FTIR and GC-MS Spectroscopic Analysis of Methanol and Chloroform Extracts of *Brenania brieyi* Root Bark

IFEOMA, F. ODO*., LAWRENCE, U. S. EZEANYIKA., VICTOR, N. OGUGUA., PARKER, E. JOSHUA. AND INNOCENT, U. OKAGU.

DEPARTMENT OF BIOCHEMISTRY, FACULTY OF BIOLOGICAL SCIENCES, UNIVERSITY OF NIGERIA, NSUKKA, ENUGU STATE, NIGERIA
*Corresponding author: odoifeoma1@gmail.com; okagu.innocent@yahoo.com

ABSTRACT

This study was aimed at the elucidation of the bioactive compounds present in methanol and chloroform extracts of Brenania brieyi root bark using Fourier transform infrared spectroscopic (FTIR) and Gas chromatography–mass spectrometry (GC-MS) techniques. The FTIR spectroscopic studies revealed the presence of these functional groups: amines, phenols, carboxylic acids, alcohols, alkenes, carbonyl, esters, and silica in the extracts. The GC-MS mass spectra of the identified compounds were compared with those of the National Institute of Standards and Technology database library. The results confirmed the presence of 16 compounds including pentadecanoic acid (17.04 %), 9, 12-hexadecanoic acid (10.18%), 9-Ocadecanoic acid (60.53 %) and octadecanoic acid (5.87 %) in methanol extract while hexadecanoic acid (21.23 %), 9-Ocadecanoic acid (54.85 %), 9, 12-Ocadecanoic acid (3.90) and Ocadecanoic acid (11.29 %) were found in chloroform extracts. The presence of these bioactive compounds in the plant offers a platform for the traditional use of B. brieyi in treatment of various diseases.

{**Citation:** Ifeoma, F. Odo; Lawrence, U. S. Ezeanyika; Victor, N. Ogugua; Parker, E. Joshua; and Innocent, U. Okagu. FTIR and GC-MS spectroscopic analysis of methanol and chloroform extracts of *Brenania brieyi* root bark. American Journal of Research Communication, 2017, 5(3): 44-54} www.usa-journals.com, ISSN: 2325-4076.

1. INTRODUCTION

Nigeria is endowed with a lot of medicinal plants used as alternate medicine for diseases of man and other animals since most of them are without side effects when compared with synthetic drugs (Anaduaka *et al*., 2013; Ashokkumar and Ramaswamy, 2014). According to Ogugua *et al*, (2013) medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Harnessing such plants could reduce expenditure on global drug

development while meeting patient's health needs (Habu and Ibeh, 2015). The analysis of these chemical constituents would help in determining various biological activities of plants. Presently, there are lot of approaches available to reach for new biologically active ingredients in medicinal plants for the preparation of safe drugs (Murugan and Mohan, 2014). A variety of techniques can be used to determine and estimate the presence of such phytocontituents in medicinal plants. Evaluation of plants by metabolomic fingerprinting can be accomplished by effective analytical tools such as HPLC with UV (DAD), ELSD, MS detection or GC-MS, HPTLC-densitometry, FT-MIR, NIR, NMR or a combination of these (Geethu *et al*., 2014). Spectroscopic and chromatography techniques are the most useful and popular tools used for this purpose (Saxena and Saxena, 2012 The Fourier transform infrared (FTIR) spectroscopy has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extracts (Maobe and Nyarango, 2013). The FTIR measures predominantly the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic "fingerprint" of the sample (Mariswamy *et al*., 2012). In addition, gas chromatography/mass spectrometry (GC/MS) has become an ideal technique for qualitative and quantitative analysis of volatile and semi volatile compounds of plant origin (Dhar *et al*., 2013).

Brenania brieyi is a flowing plant in the Rubiaceae family. It is found from Nigeria to Cameroon, Gabon and Central African Republic. It is commonly called 'mgbunsi' in Anambra State and has been recorded in botanical inventory of South Nigeria (Ebigwai *et al*., 2014) and Southeastern Cameroon (Sonke and Couvreur *et al*., 2014). It is used traditionally in the treatment of infection, fever, pain, and endocrine disorders such as menopausal complaints (Magne Nde, 2007). The present study was carried out to ascertain the bioactive compounds present in *B. brieyi* methanol and chloroform extracts with the aid of FTIR and GC-MS techniques, so as to provide an insight in its use into traditional medicine and also help in drug discovery.

2 METHODS

2.1 PLANT SAMPLE COLLECTION

Root barks of *Brenania brieyi* were collected from Abagana in Njikoka Local Government Area of Anambra State of Nigeria, between the months of June to October, 2015. The plant was identified by a taxonomist, Mr. Alfred Ozioko of Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State. Voucher specimens were retained in the Department of Plant Science and Biotechnology Herbarium, University of Nigeria Nsukka.

2.2 EXTRACTION PROCEDURES

One thousand seven hundred and ninety grammes (1790 g) of root bark of *Brenania brieyi* were extracted by maceration in chloroform-methanol (2:1) v/v for 48 h. It was filtered through

Whatman No. 4 filter paper. The filtrate was shaken with 0.2 volume water. The two layers were separated, dried and stored at 4 ºC in the refrigerator for further investigation.

2.3 DETERMINATION OF PERCENTAGE YIELD

The percentage yield of the extract was calculated using the formula below:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of powdered roots bark}} \; x \; 100$$

2.4 FOURIER TRANSFORM INFRARED SPECTROSCOPIC (FTIR) ANALYSIS

Fourier transform infrared spectroscopic (FTIR) analysis of the extracts was carried out using Shimadzu FTIR– 8400s Fourier transform infrared spectrophotometer, Japan. Methanol and chloroform extracts of *B. brieyi* were oven dried to get powders of the different solvent extracts used for FTIR analysis. The dried extracts powder (10 mg) were encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc and analysis was carried out by scanning the samples through a wave number range of 400 to 4000 cm^{-1} with a resolution of 2 cm^{-1}. FTIR analyses were performed and the different peaks present and possible chemical interactions were examined.

2.5 GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC-MS) ANALYSIS

Methanol and chloroform extracts of *B. brieyi* were analyzed with the help of GC-MS analyzer (GC-MS-QP 2010 plus Shimadzu, Japan). The carrier gas helium (99.999 %) was used at a flow rate of 1 ml per min in split mode (10:1) v/v. Methanol and chloroform extracts (8 µl) were injected into the column at 250 °C injector temperature. Temperature of oven started at 70 °C and held for 5 min. It was then raised at the rate of 10 °C per min to 280 °C without holding. Holding was allowed for 6 min at programmed rate of 5 °C per min. Temperature of ion sources was maintained at 200 °C. The injector temperature was set at 250 °C and detector temperature was set at 250 °C. The mass spectrum of compounds present in samples was obtained by electron ionization at 70 eV and detector operates in scan mode 50 to 600 Da atomic units. The MS Table was generated through ACQ mode scan within 0.5 seconds of scan interval at the speed of 666 and fragments from 30 to 350 Da was maintained. Total running was 21 minutes.

2.6 IDENTIFICATION OF COMPOUNDS

Identification of the compounds were based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. The spectrum of unknown components was compared with the spectrum of the known components stored in the NIST library. This was done in order to determine whether

this plant species contains any individual compound or group of compounds, which may substantiate its ethno-medicinal applications.

2.7 STATISTICAL ANALYSIS

Data obtained were subjected to statistical analysis and the results presented as means ± standard deviations. Differences between means were separated by one-way analysis of variance followed by *post hoc* multiple comparisons, with the least significant threshold employed at $p < 0.05$. Data analysis was done using the Statistical Product and Service Solutions (SPSS) for windows version 18.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSIONS

The percentage yield obtained were 6.8% and 9.6% for methanol and chloroform extracts respectively (Table 1). The extracts were in form of gel like brownish yellow paste. The functional groups present in *B. brieyi* root bark are amines, phenols, alcohols, carboxylic acids, alkenes, aliphatic compounds, carbonyl compounds, esters, phenyls and silica. These were confirmed by FTIR spectra study that revealed the presence of the groups:- NH_2, -CH, $-CH_3$, -C=O,- C-O, -C-C, -CH and -Si-O-Si. These agrees with the work of Maobe and Nyarango, (2013) who reported that these functional groups confirm the presence of secondary metabolites and other phytocomponents present in plants.

Table 1: The percentage yield of the extracts

Extracts	Percentage yield
Methanol	6.8 %
Chloroform	9.6 %

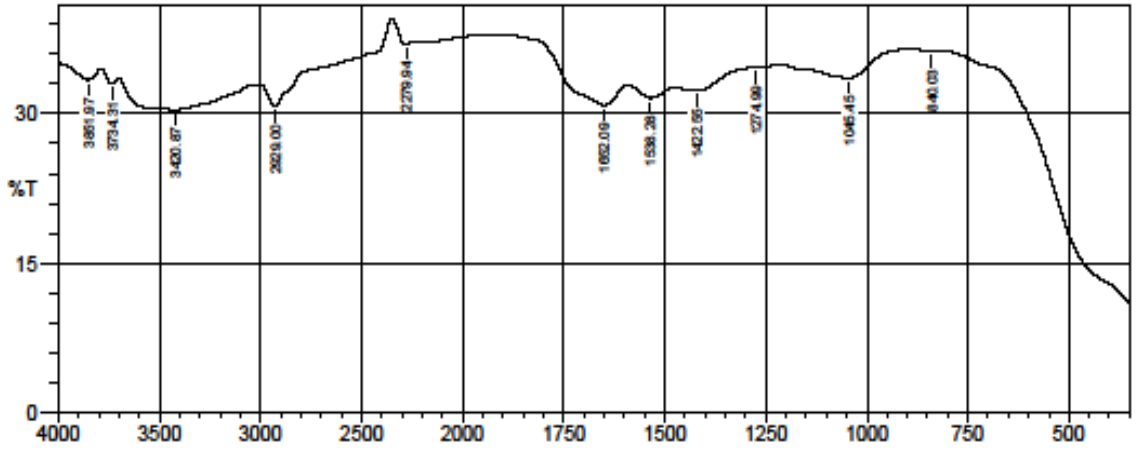

Figure 1: FTIR spectra of Methanol extract of *B. brieyi.*

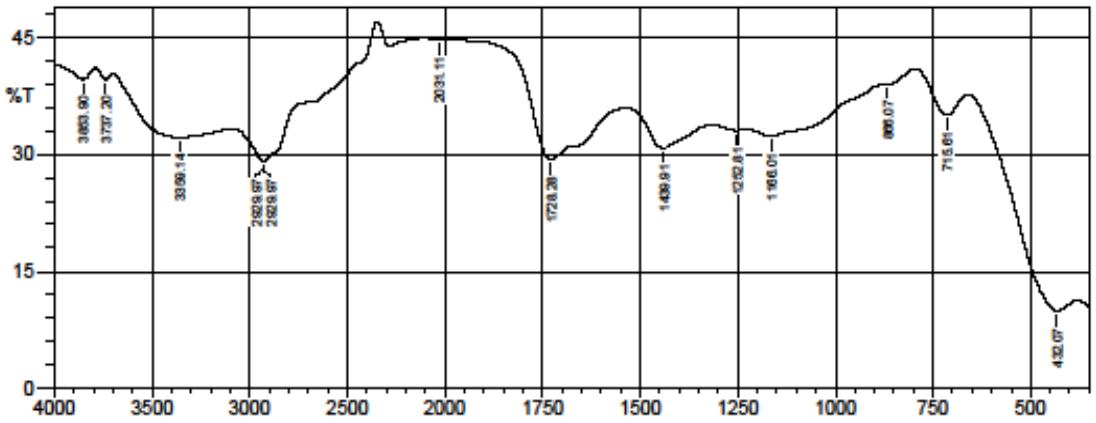

Figure 2: FTIR spectra of Chloroform extract of *B. brieyi.*

Table 2: IR Spectra band assignments for chloroform and methanol extracts *of B. brieyi*

Frequencies		Band assignments
Chloroform extract	**Methanol extract**	
3000-3880, 3700-3750, 3200-3600	3880-3900, 3700-3750, 3200-3600	OH stretch
2700-3000	2880-2950	CH stretch alkenes, carboxylic acid and alcohol
2031	2279	CH3 group stretching vibrations
1700-1750	1600-1750	C=O stretch esters
	1500-1580	CH bend
1400-1460	1400-1450	OH bend alcohols
1252	1274	C=O stretch
1150-1170	1000-1050	C-O stretch esters
850-880	840	CH bend
650-750		C-C stretch alkenes
432.07		-Si-O-Si bend, Silica

Gobalakrishnan *et al.* (2014) emphasized that the knowledge of chemical constituents of plants could be applied in the pharmaceutical industry. This study identified eight and ten compounds from methanol and chloroform extracts of *B. brieyi* respectively. Some of the identified compounds have been reported to exhibit a lot of biological activities. The most abundant compound found in both extracts as shown in Tables 3 and 4 was 9-octadecanoic acid (oleic acid). Anyasor *et al.*, (2014) and Omotoso *et al.*, (2014) reported that it has anti-inflammatory, antitumor, immunostimulory, antiandrogenic, antibacterial, antifungal, lipoxygenase inhibitory, hypocholesterolemic and cancer preventive activities. Similarly, Gnanavel and Saral, (2013) also observed antioxidant activity of this compound.

Hexadecanoic acid (palmitic acid) was the most abundant saturated fatty acid found in the plant. This compound has been reported by previous studies carried out by Rajeswari *et al.*, (2012), Anyasor *et al.*, (2014) and Omotosho *et al.*, (2014) to have antioxidant, anti-inflammatory, hypocholesterolemic, antiandrogenic, 5-α reductase inhibitor and hemolytic activities. Gobalakrishnan *et al.*, (2014); Jiang *et al.*, 2013; Mgbeji *et al.*, (2016) also observed its anticancer and antimicrobial activities respectively. The anti-inflammatory activity of n-hexadecanoic was revealed from structure and kinetic study carried out by Aparna *et al.*, (2012) to be due to its ability to inhibit PLA_2 competitively. Other compounds identified in the extracts with anti-inflammatory, antioxidant, antimicrobial and anticancer activities were octadecanoic acid, 9, 12-octadecanoic acid and tetradecanoic acids as reported by Gnanavel and saral, (2013), Rajeswari *et al.*, (2012) and Mohanambal and Murugaiah, (2015) respectively. In addition, Gobalakrishnan *et al.*, (2014) and Omotosho *et al*, (2014) also observed antieczemic, hepatoprotective, antiacne, antiarthritic and anticoronary activities of 9,12-octadecanoic acid while Atolani *et al*, (2012) observed that it reduces the risk of cardiovascular disease. Eicosanoic acid (arachidic acid) in the plant acts as modulator of numerous physiological process including reproduction, blood pressure, homeostasis and inflammation (Alhassanm *et al.*, 2014), while nonadecanoic acid has been shown to inhibit cancer growth (Mgbeje *et al*., 2016). Its conjugate form, nonadecanoic acid has been reported to prevent adult onset obesity and also improve expression of hormone sensitive lipase (Park and Park, 2010). Interestingly, squalene identified in the *B. brieyi* root bark is a strong antioxidant which protects the skin from peroxidation by acting as singlet oxygen quencher (Huang *et al*, 2009). It also has antitumor, chemopreventive, anticancer and antimicrobial activites (Rajeswari *et al*, 2012; Mohanambal and Murugaiah, 2015), cardioprotective activity, increases WBC count, reduces serum cholesterol concentration by increasing fecal excretion (Gunes, 2013). The anti-inflammatory activity of squalene is due to its membrane stabilizing properties as shown by Gunes, (2013).

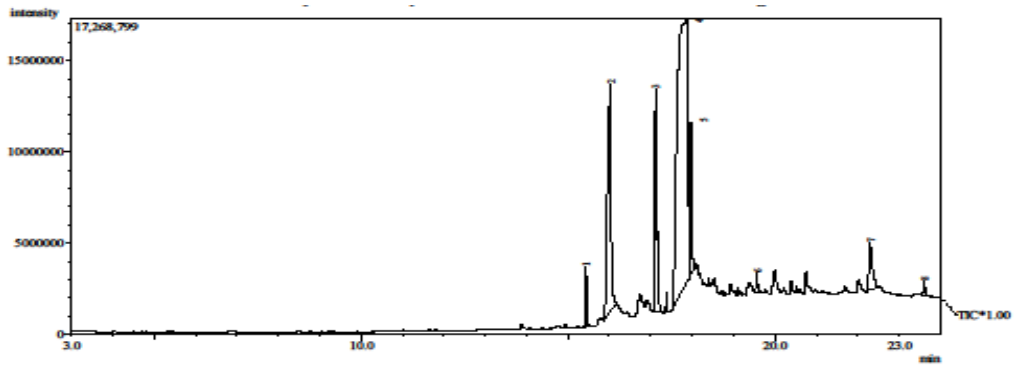

Figure 3: Chromatogram of methanol extract of *B. brieyi*

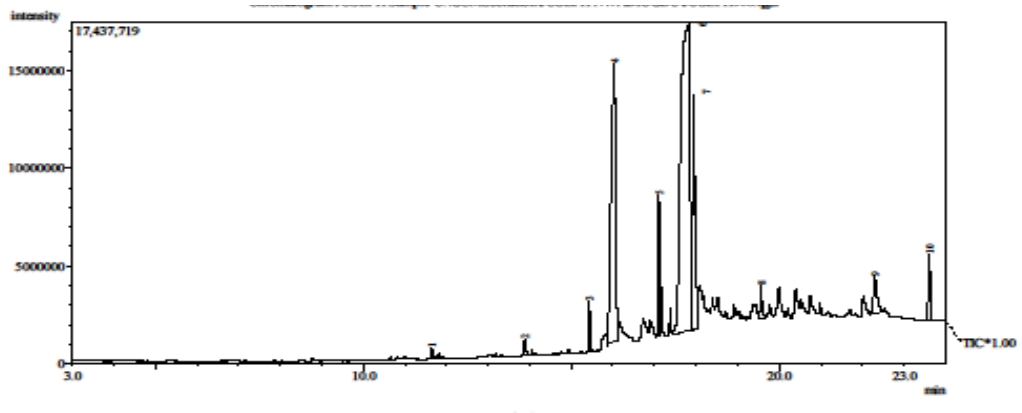

Figure 4: Chromatogram of chloroform extract of *B. brieyi.*

Table 3: Compound identified in methanol extract of *B. brieyi* by GC-MS

S/N	RT	Name of compound	Molecular formulae	Molecular weight	Area %
1	15.441	Decanoic acid	$C_{11}H_{22}0_2$	186	1.71
2	16.022	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	17.04
3	17.119	9, 12-Hexadecanoic acid	$C_{17}H_{30}O_2$	266	10.18
4	17.874	9-Ocatadecanoic acid	$C_{18}H_{34}0_2$	282	60.53
5	17.969	Octadecanoic acid	$C_{18}H_{36}0_2$	284	5.87
6	19.566	Eicosanoic acid	$C_{20}H_{40}0_2$	312	0.65
7	22.308	6,9-Pentadecadien-1-ol	$C_{15}H_{28}0$	224	3.51
8	23.611	4, 8, 12 -tetradecatrienal	$C_{17}H_{28}0$	248	0.52

Table 4: Compound identified in chloroform extract of *B. brieyi* by GC-MS

S/N	RT	Name of compound	Molecular formulae	Molecular weight	Area %
1	11.641	Undecanoic acid	$C_{11}H_{22}0_2$	186	0.51
2	13.883	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.61
3	15.439	14-methylpentadecanoate	$C_{17}H_{34}O_2$	270	1.26
4	16.038	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	21.23
5	17.107	9,12-Octadecanoic acid	$C_{19}H_{34}0_2$	294	3.90
6	17.831	9-Octadecanoic acid	$C_{18}H_{34}0_2$	282	54.85
7	17.951	Ocadecanoic acid	$C_{18}H_{36}0_2$	284	11.29
8	19.566	Nonadecanoic acid	$C_{19}H_{38}O_2$	298	1.27
9	22.307	6,9-Pentadecadien-1-ol	$C_{15}H_{28}O$	224	2.61
10	23.615	Squalene	$C_{30}H_{50}$	410	2.46

Conclusion: *B. brieyi* is endowed with a lot of bioactive compounds with known medicinal application. Hence, the wide use of the plant in traditional medicine in treating various diseases. Further research is ongoing to examine pharmacological activities of the plant especially in treatment of oxidative stress and inflammation related disorders.

Table 5: Bioactivity of phytocomponents identified in methanol and chloroform extracts of *B. brieyi* by GC-MS

Name of compound	Bioactivity	References
Decanoic acid	Antifugal, antiviral, and increases level of HDL	Duke, 1992
Pentadecanoic acid	Antioxidant	Duke, 1992
9-Ocatadecanoic acid (oleic acid)	Antifugal, antitumor, immunostimulant, antiandrogenic, antibacterial, antifungal, lipoxygenase inhibitor, 5 α reductase inhibitor, diuretic and hypocholesterolemic.	Rajeswari *et al.* 2012; Omotoso *et al.* (2014)
Octadecanoic acid (steric acid)	Antioxidant, antiviral, anticancer, antiacne and co-solvent	Gnanavel and Sarah, 2013
Eicosanoic acid (Arachidic acid)	Modulate inflammation, reproduction, blood pressure and homeostasis	Alhassanm *et al.* 2014
Undecanoic acid	Antifungal	Duke, 1992
Tetradecanoic acid (mystic acid)	Antioxidant, antifibrinogenic, cancer preventive, hemolytic, hypocholesterolemic, hemolytic, and 5-α reductase inhibitor	Rajeswari *et al.* 2012; Mohanambal and Murugaiah, 2015
Hexadecanoic acid (palmitic acid)	Anti-inflammatory, antioxidant, antiadrogenic, hypocholesterolemic, 5-α reductase inhibitor,	Aparna *et al.* 2012; Rajeswari *et al.* 2012; Anyasor *et al.* 2014; Gobalkrishnan *et al.* 2014 and Omotosho *et al.* 2014
9,12-Octadecanoic acid (linoleic acid)	Anti-inflammatory, hypochoesterolemic, cancer preventive, hepatoprotective, antihistamic, antieczemic, antiarthritic, antimicrobial and 5-α reductase inhibitor,	Gobalkrishnan *et al.* 2014; Kumar *et al.* 2010; Mohanambal and Murugaiah, 2015; Rajeswari *et al.* 2012
Nonadecanoic acid	Cancer inhibitor and anti-obesity	Mgbeje *et al.* 2016 and Park and Park, 2010.
Squalene	Anti-inflammatory, antioxidant, antitumor, immune stimulant, lipoxygenase inhibitor, antimicrobial, chemopreventive and antibacterial	Hung *et al.* 2009; Ezhilan and Neelamegam, 2012; Rajeswari *et al.* 2012; Gunes, 2013; Mohanambal and Murugaiah, 2015;

ACKNOWLEDGMENTS

The authors are highly indebted to all the people who contributed in one way or the other towards the success of this research especially Prof. Ukoha, the Dean Faculty of Physical Sciences, University of Nigeria, Nsukka for his efforts in interpreting the results.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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