

GAS CHROMATOGRAPHY MASS SPECTROMETRY ANALYSIS OF BIOACTIVE COMPOUNDS IN *MONDIA WHITEI* (HOOK. F.) SKEELS FRUIT

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Abstract

Mondia whitei fruit is consumed as a wild edible fruit. Gas-chromatography-mass spectrometry analysis of dichloromethane/methanol and methanol extracts of the fruit pulp was made to identify the bioactive compounds present. Twenty one compounds were identified from the dichloromethane/methanol extract while twenty eight compounds were identified from the methanol extract. The most abundant compounds from the dichloromethane/methanol extract were 9-octadecenoic acid (Z) -2,3-dihydroxypropyl ester commonly known as glycerol monooleate (GMO) and octadecanoic acid-2,3-dihydroxypropyl ester commonly known as glycerol monostearate (GMS). They had a relative abundance of 40.34% and 13.91% respectively. The most abundant bioactive compounds present in the methanol extract were $\alpha, \beta, \alpha, \beta$ -5-cyclohexenol, 3-O-methyl-D-glucose and oleoyl chloride which had relative abundance of 10.70%, 26.10% and 29.62% respectively. Globulol a tricyclic hydroazulene sesquiterpene, γ -Sitosterol a steroid, and n-Heptyl isocyanate a glucosinolate were also found in the methanol extract of *Mondia whitei* fruit. Compounds common to both methanol and dichloromethane/methanol extracts of *Mondia whitei* fruit include 2-Decanoic acid, 2,4,6-cycloheptatriene-1-one-4 methyl, -2,5-dimethyl-4 hydroxy-3-(2H) furanone, 4H-pyran-4-one -2,3-dihydro-3,5-dihydroxy-6-methyl, catechol, 3-O-methyl-D-glucose, 9,12-octadecadienoic acid, hexadecanoic acid-2-hydroxy-1- (hydroxymethyl) ethyl ester and oleoyl chloride. Results indicated that polarity of the solvents used for extraction influenced the relative abundance and types of compounds extracted. The compounds identified indicated that *Mondia whitei* fruit can be exploited for its nutritional, therapeutic as well as being an additive in relevant food formulations.

Keywords: *Mondia whitei* fruit, gas chromatography-mass spectrometry, bioactive compounds, extracts.

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1. INTRODUCTION

Fruits are one major dietary source of various antioxidant phyto-compounds for humans (Valvi *et al.*, 2014). Many edible fruits are very much available in the forest and wild areas and in large quantities. Most of which are not usually harvested, hence they are wasted such that their therapeutic properties and potentials as subsidiary food sources are practically unknown (Islary *et al.*, 2016). Wild fruits are safe to consume and some have been developed as medicines (Li *et al.*, 2016). The consumption of locally grown species is gaining an increasing interest, hence contributing to local community's health and welfare (Henreich *et al.*, 2006). This stems from the fact that some wild edible fruits are good sources of beneficial bioactive compounds which have different proven activities such as the ability to combat degenerative diseases.

Recently, the increased quest on the exploitation of natural compounds from fruits for medicinal purposes has received great attention due to their health-promoting effects (Abubakar *et al.*, 2015). There is a growing interest among researchers throughout the world to assess various wild edible fruits and plants for their nutritional and other features for the well being of the human society (Aberoumand and Deokule, 2009; Nazarudeen, 2010). *Mondia whitei* (Hook .F.) Skeels belongs to the family formerly *Asclepiadaceae* but now *Apocynaceae* (Lamidi and Bourobou-Bourobou, 2010). It is widely distributed in tropical Africa from Guinea through Cameroon to East Africa and wide spread in Zimbabwe and South Africa (SANBI, 2009). It grows as a climbing shrub to a height up to 8 m or more (Lamidi and Bourobou-Bourobou, 2010). It is commonly called 'Whites ginger', 'Mondia', 'Tonic root' in English, 'La racine' in French

(Lamidi and Bourobou-Bourobou, 2011), 'Umondi', 'Mundi' by the Zulus in South African (SANBI, 2009), 'Akoro' by Izzi clan in Ebonyi state, Nigeria (Amaechi and Egesi, 2017). The fruit consists of a pair of obliquely ovoid, glabrous follicles each 8-12cm x 2-4cm, green, apex rounded and many seeded (Lamidi and Bourobou-Bourobou, 2010). It dehisces to release an estimated 180-320 camose seeds that are wind dispersed (Ross, 1978). The aromatic roots are more popular for its use as food flavoring agent (Aremu *et al.*, 2011) and is used in tropical Africa for medicinal purposes (SANBI, 2009). Pharmacological studies have been carried out on both roots and leaves of *Mondia whitei* plant (Aremu *et al.*, 2011). Bioactive constituents in the ethylene chloride extract of the roots have been elucidated (SANBI, 2009), nutrient and phytochemicals of the fruits have been evaluated (Amaechi and Egesi, 2017). However, there is dearth of information on the nature of bioactive compounds in the fruit. It is in view of this that a study was conducted to evaluate and identify bioactive compounds present in dichloromethane/methanol and methane extracts of *Mondia whitei* fruit using gas-chromatography-mass spectrometry. Knowledge of the bioactive compounds present in it will help promote both cultivation, harvest and consumption.

2. MATERIALS AND METHODS

Collection and preparation of samples:

Mature *Mondia whitei* fruit was procured between the months of December 2016 and January 2017 from Abakpa market in Abakaliki, Abakaliki local Government Area of Ebonyi state, Nigeria. It was identified by a taxonomist in the Department of Plant Science and Biotechnology, Abia State University, Uturu. Whole green mature fruits were cut open to remove the seeds enclosed in a wool like structure. The edible flesh was size-reduced by cutting into smaller bits and dried under shade for 5 days. Subsequently, the dried fruit was pulverized into powder using mortar and pestle.

Extraction of bioactive compounds: This was done by methods described by Sasidharan *et al.* (2011). Five (5) grams of the pulverized *M. whitei* fruit powder sample was weighed into two labeled conical flasks respectively. 100ml methanol and 100 ml dichloromethane/methanol (1:1, v/v) were added to each conical flask containing the samples respectively and shaken vigorously. After which, each flask containing the sample and solvent were covered using aluminum foil and were allowed to stand for 24h at room temperature. Subsequently, each sample mixture was filtered through Whatman filter paper No1 into respective labeled conical flasks. The respective extracts were concentrated by evaporating excess solvent by heating in a boiling water bath. Hence, two extracts namely methanol and dichloromethane/methanol extracts of *Mondia whitei* fruit pulp were obtained. These were subjected to gas chromatography-mass spectrometry analysis for the separation and identification of bioactive compounds.

Gas chromatography-mass spectrometry analysis (GC-MS): GC-MS analysis of the fruit pulp extracts was done using Gas chromatography –mass spectrometry (Model QP 2010 series, Shimadzu, Japan) equipped with optima 5ms fused capillary column of 30mm length, 0.25 mm diameter and 0.25 μ m film thickness. Helium (99.99%) was used as carrier gas. The temperature programming was set with initial column oven temperature of 60 $^{\circ}$ C, hold time of 2 min by 120 $^{\circ}$ C to a final temperature of 300 $^{\circ}$ C with hold time of 2 min. 2 μ l of each *M. whitei* fruit pulp extract was injected respectively using a Hamilton syringe into the gas chromatogram for total ion chromatographic analysis with split injection technique (1:1). The injector temperature was 250 $^{\circ}$ C while for the mass spectrometer, the ion source temperature was 200 $^{\circ}$ C with an interface temperature of 280 $^{\circ}$ C and recorded over a scan range of 45 to 650m/z with electron impact ionization energy of 70ev. Total running time of gas chromatography-mass spectrometry analysis for the dichloromethane/methanol and methanol

extracts was 21 min. respectively. The relative percentage of each extract constituents were expressed with peak area normalization. Compounds in them were identified by mass spectrometry by comparing their retention indices and mass spectra fragmentation patterns with those stored on the computer library (i.e National Institute of Standards Technology (NIST/EPA/NIH) Mass spectral library, version 2.0). Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

3. RESULTS AND DISCUSSION

Gas chromatography-Mass spectrometry analysis of *M. whitei* fruit pulp revealed the presence of 21 compounds in the dichloromethane /methanol and 28 compounds in the methanol extracts respectively. Peak number, retention time, compound name, molecular weight molecular formula and relative abundance are stated in their various tables. Table 1 shows compounds identified in the dichloromethane/methanol extract of *M. whitei* fruit pulp which represents the hydrophobic (i.e lipophilic) fraction. 9-octadecenoic acid (Z) 2,3-dihydroxypropylester commonly known as glycerymonoleate (GMO) and octadecanoic acid-2,3-dihydroxypropyl

ester commonly known as glycerolmonostecrate (GMS) were the most abundant compounds. These constitute 54.25% of the compounds in the hydrophobic fraction. GMO eluted at peak 22 and had a relative abundance of 40.34%, while GMS eluted at peak 23 and had a relative abundance of 13.91%. GMO and GMS are added as food additives. GMO and GMS serves as a flavoring agents and adjuvant in dairy foods (CFSSAN, 2018; JECFA/FAO/WHO, 2018). The abundance of these two compounds in *M. whitei* fruit therefore makes it a good substrate from which they can be extracted and used for the purpose as a flavoring agent in the dairy industry.

Other compounds found in appreciable quantities include 3-O-methyl-d-glucose (8.86%), hexadecanoic acid 2-hydroxy -1-(hydroxyl methyl) ethyl ester (8.72%), hexanoic acid, octyl ester (6.45%), oleoyl chloride (4.84%) and 4H-pyran 4-one-2,3-dihydro-3-5-dihydroxy-6-methyl (2.89%). These consist of carbohydrate derivatives, fatty acid chloride, fatty acid esters and flavonoid. Hexadecanoic acid 2-hydroxy-1-(hydroxyl methyl) ethyl ester has been reported to have hemolytic, pesticide, flavor and antioxidant activities (Duke, 2013).

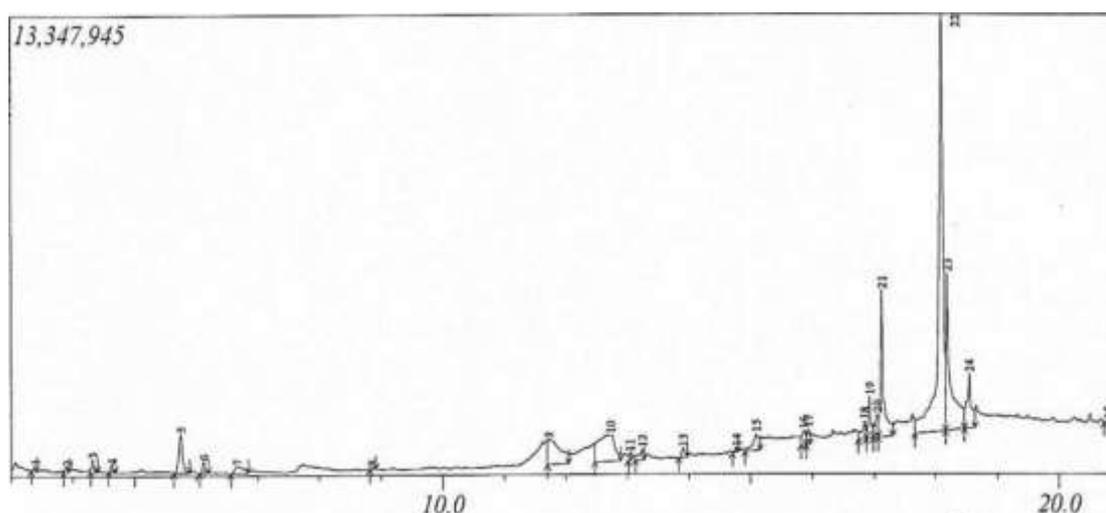


Fig.1. Gas Chromatography - Mass Spectrometry Chromatogram of Dichloromethane/Methanol Extract of *Mondia whitei* fruit pulp

Table 1. Bioactive compounds present in Dichloromethane/Methanol extract of *Mondia whitei* fruit pulp

Peak No.	Retention time (min.)	Relative abundance (%)	Compound name	Molecular weight	Molecular formula
1	3.385	0.16	2-Decanynoic acid	168	C ₁₀ H ₁₆ O ₂
2	3.908	0.27	2-Decanynoic acid	168	C ₁₀ H ₁₆ O ₂
3	4.317	0.63	2,4,6-Cycloheptatrien-1-one,4-methyl	120	C ₈ H ₈ O
4	4.633	0.33	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	128	C ₆ H ₈ O ₃
5	5.750	2.89	4H-Pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl	144	C ₆ H ₈ O ₄
6	6.125	0.53	Levomenthol	156	C ₁₀ H ₂₀ O
7	6.658	1.27	Catechol	110	C ₆ H ₆ O ₂
8	8.842	0.10	Acetic acid, 3-methyl-6-oxo-hex-2-enyl ester	170	C ₉ H ₁₄ O ₃
9	11.733	6.45	Hexanoic acid, octyl ester	228	C ₁₄ H ₂₈ O ₂
10	12.733	8.86	3-O-Methyl-d-glucose	194	C ₇ H ₁₄ O ₆
11	13.042	0.37	[1,1'-Bicyclopropyl]-2-octanoic acid,2'-hexyl-, methyl ester	322	C ₂₁ H ₃₈ O ₂
12	13.242	0.98	[1,1'-Bicyclopropyl]-2-octanoic acid,2'-hexyl-, methyl ester	322	C ₂₁ H ₃₈ O ₂
13	13.908	0.91	Phthalic acid	376	C ₂₃ H ₃₆ O ₄
14	14.767	0.59	n-Hexadecanoic acid	256	C ₁₆ H ₃₂ O ₂
15	15.092	0.21	9,12-Octadecadienoic acid (Z,Z)-	280	C ₁₈ H ₃₂ O ₂
16	15.867	2.01	9,12-Octadecadienoic acid (Z,Z)-	280	C ₁₈ H ₃₂ O ₂
17	15.933	0.82	7-Hexadecenal	238	C ₁₆ H ₃₀ O
18	16.842	1.38	7-Hexadecenal	238	C ₁₆ H ₃₀ O
19	16.925	1.19	10-Undecen-1-al, 2-methyl	182	C ₁₂ H ₂₂ O
20	17.042	2.15	9-Octadecenal,(Z)-	266	C ₁₈ H ₃₄ O
21	17.125	8.72	Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester	330	C ₁₉ H ₃₂ O ₄
22	18.100	40.34	9-Octadecenoic acid (Z)-2,3-dihydroxy propyl ester	356	C ₂₁ H ₄₀ O ₄
23	18.183	13.91	Octadecanoic acid-2,3-dihydroxy propyl ester	358	C ₂₁ H ₄₂ O ₄
24	18.542	4.84	Oleoyl chloride	300	C ₁₈ H ₃₃ ClO
25	20.983	0.11	Stigmast-5-en-3-ol, oleate	678	C ₄₇ H ₈₂ O ₂

Table 2 shows compounds identified in the methanol extract of *Mondia whitei* fruit. The major compounds identified were α 1, β 2, α 3, β 5-cyclohexenetetrol (10.70%), 3-O-methyl-D-

glucose (26.10%) and oleoyl chloride (29.62%). These constitute 66.42% of the compounds in the hydrophilic fraction. These compounds eluted at peaks 21, 22 and 30

respectively. α 1, β 2, α 3, β 5-cyclohexenetetrol was reported to be present in ethanol extract of *Zanthoxylum tertraspermum* stem bark (Ravikumar *et al.*, 2012). It is a polyhydroxy compound and is reported to have antioxidant, antimicrobial, anti-inflammatory activities (Ravikumar *et al.*, 2012). 3-O-methyl-D-glucose is a glucose analogue and it is not phosphorylated by hexokinase (Dringen and Hamprecht, 1993). Medically, it is used as a marker to assess glucose transfer by evaluating its uptake in various cells and organ systems (Dringen and Hamprecht, 1993). 3-O-methyl-D-glucose has been reported to inhibit glucose utilization and glucose oxidation (Sener *et al.*, 1999). 3-O-methyl-D-glucose is taken up by cells but not metabolized (Du *et al.*, 1999) and so can induce apoptosis of cells which is a relevant mechanism necessary to control cell proliferation and oxidative stress in cancer. Oleoyl chloride was reported to be present in hexane extract of *Psidium guajava* leaves (Alamin *et al.*, 2016). It is a fatty acid chloride and is reported to have antimicrobial activity (Alamin *et al.*, 2016). GC-MS result revealed the presence of some other compounds. These include 4H-Pyran-4-one 2,3-dihydro-3,5-dihydroxy-6-methyl. This is a flavonoid compound and has been isolated

from onion. It has been reported to modulate the activity of NF-B, hence inducing apoptotic cell death of colon cancer and modulates tumor necrosis factor - α (TN-8) (Ban *et al.*, 2007). There was also the presence of Hexadecanoic acid, -2-hydroxy-1-(hydroxymethyl) ethyl ester, γ -sitosterol and globulol. γ -Sitosterol is a steroid and has been reported that it may affect the amount and activity of components of the extrinsic apoptic pathway in human, lung and breast adenocarcinoma cells (Sundarra *et al.*, 2012). Globulol is a tricyclic hydroazulene sesquiterpene. GC-MS of Eucalyptus leaves revealed the presence of globulol and it is reported to have anti-tussive and expectorant properties (Doll-Boscardin *et al.*, 2012). There was also the presence of a glucosinolate which was n-heptyl isocyanate. Compounds common to both methanol and dichloromethane/methanol extracts of *Mondia whitei* fruit are 2-Decanynoic acid, 2,4,6-cycloheptatriene-1-one-4 methyl, -2,5-dimethyl-4 hydroxy-3-(2H) furanone, 4H-pyran-4-one -2,3-dihydro-3,5-dihydroxy-6-methyl, catechol, 3-O-methyl-D-glucose, 9,12-octadecadienoic acid, hexadecanoic acid-2-hydroxy-1-(hydroxymethyl) ethyl ester and oleoyl chloride.

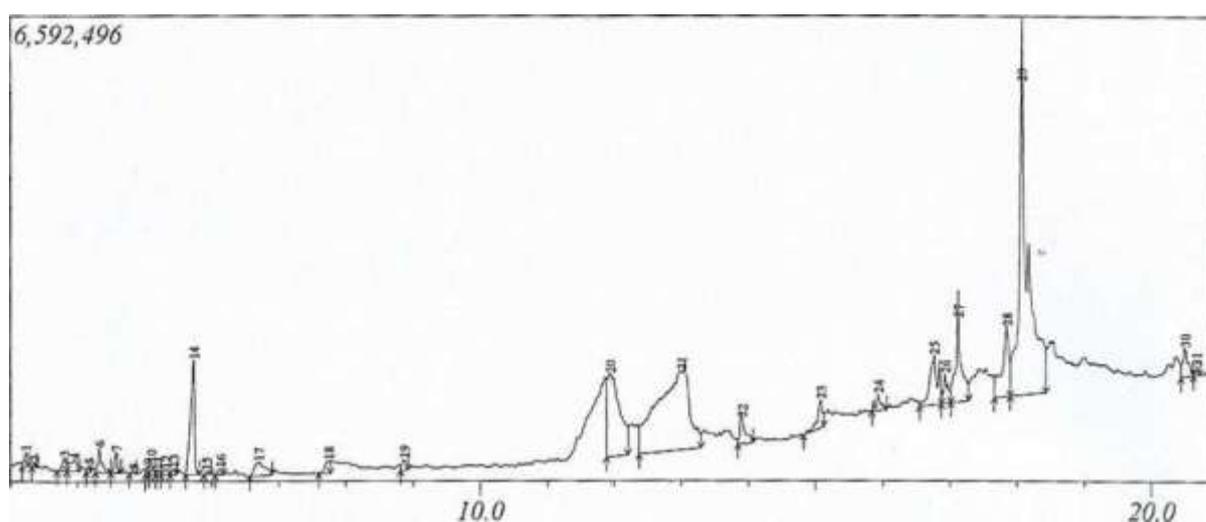


Fig. 2. Gas Chromatography - Mass Spectrometry Chromatogram of Methanol Extract of *Mondia whitei* fruit pulp

Table 2. Bioactive compounds present in methanol extract of *Mondia whitei* fruit pulp

Peak No.	Retention time (min.)	Relative abundance (%)	Compound name	Molecular weight	Molecular formula
1	3.233	0.34	2-Decanoic acid	168	C ₁₀ H ₁₆ O ₂
2	3.358	0.89	2-Decanoic acid	168	C ₁₀ H ₁₆ O ₂
3	3.808	0.08	3-Azabutyl-1-ol,4-cyclopropyl-3,3-dimethyl bromide	144	C ₈ H ₁₈ NO
4	3.958	0.50	6-Acetyl-β-d-mannose	222	C ₈ H ₁₄ O ₇
5	4.175	0.11	6-Acetyl-β-d-mannose	222	C ₈ H ₁₄ O ₇
6	4.333	0.70	6-Acetyl-β-d-mannose	222	C ₈ H ₁₄ O ₇
7	4.375	0.18	5-cis-methyl-IR-3-cis-cyclohexanediol	170	C ₇ H ₁₄ O ₂
8	4.575	0.96	2,4,6-cycloheptatrien-1-one,4-methyl-	120	C ₈ H ₈ O
9	4.825	0.60	(3-Ethoxy-4,5-dihydro-isoxazol-5-ylmethyl)-amine	144	C ₆ H ₁₂ N ₂ O ₂
10	5.042	0.75	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	128	C ₆ H ₈ O ₃
11	5.100	0.08	Imidazole, 1-methyl-2-Methylthio-	128	C ₅ H ₈ N ₂ S
12	5.200	0.24	Cycloheptanone, 2-methyl	126	C ₈ H ₁₄ O
13	5.317	0.38	5-Butyldihydro-2(3H) thiophenone	158	C ₈ H ₁₄ OS
14	5.442	0.06	n-Heptyl isocyanate	141	C ₈ H ₁₅ NO
15	5.733	0.23	1,8-Nonadien-3-ol	140	C ₉ H ₁₆ O
16	5.925	4.64	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	144	C ₆ H ₈ O ₄
17	6.133	0.20	5-Methoxypyrrolidin-2-one	115	C ₅ H ₉ NO ₂
18	6.692	1.49	Catechol	110	C ₆ H ₆ O ₂
19	7.733	0.68	p-Hydroquinone	110	C ₆ H ₆ O ₂
20	8.867	0.48	[1,1'-Bicyclopentyl]-2-one	152	C ₁₀ H ₁₆ O
21	11.933	10.71	α1,β2,α3,β5-cyclohexanetetrol	148	C ₆ H ₁₂ O ₄
22	13.042	26.10	3-O-Methyl-d-glucose	194	C ₇ H ₁₄ O ₆
23	13.900	1.58	n-Hexadecanoic acid	256	C ₁₆ H ₃₂ O ₂
24	15.067	1.54	9,12-Octadecadienoic acid (Z,Z)	280	C ₁₈ H ₃₂ O ₂
25	15.925	0.82	7-Hexadecenal	238	C ₁₆ H ₃₀ O
26	16.758	3.89	γ-Sitosterol	414	C ₂₉ H ₅₀ O
27	16.925	1.26	Cis-9-Hexadecenal	238	C ₁₆ H ₃₀ O
28	17.117	5.46	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	330	C ₁₉ H ₃₈ O ₄
29	17.850	4.79	Globulol	222	C ₁₅ H ₂₆ O
30	18.075	29.62	Oleoyl chloride	300	C ₁₈ H ₃₃ ClO
31	20.492	1.55	Stigmasta-5,22-diene-β-3-ol, acetate	454	C ₃₁ H ₅₀ O ₂

4. CONCLUSION

This study therefore has elucidated the presence of bioactive compounds and has revealed the goodness of wild edible fruit *Mondia whitei* as being useful and can be nutritionally and pharmaceutically important. Its cultivation for just the roots for culinary and therapeutic purposes can be extended to the fruits which also contain compounds which can be exploited for its nutritional, therapeutic purposes as well as incorporated as an additive in relevant food formulations.

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