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GC/Ms Analysis and Identification of Phytochemicals Present in the Fruits of Mormodica Balsamina Linn

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Abstract: *Mormodica balsamina linn is one of the medicinal plants used in Nigeria by the herbalists for the treatment of different ailments. Despite its rich pharmacological potential, so far it has not been scientifically evaluated. Hence in this study, the phytochemicals from the Mormodica balsamina seeds were extracted with ethanol and subjected to GC/MS analysis and the identification of compounds were done by comparing the chromatogram, peak value of the unknown compound with entries in NIST database. Among the 10 phytochemicals identified, 9-Octadecenoic acid or oleic acid is the most abundant compound present followed by 3,5-Dihydroxy-6-methyl 2,3-dihydro-4 H-pyran-4-one. Other compounds revealed are 2-Hydroxypropanoic acid or Lactic acid, 5- Hydroxymethylfurfural, Ethyl- alpha-d-glucopyranoside, etc. Brief analysis of some of the compounds shows the rich pharmacological potential of this medicinal plant which needs further scientific experiments to unveil some of the novel pharmacophores which might exist in this plant.*

Keywords : *pharmacophores, Mormodica balsamina, anti- diarrhea.*

I. Introduction

For ages, man has depended on plants and plant extracts as sources of food, medicine, shelter, clothing, etc. Extracts derived from plants are proven source of bioactive compounds with therapeutic indications against a wide spectrum of diseases and infections (Okwu and Ohenhe, 2010). Mormodica balsamina linn is one of such plants. It has been shown to produce secondary metabolites that display antioxidant, anti- diarrhea, neuroprotective, antiviral and antibacterial effects (Otimenyin , 2008; Thakur et al, 2009 ; Bako and Uchegbu, 2014).

Mormodica balsamina linn also known as balsam apple or African pumpkin is a climber with stem attaining 4-5mm in length (Burkill, 1995). It belongs to the family of cucurbitaleae. The plant is a perennial herb with soft stems and tendrils. The plant produces spindle shaped fruits (dark green when unripe and bright to deep orange when ripe). The seeds are embedded into a sweet edible red fleshy pulp and test like water melon (Hassan and Umar, 2006). The whole plant is used as a bitter stomachic and it's infusion is used as a wash in the management of fever and yaws (Dalziel,1937). A macerate of the whole plant is also used as a galactogogue and to massage the chest to relieve intercostals pains (Burkill,1995). The roots and stems of M. balsamina are used for the treatment of diarrhea (Otimenyin et al, 2008). In Northern part of Nigeria, the leaves are cooked as green vegetable soup for lactating mother, where it is believed to help the mother regain or regenenerate her lost blood during labour and to purify her breast milk (Hutchings et al, (1996); Roodt (1998); Bandeira et al, (2001). Work carried out by Thakur et al,2009 revealed that M. balsamina plant contains a therapeutic agent 'Momordin' which is capable of inhibiting the growth of HIV and other viruses.

Despite the rich pharmacological potential of this plant, so far it has not been scientifically evaluated. Thus, in the light of the above information the present investigation was undertaken to evaluate the phytoconstituents by GC-MS.

II. Materials And Method

Plant Materials: Plant Materials : The fruits of Momordica balsamina were collected from forests in keffi, Nassarawa state, Nigeria. Authentication of plant materials was done by Dr A. Nmeregini of Taxonomy section, Forestry Department, Micheal Okpara University of Agriculture, Umudike, Nigeria.

Sample Preparation / Extraction: The succulent part of the riped fruit were percolated in ethanol for 24 hrs and filtered. The filtrate was concentrated under reduced pressure and subjected to systematic GC and GC-MS analysis.

General Experimental Procedure:

The GC analysis were carried out in SHIMADZU JAPAN gas chromatography 5890-11 with a fused GC column (OV- 101) coated with polymethyl silicon (0.25nm x 50m) and the conditions were as follows: Temperature programming from 80- 200^{o}C held at 80^{o}Cfor 1 minute, rate 5^{o}C/min and at 200^{o}C for 20 min. FID temperature 300^{o}C, injection temperature of 250^{o}C and carrier gas nitrogen at a flow of 1ml /min, split ratio 1:75. GC- MS analysis was conducted using GCMS- QP 2010 PLUS SHIMADZU JAPAN with injector temperature of 230^{o}C and carrier gas pressure of 100kpa. The column length was 30m with a diameter of 0.25mm and the flow rate of 50ml/min. the elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra data bank. HERMLE Z 233 M-Z centrifuge Germany was used. Reagents and solvents like ethanol, chloroform, diethyl ether, hexane were all analytical grade and were procured from MERCK, GERMANY.

Component Identification:

Oil components were identified by matching the peaks with Computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks with those from literature (Brillo and Selvakymari, 2006; Okwu and Ighodaro, 2010; Uchegbu et al, 2013).

III. Results and Discussions

The GC-MS analysis of the oil from ethanol extract of Mormodica balsamia fruit showed ten peaks indicating the presence of ten compounds in the oil. Compound 1 is identified as Lactic acid with molecular formula $C_3H_6O_3$ (m/z 90). It constitutes 14.33% of the oil. The base peak occurred at m/z 45 due to the cleavage of the carboxyl fragment, COOH (m/z 45) from the compound. Compound 2 is 2,5-Dimethyl-4-hydroxy-3-hexanone, the molecular formula is $C_8H_{16}O_2$ (m/z 144). Its composition is 6.57% of the oil. Compound 3 is pyranone, 3,5-Dihydroxy-6-methyl 2,3-dihyro 4H-pyran-4-one. Its molecular formula is $C_6H_8O_4$ (m/z 144). It constitutes 16.72% of the oil. Compound 4 is identified as 5-Hydroxymethylfurfural with the molecular formula $C_6H_6O_3$ (m/z 126). It constitutes 8.96% of the oil. The base peak occurred at m/z 97 accounting for the cleavage of $C_5H_5O_2$ (m/z 97) from the compound. Compound 5 is ethyl alpha-d-glucopyranoside with molecular formula $C_8H_{16}O_6$ (m/z 208). Its percentage constituent is 9.85%. compound 7 is an ester, Ethyl palmitate with the molecular formular $C_{18}H_{36}O_2$ (m/z 284). It constitutes 3.88% of the oil. Compound 6 is a carboxylic acid, palmitic acid, with molecular formula $C_{16}H_{32}O_2$ (m/z 256). It constitutes 5.37% of the oil. The base peak occurred at m/z due to the detachment of the terminal propyl, C_3H_7 (m/z 43) fragment from the compound. Compound 8 is also a carboxylic acid, oleic acid with molecular formula $C_{18}H_{34}O_2$. It is the most abundant compound (19.40%). Compound 9 is Ricinoleic acid with the molecular formula $C_{18}H_{34}O_3$ (m/z 298). It constitutes 8.06% of the oil. Compound 10 is 3-(Benzoylthio)-2-methylpropanoic acid. Its molecular formula is $C_{11}H_{12}O_3S$ (m/z 224). The base peak occurred at m/z 105 as a result of detachment of C_7H_5O (m/z 105) fragment from the compound. It constitutes 6.87% of the oil.

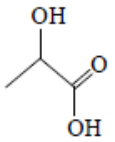
M. balsamina fruits contain high amount of oleic acid. Consumption of oleic acid from food has been associated with a decreased risk of breast cancer and reduction of blood pressure (Teras et al, 2008). n-Hexadecanoic acid was also found to be present in M. balsamina. In India, medicated oils rich in n-Hexadecanoic acid are used in the treatment of rheumatism and inflammation (Aparna et al 2012 and Smolinske and Susan, 1992).The compound, 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (DDMP) which was discovered in the seed extract of Mormodica balsamina linn has been reported to have antimicrobial, anti-inflammatory and antiproliferative activity (Rane - Zab and Anusha Bhaskar, 2012). This DDMP moiety containing soybean saponin has been shown to protect the mouse fibroblasts cells from damage by hydrogen peroxide (Yoshikoshi et al, 1996). DDMP isolated from onion have modulated the activity of NF- κB thereby inducing the apoptotic cell death of cancer cells (Ban et al, 2007, Rajasekaran et al, 2012). Ethyl alpha.-d-glucopyranoside also found in the plant has been reported to have antituberculous activity, antioxidant activity, alpha amylase inhibitory activity, hypolipemic activity and anticonvulsant (Rane – Zab and Anusha 2012). This implies that the fruit of Mormodica balsamina linn can be used to treat oedema, tuberculoses, convulsion and other ailments.

IV. Conclusion

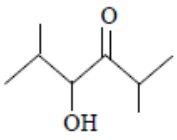
The result of the analysis shows the rich pharmacological potential of this medicinal plant which needs further scientific experiments to unveil some of the novel pharmacophores which might exist in this plant.

Table 1: GC-MS Analysis of the various Peaks from the fruits of Mormodica balsamina

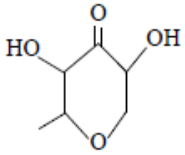
Chromatogram peak	Compound name	Molecular formular	Molecular mass	Retention Time (mins)	% content	Fragment peaks & aboundance
1	2-Hydroxypropanoic acid or Lactic acid	$C_3H_6O_3$	90	5.392	14.33	27(20%) 29(30%), 45(100%)
2	2,5-Dimethyl-4-hydroxy-3-hexanone	$C_8H_{16}O_2$	144	5.992	6.57	41(20%), 43(70%) 73(100%), 87(10%)
3	3,5-Dihydroxy-6-methyl 2,3-dihydro-4 H-pyran-4-one	$C_6H_8O_4$	144	8.750	16.72	43(100%), 58(10%), 73(30%) 101(40%), 144(70%)
4	2-furan carboxaldehye, 5-(hydroxymethylfurfural	$C_6H_6O_3$	126	10.117	8.96	29(20%), 41(70%) 53(10%), 69(40%) 97(100%), 109(15%)
5	Ethyl-α-d-glucopyranoside	$C_8H_{16}O_6$	208	15.600	9.85	42(45%), 60(100%), 73(40%), 88(10%)
6	n-Hexadecanoic acid or palmitic	$C_{16}H_{32}O_2$	256	20.125	5.37	27(20%), 41(80%) 60(90%), 73(95%) 85(30%), 98(20%) 129(30%), 256(50%)
7	Hexadecanoic acid, ethyl ester or Ethyl palmitate	$C_{18}H_{36}O_2$	284	20.392	3.88	41(30%), 57(25%) 73(151%), 88(100%) 101(50%), 157(10%), 284(10%)
8	9-Octadecenoic acid or oleic acid	$C_{18}H_{34}O_2$	282	23.017	19.40	27(15%), 41(80%) 55(100%), 69(70%), 83(60%) 97(50%), 98(30%) 264(20%)
9	(9E) 12-Hydroxy-9-octadecenoic acid or Ricinoleic acid	$C_{18}H_{34}O_3$	298	24.992	8.06	41(40%), 55(100%), 69(40%), 82(30%) 97(50%), 124(30%), 166(50%), 184(30%)
10	3-(Benzoylthio)-2-methylpropanoic acid	$C_{11}H_{12}O_3S$	224	26.200	6.87	51(10%), 77(40%) 105(100%)tt6



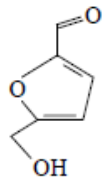
[1] 2-Hydroxypropanoic acid (Lactic acid)



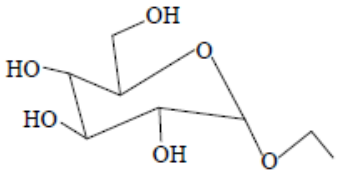
[2] 2,5-Dimethyl -4-hydroxy-3-hexanone



[3] 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one

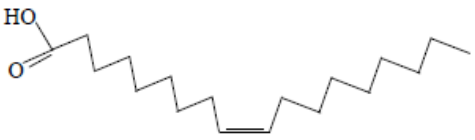


[4] 5-Hydroxymethylfurfural

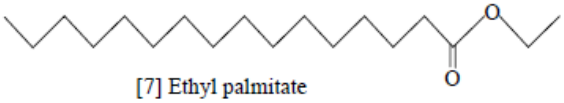


[5] Ethyl α-D-glycopyranoside

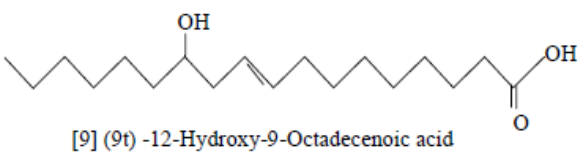
[6] n-Hexadecanoic acid



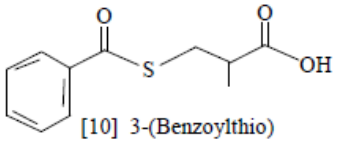
[8] 9-Octadececenoic acid (or Oleic acid)



[7] Ethyl palmitate



[9] (9t) -12-Hydroxy-9-Octadecenoic acid (or) Ricinoleic acid



[10] 3-(Benzoylthio) -2-methylpropanoic acid

Fig. 2: Structures of the compounds from GC-MS Analysis of the oil from Mormodica balsamina fruits.

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