Chemical Composition, Antibacterial and Antifungal Activities of *Anthocleista vogelii* Planch Root Bark Extracts

Chemical Composition, Antibacterial and Antifungal Activities of *Anthocleista vogelii* Planch Root Bark Extracts

Anyanwu, P. I.[1], Tarfa, F. D.[2], Shamaki, D. E.[3], and Anyanwu, G. O.[3*]

[1]Department of Community Health and Wellbeing, University of Chester, Chester, UK.
[2]Department of Medicinal Chemistry and Quality Control, National Institute for Pharmaceutical Research and Development (NIPRD), Idu Industrial Area, Abuja, Nigeria
[3]Department of Biochemistry, Bingham University, Karu, Nasarawa State, Nigeria.

Correspondence: gabrielanyanwu@binghamuni.edu.ng

ABSTRACT
This study sought to investigate the chemical composition, antibacterial and antifungal activities of ethanol extract and fractions of the root bark of *Anthocleista vogelii* Planch. The GCMS analysis of the n-hexane fraction (nHF) of *A. vogelii* revealed the presence of 12 volatile compounds. While the classes of compounds profiled in terpenoid rich fraction (TRF) using LCMS were majorly terpenoids (tarennoside, 3-Hydroxybenzaldehyde, 12-hydroxyjasmonic acid, 5-megastigmen-7-yne-3,9-diol 9-glucoside, gibberellin A2 O-beta-D-glucoside, 15-Acetoxyscirpene-3,4-diol 4-O-a-D-glucopyranoside), organic/carboxylic acids and fatty acids. The results revealed good antibacterial activity of ethanol extract (EEx) against *S. aureus* at MIC = 2 mg/mL, but moderate activity by TRF (MIC = 8 mg/mL) although, the TRF had better antibacterial activity than EEX against *E. coli* (MIC = 8 mg/mL) and *S. paratyphi* (MIC = 8 mg/mL). The TRF had similar antifungal activity with EEX against *T. rubrum* (MIC = 4 mg/mL) and *T. mentagrophytes* (MIC = 8 mg/mL), but displayed better activity against *C. albicans* (MIC = 4 mg/mL). The nHF showed no antibacterial or antifungal activities. The antimicrobial activities of extract (EEX) and fraction (TRF) of *A. vogelii* root back validates its use in traditional medicine for treatment of typhoid, urinary tract infection, food poisoning, diarrhea, stomach aches and skin diseases.

Keywords: *Anthocleista vogelii*, antibacterial, antifungal, GCMS, LCMS, terpenoids

1. INTRODUCTION
Basic bacterial and fungal infections remains a challenge in developing countries even though incidence of some infections could be prevented by maintaining high standards of hygiene. According to WHO (2004), mortality rate due to infectious diarrhoea could be as high as 56% in developing countries. Diarrhea can be caused by bacterial organisms, such as *Escherichia coli*, *Campylobacter* spp., *Shigella* spp. and *Salmonella* spp. There are far more infectious diseases caused by bacterial organisms such as *E.coli* (urinary tract infection and food poisoning), *Staphylococcus aureus* (food poisoning, impetigo cellulitis, boils, abscesses, wound infections, pneumonia and toxic shock syndrome), *Salmonella typhi* (typhoid, vomiting and diarrhea) and *Salmonella paratyphi* (paratyphoid) to mention a few of interest in this study.

In Africa, clinically diagnosed skin diseases are commonly caused by fungal infections (Havlickova *et al.*, 2008), and fungal infections significantly contribute to human morbidity and mortality (Brown *et al.*, 2012). *Trichophyton rubrum* and *Trichophyton mentagrophytes*

are among the causative agents for fungal skin infections like tinea cruris (ringworm of the groin), tinea pedis (athlete's foot), tinea unguium (onychomycosis, nail infections), while *Candida albicans* is the major causative agent of various forms of candidiasis. In Sub-Saharan Africa, the overall incidence of tinea was estimated to be 78 million in 2005 (Hay *et al.*, 2006). A study revealed *T. mentagrophytes* and *T. rubrum* in second and third positions respectively out of 5 fungal causative agents of mycotic infections among school children in Ekpoma, Nigeria (Enweani *et al.*, 1996).

Traditional healers in Nigeria use various herbal preparations to manage/treat a diversity of diseases, including many microbial infections, such as diarrhea, sore throat and gonorrhea (Olukoya, 1993). Medicinal plants are used by a relatively large proportion of the population due to non-availability of orthodox medicines in rural settlements (Olukoya, 1993), poverty, high cost of antimicrobial drugs, traditional practice handed-over from older generations, and availability of medicinal plants in the immediate environment.

The investigated medicinal plant, *Anthocleista vogelii* Planch has been extensively reviewed under the *Anthocleista* species (Anyanwu *et al.*, 2015). The bark, root and seed of *A. vogelii* are used as traditional medicines for the treatment of stomach troubles, wounds, inflammations and venereal diseases (Burkill, 1985; Jiofack *et al.*, 2010). The leaves of *A. vogelii* are used in Igala traditional medicine for the treatment of typhoid (Musa *et al.*, 2010), while the roots are traditionally used for treating throat problems (Omobuwajo *et al.*, 2008). Few studies have validated the antibacterial properties of crude extracts of the leaves and stem bark of *A. vogelii* (Olukoya *et al.*, 1993; Musa *et al.*, 2010) and little or no visible work has been done on the antifungal properties of the plant. This study sought to investigate the chemical composition, antibacterial and antifungal activities of ethanol extract and fractions of the root bark of *A. vogelii*.

2. MATERIALS AND METHODS

2.1 Collection of plant material

Fresh plant material was collected from Ovuakali, Ngor-okpala, Imo State, Nigeria. The plant was identified and authenticated by Dr. Jerome Ihuma of the Biological Sciences Department, Bingham University. Voucher specimen (GA134-7421) for *Anthocleista vogelii* Planch was deposited in the Department of Biological Sciences, Bingham University, Nigeria.

2.2 Preparation of extract and fractions

The fresh root bark of *A. vogelii* was shade-dried and ground to powder form. The plant powder (1.5kg) was extracted with absolute ethanol using Soxhlet extractor for 48h in batches. The solution was filtered with Whatman No. 1 filter paper and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40^{o}C to give ethanol extract (EEX). A portion of EEX was dissolved in n-hexane solvent for 12 hr three times, then filtered and filtrate was concentrated to give n-hexane fraction (nHF). The marc was extracted with acidified water (2 M H_2SO_4) and partitioned with chloroform three times (3 x 150ml) using a separating funnel (Harborne, 1998). The partitions of chloroform were combined and concentrated to yield terpenoid rich fraction (TRF).

2.3 Gas chromatography mass spectrometry (GCMS) analysis of fractions of A. vogelii

The GCMS analysis of n-hexane fraction of *A. vogelii* was done using Agilent GC 7890B, MS detector MSD 5977A (Agilent Technologies, USA) which is equipped with a library software (MassHunter: NIST 14.L software). Briefly, the GCMS detection involved an electron ionization system with 70 eV ionization energy and Helium gas as the carrier gas at

1 mL/min constant flow rate. The inlet temperature was fixed at 250 ^{o}C, while oven temperature was set at 100 ^{o}C for 1.5 min and then raised to 270 ^{o}C at the rate of 5 ^{o}C per min. Exactly 1 mL of diluted samples were injected and scan range was selected as 40–600.

2.4 Analysis of Terpenoid Rich Fraction (TRF) of A. vogelii by Liquid Chromatography Mass Spectrometry (LCMS-MS)

Sample of TRF was diluted 10X with methanol and analyzed using Agilent 1290 Infinity LC system coupled to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source. The solvents 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) were used with injection volume of 1.0uL. A column of Agilent Zorbax Eclipse XDB-C18, 2.1x150mm narrow-bore, 3.5 micron and 25^{0}C column temperature and 23^{0}C autosampler temperature at 0.5 mL per min flow rate were used for the separation. The ESI-MS analysis for TRF sample was run in negative polarity for 25 mins and 5 mins as post run time, mass range was 100 – 3200 m/z, and 119.03632 and 966.000725 reference ions were used. Agilent MassHunter Qualitative Analysis B.05.00 (Method: Metabolomics-July2015.m) was used to process the data.

2.5 Collection of Microbial Isolates

Typed isolates of the following microorganisms: *Escherichia coli* ATCC 25952, *Candida albicans* ATCC 2978, *Salmonella paratyphi* ATCC 9150, *Trichophyton rubrum* ATCC 28188, *Trichophyton mentagrophytes* ATCC 9533 and *Staphylococcus aureus* ATCC 25923 were obtained from the culture bank of the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.

2.6 Preparation of inoculum

Bacteria isolates were cultured on Mueller Hinton agar (MHA) plates at 37°c for 24hrs and fungi isolate were cultured on Saboraud dextrose agar (SDA) plate at 37°c for 48hrs and were sub-cultured into Muller Hinton broth (MHB) or Saboraud dextrose broth (SDB). All the media were prepared according to the manufacturer's instruction.

2.7 Minimum Inhibitory concentration determination (MIC)

The micro dilution method according to Shanmugapriya *et al.,* (2012) was employed. The 96-microtiter well was prepared by dispensing 50 μL of SDB (fungi) and MHB (bacteria) and left for 15 minutes before adding 5 μL of the bacterial or fungal suspension into each well. One hundred microlitres from the stock solution of extracts was added into the first well, then followed by two fold serial dilution down the remaining wells. The concentration of the extract/fractions were 16, 8, 4, 2, 1, 0.5, 0.25, 0 mg/mL. The last row of wells did not contain the extract thus serving as organism viability control. The plate was shaken for 20 seconds and then incubated at 37 ^{o}C for 24 h (bacteria) and 30 ^{o}C for 48 hours (fungi). After incubation, the plates were observed for the absence or presence of growth. The bioassay was performed in triplicate. MIC was the lowest concentration of the extract/fraction showing no turbidity after incubation, where the turbidity was interpreted as visible growth of the micoorganisms.

3. RESULTS

3.1 GCMS analysis of hexane fraction (HF) of A. vogelii

The n-hexane fraction of *A. vogelii* revealed the presence of twelve (12) volatile compounds (Table 1). The major compounds includes: D:C-Friedours-7-en-3-one (31.42%), 7-pentadecyne (13.40%), hexadecanoic acid (9.33%), pentadecafluorooctanoic acid (7.91%), and n-Propyl 9-octadecenoate (7.15%). Although, there are relatively about seven (7) minor

compounds, octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl- (0.86%) is the least in quantity in the n-hexane fraction of *A. vogelii*.

3.2 LCMS/MS analysis of Terpenoid Rich Fraction (TRF) of A. vogelii

The results of the LCMS analysis of TRF of *A. vogelii* revealed the presence of 30 compounds of which 21 compounds were identified and 11 compounds are unknown (Table 2). Based on the classes of compounds in TRF profiled using LCMS, terpenoids (tarennoside, 3-Hydroxybenzaldehyde, 12-hydroxyjasmonic acid, 5-megastigmen-7-yne-3,9-diol 9-glucoside, gibberellin A2 O-beta-D-glucoside, 15-Acetoxyscirpene-3,4-diol 4-O-a-D-glucopyranoside), fatty acids (3-Hydroxydodecanedioic acid, 4R-hydroxy-octanoic acid, 8-oxo-nonanoic acid), organic/carboxylic acids (malic acid, glutaconic acid, 2,3-dihydroxy-p-cumate, suberic acid, 2-hydroxy-6-oxo-7-methylocta-2,4-dienoate), alcohol (1-(3,4-Dimethoxyphenyl) ethane-1,2-diol), alkaloid (theobromine), lactone (alpha-Carboxy-delta-decalactone), Pyrazols (endo-1-methyl-N-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-N-oxide), and dithiole (3H-1,2-Dithiole-3-thione) were found. The total compound chromatogram (TCC) of LCMS analysis of TRF of *A. vogelii* showing the peak intensity and the retention time of each compound is shown on Fig. 1.

Table 1. GCMS analysis of n-hexane fraction (nHF) of *A. vogelii*

SN	RT	Compounds in saponin fraction	Area (%)	Mass
1	33.677	Hexadecanoic acid, methyl ester	9.33	270
2	37.207	9-Octadecenoic acid (Z)-,methyl ester	5.46	296
3	40.546	Hexadecanoic acid, pentyl ester	4.52	326
4	40.827	Pentadecafluorooctanoic acid, octadecyl ester	7.91	648
5	41.933	4,8,12,16-Tetramethylheptadecan-4-olide	6.36	324
6	43.574	n-Propyl 9-octadecenoate	7.15	324
7	43.914	7-Pentadecyne	13.40	208
8	46.655	3-Diazo-1-methyl-1,3-dihydro-indol-2-one	5.54	173
9	48.758	D:C-Friedours-7-en-3-one	31.42	424
10	49.029	Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-	0.86	563
11	49.355	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	4.36	342
12	50.236	Nonacos-1-ene	3.70	406

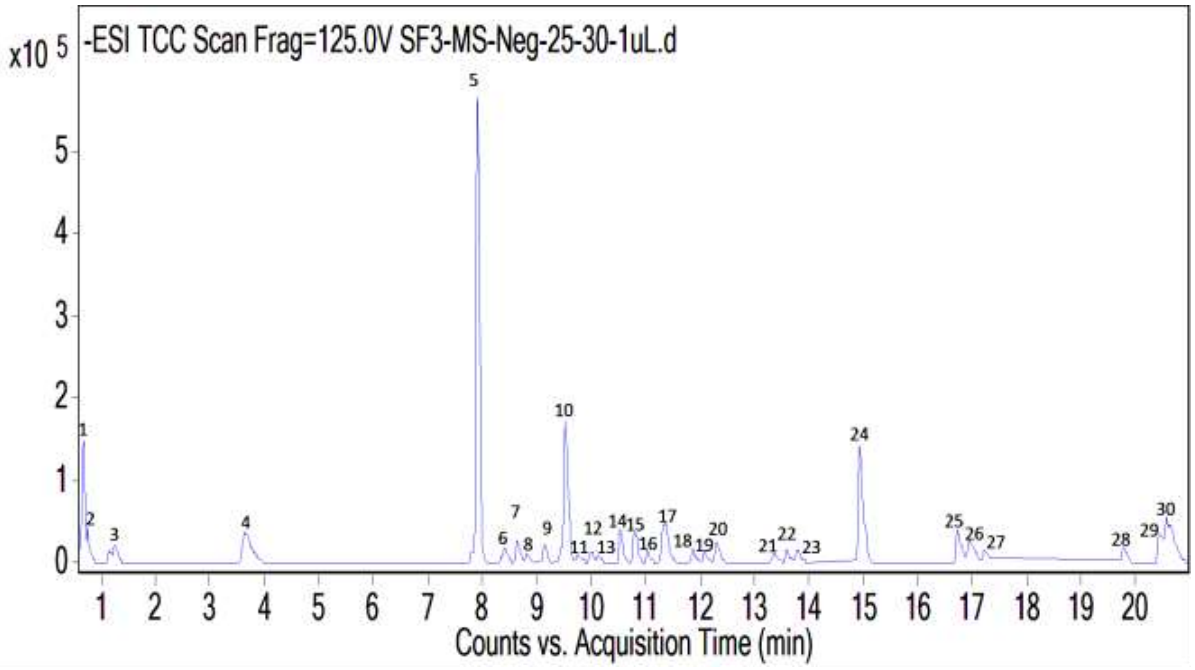

Fig. 1. Total Compound Chromatogram (TCC) of LCMS analysis of TRF of *A. vogelii*

Table 2. Compounds results of the LCMS analysis of TRF of *A. vogelii*

Peak	RT	Mass	Name	Formula	Class
1	0.68	180.06	Theobromine	C7 H8 N4 O2	Alkaloid
2	0.76	134.02	D-(+)-Malic acid	C4 H6 O5	Organic compound
3	1.26	130.03	Glutaconic acid	C5 H6 O4	Dicarboxylic acid
4	3.66	246.11	Unknown	C11 H18 O6	--
5	7.91	358.13	Tarennoside	C16 H22 O9	Iridoid monoterpenoid
6	8.42	196.07	2,3-Dihydroxy-p-cumate	C10 H12 O4	Carboxylic acid anion
7	8.63	174.09	Suberic acid	C8 H14 O4	Dicarboxylic acid
8	8.82	198.09	1-(3,4-Dimethoxyphenyl) ethane-1,2-diol	C10 H14 O4	Alcohol
9	9.15	262.14	Unknown	C13 H18 N4 O2	--
10	9.46	122.04	3-Hydroxybenzaldehyde	C7 H6 O2	Terpenoid
11	9.77	184.07	2-Hydroxy-6-oxo-7-methylocta-2,4-dienoate	C9 H12 O4	Carboxylic acid
12	10.01	246.15	3-Hydroxydodecanedioic acid	C12 H22 O5	Fatty acid
13	10.13	160.11	4R-hydroxy-octanoic acid	C8 H16 O3	Fatty acid
14	10.54	172.11	8-oxo-nonanoic acid	C9 H16 O3	Fatty acid/ Terpenoid
15	10.79	226.12	12-hydroxyjasmonic acid	C12 H18 O4	Terpenoid
16	11.04	258.14	Unknown	C9 H18 N6 O3	--
17	11.34	370.20	5-Megastigmen-7-yne-3,9-diol 9-glucoside	C19 H30 O7	Terpenoid
18	11.87	512.23	gibberellin A2 O-beta-D-glucoside	C25 H36 O11	Isoprenoids/Diterpenes
19	12.08	588.24	Unknown	C27 H40 O14	--
20	12.31	214.12	alpha-Carboxy-delta-decalactone	C11 H18 O4	Delta valerolactones
21	13.37	430.22	Unknown	C15 H30 N10	--

				O3 S	
22	13.60	486.21	15-Acetoxyscirpene-3,4-diol 4-O-a-D-glucopyranoside	C23 H34 O11	Trichothecenes/ sesquiterpene
23	13.79	572.25	Unknown	C27 H40 O13	--
24	14.94	328.19	endo-1-methyl-N-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-N-oxide	C18 H24 N4 O2	Pyrazols
25	16.74	298.22	Unknown	C15 H30 N4 S	--
26	16.95	294.22	Unknown	C18 H30 O3	--
27	17.23	294.22	Unknown	C18 H30 O3	--
28	19.79	280.24	Unknown	C18 H32 O2	--
29	20.58	356.17	Unknown	C19 H24 N4 O S	--
30	20.69	133.93	3H-1,2-Dithiole-3-thione	C3 H2 S3	Dithioles

Antimicrobial Studies

The antimicrobial activities of *A. vogelii* and the reference drug presented as MIC are represented on Table 3. The ethanol extract of *A. vogelii* (EEX) revealed relatively low antibacterial activity against *E. coli* (MIC = 16mg/mL) and *S. paratyphi* (MIC = 16mg/mL), but good antibacterial activity against *S. aureus* (MIC = 2mg/mL) compared to the antibacterial reference for each microorganism. The terpenoid rich fraction of *A. vogelii* (TRF) exhibited comparably moderate activity against *E. coli*, *S. aureus* and *S. paratyphi* with MIC value of 8 mg/mL with respect to the antibacterial reference for each microorganism. The nHx fraction of *A. vogelii* (nHx) showed no activity against all the bacterial and fungal species used in this study.

On the other hand, EEX of *A. vogelii* displayed moderate antifungal activity against *C. albicans* (MIC = 8mg/mL) and *T. mentagrophytes* (MIC = 8mg/mL), but relatively good activity against *T. rubrum* (MIC = 4mg/mL) when compared to the antifungal reference for each microorganism. The TRF had moderate antifungal activity against *T. mentagrophytes* (MIC = 8 mg/mL), but good activity against *T. rubrum* (MIC = 4 mg/mL) and *C. albicans* (MIC = 4mg/mL) compared to antifungal reference for each microorganism.

Table 3: MIC determination of extracts against test microorganisms

Microorganisms	**MIC (mg/mL)**			
	EEX	**nHx**	**CFL**	**Control**
Bacteria				
E. coli	16.0	NA	8.0	0.078
S. aureus	2.0	NA	8.0	0.010
S. paratyphi	16.0	NA	8.0	0.125
Fungi				
C. albicans	8.0	NA	4.0	0.039
T. rubrum	4.0	NA	4.0	0.078
T. mentagrophytes	8.0	NA	8.0	0.078

Note: Reference drugs- chloramphenicol (bacteria) and terbinafine (fungi); NA- no activity, which is a value >16mg.

4. DISCUSSION

The groups of phytochemicals found in the leaf, stem-bark and root bark extracts of *A. vogelii* were reported to include alkaloid, carbohydrates, saponins, flavonoids, tannin, terpenes,

steroids and phenols (Jegede *et al.*, 2011; Anyanwu *et al.*, 2013). This study gives insight to the specific compounds and their classes found in the nHF (Table 1) and TRF (Table 2) from the root bark of *A. vogelii*. The classes of compounds identified in the TRF were majorly of terpenoids, organic/carboxylic acids and fatty acids. Several studies have revealed the antimicrobial effects of terpenoids (Singh and Singh, 2003; Popova et al., 2009; Kurekci et al., 2013) which influenced our guided fraction to give TRF which contains more terpenoids including tarennoside, 3-Hydroxybenzaldehyde, 12-hydroxyjasmonic acid, 5-megastigmen-7-yne-3,9-diol 9-glucoside, gibberellin A2 O-beta-D-glucoside and 15-Acetoxyscirpene-3,4-diol 4-O-a-D-glucopyranoside.

Specifically, compounds found in TRF of *A. vogelii* have been reported to demonstrate antimicrobial activities such as: theobromine (Lakshmi and Sharmin, 2016; Nidhi *et al.*, 2015), malic acid (Rathnayaka , 2013; Gao, *et al.*, 2012), glutaconic acid (Selk *et al.*, 1982), Suberic acid (Gołebiowski *et al.*, 2015), delta valerolactones (Ferdosian and Sardari, 2013), 3H-1,2-Dithiole-3-thione (Giannini *et al.*, 2004). However, the compounds in TRF appeared to have acted synergistically to exhibit its antimicrobial activity.

Our study revealed good antibacterial activity of ethanol extract against *S. aureus* at MIC = 2 mg/mL, but moderate activity by TRF and no activity by nHex fractions of *A. vogelii*. The TRF had better antibacterial activity than EEX against *E. coli* and *S. paratyphi*, which indicated that fractionation of extracts might have led to increased concentration of active principles in the fraction. Although, Olukoya *et al.* (1993) reported no activity for ethanol extracts of *A. vogelii* against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium*, the extract concentration used in the study was undefined, hence it is difficult to compare with this study. However, in corroboration with this study, Musa *et al.* (2010) had reported antibacterial activities for ethanol, aqueous and chloroform leaf extracts of *A. vogelii* against *S. typhi* corroborating its traditional medicinal use in the treatment of typhoid fever.

The TRF had similar antifungal activity with EEX against *T. rubrum* and *T. mentagrophytes*, but displayed better activity against *C. albicans*. Although, we found no studies on the antifungal activities of extracts of *A. vogelii*, except by Tene *et al.* (2008) on the antifungal activities of xanthone compounds (1-hydroxy-3,7-dimethoxyxanthone and 1-hydroxy-3,7,8-trimethoxyxanthone) isolated from *A. vogelii* stem bark against *Candida parapsilosis*. The nHF showed no antibacterial or antifungal activities indicating that the active principles necessary for activities were absent in the fraction.

5. CONCLUSION

The antibacterial activities of extract (EEX) and fraction (TRF) of *A. vogelii* root back against *E. coli, S. aureus* and *S. paratyphi* validates its use in traditional medicine for the treatment of diarrhea, sore throat, and stomach aches. Also, the antifungal activities of the EEX and TRF of *A. vogelii* provides scientific credence to its traditional use against wounds and skin diseases. The overall display of better antimicrobial activities by TRF of *A. vogelii* compared EEX might due to the increased concentration of active principles in fraction. Thus further studies on elucidating the bioactive compounds in *A. vogelii* and screening for their antimicrobial activities is necessary.

REFERENCES

[1] Anyanwu, G.O., Onyeneke, E.C., Usunobun, U., and Adegbegi, A.J. (2013). Impact of *Anthocleista vogelii* root bark ethanolic extract on weight reduction in high carbohydrate diet induced obesity in male wistar rats. African J. Biochem. Res.. 7: 225–232.

[2] Anyanwu, G.O., Ur-Rehman, N., Onyeneke, E.C. and Rauf, K. (2015). Medicinal plants of the genus Anthocleista- a review of their ethnobotany, phytochemistry and pharmacology. J. Ethnopharmacol. 175:648-67.
[3] Brown, G. D., Denning, D. W., Gow, N. A. R., Levitz, S. M., Netea, M. G., and White, T. C. (2012). Hidden killers: Human fungal infections. Sci. Transl. Med., 4: 165rv13, 1-9.
[4] Burkill, H.M., 1985. *Anthocleista vogelii* Planch. [family LOGANIACEAE], The useful plants of west tropical Africa. Royal Botanic Gardens, Kew (K).
[5] Enweani, I.B., Ozan, C.C., and Agbonlahor, D.E. (1996). Dermatophytosis in school children in Ekpoma, Nigeria. Mycoses 39: 303–5.
[6] Ferdosian, M. and Sardari, S. (2013). A Novel Mechanistic Approach to Identify New Antifungal Lead Compounds Based on Amphotericin B Molecular Architecture. Trop J Pharm Res, 12 (2): 181-188.
[7] Gao, Z., Shao, J., Sun, H., Zhong, W., Zhuang, W. and Zhang, Z. (2012). Evaluation of different kinds of organic acids and their antibacterial activity in Japanese Apricot fruits. Afr. J. Agric. Res., 7(35): 4911-4918.
[8] Giannini, F.A, Aimar, M.L., Sortino, M., Gomez, R., Sturniollo, A., Juarez, A., Zacchino, S., de Rossi R.H, and Enriz, R.D. (2004). In vitro–in vivo antifungal evaluation and structure–activity relationships of 3H-1,2-dithiole-3-thione derivatives. IL FARMACO, 59: 245–254.
[9] Gołębiowski, M., Cerkowniaka, M., Urbanek, A., Dawgul, M., Kamyszc, W., Bogus, M.I., and Stepnowski P. (2015). Identification and antifungal activity of novel organic compounds found in cuticular and internal lipids of medically important flies. Microbiological Research 170, 213–222.
[10] Harborne, J.B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis*. 3rd ed. London: Chapman and Hall. Pp 279.
[11] Havlickova, B., Czaika, V. A., and Friedrich, M. (2008). Epidemiological trends in skin mycoses worldwide. Mycoses, 51 (Suppl. 4): 2–15.
[12] Hay, R, Bendeck SE, Chen S *et al*. Skin Diseases. In: Disease Control Priorities in Developing Countries, 2nd edn. New York: Oxford University Press, 2006: 707–22.
[13] Jegede, I.A., Ibrahim, J.A., and Kunle, O.F., 2011. Phytochemical and pharmacognostic studies of the leaf and stem-bark of *Anthocleista vogelii* (Planch). J. Med. Plants Res., 5: 6136–6139.
[14] Jiofack, T., Fokunang, C., Guedje, N., Kemeuze, V., Fongnzossie, E., Nkongmeneck, B.A., Mapongmetsem, P.M., and Tsabang, N., 2010. Ethnobotanical uses of medicinal plants of two ethnoecological regions of Cameroon. Int. J. Med. Med. Sci., 2: 60–79.
[15] Kurekci, C., Padmanabha, J., Bishop-Hurley, S. L., Hassan, E., Al Jassim, R.A.M, and McSweeney, C.S (2013). Antimicrobial activity of essential oils and five terpenoid compounds against *Campylobacter jejuni* in pure and mixed culture experiments. Intl J. Food Microbiol., 166: 450–457.
[16] Lakshmi, A. and Sharmin D.D. (2016), Comparative evaluation on antimicrobial activity of the obromine and two commercial kids fluoride toothpaste – An In vitro Study. Int J Dent & Oral Heal., 2(5): 52-56.
[17] Musa, A.D., Yusuf, G.O., Ojogbane, E.B., and Nwodo, O.F.C. (2010). Screening of eight plants used in folkloric medicine for the treatment of typhoid fever. *Journal of Chemical and Pharmaceutical Research* 2(4):7–15.
[18] Nidhi, S., Shreyan, D., Abhirup, D., Akash, R. C. and Jayanthi, A. (2015). Antimicrobial activity and cytotoxicity of *Theobroma cacao* extracts. Der Pharmacia Lettre, 7 (7):287-294.
[19] Olukoya, D.K., Idika, N., and Odugbemi, T. (1993). Antibacterial activity of some medicinal plants from Nigeria. J. Ethnopharmacol. 39: 69–72.
[20] Omobuwajo, O.R., Alade, G.O., and Sowemimo, A. (2008). Indigenous knowledge and practices of women herb sellers of south-western Nigeria. Indian J. Tradit. Knowl., 7 (3): 505–510.
[21] Popova, M.P., Chinou I. B., Marekov I. N., and Bankova V.S. (2009). Terpenes with antimicrobial activity from *Cretan propolis*. Phytochemistry 70, 1262–1271.

[22] Rathnayaka, R.M.U.S.K. (2013). Antibacterial effect of malic acid against *Listeria monocytogenes*, *Salmonella enteritidis* and *Escherichia coli* in mango, pineapple and papaya juices. American Journal of Food Technology, 8: 74-82.
[23] Selk, S. H., Pogány, S. A. and Higuchi, T. (1982). Comparative antimicrobial activity, *in vitro* and *in vivo*, of soft N-chloramine systems and chlorhexidine. Appl Environ Microbiol. 43(4): 899–904.
[24] Shanmugapriya, P., Suthagar P., Lee W.C., Roziahanim M. and Surash R. (2012). Determination of minimum inhibitory concentration of *Euphorbia hirta* (L.) extracts by tetrazolium microplate assay. Journal of Natural Products, 5:68-76.
[25] Singh, B. and Singh S. (2003). Antimicrobial activity of terpenoids from *Trichodesma amplexicaule* Roth. Phytother Res., 17(7):814-6.
[26] Tene, M., Tane, P., Kuiate, J.R., Tamokou, J.D. and Connolly, J.D.M. (2008). Anthocleistenolide: A new rearranged Nor-secoiridoid derivative from the stem bark of *Anthocleista vogelii*. Planta Med., 74: 80–83.
[27] World Health Organization. World health report 2004: changing history. Geneva: World
[28] Health Organization, 2004. p. 200.