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Characterization of *Landolphia dulcis* Ethanol Extract by Gas Chromatography - Mass Spectrometry Analysis

I.E. Otuokere[1], F.J. Amaku[1], K.K. Igwe[2] and C.A. Bosah[1]

[1]Department of Chemistry, Michael Okpara University of Agriculture
Umudike, Nigeria
ifeanyiotuokere@gmail.com

[2]Department of Vet. Biochem and Pharm, Michael Okpara University of Agriculture, Nigeria.
Umudike, Nigeria
tosmanbaba@yahoo.com

Abstract— ***Landolphia dulcis*** **is a flowering plant in the family Apolynaceae. It is a stout woody perennial climbing shrub that grows up to 10 metres. Gas chromatography – mass spectrometry analysis was carried out on the ethanol leave extract of *Landolphia dulcis.* The gas chromatogram showed the presence of nine compounds. The molecular mass of the compound were established based on the molecular ion in the mass spectra. The compounds were proposed based on comparison with National Institute of Standards and Technology (NIST) database. The suggested compounds are furan-2-ylmethanol (0.63%), 2-ethyl-2-hexen-1-al (0.61%), 7-oxooctanoic acid (61.18%), methyl 14-methylpentadecanoate (2.26%), hexadecanoic acid (5.20%), hexadecanoic acid, ethyl ester(2.76%), 9,12-Octadecadienoic acid, methyl ester (9.66%), niacin (13.84%) and 9,12,15-octadecatrien-1-ol (3.86). This study therefore justifies the traditional use of the *Landolphia dulcis* in the treatment of diseases.**

Keywords— **Bioactivity, ethanol, extract, gas chromatography, *Landolphia dulcis,* mass spectrometry**

I. INTRODUCTION

There is renewed interest in the use of plant extract as a major source of pharmaceutical ingredients [1]. Some herbal decoctions that are commonly used have been generally accepted for everyday use. A typical illustration is the use evening primrose oil by British women, to help relieve pre-menstrual tension [2]. 11% of 252 basic and essential by the World Health Organization are exclusively of plant origin or indirectly derived from natural precursors [1]. It is reported that 60% drugs in use or under clinical trials as anti-tumour and anti-infectious are of natural origin [3]. Medicinal plants have phytochemicals that are bioactive against diseases. Most Nigerians depends on plant decoction for treatment of illness because of its reliability and stability. Nearly all the tribes in the world depend on full or partial herbal medicines because of its affordability, availability, cheapness, effectiveness and low toxicity [4]. Extraction of phytocompounds from plants permits its proximate, phytochemical and vitamins to be studied [5]. Plant metabolites such as tannins, quinines, terpenoids and capsaicin are used as defensive mechanism against microorganism, insects and herbivores. These metabolites are important to man because of their medicinal value [4].

Landolphia dulcis is a flowering plant in the family Apolynaceae. It is a stout woody perennial climbing shrub that grows up to 10 metres. *L. dulcis* is known as 'utu akwara' in South-East Nigeria. It gets support from other plant by using its tendrils. *L. dulcis* can be found in west Africa countries of Senegal and Nigeria. The fruit is edible sometimes with a sweet or acidic taste. Studies have shown that the leaves contain alkaloids and saponins [6]. Cardio-tonic activities have been reported from the back extract of *L. Dulcis* [6]. Decoctions of the leafy twigs and powdered back are used in the treatment of wounds [6]. Trunk-bark and root decoction are used as a galactogogue by application to the breast [7]. Aphrodisiac activity of ethanol root extract and fractions of *L. dulcis* have been published [8]. Pictorial view of *L. dulcis* is shown in Figure 1.

Figure 1: Pictorial view of picture *L. dulcis* [12]

II. MATERIALS AND METHOD

Plant Materials

Fresh leaves of *L. dulcis* were harvested from natural habitat, in Ogbuebulle, Ikwuano, Abia State, Nigeria. The plants were identified at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

Preparation of plant extract

The plants were washed thoroughly in running water. They were dried for 15 days at room temperature and pulverized to powder using electrical grinder. The leaves of *L. dulcis* were prepared according to research reports found in literature [9, 10]. Powder leaves 50g of *L. dulcis was* soaked in 200ml of ethanol and agitated in an orbit shaker for 48 hours. After 48 hours, the mixture was filtered using whatman no 1 filter paper and then concentrated using rotary evaporator. The concentrated ethanol extract was used for GC-MS analysis.

GC-MS analysis of Landolphia dulcis

Characterization of the phytochemicals was done using GC-MS QP2010 Plus (Shimadzu, Japan) with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was 70eV. Gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60m, XTI-5).The initial column temperature was 80^{o}C for 1min, and then increased linearly at 70^{o}C min^{-1} to 220^{o}C, held for 3 min followed by linear increased temperature 10^{o}C min^{-1} to 290^{o}C for 10 min. The temperature of the injection port was 290^{o}C and the GC-MS interface was maintained at 290^{o}C. The sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min^{-1}.

Identification of phytocompoments in L. dulcis

The retention indices, peak area percentage and mass spectra molecular ion ethanol extract were compared with the database of National Institute of Standards and Technology (NIST), NIST08.LIB [11]. The name, molecular weight, formula, structure and bioactivities of the compounds were proposed.

III. RESULTS AND DISCUSSION

Gas chromatogram and mass spectra of ethanol leaves extract of *L. dulcis* are presented in Figures 2 and 3 respectively. Nine peaks were shown in the gas chromatogram (Figure 2). This suggests the presence of nine compounds in the leave ethanol extract of *L. dulcis.* The proposed compounds, retention time (RT), peak area percentage, molecular weight, molecular formula and bioactivities *are* discussed in Table 1. The structures are shown in Figures 4 - 12 respectively.

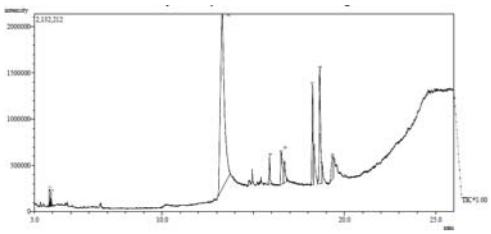

Figure 2: Gas chromatogram of ethanol leave extract of *L. dulcis*

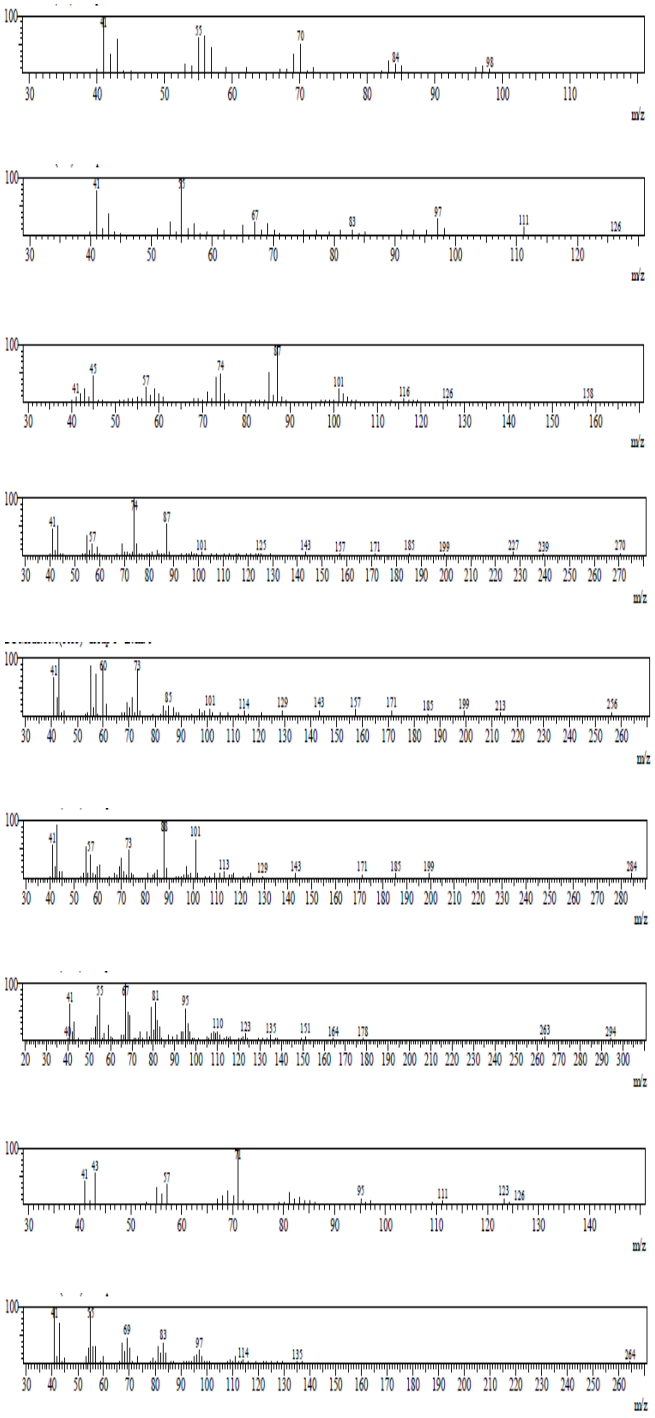

Figure 3: Mass spectra of 9 phytocompounds in in ethanol leave extract of *L. dulcis*

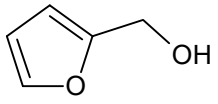

Figure 4: Furan-2-ylmethanol

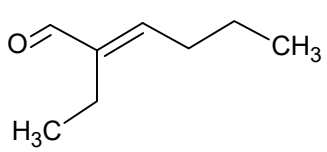

Figure 5: 2-Ethyl-2-hexen-1-al

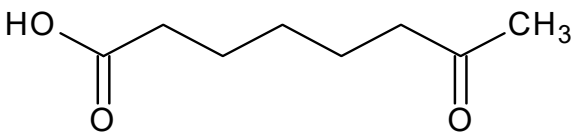

Figure 6: 7-oxooctanoic acid

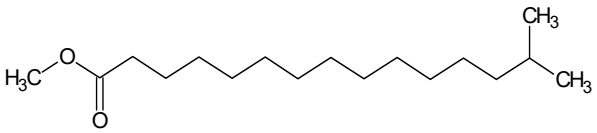

Figure 7: Methyl 14-methylpentadecanoate

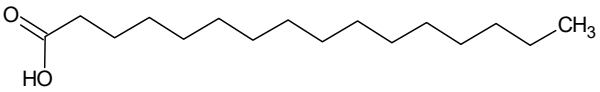

Figure 8: Hexadecanoic acid

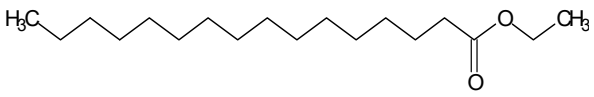

Figure 9: Hexadecanoic acid, ethyl ester

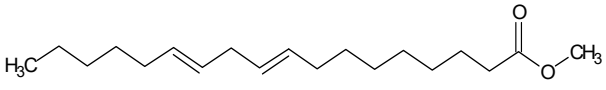

Figure 10: 9,12-Octadecadienoic acid, methyl ester

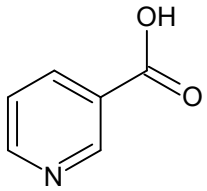

Figure 11: Niacin or nicotinic acid

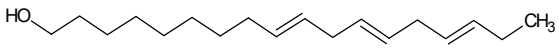

Figure 12: 9,12,15-Octadecatrien-1-ol

TABLE I
IDENTIFIED COMPOUNDS IN ETHANOL EXTRACT OF L. DULCIS

S/No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formula	Bioactivity
1	Furan-2-ylmethanol	3.824	0.63	98.09	$C_5H_6O_2$	Antioxidant
2	2-Ethyl-2-hexen-1-al	3.926	0.61	126.19	$C_8H_{14}O$	5-Alpha-Reductase-Inhibitor, Albuminurigenic, Alcohol-Dehydrogenase-Inhibitor, Aldehyde-Oxidase-Inhibitor, Aldose-Reductase-Inhibitor, Alpha-Agonist
3	7-Oxooctanoic acid	13.306	61.18	158.19	$C_8H_{14}O_3$	Acidifier, Acidulant, Arachidonic acid, Arachidonic-Acid-Inhibitor, increase Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric Acid
4	Methyl 14-methylpentadecanoate	15.898	2.26	270.45	$C_{17}H_{34}O_2$	Catechol-O-Methyl-Transferase-Inhibitor, Methyl-Guanidine-Inhibitor
5	Hexadecanoic acid	16.541	5.20	256.42	$C_{16}H_{32}O_2$	Acidifier, Acidulant, Arachidonic acid, Arachidonic-Acid-Inhibitor, increase Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric Acid
6	Hexadecanoic acid, ethyl ester	16.722	2.76	284.47	$C_{18}H_{36}O_2$	Urine-Acidifier, Urinary-Acidulant, Inhibit Production of Uric Acid, Increase Aromatic Amino Acid Decarboxylase Activity, Arachidonic-Acid-Inhibitor,
7	9,12-Octadecadienoic acid, methyl ester	18.256	9.66	294.47	$C_{19}H_{34}O_2$	Catechol-O-Methyl-Transferase-Inhibitor, Methyl-Guanidine-Inhibitor
8	Niacin or nicotinic acid	18.651	13.84	123.10	$C_6H_5NO_2$	Anticancer (Oral), Antidote (organo-P), Antidote (Organophosphorus), Orexigen
9	9,12,15-Octadecatrien-1-ol	19.380	3.86	264.44	$C_{18}H_{32}O$	Oligosaccharide Provider

Furan-2-ylmethanol acts as an antioxidant. Antioxidants have the ability to stop the oxidation of molecules. Oxidation is a process where free radicals are produced in a chain reaction that may be harmful to living cells. Investigation of dietary antioxidants on potential effects on neurodegene rative diseases have been studied [13], but have remained inconclusive [14]. A chronic infection like Parkinson's disease is treatable with a catechol-O-methyl-transferase-inhibitors. Since Methyl 14-methylpentadecanoate and 9,12-Octadecadienoic acid, methyl ester are inhibitors of catechol-O-methyl-transferase [15], they may be effective in the treatment of Parkinson's disease. Catechol-O-methyl-transferase is involved in the degradation of neurotransmitters but the inhibitors oppose the degradation of neurotransmitters

It is necessary to stop the conversion of testosterone to dihydrotestosterone (DHT) in order to stop benign prostatic hyperplasia (BPH) and androgenic alopecia. It has been reported that 2- Ethyl-2-hexen-1-al is a 5α-reductase inhibitor [15]. Clinically, 5α-reductase inhibitors are used in the treatment of conditions that are exacerbated by dihydrotestosterone (DHT) [16]. They are primarily used in the management of benign prostatic hyperplasia (BPH) and androgenic alopecia because of their antiandrogen effects. Metabolic transformation of these endogenous steroids is possible since these compounds inhibit the enzyme 5α-reductase. The major role of 5α-reductase inhibitors is the inhibition of testosterone to dihydrotestosterone (DHT).

7-oxooctanoic acid, hexadecanoic acid and hexadecanoic acid, ethyl ester have been reported to be an acidifier, acidulant and arachidonic acid inhibitor [15]. Acidifiers are chemicals that reduce the pH of the body. Acidifiers are needed for food digestion especially in patients suffering from achlorhydria. These patients are not able to secret HCl for food digestion. These phytocompounds will be beneficial since it increases gastric acid when ingested. Arachidonic acid is present in the brain, muscles, and liver [17]. Arachidonic acid is a fatty acid that is polyunsaturated in nature and responsible for the repair and growth of skeletal body tissue [18]. Arachidonic acid does not cause cancer but studies have proven that it might be a major cause of inflammation [19-22]. Additives that reduce the pH of food in order to add a tart taste and characteristic tang are called acidulants. They also have preservative and antioxidative properties[23].

9,12,15-Octadecatrien-1-ol, provides oligo saccharide [15]. It helps in cell division and cell binding. It also improves gastrointestinal health, energy levels and performance. Oligosaccharide provider simply means little or few sugar.

The breaking down of purine nucleotides leads to the formation of uric acid. High concentration of uric acid in the blood can lead to gout, diabetes and formation of ammonium acid urate kidney stones. Most of the identified phytocompounds of *L. dulcis* ethanol leave extract may help in the inhibition of uric acid.

IV. CONCLUSIONS

This study enhances the traditional usage of *L. dulcis* because of the bioactive compounds identified. Thus the GC-MS analysis is vital towards understanding the nature of the phytocompounds in *L. dulcis.*

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