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Characterization of dichloromethane and methanol extracts from the leaves of a medicinal plant: *Globimetula oreophila*

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ABSTRACT

Medicinal plants are inestimable natural resources that will continue to attract interest on drug development and their use by great majority of people across the globe for their health challenges either as curative or preventive measures. *Globimetula oreophila* of the family *Loranthaceae* is a hemi-parasitic plant that has not been explored for its acclaimed health benefits. Its ethnopharmacological uses include treatment of cancer, hypertension, diabetes, and as diuretic agent. In this study, sequential Soxhlet extraction was employed starting with dichloromethane and followed by methanol. The extracts were converted to their trimethylsilyl (TMS) and fatty acid methyl esters (FAME) derivatives and were analyzed by gas chromatography mass spectrometry (GC–MS). Fourier transform infrared (FTIR) spectroscopy was used to determine the various functional groups present in both extracts of the plant material. The GC–MS analysis revealed the presence of sterols, fatty acids, monoarylphenolics, steryl esters, sugars, sugar alcohols, carboxylic acids, dicarboxylic acids, hydroxycarboxylic acids, ketones and alkanes.

1. Introduction

Nature has provided numerous remedies to cure a variety of ailments of human kind. In fact, use of herbs had continued to rise worldwide because of the preference of consumers for natural therapies, concerns regarding undesirable side effects of modern medicines and their high cost. For many centuries, plants have been a rich source of therapeutic agents and provided basis for several synthetic drugs (Skotti et al., 2014). Despite great development of organic synthesis, currently 75% of prescribed drugs worldwide are derived from plant sources (Tan et al., 2006), indicating that plants are still a valuable source of new drugs for diseases that continue to defy orthodox medication, such as cancer. Indeed, some Nigerian herbal remedies are touted as 'cure all medicines'. The Yoruba people of South-West Nigeria calls it "gbogbonse", while the Igbo people of South-East region refers to it as "ogwonnuoria" (Odukoya, 2010). It is claimed that they are able to cure diseases ranging from diarrhea, fever, stomach upset, waist pain, arthritis, and diabetes to haemorrhoids to mention but a few (Odukoya, 2010).

The plant kingdom offers a variety of species still used as remedies for several diseases in many parts of the world such as Asia (Duraipadiyan et al., 2006; Grover et al., 2002), Africa (Khalid et al., 2012), and South America (Bolzani et al., 2012). Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cos et al., 2006).

The leaves of *Globimetula oreophila* (Oliv) van Tiegh has been identified as such a medicinal plant. *G. oreophila* belongs to the *Loranthaceae* family of parasitic mistletoes which are plants with green leaves or stem suckers that penetrate host tissue and are therefore both photosynthetic and parasitic. *Loranthaceae* is a large family of about 75 genera and over 900 species (Judd et al., 2002). The family has three terrestrial, root parasitic genera and 72 genera of aerial branch parasites (Wilson and Calvin, 2006).

Seven genera of *Loranthaceae*: *Helixanthera, Berhautia, Englerina, Globimetula, Agelanthus, Tapinanthus,* and *Phragmanthera,* with about 60 or more species are recognized in West Africa (Burkill, 1985), and the group term mistletoes is used for all these species. All these genera are found in Nigeria with the exception of *Helixanthera.* In West Africa, mistletoes are found on many tree crops of economic importance including Shea butter tree (*Vitellaria para-*

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doxa Gaertn. F.), the neem tree (*Azadirachta indica* L.), citrus species, especially sweet orange (*Citrus sinensis* L.) and grape (*Citrus paradise* L.), cocoa (*Theobroma cacao* L.), and rubber tree (*Heveabra siliensis* MuellArg) (Begho et al., 2007; Bright and Okusanya, 1998; Gill and Onyibe, 1990; Overfield et al., 1998). *G. oreophila* is usually associated with the cocoa tree.

The ethnomedicinal uses of mistletoes had, for a very long time been in the hands of very few Herbal Practitioners who claimed a general use to counter sorcery and magical powers, to treat mental conditions, sterility, and health problems associated with urino-genital system, rheumatism and pain. These hemi-parasitic plants, mistletoes of the *Loranthaceae* are widely used in various cultures in almost every continent to treat various ailments including hypertension, cancer, and diabetes, or used as a diuretic agent (Adodo, 2004; Burkill, 1985; Jadhav et al., 2010). The tea prepared from *Loranthaceae* is believed to cure bone fracture and body pains (Matsushima et al., 2006). Powdered leaves of a mistletoe and purportedly sourced from cocoa, avocado and citrus. A product of Natural Health Product Services Limited, is sold across Southern Nigeria under the trade name 'NAHEPS' *Mistletoe Tea.* NAHEPS claims that the tea is traditionally used to promote good health as it enhances natural body immunity. Mistletoes are now known as "cure all" and have been found beneficial as a drug/remedy for more than twenty health problems (Adodo, 2004).

The isolation, purification and the structural identification of a polysaccharide and peptides in the leaf cell wall of *Phragmanthera capitata* obtained from Central Africa have been reported previously (Angone et al., 2009; Fasanu and Oyedapo, 2008). Some phytochemical and antibacterial screening has been carried out on the plant (Oluwole et al., 2013). Apart from such information, *Viscum album* and other European and Asian mistletoes belonging to the *Loranthaceae* and the *Viscaceae* families have been studied; whereas there is limited documentation on the chemistry of most African mistletoes.

The most distinctive constituents of most European and Asian mistletoes are proteins, viscotoxins, lectins and carbohydrates. The low molecular weight compounds including flavonoids and phenylpropanoids of varying structural types which contribute to the anti-oxidative properties of various *Loranthaceae* extracts examined. Many reports of the presence of lower molar mass compounds, alkaloids, flavonoids, tannins and other plant constituents in the African *Loranthaceae* have not been substantiated by isolation and proper identification (Adesina et al., 2013). Although, pharmacological tests have been carried out on *Globimetula braunii* which is closely related to *G. oreophila*, such tests have not been carried out on *G.oreophila*. (Adediwura et al., 2008; Le and Zam, 2008; Okpuzor et al., 2009).

The aim of this study is to identify the yield and composition of the extracts of the leaves of *G. oreophila*. Both the CH_2Cl_2 and CH_3OH soluble extracts have been characterized by GC–MS as their trimethylsilyl (TMS) derivatives and fatty acid methyl esters (FAMES). The FTIR spectroscopy was used to support the chemical functionality of the crude extract compounds.

2. Materials and methods

2.1. Collection of plant material

G .oreophila was collected from Iwo, Nigeria. The leaves were identified and authenticated by Mr. Gabriel Ibhanesebhor and a voucher specimen deposited (Herbarium Ife number 17089) at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria

2.2. Sample preparation and extraction

The leaves were water rinsed, air-dried and pulverized into powdered form using a domestic blender. The plant materials were stored inan air-tight container. The moisture content (9.6%) of the sample was determined just before extraction. The sample (5 g, in duplicate) were extracted continuously overnight in Soxhlet apparatus with CH_2Cl_2 (150 mL) for 24 h and then CH_3OH (130 mL) for 48 h (Wei et al., 2014). Both the CH_2Cl_2 and CH_3OH extracts were evaporated to dryness to yield 4.34% and 7.39% on a dry leave basis, respectively.

2.3. GC–MS ofextract TMS derivatives

Extracts (1.0 mg, in duplicate) were weighed into GC vials to which CH_2Cl_2 (1 mL) containing anthracene as an internal standard (IS, 50 μg/mL) was added. The samples were silylated with addition of *N*,*O*-bis(trimethylsilyl)-trifluoro-acetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) (50 μL) and pyridine (50 μL) and heated for 30 min at 70 °C or continued heating until the solution became clear. The prepared TMS derivatives were analyzed by GC–MS_{EI} (FOCUS-ISQ, Thermoscientific); temperature profile: 40 °C (1 min) ramped to 305 °C (10 min) at 5 °C/min; GC capillary column (RTx-5MS, 30 × 0.25 mm Φ, Restek). The eluted compounds were identified by spectral matching with the 2008 National Institute of Standard and Technology (NIST) spectral library and known standards.

2.4. GC–MS of FAME derivatives

Extracts (5.0 mg, in duplicate) were weighed into 5 mL reactivials™ to which $CH_3OH/H_2SO_4/CHCl_3$ (1.7:0.3:2.0 v/v/v, 2 mL) was added to each vial and heated for 90 min at 90 °C. $CHCl_3$ contained 1-naphthaleneacetic acid as an internal standard (50 μg/mL). The mixture was allowed to cool after which water (1 mL) was added, shaken vigorously, centrifuged, the $CHCl_3$ layer removed, dried through anhydrous sodium sulfate and transferred to GC vials. The prepared FAME derivatives were analyzed by GC–MS (details given above): temperature profile 40 °C (1 min) to 320 °C at 5 °C/min. The eluted compounds were identified with authentic standards (C_{12} to C_{20} fatty acids) and by spectral matching with the NIST spectral library

2.5. FTIR spectroscopic analysis

The functional groups in the crude samples were determined by FTIR spectroscopy using a Nicolet iS5 Thermoscientific spectrometer using a ZnSe attenuated total reflection (ATR) probe. Spectra were collected in duplicate; the absorbance spectra were baseline corrected and averaged using Omnicv9.0 software (ThermoScientific) (Wei et al., 2013; Wei et al., 2015a).

3. Results and discussion

3.1. GC–MS analysis of the TMS derivatized CH_2Cl_2 extract

The CH_2Cl_2 extract of *G. oreophila* was analyzed by GC–MS as their TMS derivatives as shown in Fig. 1. The analysis revealed the presence of 26 different compounds and their concentrations are given in Table 1. These compounds were distributed among eight classes of organic compounds, including sugars, monoarylphenolics, fatty acids, sterols, alcohols, alkanes and triterpenoids. Glycerol, which is a product of hydrolysis of triglycerides, was detected as one of the major compounds present in the extract (112 mg/g). Free fatty acids palmitic acid (103 mg/g), octadecenoic acid (61 mg/g), octadecanoic acid (17 mg/g), and tetracosanoic