escherichia coli,* the antibacterial effect of *Massularia acuminata* on the test organism was negligible showing mean inhibitory zone diameters of 4mm, 3mm and 2mm at concentrations of 100mg/ml, 50mg/ml and 25mg/ml respectively compared to that of the Standard antibiotics; Nitrofurantoin, Augmentin and Gentamicin which gave mean inhibitory zone diameters of 16mm, 20mm and 13mm respectively when tested against the same organism thereby showing better antibacterial activity than the plant extract. *Escherichia coli* was however resistant to Ampicillin and Ceftazidime. The combination of methanol extracts of *Massularia acuminata* and *Garcinia kola* showed antibacterial activity against *Escherichia coli* with mean inhibitory zone diameters of 3mm, 2mm and 1mm respectively at concentrations ranging from 100mg/ml to 25mg/ml respectively which was less than the antibacterial activities of the Standard antibiotics which gave mean inhibitory zone diameters ranging from 10mm to 20mm. When the combined plant extracts were tested against *Staphylococcus aureus* appreciable antibacterial effect was observed with mean inhibitory zone diameters of 12mm, 10mm and 4mm at concentrations ranging from 100mg/ml to 25mg/ml compared with that of Standard Antibiotics Erythromycin, Ofloxacin, Gentamicin and Ceftriaxone which all showed a mean inhibitory zone diameter of 16mm when tested against the same organism. *Pseudomonas aeruginosa* was resistant to both of the plant extracts at all concentrations while it was sensitive to Ciprofloxacin, Gentamicin and Cefuroxime with mean inhibitory zone diameters of 20mm, 13mm and 10mm respectively. Standardized culture of *Klebsiella pneumoniae* was also resistant to both plant extracts at all concentrations used while it was sensitive to Ciprofloxacin, Gentamicin, Nitrofurantoin, Ampicillin and Ceftazidime with mean inhibitory zone diameters ranging from 16mm to 20mm respectively. The susceptibility of the test organisms to the plant extracts especially those which were resistant to the Standard antibiotics goes further to prove that medicinal plants have potential as alternative sources of antimicrobial agents in an age of increasing antibiotic resistant strains of microorganisms. The variation of the test organisms to the plant extracts in this study compared with other studies was not unexpected considering differences in strain specificity, Time of harvesting, method of storage, temperature, moisture content and drying method, extraction technique, method and conduction of antimicrobial testing are factors that could play a major role in the results obtained in the research [18]. Analysis of Variance (ANOVA) was carried out to determine if differences exist between the extracts at different concentrations. The values were statistically significant at $p < 0.05$ for antibacterial activity of *G. kola*, *M. acuminata* and the combined extracts against *Staphylococcus aureus.* The values obtained were significant for *G. kola* ($p < 0.05$) and insignificant for *M.acuminata* and the combined extracts against *Escherichia coli* ($p > 0.05$).

5. Conclusion

From the results obtained in this work it can be deduced that methanol extracts of *Massularia acuminata* and *Garcinia kola* have antibacterial effects on *Staphylococcus aureus* and *Escherichia coli,* although *Garcinia kola* showed better antibacterial activity against the Gram negative organism. Thus, the Nigerian Chewing Sticks; *Garcinia kola* and *Massularia acuminata* plant extracts may be incorporated into modern day oral hygiene products for the maintenance of oral health, they may however not be used in combination due to antagonistic effects as observed in this study.

Compliance with ethical standards

Acknowledgments

We acknowledged the support the head Lab Technologist, Mrs. Kaleb.

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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