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Phytochemical and Gas Chromatograpy-Mass Spectrometric Analyses of *Gmelina Arborea* Fruit

ABSTRACT

Phytochemical and GC-MS analyses of ethanol extract of *Gmelina arborea* fruit were carried out using standard methods. The results of phytochemical analysis revealed the presence of flavonoids, alkaloids saponins, cardiac glycosides and tannins in the sample. Seventeen chemical constituents were identified from GC-MS analysis which include ethylcyclohexane (10.8%), hept-2-ene (3.1%), ethylbenzene (14.5%), non-1-ene (14.2%), non -1-ene (9.01%), 3,5-dimethylhepta- 3,5-dien-1-yne (8.73%) (1-methylethyl) benezene (cumene)(5.61%), 3,5-dimethlhepta-3,5- dien-yne (1.30%), hexane (0.86%), hept-2-ene (2.05%), oct-2-ene (3.10%), oct-2-ene (4.68%), heptanoic acid (4.50%), oct-2-ene (0.69%), non-1-ene (10.05%), hepta-4,6-dien-2-ynoic acid (3.8%) and 2-butylpropane -1,3-diol (2.25%). Result obtained showed that the fruit extract of *Gmelina arborea* has ethylbenzene (14.5%) as the highest and oct-2-ene (0.69%) as the least chemical compounds. These relative diverse chemical constituents may be responsible for the medicinal properties of *Gmelina arborea* fruits.

Key Words: GC-MS analysis, chemical constituents, phytochemicals, *Gmelina arborea* andethanol fruit-extract

Introduction:

The use of plants in the management and treatment of diseases started with life. In recent years it has been found that many plants do have medicinal values (Sofowora, 1993).Some medicinal plants used in Nigeria include *Garcina kola* used in the treatment of asthma, *Carica papaya* used as a remedy for hypertension, *Ocimum basilicum* as a cure for typhoid fever and *Cola nitida* for treatment of pile (Nadkaru, 2008).

Gmelina arborea is moderately sized to large deciduous tree, about 30m or more in height and a diameter up to 4.5m. According to Khare (2010) and Pandy(2009) the fruit *Gmelina arborea* and leaf are used as carminative, in the treatment of headache, asthma, bronchitis, cholera, colic pain, epilepsy, rheumatism, small pox, spleen complaints, syphilis, throat swelling, poisons, cough, gonorrhea and as antidote to snake bite. The leaves are used in dyspepsia, cough, and wound treatment (Dinesh, 2009). The leaf has been reported to have anti-helmintic activity (Ambujakshi *et al.*, 2009). Offor *et al.* (2015) had reported significant ($p<0.05$) reduction in total protein and albumin levels in albino rats administered ethanol leaf-extract.

Despite the use of *Gmelina arborea*leaf and fruit for the treatment of various diseases, there is still paucity of documented data / information available regarding Gas chromatography–Mass spectrometric (GC/MS) analysis of the chemical constituents of the fruit. This study therefore evaluates the phytochemical composition and Gas chromatography–mass spectrometric (GC/MS) analysis of the chemical constituents of ethanol fruit-extract of *Gmelina arborea*.

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Figure 1: *Gmelina Arborea* Fruits (Tapsell *et al.*, 2006).

Materials and Methods

Materials

Plant Collection:

The fresh fruits of *Gmelina arborea*were collected from Presco Campus of Ebonyi State University, Abakaliki, Nigeria. The plant was identified by a taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. Some parts of the plant were also deposited in the herbarium for reference purpose.

Preparation of Plant Sample

The fruits were washed and shade dried at ambient temperature with constant turning to averts fungal growth. The dried fruits were later milled to obtained the vegetable fruit meals (VFMs) using an electric blender and was stored in 4^0C temperature in refrigerator in well labeled air-tight containers for analysis.

Preparation of *Gmelina arborea*Ethanol Fruit-Extract

Exactly 40grams of dried powdered fruit of *Gmelina arborea*were extracted successively with 300ml of ethanol in an orbital shaker for 24hours at room temperature. The extract was filtered using what-man N0.1 filter paper to remove extractable substances at every 3hrs interval. The combined extracts were then evaporated with rotary evaporator and the dried extracts were stored at 4^0C in air-tight sterile container in refrigerator.

Methods

Preliminary Phytochemical Analysis

The preliminary phytochemical screening for the presence of tannins, sapanins, alkaloids, cardiac glycosides, flavonoids and others were carried out on the ethanol fruit-extract of *Gmelina arborea*.

Test for the Presence of Tannins: This was carried out by the method of Harborne (1973)

Principle: Tannins are secondary metabolites of plant species and consist of sugar and non-sugar parts. They are capable of undergoing hydrolysis when inserted into dilute acids or boiling water to give rise to products such as polyhydroxyl phenolic compounds. They are reactive following the possession of functional groups called hydroxyl group (OH). They participate in redox reaction to give characteristics colour change on the reagent applied.

Procedure: One milliliter (1ml) of ethanol extract of the sample was collected using syringe and dispensed into test tube. Then, one milliliter (1ml) of ferric chloride ($FeCl_3$) was added to the test tube. A dirty green precipitate was

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observed which showed the presence of tannins.

Test for the Presence of Saponins: This was carried out by the method of Harborne (1973)
Principle: Saponins are glycosides with distinctive foaming characteristics. They consist of a polycyclic aglycone that is either a choline steroid or triterpeniod attached through C_3 and an ether bond to a sugar side chain. The aglycone is referred to as the sapogenin and steroid saponins are called saraponins. The ability of saponins to foam is caused by the combination of the non-polar sapogenin and the water soluble side chain (hydrophilic part), which have hydroxyl groups (¯OH) as functional group.

Procedures:
Frothing Test
Two milliliters (2mls) of the extract were diluted with 5ml of distilled water in a test tube. The mixture was stirred vigorously for about 5mins and was allowed to stand for 30minutes. Frothing which persisted for this duration indicated the presence of saponins.

Emulsion Test:
An emulsion is any thick liquid in which tiny drops of oil or fat are evenly distributed. Two to Five (2-5) drops of olive oil were added to 3mls of the sample in a test tube, stirred vigorously and allowed to stand for 30mins. Emulsification that was observed for this duration indicated the presence of saponins.

Test for Presence of Alkaloids: This was carried out by the method of Trease and Evans (1989)
Principle: Alkaloid can be detected as loose complexes following their ability to react with some reagents by producing characteristics colour changes depending on the type of reagent used. Alkaloids have an amino group (NH_2) as their functional group as in nicotine.
Procedure: Two milliliters (2mls) of the extract was collected using syringe and was dispensed into a test tube, the test tube was heated for 2mins and 5mls of hydrogen (HCl) was added and heated again and allowed to cool. The mixture was divided into A and B. To A, 2 drops of Meyer's reagent was added and white precipitate was observed which showed the presence of Alkaloids. To B, 2 drops of Dragendroff's reagent was added and the formation of red precipitate was observed which confirmed the presence of alkaloids.

Test for the Presence of Flavonoids: This was carried out by the method of Harborne (1973).
Principle: Flavonoids are colourless or pale yellow glycosides that are not soluble in non- polar solvents. They are compound that are oxidize by ethyl-acetate. They react with polar solvent to produce colour changes in accordance with the level of redox reactions that are likely to take place. Flavonoids also reacts with sodium hydroxyl group (NaOH) to form a yellow colour following the reaction of the hydroxyl group (¯OH) with the ketone functional group.
Procedure: Five milliliters (5ml) of the extract was collected using syringe and was dispensed into a test tube. Exactly 10mls of distilled water, 5mls of dilute ammonium hydroxide (NH_4OH) and few drops of tetraoxosulphate (VI) acid (H_2SO_4) were added in the test tube. A yellow colouration was observed which showed the presence of flavonoids.

Test for the Presence of Cardiac Glycoside This was carried out by the method of Harborne (1973).
Principles: Cardiac glycosides are organic compounds that are capable of undergoing hydrolysis in the presence of dilute acids, alkali or enzymes.

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Procedure: Two milliliters (2mls) of the extract was collected into a test tube and 5ml of glacial acetic acid was added and then 2mls of $FeCl_3$and 2mls of concentrated ferric acid were added too. A brown ring formation at inter phase of the mixture indicated the presence of deoxy sugar characteristics of cardiac glycosides.

Test for the Presence of Steroids and Triterpenoids:

This was carried out by the method of Harborne (1973).

Principle

Steroids are class of organic compounds with a chemical structure that contains the core of gonane or a skeleton derived from them. Usually, methyl groups are present at carbon one (C-1) and carbon three (C-3), an alkyl side chain at carbon seventeen (C-17) may also be present. Formation of red colouration if steroids is present or yellow colouration if triterpenoids is present upon addition of concentrated tetraoxosulphate (VI) acid to unknown sample in Salkwoki reagent test as a result of reaction with the functional group.

Procedure:

Five milliliters (5mls) of ethanol fruit-extract of *Gmelina arborea*was collected and dispensed into test tube, then two milliliters (2mls) of chloroform was added to the tube and then concentrated tetraoxosulphate (VI) acid (H_2SO_4) was also added. The mixture was stirred thoroughly and allowed to stand for some minutes. A red colour appeared at the lower layer of the mixture, which indicates the presence of steroids while a yellow colour was observed at the upper layer indicates the presence of triterpenoids.

GC-MS Analysis:

Procedures:

GC-Ms analysis of the ethanol extract of *Gmelina arborea*fruit was performed using Shimadzu Japan gas chromatography QP2010 plus with a fused gas chromatography (GC) column (2010) coated with poly-methyl silicon (0.25nm x 50m) and the conditions were as follows: Temperature programming from 80-200^{0}C held at 80^{0}C for 1minute, rate 5^{0}C/min and at 200^{0}C for 20min. Field ionization detector (FID) Temperature of 300^{0}C, injection temperature of 220^{0}C, carrier gas nitrogen at a flow rate of 1ml/min, split ratio of 1:75. Gas chromatography mass spectrum was conducted using GC-MS-QP 2010 plus Shimadzu Japan with injector temperature of 220^{0}C and carrier gas presence of 116.9kpa. The column length is 30m with a diameter of 0.25mm and flow rate of 50ml/min. Elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5 Kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra bank. German Hermlez 233M-Z centrifuge was used.

Component Identification

Chemical constituent of the extract was identified by matching the peak with computer Wiley Ms libraries and confirmed by those comparing mass spectra of the peaks and those from literature

Results

Result of Qualitative Phytochemical Analysis of *Gmelina Arborea* ethanol fruit-Extract.The result of the qualitative phytochemical of *GmelinaArborea* fruit ethanol extract revealed the presence of bioactive compounds such as alkaloids, flavonoids, tannins, saponins and cardiac glycosides.

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Phytochemicals	Remarks
Alkaloids	Positive
Tannins	Negative
Flavonoids	Positive
Saponins	Positive
Cardiac glycosides	Positive
Steroids	Negative

Table 1: Phytochemical Screening of Ethanol Fruit-Extract of *Gmelina Arborea*.

Results of GC-MS of Ethanol Fruit- Extract of *Gmelina Arborea* Fruits:

The ethanol fruit-extract of *Gmelina arborea* showed seventeen peaks from the GC-MS chromatogram (Figure 2). These peaks indicate the presence of seventeen compounds (1-17) in the extract as shown in Table 2. The composition of the extract comprises of non-1-ene (14.2%), ethyl-benzene (14.5%),ethyl-cyclohexane (10.8%), non-1-ene (10.05%), as the major chemical constituents.

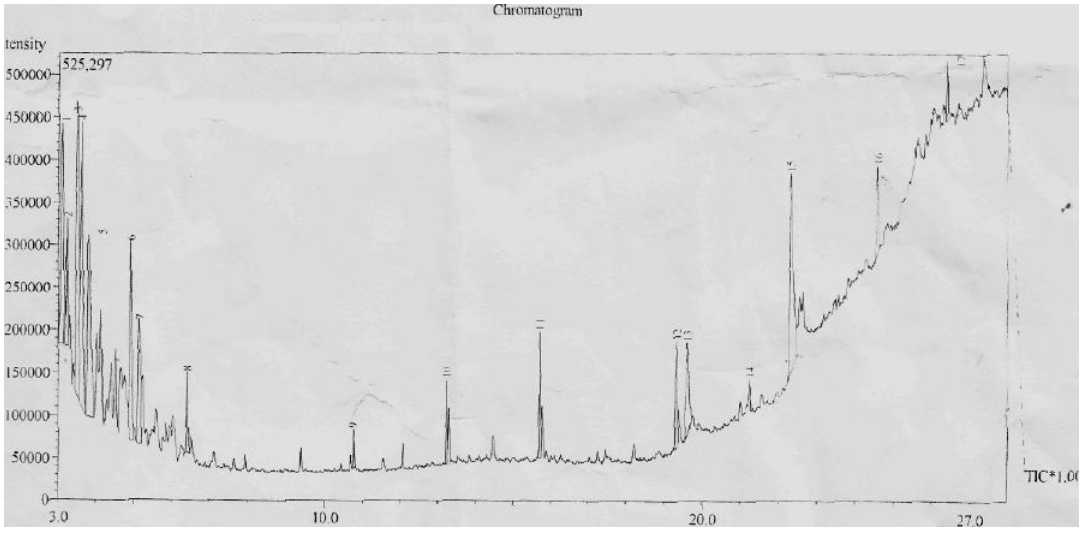

Figure 2: Chromatogram of *Gmelina arborea* Ethanol Fruits-Extract.

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Table 2: Results of GC-MS Analysis and Mass Data of *Gmelina Arborea* Ethanol fruit-Extract Showing Molecular Formula, Molecular Weight, Percentage Content, Retention Time andBase Peak.

Peaks	Compounds	Molecular Formula	Molecular Weight	Retention Time	Percentage Content	Base peak
1	Ethylcyclohexane	C_8H_{15}	111	3.093	10.8%	18
2	Hept- 2- ene	C_7H_{14}	98	3.239	3.1%	23
3	Ethylbenzene	C_8H_{10}	106	3.499	14.5%	37
4	Non – 1 – ene	C_9H_{18}	126	3.616	14.2%	22
5	Non – 1 – ene	C_9H_{17}	125	3.803	9.01%	34
6	3,5 dimethyl hepta – 3,5- dien-1-yne	C_9H_{12}	120	4.912	8.73%	27
7	(1- methylethyl) benzene (cumen)	C_9H_{12}	120	5.136	5.61%	26
8	3,5-dimethylhepta 3,5- dien -1- yne	C_9H_{11}	119	6.403	1.30%	22
9	hexane	C_6H_{13}	85	10.753	0.86%	11
10	Hept-2- ene	C_7H_{14}	98	13.214	2.05%	23
11	Oct -2- ene	C_8H_{15}	111	15.704	3.10%	23
12	Oct -2- ene	C_8H_{15}	111	19.317	4.68%	25
13	Heptanoic acid	$C_7H_{13}O_2$	129	19.603	4.50%	31
14	Oct -2- ene	C_8H_{15}	111	21.232	0.69%	29
15	Non -1- ene	C_9H_{17}	125	22.318	10.05%	45
16	Hepta – 4,6-dien-2-ynoic acid	$C_7H_5O_2$	121	24.572	3.8%%	46
17	2- butyl propane-1,3- diol	$C_7H_{16}O_2$	132	26.408	2.25%	47

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Discussion and Conclusion:

Discussion:

Phytochemical screening of *Gmelina arborea*fruit revealed that the fruit is rich in alkaloids, tannins, flavonoids, saponins and cardiac glycosides. Saponins and cardiac glycosides were found to be higher in concentration.Daya *et al*. (2012) had earlier reported high concentration of saponins and flavonoids but low concentration of phenols, glycosides and alkaloids in *Gmelina arborea*. Ayoola *et al*. (2011) also reported high level of phytochemical such as saponins and low levels of alkaloids, glycosides, steroids, flavonoids and tannins in*Tetracarpidium conophonum*. The result of this study was in correlation with the report of Aja *et al*. (2010) which revealed the presence of all these phytochemical in *Talinum triangulare* leaf in both dry and wet samples. Offor *et al*. and Aja *et al.* (2015) also reported the presence of all the phytochemicals in various concentrations in *Terminalia catappa* leaf, *Cajanus cajan* leaf and seed respectively. Aja *et al*. (2015) also had reported the rich phytochemical contents of *Dissotis rotundifolia* leaf and root. Nwali *et al*. (2013) also showed that *Bryophylum pinnatum* leaf contained low levels of phytochemicals.

The GC-MS analysis revealed seventeen different bioactive chemical constituents in ethanol fruit-extract if *Gmelina arborea*. It also revealed that fruit-extract has ethylbenzene (14.5%) as the highest and oct-2-ene (0.69%) as the least chemical constituents. Aja *et al.*(2014) identified the presence of sixteen GC-MS constituents in *Moringa oleifera* leaf with 9-octadecenoic acid (20.89%), L-(+)-ascorbic acid- 2,6-dihexadecanoate(19.66%), 14–methyl-8-hexadecenal (8.11%), 4- hydroxyl-4-methyl-2-pentanone (7.01%), 3-ethyl-2, 4-dimethylpentane(6.14%) and phytol (4.24%) as the major constituents. Nweke *et al*. (2015) also identified the presence of ten (10) GC-MS constituents of methanol leaf extract of *Vitex doniana* with 6-octadecenoic acid (24.19%) as the major and 1-tridecyne (2.4%) as the least constituents respectively. The study also correlated to the report of Uraku *et al*. (2015) on the G.C-MS constituents of essential oil from *Hyptis spicigera* leaf

Conclusion: The studyshowed that the ethanol fruit-extract of *Gmelina arborea* is rich in phytochemical. The GC-MS also revealed ethyl-benzene (14.5%) as the highest and oct-2-ene (0.69%) as the lowest chemical constituents.

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