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Chemical composition, antimicrobial and free radical scavenging activities of *Grewia pubescens*

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The phytochemical screening of n-hexane, ethyl acetate and methanol extracts of *Grewia pubescens* leaves showed the presence of alkaloids, steroids, terpenoids, flavonoids, saponins, anthraquinones, tannins, glycosides and fats and oils. The three extracts were tested on six bacterial and four fungal strains, and exhibited satisfactory inhibitory activities against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis, Pseudomona aeruginosa, Salmonella typhii, Klebsiellae pneumonae, Candida albicans, Aspergillus niger, penicillium notatum* and *Rhizopus stolonifer,* except ethyl acetate extract which showed no antifungal property on *penicillium notatum* and *Rhizopus stolonifer.* Methanol extract of the plant possessed significant antioxidant activity by exhibiting DPPH free radical scavenging property with IC_{50} of 10.26 µM, using DPPH antioxidant assay. The compounds present in the extracts were characterised using Gas chromatography-Mass spectroscopy (GC-MS). Seven, three and six compounds were revealed in n-hexane, ethyl acetate and methanol extracts of *Grewia pubescens* leaves respectively, while hexadecanoic acid is the most abundant constituent in all three extracts with corresponding percentage of abundance of 47.92, 53.20 and 61.59.

Capsule Summary: The chemical composition, antimicrobial and free radical scavenging activities of *G. pubescens* were investigated and in view of bioactivity observed, which could possibly be used in antimicrobial drugs.

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INTRODUCTION

The association of humans and animals with plants obviously originated with the beginning of life on earth, when plants supplied much of the shelter, oxygen, food and medicine needed by higher life forms. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. There is an increasing interest in the medicinal plants as a natural alternative to synthetic drugs (Ali et al., 2015; Ashraf et al., 2015; Asif, 2015a, b, c, d; Fabio et al., 2007; Hussain et al., 2016; Mensah and Golomeke, 2015; Mensah et al., 2015;). Medicinal plants have been

Table 1: Phytochemical screening of the extracts of *G. pubescens* leaves

Chemical constituents	Fractions		
	GPLH	GPLE	GPLM
Alkaloids	-	+	-
Anthraquinones	-	+	+
Glycosides	+	+	+
Steroids	+	+	+
Phenolics	-	-	-
Flavonoids	-	+	+
Tannins	-	-	+
Saponins	-	+	+
Carbohydrates	-	-	-
Terpenoids	+	+	+
Fats and Oils	+	+	+

GPLH = Hexane extract of *G. pubescens* Leaves; GPLE = Ethyl acetate extract of *G. pubescens* Leaves; GPLM = Methanol extract of *G. pubescens* Leaves

Table 2: Antimicrobial activity of hexane extract of *G. pubescens*

Isolate	mean zone of inhibition (mm)							
S. aureus	26	22	20	18	14	10	-	40
E. coli	26	22	18	16	12	10	-	38
B. subtilis	24	20	18	14	12	10	-	40
P. aeruginosa	22	18	14	12	10	-	-	38
K. pneumoniae	20	16	14	12	10	-	-	40
S. typhi	18	16	14	12	10	-	-	38
C. ablicans	18	16	14	12	10	-	-	28
A. niger	20	18	16	14	12	-	-	28
P. notatum	16	14	12	10	-	-	-	28
R. stolonifer	16	14	12	10	-	-	-	26
Conc. of extract (mg/mL)	200	100	50	25	12.5	6.25	-ve	+ve

+ve = Gentamycin10µg/ml (bacteria), Tioconazole 70% (fungi); -ve = Solvent of dilution

used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led to the discovery of novel drug candidates used against diverse diseases. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Padulosi et al., 1991). The spread of drug resistant pathogens is one of the most serious threats to the successful treatment of microbial diseases (Prabuseenivassan et al., 2007). Phytochemical is a natural bioactive compound found in plants such as vegetables, fruits, medicinal plants, flowers, leaves and root that work with and fibers to act as a defense system and protect against diseases. *Grewia pubescens* (family Maliaceae) is a shrub or sometimes scandent tree growing 8m tall and didtributed widely in the part of Nigeria. Some of the *Grewia spp.* have been used in folk medicine to cure stomach upset, skin and intestinal infections and some also have mild antibiotic properties (Chopra et al., 1956). *Grewia pubescens* is also useful for the treatment of dysentery (Joshi et al., 1980).

MATERIAL AND METHODS

Grewia pubescens leaves were collected from Amurin village, Ondo State in November 2014. The plants were identified and authenticated by Mr Bolu Ajayi of Herbarium section (UIH/001/838), department of Plant Biology, University of Ilorin and voucher specimens were deposited in the herbarium.

Sample preparation and extraction

The leaves were air-dried and grounded into fine powder. The powdered leaves were extracted using cold extraction (serial successive extraction method) as described by Das et al. (2010).

Phytochemical screening

Preliminary phytochemical screening of the crude extracts was carried out to the methods described by Pranshant et al. (2011).

Antimicrobial assay

Microorganisms: Cultures of six human pathogenic bacteria made up of four gram negative and two gram positive were used for the antibacterial assay. These were; *Salmonella typhii, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiellae pneumonae* belongs to the gram-negative, while *Bacillus subtilis* and *Staphylococcus aureus* are gram-positive bacteria. Four fungi were also utilized for the Antifungal assay. These were; *Candida albicans, Aspergillus niger, Rhizopus stolon* and *Penicillum notatum.* All the microorganisms used were clinical strains from the Medical Microbiology (University College Hospital, Ibadan) and screened in the Laboratory of Pharmaceutical Microbiology Department, University of Ibadan, Ibadan, Nigeria.

Media: Nutrient agar, Sabouraud dextrose agar, nutrient broth and tryptone soya agar were used in this study. Hexane, ethylacetate and methanol were also used in solubilizing the extracts and as negative controls in the assays.

Table 3: Antimicrobial activity of ethyl acetate extract of *G. pubescens*

Isolate	mean zone of inhibition (mm)							
S. aureus	18	16	14	12	10	-	-	40
E. coli	18	16	14	12	10	-	-	38
B. subtilis	16	14	12	10	-	-	-	38
P. aeruginosa	16	14	12	10	-	-	-	40
K. pneumoniae	14	12	10	-	-	-	-	38
S. typhi	14	12	10	-	-	-	-	40
C. ablicans	16	14	12	10	-	-	-	28
A. niger	16	14	12	10	-	-	-	28
P. notatum	-	-	-	-	-	-	-	28
R. stolonifer	-	-	-	-	-	-	-	28
Conc. of extract (mg/mL)	200	100	50	25	12.5	6.25	-ve	+ve

+ve = Gentamycin 10 µg/mL (bacteria), Tioconazole 70% (fungi); -ve = Solvent of dilution

Table 4: Antimicrobial activity of methanol extract of *G. pubescens*

Isolate	mean zone of inhibition (mm)							
S. aureus	20	18	16	14	12	10	-	40
E. coli	20	18	16	14	12	10	-	38
B. subtilis	20	18	16	14	12	10	-	38
P. aeruginosa	18	14	12	10	-	-	-	40
K. pneumoniae	16	14	12	10	-	-	-	38
S. typhi	18	14	12	10	-	-	-	40
C. ablicans	18	14	12	10	-	-	-	28
A. niger	18	14	12	10	-	-	-	28
P. notatum	14	12	10	-	-	-	-	28
R. stolonifer	14	12	10	-	-	-	-	28
Conc. of extract (mg/mL)	200	100	50	25	12.5	6.25	-ve	+ve

+ve = Gentamycin 10 µg/mL (bacteria), Tioconazole 70% (fungi); -ve = Solvent of dilution

Antimicrobial agents: Gentamycin (10 µg/mL) and Tioconazole (0.7 mg/mL) were included as standard reference drugs in the study.

Antimicrobial activity determination

Agar diffusion-pour plate method (bacteria): An overnight culture of each organism was prepared by taken two wireloop of the organism from the stock and inoculated each into the sterile nutrient broth of 5ml, each incubated for 18-24 hr at 37°C. From overnight culture, 0.1 mL of each organism was taken and put into the 9.9 mL of sterile distilled water to obtained 10^{-2} inoculum concentration of the organism. From the diluted organism (10^{-2}), 0.2 mL was taken into the prepared sterile nutrient agar cooled to about 40-45°C, then poured into sterile Petri dishes and allowed to solidify for about 45- 60 min. Using a sterile cork-borer of 8mm diameter, the wells were made according to the number of the test tubes for the experiment. For this work 8 wells were made. The graded concentrations of the extracts were put into the wells accordingly including the controls. The studies were done in duplicates to ascertain the results obtained. The plates were left on the bench for about 2 hr to allow the extract diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were incubated for 18-24 hr at 37°C.

Agar diffusion-surface plate method (fungi): A sterile sabouraud dextrose agar was prepared accordingly and aseptically poured into the sterile plates in triplicates and solidified properly. 0.2 mL of the 10^{-2} inoculum concentration of the organism was spread on the surface of the agar using a sterile Petri-dish cover to cover all the surface of the agar. Eight wells were bored using a sterile cork-borer of 8mm diameter. The graded concentrations of the extracts were put into the including the controls. All the plates were left on the bench for 2hr to allow the extract diffuse properly into the agar i.e. pre-diffusion. The plates were incubated at 25°C for 72 h.

Antioxidant activity

The ability of the samples to scavenge DPPH free radicals was assessed by the standard method adopted with suitable modifications (Sies, 1997; Sermakkani and Thangapandian, 2012). The stock solutions of extracts were prepared in methanol to achieve the concentration of 1 mg/mL. Dilutions were made to obtain concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.99 µg/mL. DPPH (2,2-diphenyl-1-hydrazine) is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity (Wong et al., 2006). The absorbance was measured in triplicate at varying concentrations and the mean absorbances were determined. Parallel to examination of the antioxidant activity of plant extracts, the value for the standard compound (Ascorbic acid) was obtained and compared to the values of the antioxidant activity and the percentage inhibitions of the serial concentrations of the methanol DPPH extracts and that of the standard was determined at different concentrations using the expression as shown in Eq. 1.

$$Inhibition\ (\%) = \left(\frac{Ac-As}{Ac}\right) \times 100 \qquad (1)$$

The IC_{50} values (Inhibition Concentration at 50%) were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm.

GC-MS analysis

GC-MS was performed with Agilent 19091GC plus automatic sampler system coupled with a quadruple mass spectrometer 433HP-5MS. Compounds were separated in HP5MS column fused with phenyl methyl silox, (length; 30m x 250µm; film thickness 0.25µm). Samples were injected at a temperature of about 250°C with a split ratio of 10:1 with a flow rate of helium 1ml/min. The extracts of *Grewia pubescens* leaves were dissolved in the respective solvent (hexane, ethyl acetate and methanol) to form solution. The analysis was done using the GC-MS machine which is made up of two major building blocks, the gas chromatography and the mass spectrophotometer. The gas chromatography uses a capillary column which depends on the column dimension as well as the phase properties. The difference in the chemical properties between the different molecules in mixture will separate the molecules as the sample travels the length of the column. The molecule take different amount of time (the retention time) to come out of (elute form) the gas chromatography. This allows the mass spectrometer downstream to capture, ionize, accelerate, deflect and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule with ionized fragments and detecting these fragments using their mass to charge ratio.

RESULTS AND DISCUSSION

The phytochemical screening of the extracts of *G. pubescens* revealed the presence of bioactive compounds of therapeutic importance. The phytochemicals present in hexane extract of *G. pubescens* leaves were steroids, glycosides, terpenoids, and fats and oils while saponins, alkaloids, flavonoids, tannins and anthraquinones were absent in the extract. Ethyl acetate and methanol extracts of *G. pubescens* leaves revealed the presence of anthraquinones, flavonoids, saponins, glycosides, terpenoids, alkaloids and steroids (Table 1). Meanwhile only methanol extract of the plant contains tannins. Compounds rich in flavonoids, terpenoids and glycosides have been reported to possess antimicrobial, anti-inflammatory and anti-diarrhoeal properties (Pranshant et al., 2011).

The antimicrobial activity was determined using the Agar broth cup difusion – pour plate method for the bacteria and the surface plate (Ditch) method for the fungi with well diameter of 8mm. The result obtained in this study showed the antimicrobial potency of the extracts of *Grewia pubescens* leaves on the test organisms. Hexane extract of the plant exhibited high antibacterial and antifungal activities on all test organisms at concentrations between 25 and 200 mg/mL, but showed low inhibition against the test bacteria and fungi at low concentrations of 6.25 and 12.5 mg/mL (Table 2). Ethyl acetate extract of *G. pubescens* leaves showed satisfactory inhibition against the bacteria isolates; *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis and Pseudomona aeruginosa,* and fungal isolates; *Candida albicans and Aspergillus niger,* at high concentrations (25–200 mg/mL) of the extract while *penicillium notatum* and *Rhizopus stolonifer* were not inhibited by the extract at all concentrations (Table 3). Meanwhile methanol extract of *G. pubescens* leaves showed higher bacterial inhibition of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* than *Pseudomona aeruginosa, Salmonella typhii* and *Klebsiellae pneumonae* at all concentrations. The extract also exhibited antifungal properties on *Candida albicans and Aspergillus niger,* at concentrations between 25 – 200 mg/mL of the extract (Table 4). The inhibition exhibited by the plant extracts gives evidence to the fact that *G. pubescens* is an antibacterial and antifungal plant and hence can be used for the treatment of illness caused by pathogenic bacteria and fungi.

Hexane, ethyl acetate and methanol extracts of *G. pubescens* leaves exhibited antioxidant activity on DPPH radicals at different concentrations, using Ascorbic acid as

Table 5: Absorbance and % inhibition using ascorbic acid as standard for DPPH antioxidant activity of crude extracts of the leaves of *G. pubescens*. Absorbance of control is 1.265

Conc (µg/mL)	A1	A2	A3	AV±SD	%I of A
1000	0.138	0.138	0.140	0.139±0.0012	89.02
500	0.150	0.150	0.150	0.15±0.000	88.14
250	0.161	0.162	0.160	0.161±0.001	87.26
125	0.180	0.180	0.180	0.180±0.000	85.79
62.5	0.193	0.195	0.194	0.194±0.001	84.26
31.25	0.245	0.245	0.245	0.245±0.000	80.67
15.62	0.311	0.311	0.311	0.311±0.000	75.440
7.81	0.453	0.452	0.454	0.453±0.001	64.18
3.9	0.782	0.781	0.78	0.781±0.001	38.26
1.95	0.991	0.991	0.991	0.991±0.000	21.66

Table 6: DPPH antioxidant activity and %inhibition of leaf extracts of GPLH, GPLE and GPLM with 0.518, 0.333 and 0.389 as absorbance of control, respectively

Antioxidant activity of GPLH					
Conc.(µg/mL)	Absorbance	Absorbance	Absorbance	Mean absorbance	% Inhibition
1000	0.231	0.234	0.232	0.232±0.0015	55.21
500	0.215	0.212	0.215	0.214±0.0017	58.69
250	0.224	0.225	0.222	0.224±0.0015	56.76
125	0.238	0.240	0.242	0.240±0.0020	53.67
62.5	0.325	0.327	0.324	0.325±0.0015	37.26
31.25	0.316	0.318	0.316	0.317±0.0012	38.80
15.62	0.353	0.352	0.352	0.328±0.0015	36.68
7.8	0.328	0.328	0.329	0.334±0.0006	35.52
3.9	0.335	0.334	0.335	0.334±0.0006	35.52
1.95	0.337	0.335	0.335	0336±0.0012	35.10
Antioxidant activity of GPLE					
Conc.(µg/mL)	Absorbance	Absorbance	Absorbance	Mean absorbance	% Inhibition
1000	0.292	0.292	0.294	0.293±0.0012	12.01
500	0.224	0.224	0.225	0.224±0.0006	32.73
250	0.226	0.224	0.223	0.224±0.0015	32.73
125	0.225	0.222	0.223	0.223±0.0015	33.03
62.5	0.219	0.221	0.218	0.219±0.0015	34.23
31.25	0.226	0.226	0.228	0.226±0.0012	32.13
15.62	0.227	0.227	0.228	0.227±0.0006	31.83
7.8	0.223	0.222	0.223	0.223±0.0006	33.03
3.9	0.228	0.228	0.228	0.228±0	31.53
1.95	0.229	0.229	0.232	0.230±0.0017	30.93
Antioxidant activity of GPLM					
Conc.(µg/mL)	Absorbance	Absorbance	Absorbance	Mean absorbance	% Inhibition
1000	0.132	0.134	0.133	0.133±0.0010	65.81
500	0.085	0.087	0.088	0.087±0.0015	77.63
250	0.114	0.115	0.114	0.114±0.0006	70.69
125	0.140	0.139	0.138	0.139±0.0010	64.27
62.5	0.172	0.169	0.170	0.170±0.0015	56. 30
31.25	0.180	0.181	0.180	0.180±0.0006	53.70
15.62	0.187	0.189	0.188	0.188±0.0012	51.67
7.8	0.198	0.197	0.198	0.198±0.0010	49.10
3.9	0.208	0.208	0.208	0.208±0	46.53
1.95	0.207	0.207	0.206	0.206±0.0012	47.04

standard antioxidant. Methanol extract of the plant showed significant inhibition of DPPH radicals at concentrations in the range of 1.95 to 500 µg/mL, by scavenging the free radicals with IC_{50} of 10.26 µg/mL, the activity was comparable with that of the control (IC_{50} 5.27 µg/mL). Hexane extract showed antioxidant activity with IC_{50} of 111.26 µg/mL (Fig. 1). Ethyl acetate of *G. pubescens* leaves revealed low inhibition of DPPH radicals (Table 5 & 6). In Gas chromatograph-Mass spectroscopy (GC-MS) analysis, seven, three and six compounds were detected in the hexane, ethyl acetate and methanol extracts of *G. pubescens* leaves respectively. The characterisation of constituents of the hexane extract of *G. pubescens* leaves by using GC-MS gave hexadecane (% abundance: 1.98), tridecane which has % abundance of 1.81, octadec-9-yne (22.48%), 7-hexadecyn-1-ol (4.94%), hexadec-1-yne (9.28%), 13-methyl methyl pentadecanoate (11.58%), and the principal constituent of the extract, hexadecanoic acid (47.92%). The fragment ions 227, 213 and 185 confirmed the molecular formula $C_{16}H_{32}O_2$ (256.42) of hexadecanoic acid (Table 7). The GC-MS analysis of ethyl acetate extract of the plant afforded five peaks where four of the peaks revealed the same constituent, 3,7,11,15-tetramethyl-2-hexadecen-1-ol (Phytol) with total percentage of abundance of 46.79.

Table 7: GC-MS analysis and the activity of hexane extract of *G. pubescens* leaves

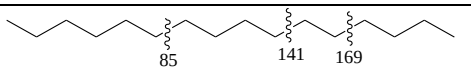
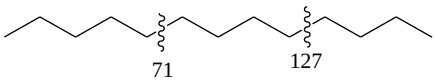
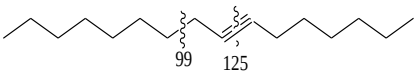
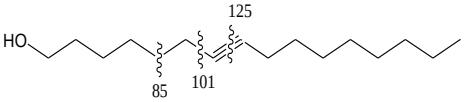
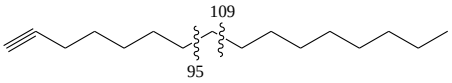
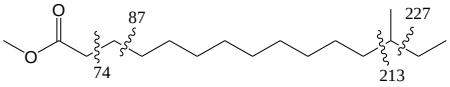
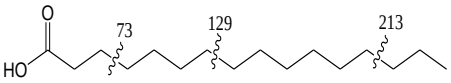
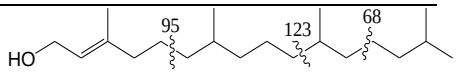
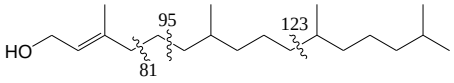
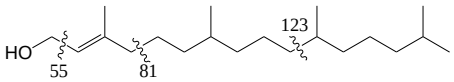
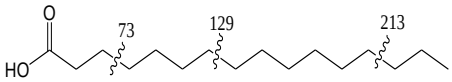
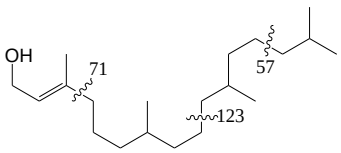
S/N	Compound	Peak area %	Molecular Formula	MW	Mass spectral fragments	Retention Time (min)	Fragmented structures
1	Hexadecane	1.98	$C_{16}H_{34}$	226.45	169, 141, 85	18.22	
2	Tridecane	1.81	$C_{13}H_{28}$	184.20	127, 93, 71	21.01	
3	9-octadecyne	22.48	$C_{18}H_{34}$	250.46	125, 99, 85	24.72	
4	7-hexadecyn-1-ol	4.94	$C_{16}H_{30}O$	238.41	161, 125, 101, 85	25.35	
5	1-hexadecyne	9.28	$C_{16}H_{30}$	222.41	193, 109, 95	26.91	
6	13-methyl methyl pentadecanoate	11.58	$C_{17}H_{34}O_2$	270.45	270, 227, 213, 185, 87, 74	26.91	
6	Hexadecanoic acid	47.92	$C_{16}H_{32}O_2$	256.42	227, 213, 185, 73, 60	28.18	

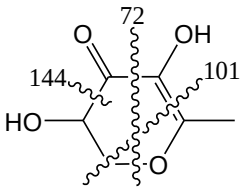
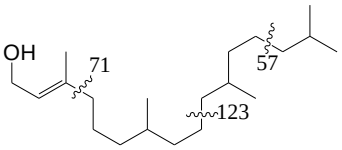
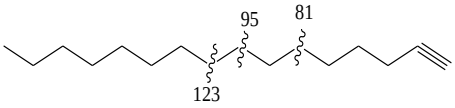
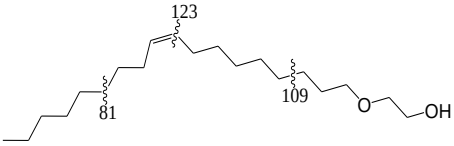
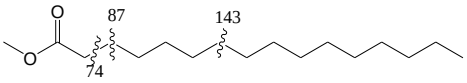
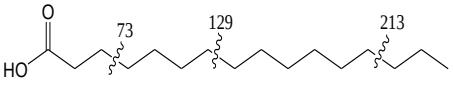
Table 8: GC-MS analysis and the activity of ethyl acetate extract of *G. pubescens* leaves

S/N	Compound	Peak area %	Molecular Formula	MW	Mass spectral fragments	Retention Time (min)	Fragmented structures
1	3,7,11,15-tetramethyl-2-hexadecen-1-ol	29.80	$C_{20}H_{40}O$	296	57, 68, 82, 95, 123	24.76	
2	3,7,11,15-tetramethyl-2-hexadecen-1-ol	5.11	$C_{20}H_{40}O$	296	57, 68, 81, 95, 123	25.38	
3	3,7,11,15-tetramethyl-2-hexadecen-1-ol	8.74	$C_{20}H_{40}O$	296	55, 81, 95, 123, 278	25.82	
4	Palmitic acid	53.20	$C_{16}H_{32}O_2$	288	73, 129, 213	28.44	
5	Phytol	3.14	$C_{20}H_{40}O$	296	57, 71, 123	31.40	

The fragment ions 57, 68, 71, 81, 82, 95, 123 and 278 correspond to a molecular formula $C_{20}H_{40}O_2$ (296).

The major compound of the extract was Palmitic acid (Hexadecanoic acid) with abundance of 53.20%. The fragment and pseudo ions 73, 129, 213 and 288 [M] correspond to their respective molecular formula -$C_3H_5O_2$, -$C_7H_{13}O_2$, -$C_{13}H_{25}O_2$ and $C_{16}H_{32}O_2$. The fragmented structures and retention time were shown in Table 8.

Table 9: GC-MS analysis and the activity of the methanol extract of *G. pubescens* leaves

S/N	Compound	Peak area %	Molecular Formula	MW	Mass spectral fragments	Retention Time (min)	Fragmented structures
1	2,3-Dihydro-3,5-dihydroxy-6-methyl 4(H)-pyran-4-one	2.83	$C_6H_8O_4$	144.13	61, 72, 101, 115, 144	24.76	
2	Phytol	16.37	$C_{20}H_{40}O$	296.53	57, 68, 81, 95, 123, 137	25.38	
3	1-Hexadecyne	3.44	$C_{16}H_{30}$	222.41	57, 69, 81, 95, 109, 123, 137, 278	25.82	
4	2-octadec-9-enyloxy-ethanol	6.00	$C_{20}H_{40}O_2$	312.53	81, 95, 123	28.44	
5	Methyl Hexadecanoate	5.91	$C_{17}H_{34}O_2$	274.04	55, 73, 87, 143, 227	31.40	
6	Hexadecanoic acid	61.59	$C_{16}H_{32}O_2$	256.42	73, 129, 213	28.18	

The GC-MS analysis of methanol extract of *Grewia pubescens* leaves revealed the presence of 2,3-Dihydro-3,5-dihydroxy-6-methyl 4(H)-pyran-4-one (2.83%), the prominent peaks are 72, 101 and 144 with molecular formular -$C_3H_4O_2$, -$C_4H_5O_3$ and -$C_6H_8O_4$ respectively. The compound has been reported to possess anti-inflammatory and anti-arthritic properties (Mythili et al., 2013). Phytol (16.37%) showed fragment ions at 68, 95 and 123 with molecular formular -C_4H_7O, -$C_6H_{11}O$

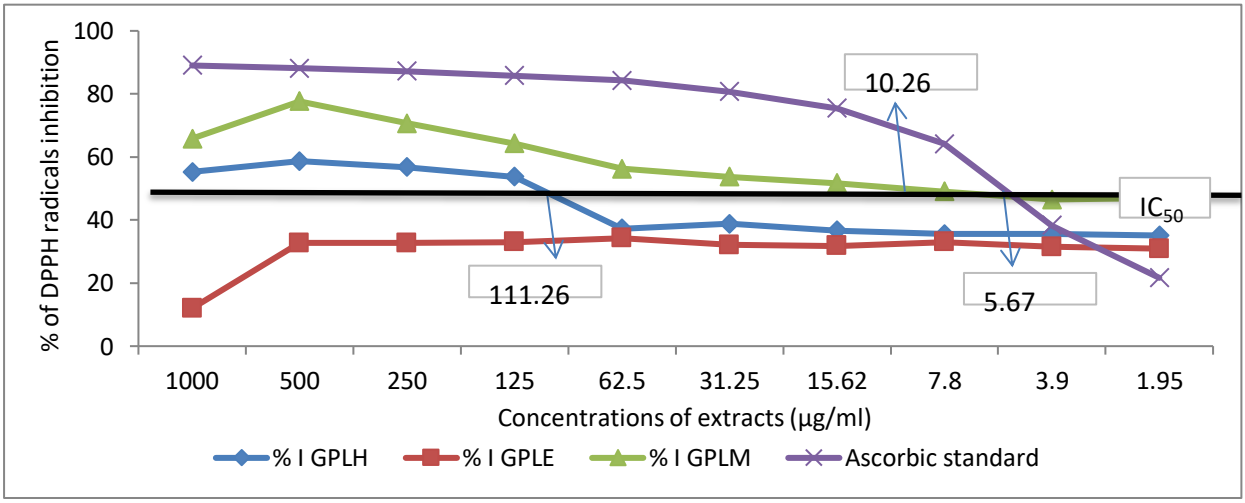


Fig. 1: Evaluation of antioxidant activity of crude extracts of the leaves of *G. pubescens*

and -C_9H_{19}, while 1-hexadecyne (3.44%) was also obtained with its fragment ions at 81, 95 and 123, which correspond to molecular formular -C_6H_9, -C_7H_{11} and -C_9H_{19} respectively. Other compounds analysed by GC-MS are: 2-octadec-9-enyloxy-ethanol (6.00%) which has prominent peaks of 81, 109 and 123 with their respective molecular formular as -C_6H_{13}, -$C_5H_{11}O_2$ and -C_9H_{18}, methyl hexadecanoate (5.91%) with fragment peaks at 74, 87 and 143 with their molecular formular as -$C_3H_5O_2$, -$C_4H_7O_2$ and -$C_8H_{15}O_2$ respectively, and the principal constituent, hexadecanoic acid (61.59%) showed fragment and pseudo ions 73, 129, 213 and 256 [M] which their respective molecular formular as -$C_3H_5O_2$, -$C_7H_{13}O_2$, -$C_{13}H_{25}O_2$ and $C_{16}H_{32}O_2$ as shown in Table 9.

CONCLUSIONS

The study has shown that the leaf extracts of *G. pubescens* have active ingredients or bioactive compounds which are able to inhibit pathogens. The observed antimicrobial efficacy of this medicinal plant on the tested organisms may be attributed to the presence of the most abundant bioactive compounds present in synergy with all other compounds present in relatively small amounts. This accounted for the use of the plant according to f folklore for the treatment of toothache, stomach disorder and other pathogenic infections. This plant may be a potential source of novel antimicrobial drugs.

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