In-vitro Antibacterial Activity of an Extract, Fractions and Terpenols from Lantana camara Linn Leaves against Selected Oral Pathogens

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Abstract

Ethnomedicinally, the leaves of *Lantana camara* (family: Verbenaceae) has a lot of applications, some of which includes in the treatment of bacterial infections. Successive and exhaustive partitioning of the crude methanol extract of the leaves (L) gave rise to the chloroform (Lc) and ethyl acetate (Le) fractions which both revealed the presence of terpenes and steroidal nucleus. Fractionation and purification of the ethyl acetate fraction (Le) led to the isolation of two long chain terpenols which were structurally elucidatedas 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol (2-Phyten-1-ol) and 3, 7, 11, 15- Tetramethylhexadeca- 2, 6, 10, 14- tetraen-1-ol (geranylgeraniol)using physical, chemical and spectral properties in comparison with literature data. Antibacterial assay of the crude extract, L (2500-10,000 $\mu g/cm^3$), its two fractions, Lc and Le (2500-10,000$\mu g/cm^3$ each) and the two isolated compounds (100 $\mu g/cm^3$) in comparison with amoxillin and ampiclox (100$\mu g/cm^3$) against selected Gram positive and Gram negative oral pathogens revealed that L and Lc exhibited no inhibitoryactivity; Le displayed a broad spectrum activity, while, the isolated compounds inhibited growth of only Gram positive pathogens.The results obtained from this study have provided some evidence for the ethnomedicinal use of the *Lantana camara* leaves as an antibacterial agent.

Keywords: Isolation, *Lantana camara*, Leaves, Oral pathogens, 2-Phyten-1-ol, Geranylgeraniol

Introduction

Natural products, such as medicinal plants have over the centuries been a source of very active therapeutic agents due to the presence of various secondary metabolites. These metabolites are known to display extensive range of bioactivities, thereby enhancing immune systems and giving resistance against several diseases in order to protect the body from harmful pathogens (Khalid *et al.*, 2018). The emergence of bacterial resistance strains to most antibacterial agents has made it pertinent to keep investigating natural products, especially plants, as possible alternatives because of their availability, cheapness and low toxicity (Patil*et al.*, 2015). *Lantana camara* Linn (Verbenaceae) is an ornamental evergreen, perennial, aromatic shrub. Though considered a noxious weed, it is a medicinal plant that possesses much potential (Mamta*et al.*, 2012). It is commonly known as Red sage/Lantana weed/Wild sage/Curse of Barbados (English), Kashinkuda (Hausa), Anya nnunu (Igbo) and Ewonadele (Yoruba). It is native to the

tropics and sub-tropics, highly invasive in many countries, growing up to a height of 1-3 m and a width of 2.5 m. Leaves are oppositely arranged, ovate in shape, rough, scabrid, bright green, hairy and aromatic when pulverized; stems possess prickles; flowers are small, clusters, most often orange/bright yellow in color with other varieties, such as white, blue, pink red and dark red, changing color with age and occurs year round. Fruits are also small, drupaceous, shinning and are either dark purple, greenish-blue, dark blue or black having two nutlets that are often dispersed by birds (Burkill, 1985; Ghisalberti, 2000; Lonare*et al.*, 2012; Vedavathi*et al.*, 2013). Traditionally, various organs of the plant are useful in different parts of the world in the treatment of different diseases, such as asthma, ulcers, rheumatism, malaria, dysentery, toothache, epilepsy, leprosy, itches, chicken pox, eczema, cataract, flu, yellow fever, skin problems, blood pressure and as an abortifacient (Burkhill, 1985; Abdullah *et al.*, 2009; Hiteshi*et al.*, 2012; Ingawale and Goswami-Giri, 2014). There has been a great variation in the phytochemical constituents of *L. camara* which differ for different climates and geographical region (Musyimi*et al.*, 2017), but generally, a significant presence of phenolic compounds, especially flavonoids and tannins, alkaloids, sterols, terpenoids, fatty acids, aromatics and glycosides (Burkhill, 1985; Mamta*et al.*, 2012; Kalita*et al*, 2012; Vedavathi*et al.*, 2013; Ingawale and Goswami-Giri, 2014; Charan and Kamlesh, 2015; Jafaar*et al.*, 2018) has been reported and several classes of these compounds have been isolated from different organs of the plant. One of the commonly isolated compounds from the plants is the triterpenoids (Lai *et al.*, 1998; Misra and Laatsch, 2000; Sharma *et al.*, 2000; Wahab*et al.*, 2003; Yadav and Tripathi, 2003; Hitesh*et al.*, 2012; Ingawale and Goswami-Giri, 2014; Patil*et al.*, 2015).The plant has antimycobacterial (Begum *et al.*, 2008), antibacterial (Patil*et al.*, 2015), analgesic (Kalyani*et al.*, 2011), antifungal (Das and Godbole, 2015; Fayaz*et al.*, 2017), antioxidant (Anwar *et al.*, 2013), wound healing (Swamy*et al.*, 2015), mosquito repellant (Akumu*et al.*, 2014) and antimicrobial (Lyumugahe*et al.*, 2017) properties to mention a few.

Terpenes /Terpenoids are small molecules synthesized by plants and are the largest and most diverse group of bioactive compounds, mostly made up of essential oils. They are made up of units of five carbon atoms in the order C_5, C_{10}, C_{15}, C_{20}, C_{25}, C_{30} and C_{40} structures, where they exist as either acyclic or cyclic (Seigler, 2012). They are known to exhibit significant antimicrobial, antibacterial, antiviral, anti-inflammatory and anticancer potentials (Mahato and Sen, 1997).This study presents the isolation and characterization of two of the terpenols of the plant, as well as investigation of the antibacterial potentials of the crude methanol extract, its

chloroform and ethyl acetate fractions and the isolated terpenolsagainst some oral pathogens in comparison with amoxillin and ampiclox.

Materials and Methods

Extraction of plant material

The leaves of *L. camara* were collected from Kakuri, Chikun Local Government Area of Kaduna State in the month of February, 2018. The leaves were air-dried, pulverized and 700 gwas cold macerated with methanol for a week until a colorless extractant was obtained. The resulting solution was filtered, concentrated *in-vacuo* and brought to dryness over a water bath. Crude MeOHextract was labeled 'L' (17 % recovery).

Preliminary qualitative screening

i) Liebermann-Burchard's test: Addition of few drops of acetic anhydride to a chloroform solution of the extract, L followed by addition of 1 cm^3 of conc. H_2SO_4 down the side of the test tube. Formation of a pink, purple or blue-violet color/green, greenish-blue color after a few minutesis indicative of terpenoids and steroidal nucleus respectively.

ii) Salkowskii's test: Addition of few drops of conc. H_2SO_4 to a chloroform solution of extract, L. Formation of a yellowor red coloration in the lower layer indicates the presence ofterpenoids and steroidal sapogenins respectively.

Thin Layer Chromatography (TLC)

Stationary phase: Silica gel 60 F_{254}pre-coated TLC sheets (0.25 mm thickness)

Mobile phases: (a) Hexane: $CHCl_3$ (4: 1), (b) hexane:EtOAc (9:1) and (c)$CHCl_3$: EtOAc (9:1)

Chromogenic reagents: All chromatograms were (i) viewed under UV light (254 and 366 nm) and developed with: (ii) I_2 vapor and (iii) Liebermann-Burchard's reagent and heated to 120°C

Solvent partitioning

A portion of extract L (90 g) was solubilized in 500 cm^3 of distilled water, homogenized and allowed to stand for an hour. The resulting mixture was filtered and the filtrate partitioned exhaustivelyand successively in a separatory funnelwith chloroform (100 cm^3 x 7)and ethyl acetate (100 cm^3 x 10) until the extractant was colorless. Both mixtures were concentrated *in-*

*vacuo*and dried at room temperature to afforda fraction labeled as the $CHCl_3$ fraction (Lc, dark green gummy mass, 0.98%) and EtOAc fraction (Le, blackish-green gummy mass, 1.7%). Both fractions were screened for the presence/absence ofterpenes and steroidal nucleus.

Isolation and characterization of compounds

The ethyl acetate fraction, Le(1.5 g) was applied to the surface of a prepared flash column packed with silica gel (60-120 mesh, 30 g, wet method) and eluted sequentially with varying proportions of increasing polarity of petroleum ether: EtOAc (100:0 to 0:100). Similar fractions were pooled based on their TLC profile and concentrated *in-vacuo* to yield 5 major sub-fractions, Le1 – Le5. Sub-fraction Le1 (obtained from pet. Ether: EtOAc, 19: 1) yielded a significant yellow spot along with some steroidal and terpenoidal spots on TLC. Further subjection of fraction Le1 to a shorter column (silica gel, mesh 230-400 nm, increasing polarity of petroleum ether: $CHCl_3$ afforded yellow oil (compound I) from pet ether: $CHCl_3$ (9:1). From a mixture of pet ether: $CHCl_3$ (4: 1), a sub-fraction which revealed a major and minor spot on TLC was purified by washing severally with methanol to which the minor spot was soluble. The insoluble major spot revealed a single spot on TLC (compound II). Both compoundswere subjected to physical, chemical and spectral characterization.IR and UV were both recorded in $CHCl_3$ using FTIR 8400 spectrometer and UV-3101PC spectrophoton respectively. ^{1}H-NMR, ^{13}C-NMR and DEPT-135 spectra were recorded in $CDCl_3$ on Jeol 400 Brukerspectrometer operating at 400 MHz, while, GC-MS was recorded using Agilent Model 7890 GC with split/splitless injector interfaced to an Agilent 5975 mass selective detector. The MS operating condition was ionization voltage 70eV and ion source 240°C. GC was fitted with a DB-5 fused silica capillary column (30 x 0.32 mm, film thickness 0.25 µm)

Antibacterial Assay

Sources of organisms

Samples from dental abscesses and tooth decay were collected from patients who presented pathological symptoms at dental unit of General Hospital Minna, Niger State, Nigeria, by rubbing the lesions with sterile swab sticks. The swab sticks were then transferred to sterile normal saline containers and transported in ice pack to the Microbiological laboratory, Federal University of Technology, Minna, Niger State, Nigeria.

Identification of clinical isolates

The identity of each strain was confirmed by standard bacteriological methods (Cheesebrough, 2010) and 16s rRNA gene sequencing. The organisms, include, Gram positive strains: *Micrococcus luteus*, *Streptococcusmutans*, *Streptococcus pneumoniae*and *Streptococcus pyogenes*; Gram negative strains: *Klebsiellapneumoniae* and *Pseudomonas aeruginosa*

Standardization of test organisms

The method of Collins *et al.*, (1995) was employed. Sterile nutrient broth (5 cm^3) was inoculated with a loopful of each organism and incubated for 24 h. 0.2 cm^3 of the 24 h culture was then sub-cultured into a 20 cm^3 sterile nutrient broth and incubated at 37°C for 5 h to standardize the culture to10^6cfu/cm^3/organism. A loopful of each standardized culture was then used for the assay.

Antibacterial testing

The antibacterial activity of crude methanol extract of *L. camara* leaves, L, its chloroform (Lc) and ethyl acetate (Le) fractions all at concentrations ranging from 2500 -10,000 µg/cm^3were tested against the oral pathogens using the agar dilution test (Collins *et al.* 1995). The activity of each test compound was compared withplates for standard control (amoxicillin and ampiclox; 100 µg/cm^3 each). Extract sterility control (ESC), organism viability control (OVC) and medium sterility control (MSC) were also prepared. All plates were incubated aerobically at 37°C for 24 h and checked for growth/no growth of organism.

Determination of minimum inhibitory concentration (MIC)

The MIC of theethyl acetate fraction (Le) was determined by microbroth dilution technique as described by (Collins *et al.*, 1995). Under septic condition, a loopful of each standardized oral pathogen was inoculated into nutrient broth containing 2500, 3000, 4000, 4500, 5000 and 10,000 µg/cm^3 of fraction Le. All were incubated for 24 h at 37°C. The MIC was determined by observing tubes with lowest concentration of fraction Le that inhibited the growth of each organism.

Determination of minimum bactericidal concentration (MBC)

The MBC ofethyl acetate fraction (Le) was determined using the Collins *et al* (1995). A loopful of each MIC tube was inoculated into solidified sterile nutrient agar and incubated at 37°C for 24 h. Plates that failed to show any visible growth after 24 h were recorded as MBC.

Results and Discussion

Subjection of ethyl acetate fraction (Le) to gas chromatography-mass spectrometry revealed the presence of fifteen major compounds: germacrene D (a sesquiterpene), diazoprogesterone (steroidal), santolinatriene (a monoterpene), lilac alcohol A and C (monoterpenes), phytol (diterpene alcohol), (Z, Z) - α- farnesene (a sesquiterpene) and geranylgeraniol (a monoterpene), while others were long chain fatty acids,fatty acid methyl esters, some steroidal, phenolic and alkaloidalcompounds.

Characterization of compound I

Physical properties: Light yellow viscous oil, 23 mg, 296 $gmol^{-1}$ and $C_{20}H_{40}O$ (both as revealed by GC-MS). **Chemical properties**: Single spotted on TLC (Hex: $CHCl_3$, 4: 1, R_f 0.55) (Hex: EtOAc, 9: 1, R_f 0.59), blue fluorescence spot under UV, bright yellow (I_2) and purple (Liebermann-Burchard's reagent). Compound was soluble in petroleum ether, $CHCl_3$, Me_2CO, EtOH; insoluble in water. **Spectral properties:FTIR (cm^{-1}):** 2924.5 (C-H stretching), 3310.8 (broad, O-H intermolecular stretch), 1558.1 (C=C stretching) and 1325.0 (C-H bending). **UV (λmax, nm):** 177 (non-conjugated C=C) and 179 (-OH). **GC-MS (m/z, intensity, %):**296 ($C_{20}H_{40}O^+$, 15%, M^+), 278 (M-H_2O, 10%), 263 (M-H_2O + CH_3, 10%),123 ($C_9H_{15}^+$, 30%), 71 ($C_4H_7O^+$,100%, base peak), 57 ($CH_3CH_2CH_2CH_2^+$, 35%) and 43 ($CH_3CH_2CH_2^+$, 40%). **^{1}H-NMR (δ ppm):** Most of the peaks appeared as clusters and were upfield indicating the presence of quite a number of methylene and methyl groups. The most de-shielded peaks were weak and ranged between δ3.78 - 4.91indicating the presence of nucleophilicgroups, -OH and alkene (Table 1). **^{13}C-NMR (δ ppm):**revealed eleven proton de-coupled peaks of which peak at C-6, C-8, C-10 and 12 was of highest intensity/bolder singlet; followed by peaks atC-16 and C-20, C-18 and C-19, indicating that there were more than one carbon atoms resonating at those frequencies. Downfield peaks at δ121.5 and 128.9 are indicative of a methine anda quaternary carbon at C-2 and C-3 respectively, while a peak at δ50.4 indicates a primary alcohol (Table 1). **DEPT (δ ppm):** revealed that of the 11 peaks displayed by ^{13}C-NMR (though a total of 20 carbons), one was quaternary (nulled), four were methine (positive), ten were methylene (negative), while, five were methyl (positive).

Table 1: ^{1}H-, ^{13}C- NMR and DEPT-135° of compound I in comparison with literature values*

Position	^{1}H (ppm)	^{1}H (ppm)*	^{13}C (ppm)	^{13}C (ppm)*	DEPT (ppm)
1	3.97 (d)	4.18	50.4 (H_2C-OH)	58.9	50.4 (neg.)
	4.22 (s)	5.05			
2	4.91 (t)	5.39	121.5 (HC=)	123.8	121.5 (pos.)
3	-	-	128.9(= C-)	139.2	Null.
4	1.90 (t)	1.94	37.4 (-CH_2)	39.6	37.4 (neg.)
5	1.22 (m)	1.31	23.5 (-CH_2)	24.9	23.5 (neg.)
	1.28				
6	1.08 (m)	1.19	34.8 (-CH_2)	37.8	34.8 (neg.)
	1.01				
7	1.25 (m)	1.40	30.8 (-CH-)	33.3	30.8 (pos.)
8	1.08 (m)	1.19	34.6 (-CH_2)	37.7	34.1 (neg.)
	1.01				
9	1. 17 (m)	1.25	23.2 (-CH_2)	24.6	23.2 (neg.)
10	1.08 (m)	1.19	34.1 (-CH_2)	37.7	34.1 (neg.)
11	1.25 (m)	1.40	30.5 (-CH-)	33.2	30.5 (pos.)
12	1.08 (m)	1.19	34.6 (-CH_2)	37.7	34.1 (neg.)
13	1.17 (m)	1.25	23.0 (-CH_2)	24.3	23.0 (neg.)
14	1.08 (t)	1.19	37.8 (-CH_2)	39.9	37.8 (neg.)
15	1.35 (t)	1.62	26.9 (-CH-)	28.1	25.9 (pos.)
16	0.90 (d)	0.91	21.9 (-CH_3)	23.2	21.9 (pos.)

17	1.40 (s)	1.79	15.5 (-CH_3)	16.3	15.5 (pos.)
18	0.81 (s)	0.87	20.7 (-CH_3)	21.0	20.7 (pos.)
19	0.83 (s)	0.89	20.7 (-CH_3)	21.0	20.7 (pos.)
20	0.88 (s)	0.91	21.9 (-CH_3)	23.2	21.9 (pos.)

Keys:s, singlet;d, doublet; t, triplet; m, multiplet;pos., positive peak; neg., negative peak; null, nullified peak; *ACD/ChemDraw (Product Version 15)

A comparative study of the obtained physical, chemical and spectroscopic data of compound I with those published in literature, revealed to be 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol/ 3, 7, 11, 15-tetramethylhex-2-en-1-ol/2-Phyten-1-olas shown in Figure 1

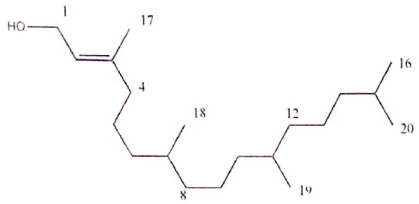

Figure 1: Structure of Compound I (3, 7, 11, 15 -Tetramethyl-2-hexadecen-1-ol)

The compound,2-Phyten-1-ol is an acyclic diterpene unsaturated alcohol (aliphatic diterpenoid alkene alcohol) commonly found in plants, a building block of the chlorophyll molecule and a precursor for the manufacture of vitamins E and K1 (Byju*et al.*, 2013). It is useful as a food additive and exhibits significant biological activities, such as anti-spasmodic (Pongprayoon*et al.*, 1992), anti-cancer (Lee *et al.*, 1999), anti-tubecular (Saikai*et al.*, 2010), anti-convulsant (Costa *et al.*, 2012), anti-mycobacterial (Bhattacharya and Rana, 2013) and anti-schistosomal properties (de Morae*set al.*, 2014). The compound has been isolated from several plants (Mcneil*et al.*, 2012; Passos*et al.*, 2012; Thakor*et al.*, 2016; Phantangare*et al.*, 2017).

Compound II

Physical properties: A colorless viscous volatile oil, 18 mg, 290.5 $gmol^{-1}$ and $C_{20}H_{34}O$ (both as revealed by GC-MS). **Chemical properties**: Single spotted on TLC (Hex: $CHCl_3$, 4: 1, R_f 0.58) (Hex: EtOAc, 9: 1, R_f 0.61), blue fluorescence spot under UV, bright yellow (I_2) and purple (Liebermann-Burchard's reagent). Compound was soluble in petroleum ether, $CHCl_3$,

CH_2Cl_2, Me_2CO, slightly soluble in EtOH and insoluble in water. **Spectral properties: FTIR (cm^{-1}):** 3321 (broad, O-H intermolecular stretch), 3079 (=C-H stretch), 1640 (C=C stretching) and 1452 (C-H bending). **UV (λmax, nm):** 184 (-OH) and 201(C=C). **GC-MS (m/z, intensity, %):** 290.5 ($C_{20}H_{34}O^+$, 10%, M^+), 273 ($M-H_2O$, 10%), 258 ($M-H_2O + CH_3$, 15%), 69 ($C_4H_5O^+$, 100%, base peak), 81 ($C_5H_7O^+$, 52%), 55 ($C_4H_7^+$, 25%), 41 ($C_3H_5^+$, 58%) and 31 (CH_2OH^+). **^{1}H-NMR (δ ppm):** The most de-shielded peak at 5.11 ppm (a weak triplet) was assigned to the sp^2 hybridized methine carbon atom at C-2 neighboring the C-OH group at C-1, followed by a very weak triplet at4.99 ppm which was also assigned to sp^2 hybridized methine carbon atoms at C-6, C-10 and C-14;while a weak singlet at 4.88 ppm and a doublet at 4.04 ppm were assigned to the –OH group and hydroxylic proton at C-1 respectively. Shielded peaks of which most were sharp singlets were attributed to methylene peaks at 1.90 ppm (C-4, C-5, C-8, C-9, C-12 and C-13), while, peaks at 1.53 ppm was assigned to methyl peaks at C-17, C-18 and C-19) as shown in Table 2. **^{13}C-NMR (δ ppm):** This revealed thirteen proton de-coupled peaks of which peaks at C-4, C-8 and C-12, C-5 and C-9, C-6 and C-10, C-7 and C-11, C-17, C-18 and C-19 were of higher intensities, indicating that more than one carbon atoms were resonating at those frequencies. The most de-shielded peak at 139.5 ppm was assigned to the tertiary carbon atom bearing a sp^2 and sp^3 hybridized carbon atoms at C-3.Other de-shielded peaks ranged from 133.8 to 120.2 ppm, all peaks of high intensities,indicating the presence of several sp^2 hybridized carbon atoms in the molecule (C-2 and C-3, C-6 and C-7, C-10 and C-11, C-14 and C-15). Another de-shielded peak at 51.4 ppm was assigned to a carbon atom bearing a nucleophilic –OH group (C-1). Other peaks which were more shielded were assigned to methine, methylene and methyl carbons respectively (Table 2). **DEPT (δ ppm)** revealed that of the 13 peaks displayed by ^{13}C-NMR (though a total of 20 carbons), four were quaternary (nulled), four were methine (positive), seven were methylene (negative), while, five were methyl (positive).

A comparative study of the obtained physical, chemical and spectroscopic data of compound II with those published in literature, the compound was identified as 3, 7, 11, 15-Tetramethylhexadeca- 2, 6, 10, 14- tetraen-1-ol/tetraprenol/Geranylgeraniol/Geranylgeraniol as shown in Figure 2

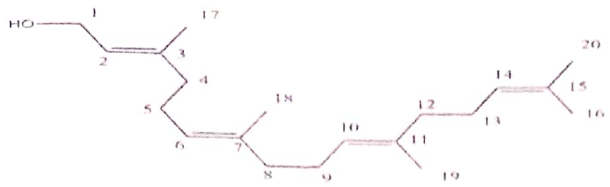

Figure 2: Structure of compound II (Geranylgeraniol, GGOH)

The compound, geranylgeraniol is a long chain diterpene alcohol/isoprenoid/ polyprenol that has been isolated from some plants and is involved in various physiological processes (Ho *et al.*, 2018). The compound reportedly exhibited bactericidal activity against *S. aureus* (Inoue *et al.*, 2005), anti-mycobacterium and anti-leshmanial activities (Vik*et al.*, 2007). The volatile nature of the compound makes GGOH a valuable starting material for perfumes and pharmaceutical products (Tokuhiro *et al.*, 2009).

Table 2: ^{1}H-, ^{13}C- NMR and DEPT-135° of compound II in comparison with literature values*

Position	^{1}H (ppm)	^{1}H (ppm)*	^{13}C (ppm)	^{13}C (ppm)*	DEPT (ppm)
1	4.04 (d)	4.18	51.4 (-CH_2OH)	58.9	51.4(neg.)
	4.88 (s)	5.05			
2	5.11 (t)	5.39	120.7 (-CH)	124.6	120.7 (pos.)
3	-	-	133.8 (-C-CH_3)	139.5	Null.
4	1.90 (s)	2.00	36.9 (-CH_2)	39.7	36.9(neg.)
5	1.90 (s)	2.00	25.8 (-CH_2)	26.7	25.8(neg.)
	1.81				
6	4.99 (t)	5.20	121.1 (-CH)	124.3	121.1 (pos.)
7	-	-	132.4 (-C-CH_3)	135.7	Null.
8	1.90 (s)	2.00	36.9 (-CH_2)	39.7	36.9(neg.)
9	1.90 (s)	2.00	25.8 (-CH_2)	26.7	25.8(neg.)
	1.81				

10	4.99 (t)	5.20	120.7 (-CH)	124.3	120.7(pos.)
11	-	-	132.4 (-C-CH_3)	135.7	Null.
12	1.90 (s) 1.83	2.00	36.9 (-CH_2)	39.7	36.9(neg.)
13	1.90 (s) 1.81	2.00	25.1 (-CH_2)	26.4	25.1(neg.)
14	4.99 (s)	5.20	120.2 (-CH)	123.5	120.2(pos.)
15	-	-	129.9(-CH-CH_3)	132.0	Null.
16	1.65 (s)	1.82	22.5 (CH_3)	24.6	22.5 (pos.)
17	1.53(s)	1.79	15.8 (CH_3)	16.4	15.8 (pos.)
18	1.53 (s)	1.79	15.8 (CH_3)	16.4	15.8(pos.)
19	1.53 (s)	1.79	15.8 (CH_3)	16.4	15.8(pos.)
20	1.44 (s)	1.70	17.2 (CH_3)	18.6	17.2(pos.)

Keys: s, singlet; d, doublet; t, triplet; pos., positive peak; neg., negative peak; null, nullified peaks; *ACD/ChemDraw (Product Version 15)

Table 3: Antibacterial activity of ethyl acetate fraction of *Lantana camara*leaves (Le)

Test Organisms	Concentration (µg/cm^3) of test compounds against test organisms							
	**Ethyl acetate fraction (Le)						Ampiclox	Amoxicillin
	2500	3000	4000	4500	5000	10,000	100	100
Gram positive								
M. luteus	-	-	-	-	+*	+	+	+
S. mutans	-	-	-	+*	+	+	+	+

S. pneumoniae	-	-	-	-	+*	+	+	+
S. pyogenes	-	-	+*	+	+	+	+	+
Gram negative								
K. pneumoniae	-	-	-	-	+*	+	+	+
P. aeruginosa	-	-	-	-	+	+	+	+

Keys: +, activity; -, no activity, *, MBC, **the fraction was only static on *P. aeruginosa*, but cidal on all other test organisms

Table 4: Antibacterial activity of the terpenols from the ethyl acetate fraction of *Lantana camara* leaves (Le)

Test Organisms	Activity of test compounds against test organisms at 100 µg/cm^3			
	Compound I	Compound II	Ampiclox	Amoxicillin
Gram positive				
M. luteus	+	+	+	+
S. mutans	+	+	+	+
S. pneumoniae	+	+	+	+
S. pyogenes	+	+	+	+
Gram negative				
K. pneumoniae	-	-	+	+
P. aeruginosa	-	-	+	+

Keys: +, activity; -, no activity

The crude methanol extract (L) and the chloroform fraction (Lc) of the leaves of *Lantana camara* from concentrations ranging from 2500 to 10,000 µg/cm^3 displayed no activity against any of the tested oral pathogens, an indication that the extract and $CHCl_3$ fraction might not be useful in the treatment of oral infections and other infections caused by such organisms. The EtOAc fraction inhibited the growth of all oral pathogens tested. The fraction was cidal on all

the test organisms, except on *P. aeruginosa*, supporting the ethnomedicinal uses of the plant. The observed inhibitory effects may be due to the presence of mid-polar bioactive compounds which all probably acted synergistically (Doughari and Obidah, 2008). Although, the activity displayed by the fraction was at higher concentrations in comparison to that exhibited by the standard drugs at 100 µg/cm^3 (Table 3); sometimes the antibacterial activity of a fraction could be enhanced at lower concentrations with further fractionation and purification (Ndip*et al.*, 2009).

The two oils isolated from the ethyl acetate fraction of the plant (compounds I and II) both inhibited the growth of the Gram positive oral pathogens only in comparison to the standard drugs all at 100 µg/cm^3 (Table 4); an indication that both compounds do not possess broad spectrum activity or might become enhanced at higher temperature. Long chain diterpenes possessing the –OH group(s) have been reported to exhibit good bactericidal activity against some Gram positive strains at very low concentrations (Inoue *et al.*, 2005, Vik*et al.*, 2007).

Conclusion

The ethyl acetate fraction (Le) at concentrations ranging from 4000 – 10,000 µg/cm^3 exhibited a broad spectrum activity against the oral pathogens, while, and the two long chain terpenols isolated from it; 2-Phyten-1-ol (compound I) and Geranylgeraniol(compound II) both inhibited the growth of Gram positive oral pathogens. The results obtained from this study have provided some evidence for the ethnomedicinal use of the leaves of *L. camara* as an antibacterial agent.

References

Abdullah, M. A., Hassandarvish, P., Ali, H. M., Noor, S. M. & Mahmoud, F. H. et al. (2009).Acceleration of wound healing potential by *Lantana camara* leaf extract in experimental rats.*J. Med. Sci.* 3, 75-79

Akumu, E. O., Anthoney, S. T., Mutuku, C. N., Jackie, K. O. & Edwin, M. (2014).Phytopharmacological evaluation of *L. camara* leaves' smoke.*Int. J. of Pharmacy and Biological Sciences*, 4 (2), 28-34

Anwar, F., Shaheen, N., Shabir, G., Ashraf, M., Alkharfy, K. M. &Gilani, A. H. (2013).Variation in antioxidant activity and phenolic and flavonoid contents in the flowers and leaves of Ghaneri (*Lantana camara* L.) as affected by different extraction solvents.*Int. J. of Pharmacology*, 9 (7), 442-453

Begum, S., Wahab, A. &Siddiqui, B. S. (2008).Antimycobacterial activity of flavonoids from *Lantana camara* Linn.*Nat. Prod. Res.*, 22 (6), 467-470

Bhattacharya, A. K. &Rana, K. C. (2013).Antimycobacterial agent, (E)-phytol and lauric amide from the plant *Lagasceamollis.Indian J. Chem.* 52B, 901-903

Burkill, H. M. (1985). The *useful plants of West Tropical Africa* vol. 5. Royal Botanical Gardens, Kew

Byju, K., Vasundhara, G., Anuradha, V., Nair, S. M. & Kumar, N. C. (2013). Presence of phytol, a precursor of vitamin E in *Chaetomorphaantinnina.Mapana J. Sci.*, 12 (2), 57-65

Charan, S. &Kamlesh, C. (2015).Micropropagation and analysis of phytochemical profile of *Lantana camara* whole plant extraction.*World J. of Pharm. and Pharm. Sci.* 4 (8): 1907-1919

Cheesebrough, M. (2010).*Distinct Laboratory Practice in Tropical Countries*.Part 2. UK: Cambrigde University Press, pp. 35-38

Collins, C. H., Lynes, P. M. & Grange, J. M. (1995).*Microbiological Methods*. 7th ed. UK: Butterworth Heinemann, Ltd., pp. 175-190

Costa, J. P., Ferreira, P. B., Sousa, D. P., Jordan, J.&Freitas, M. (2012).Anticonvulsant effect of phytol in a pilocarpine model in mice.*Neurosci.Lett.*, 523, 115-118

Das, L. &Godbole, S. (2015). Antifungal and phytochemical analysis of *Lantana camara*, *Citrus limonum* (Lemon), *Azadirachtaindica* (Neem) and *Hibiscus rosasinensis* (China Rose). *J. of Pharmacy Research*, 9 (7), 476-479

de Moraes, J., de Oliveira, R. N., Costa, J. P., Junior, A. L. G., de Sousa, D. P., Freitas, R. M., Allegretti, S. M. & Pinto, P. L. S. (2014). Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected tropical disease - *Schistosomiasismansoni.PLOS Neglected Tropical Diseases* 8 (1), e2617

Doughari,J. H. &Obidah, J. S. (2008). Antibacterial potentials of stem bark extracts of *Leptadenialancifolia* against some pathogenic bacteria. *Pharmacology on line*, 3, 172-180

Fayaz, M., Bhat, M. H, Fayaz, M., Kumar, A. & Jain, A. K. (2017). Antifungal activity of *Lantana camara* L. leaf extracts in different solvents against some pathogenic fungal strains. *Pharmacologia*, 8, 105-112

Ghisalberti, E. L. (2000). *Lantana camara* L. (Verbenaceae).*Fitoterapia*, 71, 467-486

Hitesh, H.S., Mayukh, B. & Mahesh, A. R. (2012).Isolation and characterization of chemical constituents of aerial parts of*Lantana camara.Int. J. of Pharm. Res. and Bio-Sci.*, 1 (6), 198-207

Ho, H. J., Shirakawa, H., Giriwono, P. E., Ito& Komai, M. (2018). A novel function of geranylgeraniol in regulating testosterone production. *Biosci.Biotechnol.Biochem.*, 82 (6), 956-962

Ingawale, G. S.&Goswami-Giri, A. S. (2014).Isolation and characterization of bioactive molecule from *Lantana camara.Asian J. Res. Chem.*, 7 (3), 339-344

Inoue, Y., Hada, T., Shiraishi, A., Hirose, K., Hamashima, H. & Kobayashi, S. (2005). Biphasic effects of geranylgeraniol, terprenone and phytol on the growth of *Staphylococcus aureus.Antimicrobial Agents and Chemotheraphy*, 49, 10.1128/AAC. 49.5. 1770-1774. 2005

Jafaar, N. S., Hamad, M. N., Alshammaa, D. A &Abd, M. R. (2018). Preliminary phytochemical screening and high performance thin layer chromatography [HPTLC] detection of phenolic acids in *Lantana camara* leaves cultivated in Iraq.*Int. Res. J. of Pharm.*, 9 (7), 59-64

Kalita, S., Kumar, G., Karthik, L. &Rao, K. V. B. (2012).A review on medicinal properties of *Lantana camara* Linn.*Res. J. Pharm. and Tech.*, 5 (6): 711-715

Kalyani, L., Lakshmana, R. & Mishra, U. S. (2011).Antibacterial and analgesic activity of leaves of *Lantana camara.International Journal of Phytomedicine*, 3, 381-385

Khalid, S., Shahzad, A., Basharat, N., Abubakar, M.& Anwar, P. (2018).Phytochemical screening and analysis of selected medicinal plants in Gujrat.*J.PhytochemistryBiochem*, 2,108

Lai, J. S., Chan, Y. F., Huang, K. F. & Chin, I. (1998).Triterpenoids, flavonoids and a mixture of campesterol, stigmasterol and β-sitosterol from the stem of pink flowering taxa of *Lantanacamara.Pharm. J. (Taipei)*, 50, 385-392S

Lee, K. L., Lee, S. H. & Park, K. Y (1999). Anticancer activity of phytol and eicosatienoic acid identified from *Perilla* leaves. *J. Korean Soc. Food Sci. Nutr.*, 28, 1107-1112

Lonare, M. K., Sharma, M., Hajare, S. W. &Borekar, V. I. (2012).*Lantana camara*: Overview on toxic to potent medicinal properties. *Int. J. Pharm. Sci. Res.*, 3 (9), 3031-3035

Lyumugahe, F., Uyisenga, J. P., Bayingana, C. &Songa, E. B. (2017). Antimicrobial activity and phytochemical analysis of *Vernoniaaemulans*, *Vernoniaamygdalina*, *Lantana camara*and *Markhamialutea* leaves as natural beer preservatives. American *J. of Food Tech.*, 12 (1), 35-42

Mahato, S. B. &Sen, S. (1997). Advances in terpenoid research 1990-1994. *Phytochemistry*, 44, 1185-1236

Mamta, S., Jyoti, S. &Sarita, K. (2012). A brief review on: Therapeutic values of *Lantana camara* plant. *Int. J. of Pharm. & Life Sci.* (IJPLS), 3 (3), 1551-1554

Mcneil, M. J., Porter, R. B. & Williams, L. A. (2012).Chemical composition and biological activity of the essential oil from Jamaican *Cleome serrata.Nat Prod. Comm.*, 7, 1231-1232

Misra, L. &Laatsch, H. (2000).Triterpenoids, essential oil and photooxidative 28-13-lactonization of oleanolic acid from *Lantana camara.Phytochemistry*, 54, 969-974

Musyimi, D. M., Opande, G. T., Chesire, J., Sikuku, P. A. &Buyela, D. K (2017).Antimicrobial potential and screening of phytochemical compounds of *Lantana camara* Linn. IJRDO- *J. of Biological Sciences*, 3(6), 18- 35

Ndip, R. N., Ajonglefac, A, N., Wima, T., Luma, H. N., Wirmun, C. &Efange, S. M. (2009). In-vitro antimicrobial activity of *Ageratum conyzoides* (Linn.) on clinical isolates of *Helicobacter pylori.African Journal of Pharmacy and Pharmacology*, 3 (11), 585-592

Passos. J. L., Barbosa, L. C., Demuner, A. J., Alvarenga, E. S., Silva, C. M. &Barreto. R. W. (2012). Chemical characterization of volatile compounds of *Lantana camara* L. and *Lantana radula* Sw. and their antifungal activity.*Molecules*, 17, 11447-11445

Patil, G., Khare, A. B., Huang, K. F. & Lin, F. M. (2015). Bioactive chemical constituents from the leaves of *Lantana camara* L. *Indian J. of Chemistry*, 54B, 691- 697

Phatangare, N. D., Deshmukh, K. K., Murade, V. D., Hase, G. J. &Gaje, T. R. (2017). Isolation and characterization of phytol from *Justiciagendarussa*Burm.f. –An anti-inflammatory compound.*Int. J. of Pharmacognosy and Phytochemical Research*, 9 (6), 864-872

Pongprayoon, U., Baeckstreom, P., Jacobson, U., Lindstreom, M. &Bohlin, L. (1992). Antispasmodic activity of β-datascenone and e-phytol isolated from *Ipomoea pescaprae*. *PlantaMedica*, 58, 19-21

Saikai, D., Parihar, S., Chanda, D.*et al.*, (2010).Anti-tubecular potential of some semisynthetic analogues of phytol.*Bioorg. Med. Chem. Lett.*, 20, 508-512

Seigler, D. S. (2012).*Plant secondary metabolism*. Springer Science and Business Media pp. 668

Sharma, O. P., Singh, A. & Sharma, S. (2000). Levels of lantadenes, bioactive pentacyclictriterpenoids in young and mature leaves of *Lantana camara* var. aculeate.*Fitoterapia*,71, 487-491

Swamy, M. K., Sinniah, U. R. &Akthar, M. S. (2015). *In vitro* pharmacological activities and GC-MS analysis of different solvent extracts of *Lantana camara* leaves collected from tropical region of Malaysia. *Evidence-based Complementary and Alternative Medicine*, 2015, 1-9

Thakor, P., Mehta, J. B., Patel, R. R. &Thakkar, V. R. (2016). Extraction and purification of phytol from *Abutilon indicum*: cytotoxic and apoptotic activity. *RSC Advances*, 6 (54), 48336-48345

Tokuhiro, K., Muramatsu, M., Ohto, C., Kawaguchi, T., Obata, S., Muramoto, N., Hirai, M., Takahashi, H., Kondo, A., Sakuradani, E. & Shimizu, S. (2009). Overproduction of geranylgeraniol by metabolically engineered *Saccharomyces cerevisiae*. Appl. Environ.Microbiol.,75 (17), 5536-5543

Vedavathi, T., Bhargavi, K., Swetha, G. &Mythri, K. (2013).Estimation of flavonoid, phenolic content and free radical scavenging activity of fresh unripe fruits of *Lantana camara* Linn.(Verbenaceae).*Int. J. of Res. in Pharmacology and Pharmacotherapeutics*, 2 (issue 1), 286-294

Vik, A., James, A. &Gundersen, L. L. (2007). Screening of terpenes and derivatives for antimycobacterialactivity ; identification of geranylgeraniol and geranylgeranyl acetate as potent inhibitors of *Mycobacterium tuberculosisin-vitro*. *PlantaMedica,* 73 (13), 1410-1412

Wahab, A., Siddiqui, B. S. & Begum, S. (2003). Pentacyclictriterpenoids from the aerial parts of *Lantana camara*.*Chem Pharm Bull (Tokyo)*, 51, 134-137

Yadav, S. B. &Tripathi, V. (2003).A new triterpenoid from *Lantana camara*.*Fitoterapia* 74, 320-321