Gas Chromatography-Mass Spectrometry Characterization of W. *lateritia* Leaves Decoction

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ABSTACT
Characterization of plants to unravel the bioactive components is a necessary step towards the building of knowledge on the natural endowment of every plant which will help for its proper utilization/ application. In this study, the gas chromatography –mass spectrometry (gc-ms) characterization of *Whitfieldia lateritia* leaves decoction was carried out. The leaves were harvested fresh and subjected to decoction extraction technique. The compounds were identified by gas chromatography with mass-spectrometry (gc-ms) analysis and characterized by comparison through the NIST02 and Wiley275 library search software. The result of the gc-ms analysis of the *W.lateritia* leaf decoction showed 12 bioactive compounds. They include the following undec-10-enoic acid; n-tetradecanoic acid; 1-penta decanecarboxylic acid**;** (z)-9-octadecanoic acid methyl ester; (z)-9-octadecanoic; glycerol-1,3-dipalmitate; (z,z)-9,12-octadecdienoic acid-2,3,dihydroxypropyl ester; 14-methyl-8-hexadecyn-1-ol; palmitic acid, beta monoglycerol; n-octyl phthalatate; 14-methyl-8-hexadecyn-1-ol and cyclobutyl-4-nitrobenzoate**.** The bioactive compound with the highest percentage occurrence is (z)-9-octadecanoic (39 %) while the n-tetradecanoic acid is the least with 1.20%. The finding in this study showed that the compounds identified belong to four major classes; carboxylic, esters, alcohols and amino compound. These compounds might be responsible for the medicinal activities attributed to the *W.lateritia* leaves decoction.

Keywords: *Whitfieldia lateritia*, palmitic acid, mass-spectrometry, undec-10-enoic acid, decoction

Introduction
The uses of plant for medicinal purposes have been an age long practice, and have remained the major sources of nutrient and non nutrient molecules (Aja *et al.*, 2016 and Okorie *et al.*, 2020).

Numerous health benefits plants such as antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens have been documented (Aja *et al*., 2016). The contributions of various plants' part (roots, stem, leaves, flower and seeds) to the maintenance of cellular health had been reported (Ohshima *et al*., 2006). Dietary antioxidants such as carotenoids, vitamins C, E and selenium play significant roles in DNA and cell maintenance and repair through different mechanisms including free radical scavenging activity (Ujang, 2008). Significant restorations of hepatorenal dysfunction, antianaemic, antioxidant, antitumoral, antiviral and antibiotic activities are frequently reported for plant bioactive components (Uraku *et al*., 2019; Ngozi *et al*., 2017). Reports on the efficacy of most plant extracts in the treatment of various ailment such as anaemia, diahrrioa and hepatotoxic restoration are common (Priyanka *et al.*, 2015 and Sherry *et al*., 2016). Many plants, however, may not be safe for consumption by human and animals because some bioactive component that may be toxic (Ilka and Jose, 2004). Documentary evidence has shown that plants represent one of the most important sources of bioactive compounds used for drug development, with up to 40% of modern drugs being derived from them (Rajasekaran, 2013). It is therefore important to characterize the different functional components present in plants, especially the under-studied and under-utilized species, for their nutritional and bio-pharmacological activities. *Whitfieldia lateritia* is one the numerous plants that are under-studied. It belong to the family, *Acanthaceae* which contains ten (10) species (Hutchusion and Dalziel, 1973). It is an evergreen plant that can easily be identified by its well developed leaves arranged alternately along the stem (Aja *et al.*, 2016). Most inhabitants in the remote villages of the South-Esatern Nigeria use the decoction for the treatment of anaemia and high fever (Okorie *et al*., 2020 and Aja *et al*., 2015). The use of the *W. lateritia* leaves decoction for medicinal purpose without proper characterization of the leaves decoction and documentation is the basis for this study.

Methods

Plant materials

Whitfieldia lateritia plant was harvested from forest of Ishiagu, Ebonyi State, Nigeria. The plant specimens were collected during the Month of October, 2019, identified and authenticated by a Taxonomist. A voucher specimen of leaves has been deposited in the Department of Botany University of Port Harcuort.

Preparation of decoction

Fresh leaves of *W. lateritia* were collected, washed with running tap water, and then 100 g was weighed into a 500 ml beaker containing 300 ml of eva table water. It was then boiled for 15 minutes, and then cooled and sieved with cheese cloth. The filtrate was concentrated in a water bath and the percentage yield calculated using the formula:

$$\%\text{ yield} = \frac{weight\ of\ beaker + extract - weight\ of\ empty\ beaker}{\text{initial weight of sample used}} \text{ x } 100$$

The extract was stored in the refrigerator and used for the gc-ms analysis.

Identification of chemical constituents

The bioactive compounds present in different solvent extracts of the *W.lateritia* leaves were analyzed and identified based on GC retention time on Rtx®-5MS fused silica capillary column.

The mass spectra were matched with computer matching with those of standards (NIST 2005 v.2.0 and Wiley Access Pak v.7, 2003 of GC-MS systems).

Results

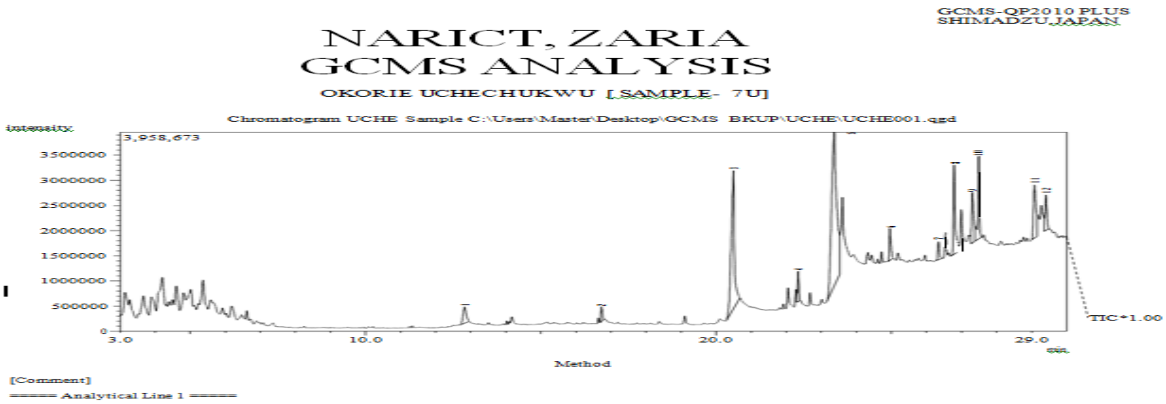
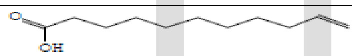
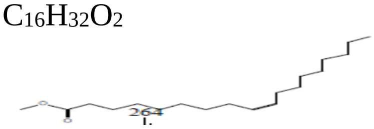
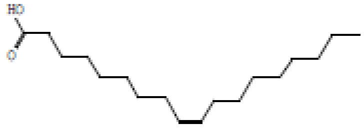
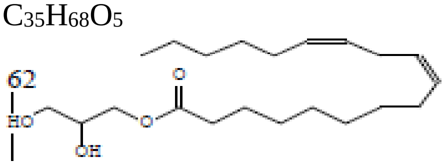
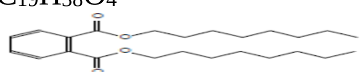
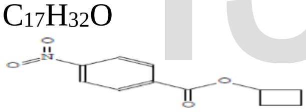


Figure 1: The chromatogram of the gc-ms of the *W.lateritia* leave decocotion

Table 1 GC-MS result of *W.lateritia* leave decoction

S/N	Compounds	Mol.wt	Retention time	Base peak	Area (%)
1	 undec-10-enoic acid $C_{11}H_{20}O_2$	18.41	12.825	55.05	2.50
2	 n-tetradecanoic acid $C_{14}H_{28}O_2$	228	16.733	73.00	1.20
3	 1-penta decanecarboxylic acid $C_{16}H_{32}O_2$	256	20.492	73.05	21.62
4	 (z)-9- octadecanoic acid methyl ester $C_{19}H_{36}O_2$	296	22.352	55.00	1.96
5	 (z)-9-octadecanoic $C_{18}H_{34}O_2$	282	23.375	55.05	39.37
6		568	24.950	57.05	2.56

7	glycerol-1,3-dipalmitate $C_{35}H_{68}O_5$  (z,z)-9,12-octadecdienoic acid-2,3,dihydroxypropyl ester $C_{21}H_{38}O_4$	354	26.333	67.00	1.45
8	 14-methyl-8-hexadecyn-1-ol $C_{17}H_{32}O$	252	26.783	81.00	7.50
9	 palmitic acid ,beta monoglycerol $C_{19}H_{38}O_4$	330	27.308	43.00	5.29
10	 n-octyl phthalatate $C_{24}H_{38}O_4$	390	27.483	149.05	6.43
11	 14-methyl-8-hexadecyn-1-ol $C_{17}H_{32}O$	252	29.075	81.00	7.02
12	 cyclobutyl-4-nitrobenzoate $C_{11}H_{11}NO_4$	221	29.408	70.10	3.11

The result (Table 1) of the GC-MS analysis of the *W.lateritia* leaf decoction shows 12 bioactive compounds. They include the following undec-10-enoic acid; n-tetradecanoic acid; 1-penta decanecarboxylic acid**;** (z)-9- octadecanoic acid methyl ester; (z)-9-octadecanoic; glycerol-1,3-dipalmitate; (z,z)-9,12-octadecdienoic acid-2,3,dihydroxypropyl ester; 14-methyl-8-hexadecyn-1-ol; palmitic acid, beta monoglycerol; n-octyl phthalatate; 14-methyl-8-hexadecyn-1-ol and cyclobutyl-4-nitrobenzoate**.** The bioactive compound with the highest percentage occurrence is (z)-9-octadecanoic (39 %) while the n-tetradecanoic acid is the least with 1.20%. It was observed that the compounds identified belong to four major classes; carboxylic, esters, alcohols and amino compound.

Discussion

The gc-ms analysis of the decoction extract of the *Whitfieldia lateritia* leaf revealed relative abundance of different phytochemical compounds (Table 1). These bioactive compounds were idnetified based on their retention time and base peak. GC-MS analysis showed that twelve bioactive compounds were present in the decoction; they are as follows: Undec-10-enoic acid; n-Tetradecanoic acid; 1-Penta decanecarboxylic acid; (z)-9- Octadecanoic acid methyl ester; (z)-9-

Octadecanoic; Glycerol-1,3-dipalmitate; (z,z)-9,12-Octadecdienoic acid-2,3,dihydroxypropyl ester; 14-Methyl-8-hexadecyn-1-ol; palmitic acid, beta monoglycerol; n-octyl phthalatate; 14-Methyl-8-hexadecyn-1-ol; cyclobutyl-4-nitrobenzoate. Our observation is in tandem with that of Aja *et al*., (2016) who had documented carboxylic acids, which were similar to our finding, from crude ethanol extract of *Whitfieldia lateritia* leaves. The practice of boiling plant materials to obtain the decoction is an age long practice. Even though this practice is economical due to its low cost in terms of instrumentation and reagents, it may be an inefficient process given that ingredients may be damaged during the prolonged heating of substances, and other ingredients may be oxidized and then loose activity; some volatile oil may also be lost (Mohamed *et al.*, 2010). The heat treatment performed during preparation of decoction accelerate the mechanism of the diffusional process during the extraction from plants which normally reduced the surface tension and viscosity of the solvent helping it to reach the active sites inside the plant cell matrix. Furthermore, high temperature can decrease the cell barrier by weakening integrity of the cell wall and membrane allowing the solvent easy access to get in contact with phytochemical compounds (Mohamed *et al.*, 2010). Undec-10-enoic acid is used in the synthesis of chemicals such as fine chemical fragrance, flavors, cosmetic and polymer. When in salt form (Zn/Ca) it can be used for personal care because of its natural antimicrobial and preservatives properties which is attributed to its bifunctional nature - possession of ester function and terminal double bond at the end of the chain. The fatty acid, n-tetradecanoic acid is credited to being a lipid anchor in biomembranes and used for topical medicinal preparations. 1-penta decanecarboxylic acid has been found to possess Anti-inflammatory, antioxidant, nematicide, antiandrogenic, 5-Alpha reductase inhibitor, and potent mosquito larvicid (Oladayo *et al*., 2019). Its presence in the adipose tissue of humans is reported to be a good biological marker of long-term milk fat intake in free-living individuals in populations with high consumption of dairy products. (z)-9- Octadecanoic acid methyl ester has application in soap making, gear oils ink solvents, jet lubricants, stabilizers and flavorings (Anand, 2015). Plants containing (z)-9-octadecanoic, glycerol-1,3-dipalmitate, (z,z)-9,12-octadecdienoic acid-2,3,dihydroxypropyl ester and 14-methyl-8-hexadecyn-1-ol have been shown, based on the works of Surya (2018) on Sumbawa leaves oils and Wan-suriyani (2018) for the decoction of *Abrus Precatorius* leaves, to possess nerve tonic property and treat wounds including swellings due to their anti-inflammatory potentials, antispasmodic and antipyretic. This may be one of the basis for the application of *Whitfieldia lateritia* leaves for antipyretic and anti-anaemic purposes in folklore medicine. The toxic property of (z)-9-octadecanoic against *P. Falciparum* has been described to occur through targeting of the topoisomerase IB enzymes, in particular LdTopIB (Néstor, 2013). 1, 3-dipalmitin which was observed in the present study, is primarily located in the membrane and can also be found in the extracellular space. 1, 3-dipalmitin exists in all living species, ranging from bacteria to humans (HMDB, 2018) and may influence the intestinal microbiome and contribute in complex ways to neurological and immune system development through roles in cell signaling and regulation of gene expression (HMDB, 2018)). Cis, cis -9, 12-octadecdienoic acid-2, 3, dihydroxypropyl ester tend to be minor components of most plant and *animal* tissues, and are said to possess strong detergent properties which would have a disruptive *effect* on cellular membranes. This compound, however, is necessary for the brain development, especially, at the level of nerve growth and synaptogenesis (Ackermann *et al.*, 2015). Although it occurs throughout a healthy person's lifespan, an explosion of synapse formation occurs during early brain development, affecting the membrane fluidity; thus influencing neurotransmitter receptor activity (Ackermann *et al.*, 2015). The 14-methyl-8-hexadecyn-1-ol is known to possess anticancer activity (Anand, 2015). N-octyl phthalate and cyclobutyl-4-nitrobenzene were only found in the decoction. N-octyl phthalate is associated with histological changes in hepatic architecture, decreased gamma-glutamyl-transpeptiase activity and thyroid toxicity (CDC-ASTSDR, 2005). Cyclobutyl-4-nitrobenzene has antibacterial potential through binding to bacterial DNA gyrase (GyrA) and/or Topoisomerase IV (ParC) stabilize the cleaved complex, thus inhibiting overall gyrase function, leading to cell death. In addition, cyclobutane compounds such as amino acids, peptides and nucleosides had been reported to display protective properties against UV radiation. Previous finding had shown that cyclobutane units are basic structural element in a wide range of naturally occurring compounds in bacteria, fungi, plants, and marine invertebrates and are impotant source of leads in drug discovery (Anastasia *et al*., 2008).

Conclusion

Twelve (12) bioactive compounds (belonging to four major classes: carboxylic, esters, alcohols and amino compound) were found in the *W. lateritia* leave decoction which might justify the application the leaves of this plant for the treatment anaemia in the local communities in the South-Eastern , Nigeria.

Acknowledgement

We acknowledge National Research Institute for Chemeical Technology for helping us to run our gc-ms analysis

Funding

The research work was funded by the authors

Conflict of interest

The authors declare no conflict of interest.

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