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GC-MS Determination of Bioactive Components of *Napoleona imperalis* P. Beauv

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ABSTRACT

Napoleona imperalis, P. Beauv (Lecythidaceae) known as Ntum in Akaeze and Ikwuano dialect of Igbo language of Nigeria is traditionally used as local sweetener. The present investigation deals with GC-MS analysis of methanol extract of the above said plant. Twelve compounds were identified and of particular interest is 2, 5-dimethyl-2, 4-dihydroxy-3(2H)-furanone.

Key words: *Napoleona imperalis*, GC-MS analysis, mass spectrum, sweetener

INTRODUCTION

Though, *N. imperialis* is one of the lesser known plants, its economic importance has partially been reported by Dalziel (1955). These include the use of the fruit sugary pulp as desserts, the roots for medicinal purposes and the twigs as traditional chewsticks (Irvine, 1961). The plant is commonly known as Ntum in the Ikwuano dialect of Igbo language of Nigeria and is traditionally used as local sweetener. Glycosides containing a polycyclic aglycone moiety of either C_{27} steroid or C_{30} triterpenoid (collectively known as sapongenins) attached to a carbohydrate have been reported in *Napoleona imperalis* seed (Ukpabi and Ukpabi, 2003). *Napoleona imperialis* are rich sources of commercial hemolytic saponins and feed ingredient (Dong *et al*., 2011). The methanol extract of *Napoleona imperialis* has shown antibacterial and wound healing properties (Chah *et al*., 2006). Taking into consideration the economic importance of this plant, the methanol extract of fruit of *Napoleona imperialis* were analyzed for the first time using GC-MS. This study will help to identify the compounds with flavourant properties. GC-MS is one of the best techniques to identify the bioactive constituents of long chain, branched chain hydrocarbons, alcohols, acids and ester etc.

MATERIALS AND METHODS

This study was carried out at the Chemical Science Laboratory Evangel University Akaeze from 2012-2013.

Collection and identification of plant materials: Ripened fruit of *Napoleona imperalis* was collected from uncultivated farmland located at southern parts of Nigeria. The plant sample was identified and authenticated at Biological Science Department herbarium, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

Sample preparation and extraction procedure: The fresh ripened fruit pulp was ground into fine slurry using a blending machine. Required quantity of pulp was weighed and transferred to stoppard flask and treated with methanol until the pulp was fully immersed. The flask was shaken hourly for the first 6 h and then it was kept aside and again shaked after 24 h. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using a rotatory evaporator. The final residue, thus obtained, was then subjected to GC-MS analysis.

GC-MS analysis: GC-MS analysis of these extracts was performed using a Perkin-Elmer GC Clarus 500 system and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a elite-I, fused silica capillary column (30×0.25 mm 1D X 1 μMdf, composed of 100% dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 mL min^{-1} and an injection volume of 2 μL was employed (split ratio of 10:1); injector temperature 250°C and ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C min^{-1} to 200°C then 5°C min^{-1} to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 sec and fragments from 45-450 Da. Total GC running time was 36 min. The relative (%) amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a turbomass. 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 spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS

The components present in the methanol extract of fruit of *Napoleona imperalis* were identified by GC-MS analysis (Fig. 1). The active principles with their Retention Time (RT), molecular formula, Molecular Weight (MW) and base peak (%) in the methanol extract of fruits of *Napoleona imperalis* are presented in Table 1, while spectral data for the compounds are depicted in Table 2. Twelve compounds were identified in methanol extracts of *Napoleona imperalis*

Table 1: Components detected in crude methanol extract of *Napoleona imperalis*

RT	Name	Formula	Base peak (%)	Molecular wieght
3.975	2,3-Butanediol	$C_4H_{10}O_2$	44.95	90
5.783	2-ethyl-3-oxobutanoic acid	$C_7H_{12}O_3$	42.95	144
5.967	Dihydro-2(3H)-furanone	$C_4H_6O_2$	41.95	86
6.767	2-Hydroxy-2-cyclopent-1-one	$C_5H_6O_2$	98.10	98
8.592	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone	$C_6H_8O_4$	42.95	144
10.492	2,3-Dimethyl-1,4-dioxene	$C_6H_{10}O_2$	42.95	114
13.650	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	$C_6H_8O_4$	42.95	144
15.808	5-Hydroxymethyl-2-furanldehyde	$C_6H_6O_3$	40.95	126
27.600	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	43.00	256
28.767	(Z)-6-Octadecenoic acid	$C_{18}H_{34}O_2$	55.00	282
29.625	Methyl undecenate	$C_{12}H_{22}O_2$	55.00	198
31.067	Bis(2-ethylhexyl)-3-nitrophthalate	$C_{24}H_{37}NO_6$	57.05	435

Table 2: Spectroscopic data of chemical constituents of *Nepoleona imperalis*

Compound	Spectral data (MS[m/z(%)])
2, 3-Butanediol	45(100), 57(14), 43(11), 75 (0.5), 90 (0.4)
2-Ethyl-3-oxobutanoic acid	43(100), 87(45), 15(42), 102(40), 59(20), 70(15), 113(10), 144(0.2)
Dihydro-2(3H)-furanone	42(100), 41(50), 28(45), 86(36), 56(32), 29(25)
2-Hydroxy-2-cyclopent-1-one	98(100), 55(70), 42(52), 41(40), 69(35), 43(30)
2, 4-Dihydroxy-2, 5-dimethyl-3(2H)-furanone	43(100), 101(75), 73(62), 55(50), 144(40)
2, 3-Dimethyl-1, 4-dioxene	43(100), 114(18), 69(0.4), 86(0.2)
3, 5-Dihydroxy-6-methyl-2, 3-dihydro-4H-pyan-4-one	43(100), 44(70), 144(50), 101(45), 73(34), 72(30)
4,5-Dimethyl-4-hexen-3-one	41(100), 69(98), 97(90), 126(35), 27(32), 53(15), 70(5.4)
n-Hexadecanoic acid	43(100), 73(98), 60(84), 58(82), 41(76), 56(70), 256(46), 129(40), 85(30), 98(26), 213(20), 115(15), 227(10)
(Z)-6-octadecenoic acid	55(100), 41(72), 69(70), 83(60), 42(58), 97(50), 57(46), 98(35), 28(30), 60(28), 264(22), 282(0.2)
Methyl undecenoate	55(100), 74(98), 41(75), 87(60), 69(45), 97(40), 124(30), 166(12), 149(100), 137(8.0)
Bis(2-ethylhexyl)-3-nitrophthalate	57(100), 112(78), 70(65), 41(60), 194(42), 212(25), 149(20), 177(10), 324(0)

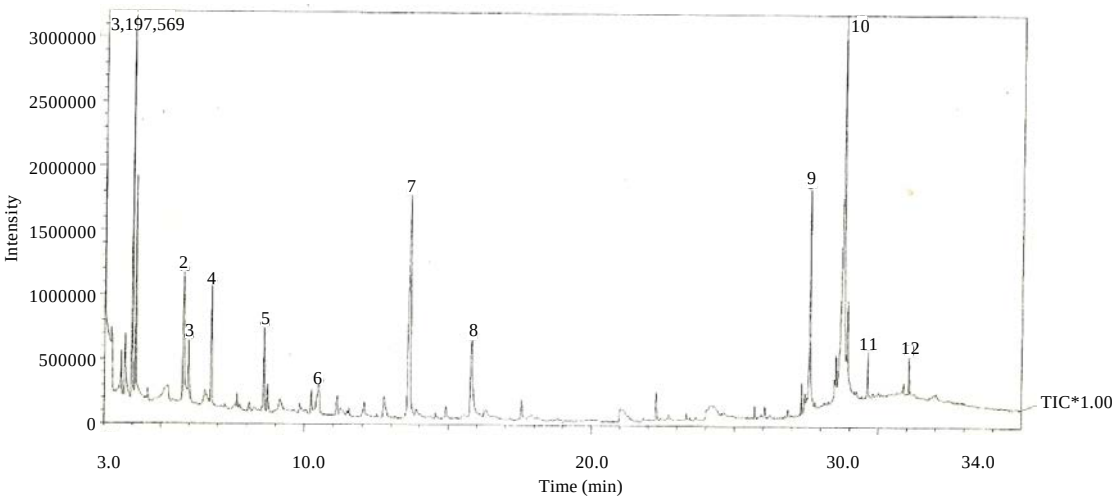

Fig. 1: GC-MS chromatogram of methanol extract of *Nepoleona imperalis* fruit

fruit. The prevailing compounds of interest are 2, 4-dihydroxy-2, 5-dimethyl-3(2H)-furanone, n-hexadecanoic acid and (Z)-6-octadecenoic acid. Figure 2-4 shows their mass spectrum and structures, respectively.

DISCUSSION AND CONCLUSION

In this present study, 12 compounds have been identified from methanol extract of the fruits of *N. imparalis* by Gas Chromatograph-Mass Spectrometer (GC-MS) analysis. Among the identified phytochemicals, 2, 4-dihydroxy-2, 5-dimethy-3(2H) furanone has a flavourant property comparable to those of steviol glycoside (Dong *et al.*, 2011). Dong *et al.* (2011), reported that crude stevia leaves are 10-15 times sweeter than table sugar while steviosides or the refined stevia extract

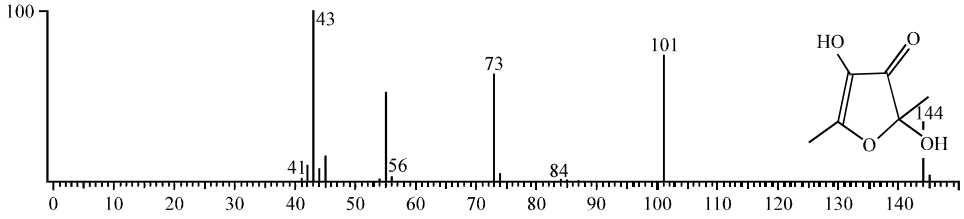

Fig. 2: Mass spectrum of 2, 4-dimethyl-2, 5-dihydroxyl-3 (2H)-furanone

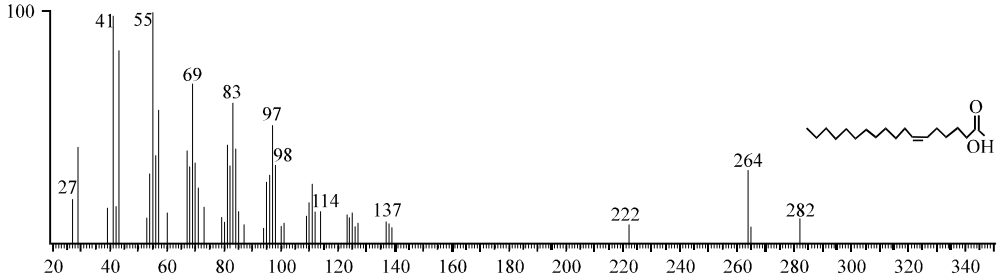

Fig. 3: Mass spectrum of [Z]-6-octadecenonic acid

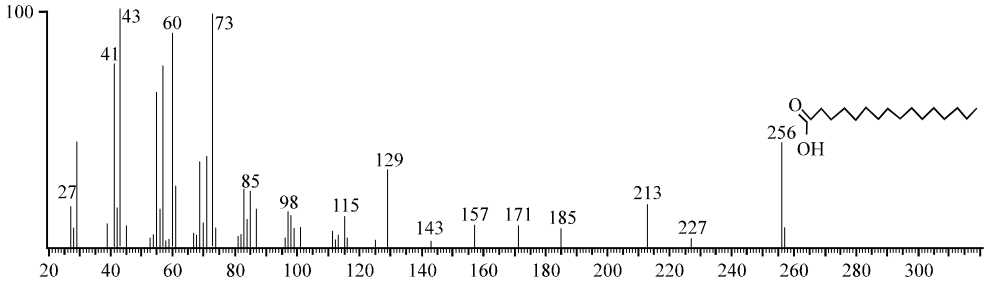

Fig. 4: Structure of n-hexadecanoic acid

is reported to be 200-300 times sweeter than table sugar. Others are (Z)-6-octadecenoic acid and n-hexadecanoic acid with antioxidant activity (Lalitharani *et al.*, 2009). Some unknown flavour compound were isolated by Gas Chromatograph-Mass Spectrometer (GC-MS) and variety of functionalities including alcohols, ketones, lactone, aldehydes, alphatic carboxylic acids and esters were identified as constituents of *Nepoleona imperalis* fruit flavour for the first time. Moreover, compounds not previously reported in *Nepoleona imperalis* fruit flavour 2, 5-Dimethyl-2, 4-dihydroxy-3(2H)-furanone or any known plant was identified. The organoleptic properties of 4-hydroxy, 2, 5-dimethyl-3(2H)-furanone and 4-methoxy-2, 5-dimethyl-3(2H)-furanone (mesifuran) as well as their importance for fruit flavour are well documented in literature (Nijssen, 1996; Mayerl *et al.*, 1989). This type of GC-MS analysis is the first step towards understanding the nature of active principles in the plant and this study will be helpful for further detailed study.

Figure 2 describes the mass spectrum of 2, 4-dimethyl-2, 5-dihydroxyl-3 (2H)-furanone with a molecular mass of 144 (M/Z 144 M^+) and base peaks at M/Z = 43, respectively. From the mass spectra, it was observed that cleavage of the bond beta to the carbonyl moiety (ring opening) and

subsequent cleavage on the bond alpha to the carbonyl moiety resulted to the fragment peak at M/Z = 101. This was due to the elimination of methyl radical and carbon monoxide molecule which resulted in rearrangement as is seen in the peak at M/Z = 101. Further fragmentation resulted to the loss of another carbon monoxide molecule at M/Z = 73. This, later, gave as intense peak at M/Z = 55 which was due the elimination of H_2O molecule. The peak at M/Z = 43 was due to the loss of an aldehyde (CH_2CHO) group from the parent molecular ion. This compound is the predicted compound of interest and further assay is needed to authentic it.

Figure 3 depicts the structure of [Z]-6-octadecenonic acid with molecular mass of 282 (M/Z 282 M^+). From the mass spectra, diagnostic peaks were as seen at M/Z = 264 which was due to loss of water molecule. Others are fragment peaks at M/Z = 222 which was due to the loss of $CH_2 = C = O$ and M/Z = 98 resulting from loss of 124 mass unit (CH_3-$(CH_2)_3$-CH = CH-CH_2-CH = CH_2). An alkyl group from the compound form C_7H_{14} molecule. Further cleavage resulted in the fragments at M/Z = 55 and M/Z = 69. These were due to loses of propylene group and ethyl group [CH_2 = CH-CH_2] and [CH_3-CH_2], respectively. However, it could be noticed that the loss of propylene radical was favoured because it has the highest percentage abundance at M/Z = 55 against the ethyl radical at M/Z = 69 with about 72% abundance. The loss of methylene radical CH_2 revealed a strong peak at M/Z = 41.

Figure 4 showed the structure of the compound n-hexadecanoic acid from crude extract. The molecular mass is indicated as (M/Z 256 M^+) and base peak at M/Z = 43. From the mass spectrum, useful peaks includes fragmentation peak at M/Z = 227 which was due to the loss of ethyl radical and at M/Z = 213, loss due to propyl radical with a mass unit of 43 ($CH_3CH_2CH_2$). Subsequent cleavage includes the elimination of ethene molecule (C_2H_4) at M/Z = 185, 157 and 129, respectively. Carbon dioxide molecule was lost at M/Z = 85, which corresponds to a mass unit of 44 (CO_2). The base peak at M/Z = 43 was due to the loss of propyl radical (CH_2 = CH-CH_2).

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