Quest Journals
Journal of Research in Pharmaceutical Science
Volume 11 ~ Issue 4 (Jul.- Aug.2025) pp: 22-27
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

Evaluation of the Antibacterial activity of a Nigerian Chewing Stick*(Lantana Camara)*linn Ethanolic Extract Against Multi-Drug-Resistant Oral Bacteria

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ABSTRACT: *The detrimental effects of oral diseases on human health, as well as the emergence of multi drug resistant (MDR) bacteria in oral and dental health care are issues of great concern. Hence, this study aims to evaluatethe antimicrobial effects of a Nigerian chewing stick (Lantana camara) extract against multi-drug-resistant oral bacteria and their prospect for use in formulating oral healthcare products. Bacteria were obtained from the Department of Pharmaceutical Microbiology and Biotechnology laboratory of the Nnamdi Azikiwe University Awka, Anambra State Nigeria. Their antibiogram determined using the Kirby Bauer disc method using standard gram-positive antibiotic sensitivity discs. Plant materialwas extracted by maceration with 70% ethanol. The bioactive components were identified using Gas chromatography mass spectrometry (GCMS). Antimicrobial activity of the extracts was assessed using agar well diffusion method. The organisms were all identified to be MDRs. Phytochemical analysis using GCMS, revealed that the Lantana camara extract contained twelve major bioactive compounds including; 2-Furancaboxaldehyde, Apha-Tepheneol, 2-methoxy-4-vinylphenol, 1-cylopro azulen-7-ol, Neophyticiene, Hexadecanoic acid. Others are Phytol, 9-octadecenoic acid, Cis-Vaccenic acid, Oleic acid,Beta.amyrin and 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)]-. Results showed that the extract at MIC of 25mg/ml had an Inhibition zone diameter (IZD) of 10.5mm against M.sciuri and 13.0mm against E. caseliflavus. On the other hand, at MIC of 50mg/ml, it had an IZD of 15.0mm against E.galinarum and 12.5mm against G. creatinolyticus. The study showed that ethanolic extract of L.camaratwigs is capable of inhibiting the growth and proliferation of some multidrug resistant oral bacteria and can be developed and incorporated into oral hygiene products.*

Keywords*: Oral bacteria, dental health, antimicrobial, Chewing-stick, Phytochemical, MDR.*

R*eceived 09 July, 2025;* ***R****evised 21 July, 2025;* ***A****ccepted 23 July, 2025 © The author(s) 2025.*
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I. INTRODUCTION

Plants are a major source of medicines as they have been used throughout human history. (Abdallah*et. al.,* [1]). So many countries from the developing world are still dependent on medicinal plants for treating the sick amongst them (Popoola, [2]). The world health organization (WHO) has defined medicinal plants as plants whose part or whole contains substances that are being used as therapeutic or are precursor for the synthesis of useful drugs, (Agbulu*et al* [3]). Traditional medicine practice is an important part of health care delivery system in most parts of the developing world. (Oyebode, *et al,* [4]), and is a source of primary health care to 80% of the world's population (Maylin Meincke, [5]),(Chali *et al*, [6]). According to the World Health Organization WHO, up to 80% of the population in Africa depends on traditional herbal medicine for primary health care, accounting for about 20% of the overall drug market (Okaiyeto and Oguntibeju, [7]: Mbali, *et. al*, [8], Amaeze*et. al,* [9]). Many traditional healing herbs and their parts have been shown to have medicinal value and can be used to prevent, alleviate or cure human diseases including oral infections (Tahir, *et. al.*, [10])

Oral hygiene is the practice of keeping the mouth clean and healthy by brushing or flossing to prevent tooth decay and gum diseases. (Agbulu*et. al*., [3]). Oral hygiene can also be defined as the general mouth cleanliness. There are various methods of cleaning to make it hygienic (Fagbule, *et. al;* [11] Okoroafor, *et. al*, [12]; Osuh, *et. al*, [13]). The two methods employed by Nigerians to remove debris and biofilm from the mouth and tooth surface are by tooth brush and paste or by the use of parts of various plants native to West Africa referred to as "African chewing sticks" otherwise known as Traditional tooth brushes, (Umoh, *et. al*, [14]).

Since the discovery of multi drug resistance in bacteria, the efficacy of some common antimicrobials is failing but some medicinal plants can offer the alternatives since these plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoidsetc.,some of which have been found to have antimicrobial properties. (Shittu *et al*, [15]). There is the need to discover medicinal plants with newer and effective antimicrobial agents/compounds with novel mechanism of action, increased therapeutic and prophylactic activities that would combat the burden of antimicrobial resistance which are cheap and readily abundant as possible sources of antimicrobial agents, (Shittu *et al*, [15]). Medicinal plants are known to contain several different phytochemicals (i.e. secondary metabolites) that may act individually, additively or in synergy to improve human health. (Mohammad et*. al.*, [16]; Srinivasahan& Durairaj, [17]).

***Lantana camara* Linn**

Lantana camara belongs to the family Verbanaceae described by Linnaeus in 1753 to contain seven species with six from South Africa and one from Ethiopia. Lantana is derived from the Latin word Lento which means to bend gotten from the ancient Latin name of the genius Viburnum lantana and is in most cases known to be native to tropical and subtropical America, though a few taxa are indigenous to Africa and tropical Asia. Lantanais a genus of about 150 species.*Lantana camara* commonly referred to as red or wild sage and is the most widely spread species of this genus (Aamir, [18]).

The aim of this study is to evaluate the antimicrobial activity of *Lantana camara*ethanolic stem extract against multidrug resistant oral bacteria

II. MATERIALS & METHODS

2.1Instruments and Materials Used for the Study

The instruments and equipment used in course of this research study, include: Incubator Model gp/50/ CLAD/250/HYDChina, Micro pipettes (Thermo Scientific, USA), Syringes (BD Bioscience), Refridgerator (Nexus, China), Brookfield Viscousity.USA), Electronic weighing balance (Ohaus Corp., USA) Equitron Partially Automatic Autoclave (Msedica Instrument Manufacturing CO., India), Light Microscope (Nikon Esol, Japan), pH meter (PHS-25 Helmreasinn), China.

2.2 Culture Media and Reagents

All culture media used for the work were prepared in accordance to the instructions given by the manufacturers. Culture media used were Nutrient broth (Oxoid, Uk), Nutrient Agar (Oxoid, Uk), Blood Agar (Oxoid, Uk), Mueller Hinton broth (Oxoid, Uk), Mueller Hinton Agar (Oxoid, Uk), Dimethyl Sulfoxide (Sigma – Aldrich Co., Germany), Ethanol (BDH Chemicals Ltd, England), Sheepblood (Hidden impact diagnostics, Abuja), hydrogen peroxide (BDH Chemicals Ltd, England).

2.3 Antibiotics Used

In vitro diagnostics (IVD), gram positive antibiotic dics (Celtech Diagnostics, Belgium Code BD R002), were used. This includes Imipenem Cilastatin (IMP, 10µg), Cefuroxime (CXM 30µg), Erythromycin (ERY, 15µg), Gentamycin (GN, 10µg), Azithromycin (AZN, 15µg), Amoxacillin Clavulanate (AUG, 30µg), Cefotaxime (CTX, 25µg), Ceftriaxone Subactam (CRO, 45µg), Cefexime (ZEM, 5µg), Levofloxacine (LBC, 5µg) and Ciprofloxacin (CIP, 5µg).

2.4 Source of Bacterial Isolates:

Oral bacteria were obtained from the deparrttment of PharrmaceuticalMicrrroiolggy and Biotechnologgylaborrattory, Nnamdi Azikiwe University from a previous study carried out on patients attending dental clinics in Awka.

2.5 Isolation Cultivation and Preservation of Bacterial isolates

Bacterial isolates were aseptically inoculated into sterile media such as Nutrient broth and sub cultured by streaking onto the surface of sterile Blood agar medium, thereafter the well labeled petri dishes were incubated at 37ºC for 24 hours. Pure isolates of the bacteria were obtained by sub culturing into fresh medium to obtain a pure culture. The pure cultures obtained were then sub cultured into agar slants labeled and stored in the refrigerator at 4ºC for further use.

2.6 Collection and of plant samples.

Twigs of the plans used for the study which is, *Lantana camara* was obtained from locals in Awka metropolis, Anambra State .The plant is known to be used as chewing-sticks and have good tooth cleaning as well as analgesic effects according to those in the study community .Plant identification and phytochemical screening was carried out at the department of Pharmacognosy and Phytotherapy, Unizik by Dr. InnocentMary and the voucher number PCG/474/V/029, was assigned to the plant..

2.7 Preparation of Plant Samples

This was done by cutting them into small pieces and shade drying for two weeks. The dried samples were powdered by grinding in a Wiley mill. The powdered samples were then stored in sterile screw capped bottles for subsequent use. Extraction of the plants was carried out using the method described by Harborne [19] with slight modification. One hundred grams (100g) of the powdered plant material was suspended in 500ml of an aqueous-ethanol solvent by cold maceration for 72hrs, with fresh replacement of solvent at 24hrs interval. This was done to ensure exhaustive extraction. During the extraction, the extraction jar was agitated intermittently with the use of an orbital shaker. The extract solution, was filtered using a Whatmann No.1 filter paper. The resultant filtrate was concentrated in a Rotary evaporator at 50^{o}C and the concentrated extract was further dried over a water bath at 50^{o}C. The dried extract was kept in a refrigerator prior to use.

2.8Minimum Inhibitory Concentration (MIC)

Using the agar well diffusion method, 0.1 ml standardized 24-hour old culture of the bacteria were seeded into 20 ml of sterile molten Mueller Hinton agar into Mac Cartney bottles. The content of each bottle was then mixed thoroughly by shaking/rotating gently. The content of each bottle was then aseptically poured into sterile petri-dishes and allowed to solidify. Wells were then made using sterile cork borer (5mm). The resulting wells were then inoculated with 2 ml of the various concentrations of each of the test plant extract, e.g 100 mg/ml, 50mg/ml, 25 mg/ml, 12.5 mg/ml etc, finally, 2 ml of Listerine to serve as positive control and 2 ml DMSO to serve as negative control. These were done in triplicates and incubated at 37^{o}C for 24 hours. At the end of incubation, the plates were observed for inhibition zones around the wells and the lowest dilution that inhibits the growth of the bacteria was then taken as the minimum inhibitory concentration. The diameter was measured and the mean inhibition zone diameter was taken and recorded to the whole millimeter (mm).

2.9Standardization of culture

The pure isolates obtained were inoculated into 0.1% peptone water using a sterile wire loop and the inoculums size adjusted to 0.5 Mac farland's standard measuring 1×10^{8} cfu/ml.

Identification of Isolates

The isolated pathogens were identified and screened using basic standard microbiological techniques eg Gram stain, biochemical tests using etc. in the department off Pharmaceutical Microbiology and Biotechnology Nnamdi Azikiwe University laboratory, Awka. Molecular studies such as gene sequencing, 16S rDNA sequencing, polymerase chain reaction (PCR) was be carried out at the Iqaba Biotech West Africa Ltd Ibadan Nigeria.

2.10Antibiotic Sensitivity Tests

After incubating pure culture isolates, 0.1ml of standardized cultures were inoculated into sterile cooled molten Mueller Hinton agar of 20ml in Mac cartney bottles respectively. The content of each bottle was thoroughly mixed and aseptically poured into sterile petri dishes and allowed to solidify. A sterile forcep was used to place standard antibiotic (Gram positive) discs on the surface of the agar. The discs were gently pressed down for proper adherence to the agar. The antibiotic discs used were Imipenem (IMP, 10µg), Cefuroxime (CXM, 30µg), Erythromycin (ERY, 15µg), Gentamycin (GN, 10µg), Azithromycin (AZN, 15µg), Augmentin (AUG, 30µg), Cefotaxime (CTX,25µg), Ceftriaxone (CRO, 45µg), Cefexime (ZEM, 5µg), Levofloxacin (LBC, 5µg), and Ciprofloxacin (CIP. 5µg), were placed on the agar plates equidistant to each other according to modified Kirby Bauer disc diffusion method as recommended by Clinical laboratory Standards Institute (CLSI). This procedure was carried out in duplicates. The plates were incubated at 37^{o}C for 24 hours. Thereafter, inhibition zone diameter (IZD) was measured in millimeter with a transparent ruler and the mean inhibition zone diameter was recorded to the nearest whole millimeter. Using the interpretative chart derived from inhibitory zone diameter of standard organisms according to Cheesbrough [20]), the IZD of each antibiotic disc against the isolates was interpreted as being "Resistant", "Intermediate" or "Susceptible". Only resistant strains were used for further studies.

2.11Molecular Analysis.

It is a well-known fact, that some bacterial isolates cannot be Taxonomically identified from only their Phenotypic characteristics. Hence, bacterial isolates are characterized by sequencing their 16S rDNA. The universal primers 27F and 1492R are used to amplify the 26S target region. (Lane *et.al*., [21])

Genomic DNA was extracted from the multidrug resistant bacterial cultures used for the study. This was achieved using the Quick-DNA Faryol/Bacteria Miniprep Kit.(Zymo Research, catalogue No. D6005. The 16S target region was amplified, using the One-Taq Quick-Load2X Master Mix (NEB, catalogue No. M0486). These primers have been presented in the table below. The PCR products were run on a gel and enzymatically cleaned up using the EXOSAP method. The extracted fragments were sequenced in the forward and reverse directions (Nimagen Brilliant dye, Terminator cycle sequencing kit V3.1, BRD 3-100/1000) and purified with Zymo research, ZR-96 DNA sequencing clean up kit, catalogue No. D4050. The purified fragments were analysed from the ABI 3500xl Genetic analyser. (Amplified Biosystems, Thermo Fisher Scientific) for each reaction for every sample as listed below.

Bioedit sequence alignment Editor version 7.2.5 was used to analyse the ab1 files generated by the ABI 3500xl genetic analyser and results were obtained by a BLAST search. (NCBI) as described by Atschul*et. al*., [22].

III. RESULTS

3.1 Table 1: Showing the percentage occurrence of isolated Multidrug Resistant Strains (MDS)

Name of organism	Percentage occurrence (%)
Enterococcus caseliflavus	30.8
Enterococcus galinarum	7.7
Glutamicibactercreatinolyticus	15.4
Mammalicoccussciuri	46.2

Table 1 above showed the percentage occurrence of the isolates that are multidrug resistant strains. From the *Mammalicoccussciuri* has the highest occurrence of 46.2 percent while *Enterococcus galinarum* got the lowest occurrence of 7.7 percent.

3.2 Table 2: Showing oral bacterial isolates resistant to drugs of different classes of antibiotics (Multi Drug Resistant Bacteria)

S/N	Isolates	CXN	GN	CTX	CRO	ZEM	LBC	CIP	IMP	OFX	ERY	AZN	AUG
1	***Mammalicoccussciuri***	+	+	+			+	+	_	+	+	+	+
2	***Enterococcus caseliflavus***	+	+	+	+	+		+	+	+	+	+	_
3	***Enterococcus caseliflavus***	_	+	+	+	+	+	+		+	+	+	+
4	***Enterococcus caseliflavus***	_	+	+	+	+	+	+	_	+	+	+	+
5	***Mammalicoccussciuri***	+	+	_	+	_	+	_	_	+	+	_	_
6	***Enterococcus caseliflavus***	_	+	_	+	_	+	+	_	+	_	_	_
7	***Mammalicoccussciuri***	+	+	+		+	+	+	_	+	+	_	+
8	***Mammalicoccussciuri***	_	+	+	+	_	+	+	_	+	_	_	_
9	***Enterococcus galinarum***	_	+		+	_	+	+	_	+	_	+	+
10	***Mammalicoccussciuri***	_	+	_	+	_	+	+	+	+	+	_	_
11	***Mammalicoccussciuri***	+	+	_	+	_	+	+	_	+	_	+	_
12	***Glutamicibactercreatinolyticu***	+	+	_	+	_	+	+	_	+	_	+	_
13	***Glutamicibactercreatinolyticu***	+	+	_	+	_	+	_	_	+	+	+	_

Table 2 above showed the bacteria isolates and the antibiotics to which they are resistant to and as well as those they are sensitive to. The antibiotics are from different classes of antibiotics. Only gentamycin and ofloxacin were active to all the bacterial isolates.

Key: CXN: Cefuroxi1ne; GN: Gentamicin; CTX: Cefotaxime; CRO: Ceftriaxone; ZEM: Cefoxitin; LBC: Levofloxacin; CIP: Ciprofloxacin; IMP: hni12ene1n; OFX: Ofloxacin; ERY: Erythromycin; AZN: Azithro1nycin; AUG: Augmentin; (-) = Resistant; (+) = sensitive

3.3 Table 3: the antibiotics used in the study and their classes

Code	Antibiotic	Class of antibiotic
CXN	Cefuroxime	2nd Cephalosporin
GN	Gentamycin	Aminoglycosides
CTX	Cefotaxim	3rd Cephalosporin
CRO	Ceftriaxone	3rd Cephalosporin
ZEM	Cefixime	3rd Cephalosporin
LBC	Levofloxacin	Fluoroquinolone

CIP	Ciprofloxacin	Fluoroquinolone
IMP	Imipenem	Carbapenem
OFX	Ofloxacin	Fluoroquinolone
ERY	Erythromycin	Macrolides
AZN	Azithromycin	Macrolides
AUG	Augmentin	B-lactam (Penicillin)

Table 3 above showed the different antibiotics tested with the class of antibiotics the belonged to

3.4 Table 4: Showing the IZD at different MIC of *Lantana camara* extract against the MDR isolates (the antibacterial activity of the extract on the bacterial isolates)

		Different Minimum inhibitory concentrations (MIC)				
		100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
S/N	Isolate	IZD (mm)	IZD (mm)	IZD (mm)	IZD (mm)	IZD (mm)
1	*M. sciuri*	21.5±0.5	11.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0
2	*E. caseliflavus*	23.0±1.0	11.0±1.0	10.5±0.5	0.0±0.0	0.0±0.0
3	*E. caseliflavus*	24.5±0.5	15.5±0.5	13.0±0.0	0.0±0.0	0.0±0.0
4	*E. caseliflavus*	22.0±0.0	11.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0
5	*M. sciuri*	12.5±0.5	11.5±1.0	10.0±0.0	0.0±0.0	0.0±0.0
6	*E. caseliflavus*	20.5±0.5	14.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0
7	*M. sciuri*	20.0±0.0	12.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
8	*E. galinarum*	19.0±1.0	13.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
9	*M. sciuri*	15.5±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
10	*M. sciuri*	20.5±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
11	*M. sciuri*	13.5±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
12	*G creatinolyticus*	15.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
13	*G. creatinolyticus*	21.0±1.0	12.5±0.5	0.0±0.0	0.0±0.0	0.0±0.0

From the result in table 4 above, at MIC of 50mg/ml all the organisms were inhibited by the plant extract with highest inhibition against *E. caseliflavus* with IZD of 24.5mm. however at MIC of 25mg/ml, the extract was active against isolates 2, 3 and 5 as shown above and was not active against all the other isolates. At MIC of 12.5mg/ml and 6.25mg/ml there were no inhibition produce by the extract on the growth of any of the isolates

3.5 Table 5: compounds characterized from*L. Camara*with quality factor ≥ 90%

S/N	RT mins	Compound Name	MW	Molecular Formula	% Conc.	Quality Factor (%)	Biological Activity	References
1	3.319	2-Furancarboxaldehyde, 5-methyl-	110	C6H6O2	1.249	91	anti-inflammatory, Antioxidant Anti-sickling	Osheiza*et al*., 2005 [23]
2	4.645	α-Terpineol	154	C10H18O	0.886	93	antioxidant, anti-inflammatory, antimicrobial, anticancer, analgesic, gastroprotective, cardioprotective, neuroprotective, and antidiarrheal.	Gulzar and Mohammad, 2023 [24]
3	6.308	2-Methoxy-4-vinylphenol	150	C9H10O2	0.736	93	Antimicrobial, Antibacterial, Anti-inflametory anticancer	Eri *et al*., 2023 [25]
4	8.074	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)]-	220	C15H24O	0.789	97	Antimicrobial, antibacterial.	Mansureh *et al.,* 2024 [26]
5	8.628	Neophytadiene	278	C20H38	1.190	99	analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant compound	Gonzalez-Rivera *et al.*, 2023 [27]
6	9.783	Hexadecanoic acid, methyl ester	270	C17H34O2	1.277	98	antioxidants,anti-inflametry, antibacteriahypocholesterolemic, nematicide, and pesticide,	Ravi *et al*., 2017 [28]
7	10.229	n-Hexadecanoic acid	256	C16H32O2	22.05	99	antioxidants,anti-inflametry, antibacteriahypocholesterolemic, nematicide, and pesticide,	Ravi *et al.,* 2017 Ganesan *et al.,* 2022 [29]
8	10.972	Phytol	296	C20H40O	2.629	98	anticancer, antioxidant, and antimicrobial, anti-inflametory, *anxiolytic, cytotoxic, metabolism-modulating, antioxidant, autophagy,apoptosis-inducing*, antinociceptive	Islam *et al.,* 2018 [30]

9	11.492	9-Octadecenoic acid, (E)-	282	C18H34O2	35.81	98	Antibacterial	Stenz L, *et al.* 2008 [31]
10	11.972	cis-Vaccenic acid	282	C18H34O2	0.687	98	Antimicrobial.	Ayşenur, 2024 [32]
11	12.292	Oleic Acid	282	C18H34O2	1.244	92	Antimicrobial	Dilika F, *et al*. 2000 [33]
12	15.858	β-Amyrin	426	C30H50O	2.418	99	antidiabetic, anti-inflammatory, antiarthritic, and anticancer	Gonzalez *et al.*, 2023[27]

Table 5 showing the compounds identified with the GC- MS from the extract, with quality of 90% and above.

IV. Discussion

Examination of the results above showed that there are various oral bacteria resistant to most of the conventional antibiotics.

Information gotten from the cultural, morphological, biochemical and molecular identification of the oral bacteria of the dental patients during the study, revealed there were thirteen (13) multidrug resistant species (MDS), six (6) species of *Mammalicoccussciuri* with accession number CP041879.1, four (4) species of *Enterococcus caseliflavus* with accession number CP0446123.1, one (1) species of *Enterococcus galinarum* with accession number MT 597704.1 and two (2) species of *Glutamicibactercreatinolyticus* with accession numbers KY814644.1 and CP034412.1 respectively. It could be deduced from these results that *M.sciuri, E. caseliflavus*, *E. galinarum* and G.*creatinolyticus*, can all be considered as transient oral bacteria, which have similar cultural, morphological and biochemical characteristics but differ in their molecular characteristics or gene sequence. This affirms the report of Komiyame*et..al*., 2016 [34], stating that *Enterococcal species* are transient oral bacteria and prevalent in various population groups including children, adolescents, adults and the elderly.

The results of the antibiotic sensitivity study in table 2 showed that*Enterococcal species* were sensitive to Gentamycin, Ceftriaxone, Levofloxacin, Ofloxacin, Cefotaxime, Erythromycin, Ciprofloxacin and Azithromycin but were resistant to Cefuroxime and Imipenem. However, *E galinarum*was found to be resistant to Erythromycin and Azithromycin unlike *E. caseliflavus*. *M.sciuri* was found to be sensitive to Gentamycin, Levofloxacin, Ciprofloxacin, and Ofloxacin but resistant to Cefixime, and Imipenem. Similarly, *G. creatinolyyicus* was noted to be sensitive to Cefuroxime, Gentamycin, Ceftriaxone, Levofloxacin and Ofloxacin but partially sensitive to Ciprofloxacin, Erythromycin and Azithromycin. However, they were resistant to Cefixime, Imipenem and Augmentin.

Phytochemical analysis using GCMS, revealed that *Lantana camara*extract contained bioactive compounds like alkaloids, saponins, flavonoids, tannins, anthocyanins, etc.,with antimicrobial and antibacterial activities and this corroborates with the report of Lonare *et.al*., 2012 [35].

The antibacterial activity results of the plant extracts used for the study as shown in table 4 gave an IZD of 10.0mm at an MIC of 25mg/ml for *M. sciuri*, 13.0mm at MIC of 25mg/ml for *E. caseliflavus*, 13.0mm at MIC of 50mg/ml for *E. galinarum* and 13mm at an MIC of 50mg/ml which implies that *E. caseliflavus* and *M. sciuri* are more susceptible to the plant extract whereas *E galinarum* and *G. creatinolyticus* are more resistant to the extract.

GCMS analysis revealed that *Lantana camara*extract contained 38 compounds withtwelve major bioactive compounds with quality factor of 90% and above as shown in table 5.These compounds with quality of 90% and above are; 2-Furancaboxaldehyde, Apha-Tepheneol, 2-methoxy-4-vinylphenol, 1-cylopro azulen-7-ol, Neophyticiene, Hexadecanoic acid. Others are Phytol, 9-octadecenoic acid, Cis-Vaccenic acid, Oleic acid and Beta.amyrin. However, 9-octadecenoic acid had the highest peak with retention time of 11.49 minutes. This result is consistent with the results of Ravi *et al*, (2017) [27] and Ganesan *et al*, (2022) [29] who both reported the presence of these compounds.

V. CONCLUSION

The result of this study justified the use of the plant *Lanttanacamarra* as Chewing-stick in Anambra state, Nigeria. The presence of numerous bioactive compounds in the analyzed plant extract which demonstrated antibacterial activity against all isolated MDRs, (*M. sciuri*, *E. caseliflavus*, *E. galinarum*, and *G. creatinolyticus*) at varying concentrations confirm its antibacterial potential. Hence the extract of *L. Camara*has very good prospect to be used and incorporated into oral hygiene products formulations.

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