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GAS CHROMATOGRAPHY-MASS SPECTROSCOPY AND FOURIER TRANSFORM INFRARED PROFILING OF THE BIOACTIVE COMPOUNDS PRESENT IN METHANOL LEAF EXTRACT OF *SOLANUM AETHIOPICUM* FROM IMO STATE, NIGERIA

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ABSTRACT: *The study aimed to access the bioactive compounds and functional groups present in Solanum aethiopicum using the Gas Chromatography coupled with mass spectrometer (GCMS) and Fourier Transform Infrared Spectroscopy (FTIR). The GCMS showed Solanum aethiopicum showed a total of thirty-seven bioactive compound with the highest abundance being Squalene (21.13%), Palmitoleic acid (16.63%), 13-Octadecenal (16.63%) and 9-Octadecenoic acid (17.62%). Fourier Transform Infrared Spectroscopy of Solanum aethiopicum showed the presence of functional groups which include alkanes, alkenes, alkynes, primary (1^0) and secondary (2^0) amines, amides, imino, epoxy, peroxides, carboxylic esters, ethers, primary (1^0), secondary (2^0) and Tertiary (3^0) alcohols, phenols, aldehydes, thiols, cyanate, isocyanates and alkyl halides. These bioactive compounds with reference to literature revealed a variety of biological functions spanning across antibacterial, anti-inflammatory, anti-oxidant, analgesic, antimalarial, antifungal, anti-tumour, neuroprotective, anti-tumour, anti-cancer, anti-seizure, anti-allergy, anti beta-amyloid protein aggregation.*

KEYWORDS: *Solanum aethiopicum*, GCMS, FTIR, Phytochemicals.

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INTRODUCTION

The use of herbs in treating ailments is as old as mankind (Petrovska, 2012). Human instincts were the major drive to the beginning of the use of plants as medicine (Stojanoski, 1999). Plant use in traditional medicine and by extension orthodox medicine is globally on the increase and this is majorly attributed to the active phytochemicals present.

Solanum aethiopicum belongs to the Solanaceae family. It is often called African eggplant, bitter tomato, scarlet eggplant and garden eggs. The specie *Solanum aethiopicum* is believed to have an origin from Africa and was domesticated from the wild *Solanum anguivi*, through the semi-domesticated *Solanum distichum* both of which are found in tropical Africa (Anaso, 1991; Lester, 1998). *Solanum aethiopicum* is one of the most widely grown and consumed vegetable plant species in Africa and contains a great amount of nutrients (Kamga, Kouamé, Atangana, Chagomoka & Ndango, 2013). *Solanum aethiopicum* is an important food source as the fruits and leaves are used to prepare stews and soups. Moreso it is also used to treat certain diseases due to its phytochemical constituents, and also a rich source of important macronutrients and micronutrients (Han, Opoku, Bissah & Su, 2021). *Solanum aethiopicum* grows to about 2.5 m in height and is often a branched deciduous shrub. On the stems, the leaves are alternately arranged and have smooth or lobed margins. Leaf-blades can reach a length of up to 30 cm and a width of 21 cm. The leaves' petioles are oval or elliptical, reaching a length of up to 11 cm (Bukenya & Carasco, 1999).

The species *S. aethiopicum* can be classified with respect to its use into four distinct groups. They are the Gilo, Shum, Kumba and Aculeatum group (Lester & Daunay, 2003). The African eggplant is often grown as an annual, but generally a perennial, plant with stems that become more or less woody and persist. It grows well in deep, well-drained soils. Cultivation of African eggplant is mainly dependent on the rain, but irrigation can be applied during the dry seasons. The African eggplant requires a pH of 5.5–6.8, and thrives well at daytime temperatures ranging between 20–30 ◦C, but it can tolerate 10–40 ◦C. It cannot tolerate very cold or water-logged conditions (James *et al.,* 2010).

A variety of phytochemical constituents are determined using qualitative methods. GCMS aims at using both the quantitative and qualitative methods to determine plant phytochemical constituents. FTIR is used to access the functional groups (chemical bonds) present in an unknown plant. This study aimed at evaluating the phytochemical constituents of *Piper guineense* and *Solanum aethiopicum* ethanol extracts from Imo state, Nigeria.

MATERIALS AND METHODS

Plant collection, Preparation and Processing

Fresh plant leaves of *Solanum aethiopicum* were purchased at Relief market located in Owerri-Municipal Local Government Area of Imo state. The plant was identified by a plant taxonomist, Dr. Udoka Emmanuel of the Department of Forestry and Wildlife, Michael Okpara University of Agriculture Umudike (MOUAU), Umuahia, Abia State.

The leaves of *Solanum aethiopicum* were washed with tap water and subsequently air-dried for a duration of three weeks at ambient temperature under the shade while being spread-out. The

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samples were ground into fine particulate matter using an industrial-grade grinding machine. They were then placed in containers that were clearly labeled to facilitate identification and storage until needed for subsequent analysis.

Extraction

The leaves underwent uniform preparation techniques to produce methanol extracts using 99.8% methanol in the cold maceration technique. 130 grams of ground plant samples were dissolved in 1000 ml methanol in a volumetric flask in order to fully submerge the plant material. It was uniformly stirred for 5 minutes and left to stand for 72 hours. The solution was subjected to filtration, and water bath evaporation at 650 C to form a sticky concentrate. The concentrates (extracts) were appropriately labeled and stored in a refrigerator at a temperature of 4°C.

GC-MS Analysis of the phytochemical constituents

The GC (Agilent 6890N) and MS (5975B MSD) were used in the analysis. Both were equipped with DB-5ms capillary columns (30m×0.25mm; film thickness 0.25μm). The initial temperature was set at 40^{o}C but increased to 150^{o}C at the rate of 10^{0}C/min. The temperature was further increased to 230^{o}C at the rate of 5^{o}C/min. The process continued till the temperature reached 280^{o}C at the rate of 20^{o}C/min and was then held for 8 minutes. The injector port temperature remained constant at 280^{o}C and detector temperature was 250^{o}C. 99.995% pure helium was used as the carrier gas with a flow rate of 1 ml/min. Split ratio and ionization voltage were 110.1 and 70eV respectively (Buss & Butler, 2010).

Identification of Phytochemical Constituents

The National Institute of Science and Technology 2014 (NIST, 2014) database was used to identify the unknown components in the extract by comparing the unknown peak value and chromatogram from the GC-MS against the known chromatogram, peak value from the NIST library database. The details about the molecular formula, molecular weight, retention time and percentage content were also obtained.

Procedure for FTIR Spectroscopy

Buck scientific M530 USA was used for the analysis. This instrument was equipped with a detector of deuterated triglycine sulfate and a beam splitter of potassium bromide. The Gram A1 software was used to obtain the spectra and to manipulate them. 1.0g of sample and 0.5ml of nujol were mixed properly and placed on the salt pellet. FTIR spectra were obtained at frequency regions of 4000-6000 cm-1 and co-added at 32 scans and at 4 cm-1 resolution. FTIR spectra were displayed as transmitter values (VanderWeerd, Heeren and Boon, 2004). The wavenumbers at different peaks were used to extrapolate the diverse functional groups present in *Piper guineense* and *Solanum aethiopicum* (Nandiyanto, Oktiani & Ragadhita, 2019).

Results

Extract yield

$$\text{Yield (\%)} = \frac{20.91\text{g}}{130.00\text{g}} \times 100$$

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Yield =15.38%

The table 1.1 showed that Squalene (21.13%) and Palmitoleic acid (16.63%), 13-Octadecenal (16.63%) and 9-Octadecenoic acid (17.62%).were the most prevalent phytochemicals in *Solanum aethiopicum* amongst other phytochemicals present.

FTIR analysis in Table 1.2, revealed the presence of functional groups in *Solanum aethiopicum* which include alkanes, alkenes, alkynes, primary (1^0) and secondary (2^0) amines, amides, imino, epoxy, peroxides, carboxylic esters, ethers, primary (1^0), secondary (2^0) and Tertiary (3^0) alcohols, phenols, aldehydes, thiols, cyanate, isocyanates and alkyl halides.

Table 1.1: GCMS Phytochemicals for *Solanum aethiopicum*

S/N	Class of compound	RT	Area (%)	Bioactive Compound	MW	MF
1	Triterpenoids	36.267	21.13	Squalene	410.7	$C_{30}H_{50}$
2	Fatty acid	25.895	0.71	Oleic acid	282.461	$C_{18}H_{34}O_2$
3	Alpha and beta-adrenergic agonist	23.877	0.13	Epinephrine, 1,5-dimethyl epinephrine	183.204	$C_9H_{13}NO_3$
4	Alkane	27.320	1.36	Pentadecane	212.42	$C_{15}H_{32}$
5	Amine	30.003	0.05	7H-purin-6-amine	135.13	$C_5H_5N_5$
6	Alkane	26.212	1.71	Eicosane	282.54	C20H42
7	Fatty acid	34.683	17.62	9-Octadecenoic/Stearic acid	282.5	$C_{18}H_{34}0_2$
8	Alkene	34.173	1.35	1-Nonadecene	266.5	$C_{19}H_{38}$
9	Fatty acid	32.704	16.63	Palmitoleic acid	256.43	$C_{16}H_{30}O_2$
10	Aldehyde	32.704	16.63	13-Octadecenal	266.5	$C_{18}H_{34}O$
11	Fatty acid	24.400	0.15	Decanoic acid	172.26	$C_{10}H_{20}0_2$
12	Alkane	31.100	0.37	Cyclotetradecane	196.37	$C_{14}H_{28}$
13	Alkene	32.909	5.98	1-Docosene	308.60	$C_{22}H_{44}$
14	Alkane	31.100	0.37	Heptadecane	240.471	$C_{17}H_{36}$
15	Alkane	22.754	0.59	Hexadecane	226.445	$C_{16}H_{34}$
16	Alkane	26.953	0.43	Nonadecane	268.518	$C_{19}H_{40}$
17	Alkane	26.411	0.49	2-methylhexacosane	380.70	$C_{27}H_{56}$
18	Precursor to hormone	25.895	0.71	Estra-1,3,5 (10)-trien-17-beta-ol	334.4	$C_{23}H_{26}0_2$
19	Fatty alcohol	25.542	0.41	Aspidospermidine-17-ol,1-acetyl-19,21-epoxy-15,16-dimethoxy	414.5	$C_{23}H_{30}N_2O_5$
20	Alkane	27.320	1.36	Octadecane	254.49	$CH_3(CH_2)_{16}CH_3$
21	Alkane	31.348	0.33	1-bromo triacontane	501.7	$C_{30}H_{61}Br$
22	Carboxylic acids	24.400	0.15	7-methyl-Z-tetradecen-1-ol acetate	268.43	$C_{17}H_{32}O_2$
23	Aldehyde	6.967	0.67	E-15-Heptadecenal	252.4	$C_{17}H_{32}O$
24	Alkane	26.212	1.71	Octacosane	394.8	$C_{28}H_{58}$
25	Alkane	25.222	0.81	Heneicosane	296.6	$C_{21}H_{44}$

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26	Fatty acid	24,943	0.38	Cis-5-Dodecenoic acid	198.30	$C_{12}H_{22}O_2$
27	Alkane	24.243	0.08	Tetrapentacontane, 1,54-dibromodecane	917.24	$C_{54}H_{108}Br_2$
28	Fatty alcohol	20.290	0.05	1-heptanol	116.20	$C_7H_{16}O$
29	Aldehyde	17.566	0.04	2-heptanal	114.19	$C_7H_{14}O$
30	Alkane	9.168	0.57	Undecane	156.31	$C_{11}H_{24}$
31	Ester	9.168	0.57	Carbonic acid, nonyl prop-1-en-2-yl ester	228.33	$C_{13}H_{24}O_3$
32	Carboxylic acid	7.583	0.78	Cyanoacetic acid	85.06	$C_3H_3NO_2$
33	Carboxylic acid	7.093	0.63	Dichloroacetic acid, 2 pentadecyl ester	128.94	$C_2H_2Cl_2O_2$
34	Alkane	27.477	1.16	Cyclohexadecane	224.42	$C_{16}H_{32}$
35	Alkane	26.411	0.49	Dotriacontane	450.90	$C_{32}H_{66}$
36	Alkane	17.684	0.20	Tetradecane	198.39	$C_{14}H_{30}$
37	Phthalates	30.003	0.05	Dibutyl Phthalate	278.34	$C_{16}H_{22}O_4$

Table 1. 2: FTIR for *Solanum aethiopicum*

S/N	Wavenumber (cm-1)	Bond/Mode of Vibration	Functional group
1	3835.48	O-H Stretch	Alcohols, Phenols
2	3702.799	N-H stretch	Amide
#3	3520.527	N-H Stretch	Amine
4.	3432.179	O-H Stretch N-H Stretch	Dimeric alcohols Heterocyclic Amines
5.	3247.555	O-H Stretch	Alcohols, Phenols
6.	2846.22	C-H Stretch $O-CH_3$ C-O N-CH3 Stretch	Methyne, Methyl Methoxy Aldehyde Methylamino
7.	2687.900	S-H Stretch	Thiols
8.	2528.652	S-H Stretch	Thiols
9	2245.355		Medial Alkyne Cyanate Isocyanate
10	2130.733	C≡C Stretch -NCS Stretch	Terminal Alkyne Isothiocyanate
11	1651.56	N-H bend -N=N-	Secondary (2^0) amine Open chain azo
13	1295.598	O-H bend C-N Stretch C-N Stretch	Primary(1^0)/Secondary (2^0) Alcohol Primary(1^0)/Secondary (2^0) Aromatic Amine
14	1177.917	C-N Stretch C-N Stretch	Tertiary (3^0) Amine 2^0 Amine
15	880.6337	C-O-O Stretch	Peroxides Epoxy and oxirane rings

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16	732.5869	-C-Cl Stretch $(-CH_2)_n$ Aromatic C-H bend	Alkyl halides Methylene Aryl

DISCUSSION

GC-MS profile of *Piper guineense* and *Solanum aethiopicum* revealed the presence of a number of secondary metabolites. Squalene (21.13%) has been reported to possess neuroprotective, antioxidant, anti-tumour and anticancer properties (Ryszard, 2009; Lozano-Grande, Gorinstein, Espitia-Rangel, Davila-Ortiz & Martinez-Ayala, 2018; Gaudin *et al*., 2015). Oleic acid (19.00%) possesses neuroprotective, anti-inflammatory, anticancer, antiandrogenic, antibacterial, dermatitigenic and hypocholesteremic properties (Sreekumar, Ramesh & Vijaykumar, 2014; Awa, Ibrahim & Ameh, 2012; Song *et al*., 2019; Ubaid *et al*., 2020). Palmitoleic acid (16.63%) shows anti-inflammatory, antibacterial and antifungal properties (Odiase-Omoighe & Agoreyo, 2022; Ojinnaka, Kenne & Abbey, 1992). Epinephrine (0.13%) is a known neurotransmitter that helps the cholinergic pathway (Bylund, 2007), as 7H-purin-6-amine (0.05%) is a neuroprotective compound (World Intellectual Property Organization, 2010). Eicosane (1.71%; 1.23%) has active anti-inflammatory, analgesic, anticancer properties (Okechukwu, 2020; Tiloke, Anand, Gengan, & Chuturgoon, 2018). 9-Octadecenoic acid (17.62%:10.13%) has been reported to possess good antibacterial and anti-inflammatory properties (Adegoke, Jerry & Ademola, 2019; Imad, Huda, & Ghaidaa, 2016). 13-Octadecenal (16.63%) is potent as an antibacterial and antimicrobial (Ajanaku et al, 2018). Hexadecane (3.27%), Heptadecane (0.31%) and Nonadecane (0.43%) have antibacterial properties (Balmurugan, Selvam, Thinakaran & Sivakumar, 2013; Tyagi & Agarwal, 2017). Nonadecane (0.43%) also has antioxidant, antimicrobial, antimalarial and anti-toxic effects (Banakar & Jayaraj, 2018). Heptadecane (0.31%) also has strong anti-inflammatory properties (Kim *et al*., 2013) Decanoic/Capric acid (0.15%) has been known to be antibacterial, anti-inflammatory, anti-seizure and reduce oxidative stress, regulate signaling pathways (Yang *et al*., 2018:Chang *et al*., 2016: Huang *et al*., 2014,). 1-Docosene (5.98%), 2-methylhexacosane(0.49%) and Dibutyl phthalate (0.05%) possess antibacterial properties (Togashi *et al*., 2007; Sahar & Aida, 2018; Khatiwora, Adsul, Kulkarni, Deshpande & Kashalkar, 2012). Aspidospermidine-17-ol,1-acetyl,19,21-epoxy-15,16-dimethoxy (0.41%) has antimicrobial properties (Olubunmi & Anthony, 2017; Safwat, Hamed & Moatamed, 2018). Cis-5-Dodecenoic acid (0.38%) and 1-heptanol (0.05%) possess active antibacterial properties (Rouis-Soussi *et al*., 2014; Renugadevi, Valli & Moin, 2021; Ingram & Vreeland, 1980). Tetrapentacontane-1,54dibromodecane (0.08%) is a molecule that has active antioxidant properties (Ijaz *et al*., 2008). Cyclohexadecane (1.16%) has active antibacterial and antifungal properties (Kumari, Menghani & Mithal, 2019). Tetradecane (0.20%; 0.47%) has antibacterial (Rahbar *et al*., 2012) and antifungal properties (Guo *et al*., 2008). Estra-1,3,5 (10) trien-17-beta-ol (0.71%) is a precursor to the hormone estrogen which is implicated in menopausal women and AD pathogenesis (Zhao *et al*., 2015). E-15-heptadecenal (0.67%) is reported to have anti-inflammatory, antibacterial, antifungal, antioxidant, anticancer properties (Kumar, Anburaj, Subramanian, Vasantha & Selvam, 2011). 1-nonadecene (1.35%) exhibits antioxidant, anti-inflammatory and anticancer properties (Sivakumar & Balaraman, 2018). Octacosane (1.71%) has antioxidant, wound healing, and larvicidal properties (Balachandran *et al*., 2023; Rajkumar & Jebanesan, 2003). Carbonic acid, nonylprop-en-2-yl ester (0.57%) has active antibacterial properties (Mitchell *et al*., 2017). Cyanoacetic acid (0.78%)/its

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derivatives possess active antibacterial properties and are used as scientific probes for medical purposes (Mohareb, El-Arab & El-Sharkawy, 2009; Teppang, Ehrlich & Yang, 2020). Anticancer, anti-inflammatory and hepatoprotective properties have been reported for 7-methyl-Z-tetradecen-1-ol acetate (0.15%) (Al-Mawla & Abu-Serag, 2019; Hameed, Hussein, Kareem & Hamad, 2015). Undecane (0./57%; 0.09%) also has anti-inflammatory and anti-allergy properties (Choi, Kang & Park, 2020). Dotriacontane (0.49%) has antioxidant, antimalarial, anti-tumour and antiprotozoal activities (Gallo & Sarachine, 2009). 2-Heptanal (0.04%) has antimicrobial and antifungal properties (Ji, Kang & Baik, 2017; Li *et al*., 2021). Hexacosane (1.23%) has antibacterial properties (Das, Das, Bhavya & Nivashini, 2020; Rukiyat, Garba & Labaran 2015). Heneicosane (0.36%) has pesticidal, antimicrobial and antioxidant properties (Rhesto, Shubharani, Roopa & Sivaram, 2020; Nandhini, Sangareshwari & Kumari 2015; Otieno, 2016). Dichloroacetic acid, 2 pentadecyl ester (0.63%) and its salt derivatives have antidiabetic and antitumour properties, they are also used to therapeutically treat congenital lactic acidosis, a rare medical condition (Stacpoole, Gilbert & Neiberger, 2008; Michelakis, Sutendra, & Dromparis., 2010; Stacpoole & Greene, 1992).

FTIR analysis revealed the presence of functional groups in *Solanum aethiopicum* which include alkanes, alkenes, alkynes, primary (1^0) and secondary (2^0) amines, amides, imino, epoxy, peroxides, carboxylic esters, ethers, primary (1^0), secondary (2^0) and Tertiary (3^0) alcohols, phenols, aldehydes, thiols, cyanate, isocyanates and alkyl halides.

IMPLICATION TO RESEARCH AND PRACTICE

This study has demonstrated that *Solanum aethiopicum* which is a common culinary vegetable is a rich source of phytochemicals which span across terpenoids, purines, alkanes, mono-unsaturated omega-9-fatty acid and monounsaturated omega-7-fatty acid and have bioactive potentials.

CONCLUSION

The presence of various bioactive compounds in appreciable concentrations in *Solanum aethiopicum* has revealed its potential biological, pharmacological and industrial applications. The bioactive compounds in the methanolic extracts exhibited important biological functions which include neuroprotective, antitumour, anti-cancer, antioxidant, analgesic, antibacterial, antifungal, antimalarial, anti beta-amyloid protein aggregation and anti-inflammatory properties, linked to their rich phytochemical composition which include terpenoids, purine, alkanes, mono-unsaturated omega-9-fatty acid and monounsaturated omega-7- fatty acid.

This research validates the use of these plants as indigenous edible vegetables as they are rich in bioactive compounds which are biologically important to man and can be used in drug development.

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