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## PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS PRESENT IN HYDROETHANOLIC LEAF EXTRACT OF *SOLANUM AETHIOPICUM* (L)

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### ABSTRACT

*Solanum aethiopicum* is a popular traditional vegetable cultivated widely in tropical Africa. It is a species of garden egg grown mostly for the nutritional and medicinal values of its leaves and fruits. This research was carried out to investigate the phytochemicals and GC-MS analysis of the hydroethanolic leaf extract of *Solanum aethiopicum*.

Fresh, healthy, and young leaves of the plant were collected from a farm in Omuokiri in Aluu, Port Harcourt, Rivers State, Nigeria. These collected leaves were air-dried and grounded into a powdered form and subjected to selective sequential extraction using water and ethanol to obtain a hydroethanolic extract. The extract was then subjected to qualitative and quantitative phytochemical analyses as well as gas chromatography and mass spectrometry (GC-MS), to determine its bioactive constituents.

The quantitative determination of the different biologically active compounds from the hydroethanolic leaf extract of the plant using gas chromatography-mass spectrometry revealed the presence of fifteen (15) bioactive and chemical entities of known biological activities. These chemical entities found in the leaf of the studied plant were found to vary in their molecular weights and amounts present and were considered to be biologically and pharmacologically important.

The study has established the chemical composition of the leaf of *Solanum aethiopicum* and therefore provides the basis for accounting for its anti-cancerous, anti-inflammatory, anti-diabetic, weight reduction, antioxidant, anti-inflammatory, cardio-protective, and immunomodulatory activities.

### KEYWORDS

*Solanum aethiopicum*, Phytochemicals, GC-MS analysis, Hydroethanolic and Bioactive compounds.



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## INTRODUCTION

Plants play a significant role in the prevention and treatment of diseases and are also useful in the prevention and reduction of the adverse effects of conventional treatments.<sup>[1]</sup> They are also a very good source of chemical compounds of biological and pharmacological importance. Historically, plants are sources of successful drugs and will continuously be important for screening of new pharmacologic compounds.<sup>[2]</sup> According to the World Health Organization (WHO), about 80% of the population of developing countries still relies on traditional medicines (mostly plant drugs) for their primary health care needs.<sup>[3]</sup> Plants are a rich source of secondary metabolites with interesting biological activities.

*Solanum*, a widespread plant genus of the family Solanaceae, has over 1000 species worldwide with at least 100 indigenous species in Africa and adjacent islands, which include a number of valuable crop plants and some poisonous ones.<sup>[4]</sup> In Nigeria, it is represented by some 25 species including those domesticated with their leaves, fruits or both eaten as vegetables or used in traditional medicine.<sup>[5, 6]</sup> Among these species is the *S. aethiopicum* L. (African eggplant), which is widely cultivated in Nigeria and across the African continent.<sup>[7,8]</sup>

This study was therefore aimed at determining the active ingredients in the hydroethanolic leaf extract of *Solanum aethiopicum* using GC-MS, so as to establish the basis for the numerous pharmacologic actions associated with it. It is our hope therefore that attempting a phytochemical characterization of the hydroethanolic leaf extract of the plants will form the basis for its anecdotal use by traditional medicinal practitioners.

## MATERIALS AND METHODS

### Plant collection and Preparation of plant extracts

Fresh, healthy, and young leaves of *Solanum aethiopicum* were collected from a farm in Omuokiri in Aluu, Port Harcourt, Rivers State, Nigeria. The plant was identified and authenticated by a plant taxonomist, Dr Suleiman from the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt. Voucher sample was deposited in the herbarium and the voucher number: UPHS0474 was obtained.

The leaves were cleaned and shade dried for a week and then ground to a fine powder using a motorized electric grinder. About 500 g of the dry powder was extracted with water (20%) and ethanol (80%) at 70°C by continuous hot percolation using Soxhlet apparatus. The extraction was continued for 24 hours after which the hydroethanolic extract was then filtered and kept in a hot air oven at 40°C for 24 hrs to evaporate the water and ethanol from it. A dark brown residue was obtained. The residue was kept separately in airtight containers and stored in a deep freezer.

### Phytochemical analysis tests

The phytochemical analysis of the hydroethanolic extract of *Solanum aethiopicum* leaves for secondary metabolites was done using standard methods.<sup>[9]</sup>

### Gas Chromatography-Mass spectrometry (GC-MS) analysis

GC-MS technique was used for the analysis of the leaf of the plant extract for the present study. The gas chromatography (GC) portion (Agilent technologies, United States of America, Model number 7890 (B) was coupled to a mass spectrometer (MS) (Agilent technologies, United States of America, Model number 5975(B). The procedure adopted for the GC-MS analysis of the plant extract for this study is consistent with that previously reported.<sup>[10,11]</sup>

The analysis of result of GC-MS was done using the database of National Institute Standard and Technology which consists of over 60,000 patterns.<sup>[12]</sup> The spectrum of the unknown constituent obtained was compared with the spectrum of the known components found in the NIST library. An attempt was also made to establish the names of the compounds found in the extracts, as well as their molecular formula, molecular weights and biological activities.

## RESULTS

**Table 1: Phytochemical screening of the hydroethanolic leaf extract of *Solanum aethiopicum***

Phytochemicals	Qualitative Analysis	Quantitative Analysis
Tannins (Mg/100g)	+++	980
Alkaloid (%)	+	9.2
Flavonoids (%)	+	8.0
Saponin (%)	++	14.0
Cardiac Glycosides (Mg/100g)	++	700
Cyanogenic glycosides (Mg/100g)	+	6.2
Phenols (Mg/100g)	+	2.1
Coumarins (%)	++	0.8

+ = sparingly present; ++ = moderately present; +++= abundantly present

**Table 2: Possible biologic effects of Phytochemicals in the hydroethanolic leaf extract of *Solanum aethiopicum***

Phytochemicals	Possible biologic actions
Tannins	Decrease in feed intake, growth rate, feed efficiency, net energy mobilization and protein digestibility. <sup>[13]</sup> Decreased efficiency in converting absorbed nutrients to new body substances Protection of cellular oxidative damage, including lipid peroxidation via the inhibition of generation of superoxide radicals. <sup>[13]</sup> Acceleration of blood clotting, reduces blood pressure, decreases serum lipid profile and modulates immuno responses. <sup>[13]</sup>
Alkaloid	Antimalarial, antimicrobial, antidiabetic, anti-cancerous and antioxidant. <sup>[14]</sup>
Flavonoids	Antioxidant, anti-inflammatory, anti-cancer and antidiabetic. <sup>[15]</sup>
Saponin	Used as antidote against lead poisoning and found to be hepatoprotective. <sup>[16]</sup> Antiobesity, antipyretic, diuretic, reduction in fat absorption, sedative and hypercholesterolemic. <sup>[17]</sup>
Cardiac Glycosides	Antitumor activity. <sup>[18]</sup> Inhibitory activity against rhinovirus. <sup>[19]</sup> Treatment of cardiac failure. <sup>[20]</sup>
Phenols	Antioxidant and anti-inflammatory. <sup>[21]</sup> Antidiabetic and hypercholesterolemic. <sup>[22]</sup>
Coumarins	Antimutagenic, anticoagulant, anti-inflammatory, bacteriostatic. <sup>[23,24]</sup> Cancer preventive. <sup>[25]</sup>

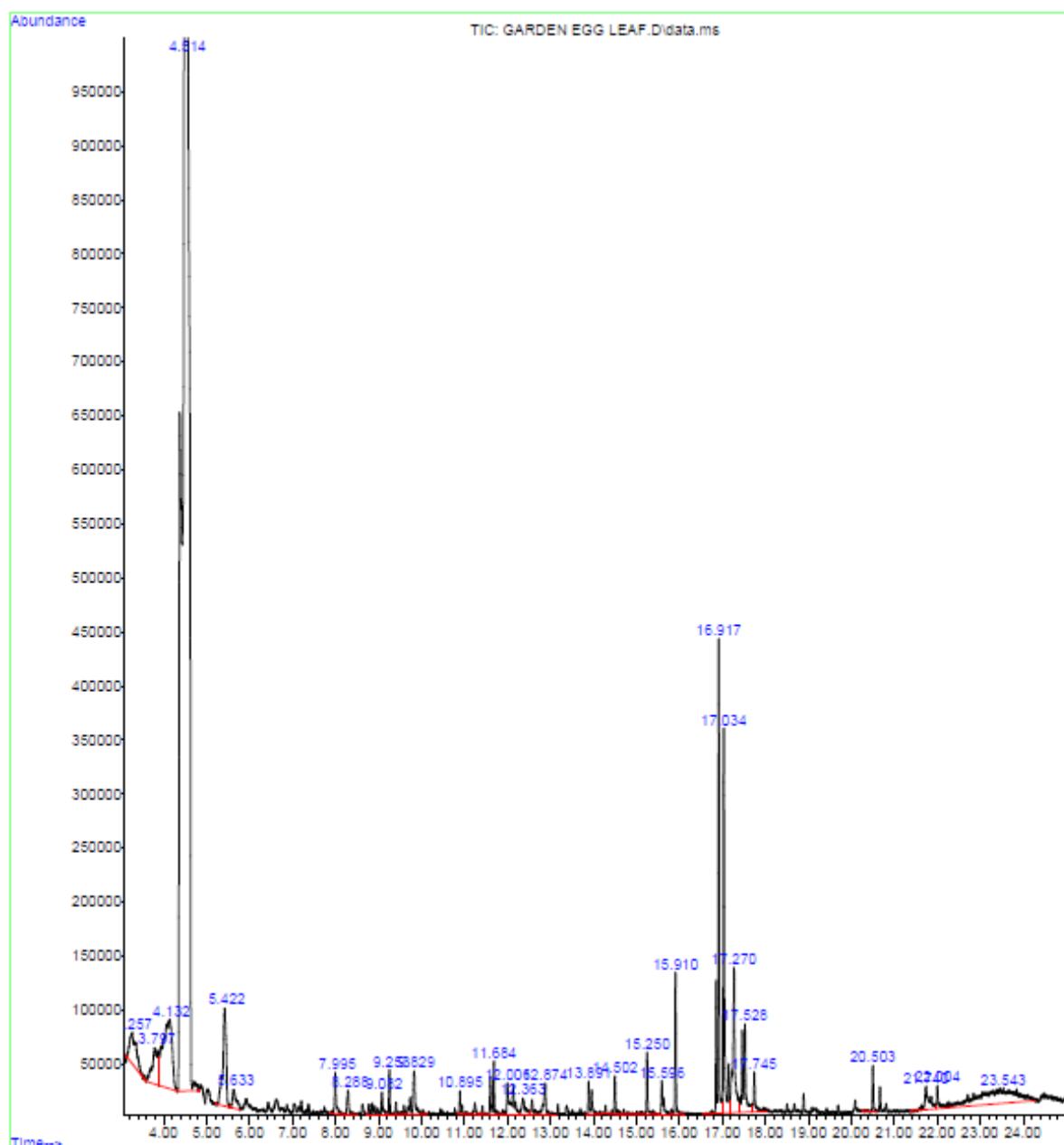
**Table 3: Physical properties of bioactive compounds in the hydroethanolic leaf extract of *S.aethiopicum*.**

Name of compound	Retention time (RT) (Minutes)	Molecular formula	Molecular weight (g/mol)	Peak Area (%)	CAS Registry Number
D-Limonene	4.51	C <sub>10</sub> H <sub>16</sub>	136.3	64.03	005989-27-5
1,2,3-Propanetriol	4.13	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	229.2	4.38	000056-81-5
11-Octadecenoic acid	16.92	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.5	3.60	001937-63-9
2-Hexadecen-1-ol	17.03	C <sub>16</sub> H <sub>32</sub> O	240.4	3.31	000150-86-7
6-Octadecenoic acid	17.27	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.5	2.33	997498-18-6
1,3-Cyclopentadiene	3.80	C <sub>5</sub> H <sub>6</sub>	66.1	1.62	003141-02-4
L- Furan.	3.26	C <sub>4</sub> H <sub>4</sub> O	71.9	1.48	001708-29-8
Cyclohexadecane	11.68	C <sub>16</sub> H <sub>32</sub>	224.4	0.77	000295-65-8
Hexadecanoic (palmitic) acid	15.25	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.4	0.64	000112-39-0
2-Methylpiperidine	9.08	C <sub>6</sub> H <sub>13</sub> N	99.2	0.60	000109-05-7
Octadecanoic acid	17.75	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284.0	0.55	000111-61-5

Trifluoroacetic acid (TFA)	13.89	CF <sub>3</sub> COOH	114.0	0.50	959010-23-2
Pentadecyl ester (Hexanoic acid)	13.89	C <sub>21</sub> H <sub>42</sub> O <sub>2</sub>	326.6	0.50	959010-23-2
Diethylsulphate	12.36	C <sub>4</sub> H <sub>10</sub> SO <sub>4</sub>	154.2	0.41	000064-67-5
Caffeine	14.50	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>	194.2	0.37	000058-08-2

**Table 4: Possible biologic effects of some identified compounds in the hydroethanolic leaf extract of *S. aethiopicum*.**

S/No	Name of compound	Possible Biologic Effects
1	D-Limonene	Antioxidant, antidiabetic, anti-inflammatory, cardioprotective, gastroprotective and immunomodulatory. [26]
2	1,2,3-Propanetriol	Diuretic, hyperosmotic, laxative and stool softener. [27]
3	11-Octadecenoic acid (Vaccenic Acid)	Predominant fatty acid comprising trans-fat in human milk . [28] Capable of lowering total cholesterol, LDL cholesterol and triglyceride levels in rats. [28]
4	2-Hexadecen-1-ol	Cancer-preventive. [25] Antimicrobial, anti-inflammatory and diuretic. [29]
5	6-Octadecenoic acid	Antioxidant and antimicrobial. [30]
6	1,3-Cyclopentadiene	Used to make resins, insecticides, fungicides and other chemicals.
7	L- Furan.	CNS depressant and analgesic activities. [31]
8	Cyclohexadecane	Insecticidal, antioxidant and anticancer. [32, 33]
9	Hexadecanoic (palmitic) acid	Antioxidant, antimicrobial, 5-alpha reductase- inhibitor, anti-fibrinolytic, hemolytic, antiallopecic, lubricant and nematicide. [25]
10	2-Methylpiperidine	Analgesic. [34]
11	Octadecanoic acid	Hypercholesterolemic. [25]
12	Trifluoroacetic acid (TFA)	Used in organic synthesis . [35] Versatile solvent for NMR spectroscopy and a calibrant in mass spectrometry. [36]
13	Pentadecyl ester (Hexanoic acid)	Flavouring, emulsifying, cleansing and buffer agent . [37]
14	Diethylsulphate	A carcinogenic agent, an apoptosis inducer, an alkylating agent and a mutagen.
15	Caffeine	Diuretic and increases rennin secretion. [38] Boosts alertness and memory by acting on the central nervous system . [39] Increases muscular strength, power and may improve muscular endurance. [40] Associated with cautions, enrichment, mood and an increase in awakening . [41]



**Fig 1:** Chromatogram obtained from GC-MS screening of the hydroethanolic leaf extract of *S.aethiopicum*

## DISCUSSION OF RESULTS

### Phytochemical analysis

The result of the phytochemical analysis of the hydroethanolic leaf extract of *S. aethiopicum* is shown in Table 1. The results revealed the presence of tannins, flavonoid, Saponin, cardiac glycosides, phenols and coumarins. The possible biologic actions of the phytochemicals are presented in table 2.

### Gas Chromatography-Mass spectrometry (GC-MS) analysis

The GC-MS analysis of the hydroethanolic leaf extract of *Solanum aethiopicum* revealed the presence of 15 compounds of known biological activities (Tables 3 and 4). The chromatogram obtained from the GC-MS screening of the hydroethanolic leaf extract of *S.aethiopicum* is shown in figure 1. From the GC-MS results, it was found that the leaf extract of *S.aethiopicum* contains D-Limonene, 1,2,3-Propanetriol, 11-Octadecenoic acid, 2-Hexadecen-1-ol, 6-Octadecenoic acid, 1,3-Cyclopentadiene, L-Furan, Cyclohexadecane, Hexadecanoic (Palmitic) acid, 2-Methylpiperidine, Octadecanoic acid, Trifluoroacetic acid, Pentadecyl ester (Hexanoic acid),

Diethylsulphate and Caffeine. The above compounds were identified based on their peak area (percentage abundance), retention time (RT) value, molecular weight, molecular formula and CAS Registry Number (Table 3).

D-Limonene is a solvent of cholesterol. It is the most abundant bioactive component with a peak area of 64.03%, retention time (RT) of 4.51 minutes, a molecular formula of  $C_{10}H_{16}$  and its molecular weight is 136.3g/mol. It has antioxidant, antidiabetic, anti-inflammatory, cardio-protective, gastro-protective and immuno-modulatory effects. [26]

1, 2, 3-Propanetriol is a sweet, hygroscopic, viscous liquid, widely distributed in nature as a constituent of many liquids. It has a peak area of 4.38%, retention time (RT) of 4.13 minutes, a molecular formula of  $C_6H_{12}O_6$  and its molecular weight is 229.2g/mol. This compound has been reported to have diuretic, hyperosmotic, laxative and stool softening activities. [27]

11-Octadecenoic acid (Vaccenic Acid) is a predominant fatty acid comprising trans-fat in human milk. [28] It has a peak area of 3.60%, a retention time (RT) of 16.92 minutes,  $C_{19}H_{36}O_2$  molecular formula and a molecular weight of 296.5g/mol. It is capable of lowering total cholesterol, LDL cholesterol and triglyceride levels in rats. [28]

2-Hexadecen-1-ol is an alcohol with a peak area of 3.31%. It has 17.03 RT value,  $C_{16}H_{32}O$  molecular formula and a molecular weight of 240.4g/mol. It has cancer-preventive [25], antimicrobial, anti-inflammatory and diuretic effects. [29]

6-Octadecenoic (petroselinic) acid is a fatty acid which occurs naturally in various animal vegetable fats and oils. Its peak value in the leaf of the studied plant is 2.33%. It has a retention time of 17.27 minutes,  $C_{18}H_{34}O_2$  molecular formula and a molecular weight of 282.5 g/mol. It has antioxidant and antimicrobial activities. [30]

1,3-Cyclopentadiene is a colourless compound with a sweet odour, used to make resins, insecticides, fungicides and other chemicals. It has a retention time (RT) of 3.80 minutes, a molecular formula of  $C_5H_6$  and its molecular weight is 66.1g/mol. It has antimicrobial, antifungal and activities.

L-furan is a colourless, flammable and highly volatile liquid, slightly soluble in water with a strong, ethereal and chloroform-like odour. In the leaf of the studied plant, it has a peak area of 1.48%, a retention time of 3.26 minutes. Its molecular formula is  $C_4H_4O$  while its molecular weight is 71.9g/mol. It has CNS depressing and analgesic activities. [31]

Cyclohexadecane is a saturated hydrocarbon with a peak area of 0.77%, a retention time of 11.68 minutes, a molecular formula of  $C_{16}H_{32}$  and its molecular weight is 224.4g/mol. It has insecticidal, antioxidant and anticancer activities. [32, 33]

Hexadecanoic (Palmitic) acid is one of the most common saturated fatty acids found in animals, plants and microorganisms. In the leaf of *Solanum aethiopicum*, it has a peak area of 0.64%, a retention time of 15.25 minutes, a molecular formula of  $C_{16}H_{32}O_2$  and its molecular weight is 256.4g/mol. It has antioxidant, antimicrobial, anti-fibrinolytic, haemolytic, antiallopecic, lubricant and nematicide properties. [25]

2-Methylpiperidine is an organic compound with a retention time (RT) of 9.08 minutes, a molecular formula of  $C_6H_{13}N$  and its molecular weight is 99.2g/mol. It has an analgesic activity. [34]

Octadecanoic (Stearic) acid is a saturated fatty acid with an 18-carbon chain. It has a peak area of 0.55%, retention time (RT) of 17.75 minutes, a molecular formula of  $C_{18}H_{36}O$  and its molecular weight is 284.0g/mol. It is hypercholesterolemic in action. [25]

Trifluoroacetic acid (TFA) is an organofluorine colourless liquid with a vinegar-like odour. It has a peak area of 0.50%, a retention time of 13.89 minutes, a molecular formula of  $CF_3COOH$  and a molecular weight of 114.0g/mol. It is used in organic synthesis, [35] acts as a versatile solvent for NMR spectroscopy and useful as a calibrant in mass spectrometry. [36]

Pentadecyl ester (Hexanoic acid) is a straight-chain saturated fatty acid which can be formed from the breakdown of either arachidonic acid or linoleic acid. It has a peak area of 0.50%, a retention time of 13.89 minutes, a molecular formula of  $C_{21}H_{42}O_2$  and a molecular weight of 326.6g/mol. It acts as a flavouring, emulsifying, cleansing and buffer agent. [37]

Diethylsulphate is a colourless, corrosive and odourless liquid with a peppermint odour. It has a peak area of 0.41%, a retention time of 12.36 minutes, a molecular formula of  $C_4H_{10}SO_4$  and a molecular weight of 154.2g/mol. It is a carcinogenic agent, an apoptosis inducer, an alkylating agent and a mutagen.

Caffeine is a natural stimulant seen in some beverages like cocoa, coffee and tea. It has a peak area of 0.37%, retention time (RT) of 14.50 minutes, a molecular formula of  $C_8H_{10}N_4O_2$  and its molecular weight is 194.2g/mol. It is diuretic, increases blood flow and rennin secretion. [38] It also boosts alertness and memory by acting on the central nervous system. [39] It increases muscular strength and power and may improve muscular endurance. [40] It also increases awakening [41] and has been found to play a significant role in pregnancy insomnia, depression, psychosis, cardiovascular disease and spontaneous abortion.

## CONCLUSION

From the present study, 15 compounds of known biological activities were identified from the hydroethanolic leaf extract of *Solanum aethiopicum* (L) using Gas-chromatography–Mass spectrometry (GC-MS) analysis. The results revealed the presence of some very important and medicinally relevant constituents in the leaf of the plant studied. The chemical identification of the plant constituents was conducted based on their peak area, retention time (RT), molecular formula, molecular weight and CAS registry number. The investigation of the presence of these identified phytoconstituents is useful in validating the antioxidant, antidiabetic, anti-inflammatory, cardio-protective, gastro-protective, immuno-modulatory, anticancer, diuretic, weight-reduction, hypercholesterolemic, antimicrobial, anti-fibrinolytic, haemolytic and antialopepic properties associated with the leaf of the plant studied. The outcome of this study further presents the hope for the development of many more novel therapeutic agents from the plant which in future may serve for the production of synthetically improved therapeutic agents. Therefore the leaf extract of this plant is strongly recommended to be used as a source of herbal drug.

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