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Phytochemical Screening and GCMS Profile of Bioactive Natural Products in Ethanolic Root Extract of *Uvaria ovate*

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ABSTRACT: Phytochemical and gas chromatography and mass spectrometry (GCMS) analysis of the ethanolic extract of *Uvaria ovata* (Uo) roots was carried out. Phytochemical analysis showed high amounts of alkaloids, tannins and flavonoids, moderate amounts of saponins, terpenes and cardiac glycosides, while steroids and phenols were found in little quantities. Gas chromatography and mass spectrometry analysis revealed the presence of eleven compounds, of which D-Streptamine and Cystine were prominent. The aforementioned bioactive compounds have been shown to possess medicinal potential that could prove valuable in formulating therapeutic drugs.

Keywords: *Uvaria ovata*, Ethanol extract, Phytochemicals, Bioactive natural products, GCMS, Pharmacological activity

Introduction

The use of herbal medicinal plants in treating human disease conditions cannot be overemphasized. It has become an area of great research among medical practitioners, orthodox medical researchers and pharmaceutical

companies, as their therapeutic values are now recognized. Many centuries ago, herbs were used to treat ailments throughout the world. Then people moved towards advanced medical science and drugs, abandoning herbalism to the rural dwellers. However, in recent years, there has been a surge in the utilization of herbal formulations to treat various disease states. It is estimated that about 88% of the world population relies on herbal medicine for the treatment of various diseases (Marshin *et al*., 2013). This renewed interest in herbal medicine may be due, in part, to the plethora of side effects and exorbitant costs associated with the use of current drugs available for the management of gastrointestinal disorders. Plants also form a vital part of the advances made by modern medicine, thereby making the exploitation of wild plants for medicinal purposes a globally accepted concept (Oluwole and Peter, 2011). Medicinal plants are distributed worldwide, but they are most abundant in tropical countries. The majority of Africa lies in the tropics, where about 2/3 of the world's flora are domiciled (Lewis, 2001). Among all the families of the plant kingdom, members of the annonaceae family have been used for centuries in folk medicine (Doyle and Le Thomas, 2012). *Uvaria* is a genus in the family (LinLin *et al*., 2009). The family contains several hundred genera and thousands of species. It is the largest family in Magnoliales order. Of all the genera, only four (including *Uvaria*) produce edible fruit (Parmar *et al*., 1994).

Very little literature exists about the species *Uvaria ovata*, but it is a shrub that grows about 1-2m tall. The branches are characterized by rusty hairs, ovate-shaped leaves, short, two-flowered, leaf-opposed peduncles, six oval-oblong unequal petals in many stamens, and yellow-coloured flowers which are borne singly in small clusters (Vahl ex Dunal, 2019). *Uvaria* is derived from the Latin word "uva", which means grape. It was so named because the fruits of some of its species resemble grapes. The name "ovata" was gotten from the fact that the leaves of this shrub were ovate in appearance. The plant is referred to as "ire" in the Igbo Language of Southeastern Nigeria. *Uvaria ovata* is predominantly found in savannah regions, confined mostly to West Africa. The plants are also common in coastal thickets, gravelly soil, rocky and mountainous areas and forests, spanning the entire breadth of West tropical Africa, from Sierra Leone to Benin (Burkil, 2004). It is classified as follows:

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Magnoliales
Family: Annonaceae
Genus: *Uvaria*
Species: Ovata

Phytochemistry involves studying naturally occurring biologically active chemical compounds contained in certain plant extracts and act as natural defence systems, as well as provision of colour, flavour and aroma. Gas chromatography and mass spectrometry had recently become a vital technological tool for assessing secondary metabolites in plants. Presently, very limited study has been carried out on *Uvaria ovata*. However, pharmacological studies on *Uvaria* species have intensified in the last few years. The first phytochemical study of this genus was reported as late as 1968 (Parmar *et al*., 1994). The screening revealed the presence of carbohydrates, lipids, amino acids, essential oils, terpenes, polyhydroxychalcones, lactones, esters and phenolics (Parmar *et al*., 1994). A review by Abu *et al*. (2018) on roots, stems, leaves and fruit of some selected *Uvaria* species showed a broad spectrum of physiologically-active compounds, including essential oils, flavonoids, alkaloids, and annonaceous acetogenins. Previous phytochemical analysis of *Uvaria* species by Okwuosa *et al*. (2012) isolated varying degrees of terpenoids, alkaloids , glycosides, tannins, saponins, acids, lipids and oils, etc., from the plant. These metabolites may be responsible for their numerous pharmacological activities.

Uvaria species from the family Annonaceae are plants associated with a plethora of health benefits. They have been used extensively as traditional medicine for the management of a wide range of diseases. A study by Idam and Ebisintei (2022), revealed that *Uvaria ovata* roots showed significant gastroprotection against aspirin-induced gastric ulcers. Extracts were made from several *Uvaria* species such as *Uvaria ovata*, *Uvaria dependens*, *Uvaria faulknerae*, *Uvaria kirkii*, *Uvaria leptocladon*, *Uvaria lucida*, *Uvaria pandensis*, *Uvaria scheffleri*, etc., where several active compounds were isolated, including uvaretin and indole which showed antimalarial activities (Zofou and Titanji, 2013). A study to investigate the wound-healing potential of seventeen medicinal plants, including *Uvaria ovata*, was carried out. The findings at the conclusion of the study showed the proliferation of fibroblasts in the scratch assay. The wound-healing effect was attributed to the rutin and quercetin isolated from the extract (Freiesleben *et al*., 2017). *Uvaria* roots are components of various anticancer regimens. According to previous literature, diverse species of the *Uvaria* genus revealed promising cytotoxic effects against several human cancer cell lines, as demonstrated by spectrophotometric analysis. Uvariganols and acetogenin uvagrinin isolated from *Uvaria* species showed cytotoxicity against human leukemia and colon adenocarcinoma cell lines. The extracts were found to inhibit the mutagenicity of promutagens by interacting with the metabolic pathway involved in the activation of procarcinogens (Padma *et al*., 2020). *Uvaria* plants are believed to facilitate the amelioration of the pains of childbirth. The plant extracts exhibited uterine contraction activity in a study carried out on guinea pigs (Okwu and Iroabuchi, 2009). Igoli *et al*. (2005), researched a polyherbal formula comprising eighty-nine species of plants, including *Uvaria* species, and discovered that it possessed laxative effects. The leaves and roots of *Uvaria* plants, macerated together, have been used traditionally in the treatment of cough, renal pain, and pain in the thoracic region. Root infusions with *Tasmannia lanceolata* and gin are used in the treatment of abdominal pain (Burkil, 2004). Cerebral diseases are managed locally in children by applying root and leaf infusions of *Uvaria* plants to the fontanelle. The roots, boiled with spices, are used in rural communities of West Africa to treat jaundice (Burkil, 2004). James *et al*. (2013), proved that some *Uvaria* plants possess antivenom activity by carrying out *in vitro* and *in vivo* studies which highlighted the neutralizing activity of *Uvaria* leaf fractions on the venom of *Naja nigricollis* in albino rats.

The aim of this study is to identify the phytochemical components of *Uvaria ovata* root extract by both preliminary studies and a detailed gas chromatography-mass spectrometry (GC-MS) analysis of the bioactive compounds it contains.

Materials and methods

Sample identification and collection: Fresh roots of *Uvaria ovata* were collected from fallow grassland in Umuahia, Umuahia Local Government Area of Abia State, Nigeria, and they were authenticated at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria, where it was assigned the voucher number MOUAU/ZEB/HERB/2019/021.

Preparation of plant extract: The root extract was prepared according to the method earlier adopted by Jensen, (2007). The collected fresh roots of *Uvaria ovata* were dried under shade for 14 days, after which they were pulverized to powder using a manual blender. Fifty grams of the powdered sample were introduced into the extraction chamber of the Soxhlet extractor, and extraction was done using ethanol as solvent. The temperature was maintained at 60^0C throughout the extraction period of 48 hours. At the end of the period, the collected

extract in ethanol was dried in a laboratory oven at 40^{o}C to obtain an extract of 9.4g, which was preserved in a freezer until needed, after ascertaining its percentage yield.

$$\% \text{ Yield} = \frac{X}{Y} \times 100\%$$

where X = weight of prepared and dried extract

Y = weight of powdered plant material before extraction

1g of dried extract was dissolved in 10ml of water to give a stock solution of 0.1g/ml (100mg/ml).

Determination of the phytochemical components of the extract: Preliminary Phytochemical studies of the extract were carried out according to the method of Trease and Evans, (1989). This method was also revised and employed by Deka and Kalita, (2012).

Test for tannins: 1.0 ml of the extract was diluted with 4.0 ml of distilled water in a ratio 4:1 followed by the addition of a few drops of $FeCl_3$ solution. The mixture was then observed for colour change. A blue and green colouration confirmed the presence of tannins (Trease and Evans, 1989).

Test for saponins: 5 ml of distilled water was added to 2.0 ml of the extract. The mixture was shaken vigorously for 2 minutes. Few drops of olive oil were added. The formation of an emulsion indicated the presence of saponins (Deka and Kalita, 2012).

Test for flavonoids: 2.0 ml of 5% NaOH was added to the extract and observed. The formation of yellow colouration indicated the presence of flavonoids (Deka and Kalita, 2012).

Test for alkaloids: Few drops of Dragendorf reagent were added to 2.0 ml of the extract. Formation of an orange colour indicated the presence of alkaloids (Trease and Evans, 1989).

Test for steroids: Test for steroids was carried out according to the method of Sofowora, (1993). 2ml of acetic anhydride was added to 0.5g of *Uvaria ovata*, and 2ml of sulphuric acid was added by the sides of the test tube. A violet or blue-green coloration indicated the presence of steroids.

Test for phenols: A little quantity of *Uvaria ovata* was mixed with distilled water in a test tube and warmed followed by the addition of 2ml of Ferric chloride solution. The formation of green or blue colour indicated the presence of phenols (Trease and Evans, 1989).

Test for glycosides: About 0.5ml of *Uvaria ovata* was added to 1ml of glacial acetic acid containing traces of Ferric chloride in a test tube. To this solution 1ml of concentrated sulphuric acid was added and observed for the formation of reddish-brown color at the junction of the two layers with the upper layer turning bluish-green, this indicated the presence of glycosides (Trease and Evans, 1989).

GCMS evaluation of Uvaria ovata root extract: The characterization of the phytochemicals in *Uvaria ovata* was done using GC-MS QP2010 Plus (Shimadzu, Japan), while the identification of the phytochemicals in the sample was carried out using a QP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was set at 70eV. Gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60 m, XTI 5).The initial column temperature was 80^{o}C for 1min, and was later increased linearly at 70^{o}C min-1 to 220^{o}C, held for 3 min followed by a linear increased temperature 10oC min-1 to 290^{o}C for 10 minutes. 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. The identification of compounds was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS.

Identity of the active components in the extract was by comparison of their retention indices, peak area percentage and mass spectra fragmentation pattern with those stored on the National Institute of Standards and Technology (NIST) digital library data and also with published Literature. NIST08.LIB, (Stein, 1990), WILEY

8 LIB, (Lafferty, 1986), library sources were used for matching the identified components from the plant material. After adhering to the aforementioned procedure, the name, molecular weight, formula, structure and bioactivities of the compounds were ascertained.

Results

The phytochemical components in *Uvaria ovata* root extract are presented in Table 1. Alkaloids, tannins and flavonoids were found in high quantities, while saponins, terpenes and cardiac glycosides existed in moderate amounts. Other phytocomponents including steroids and phenols were only present in little quantities.

$$\text{Percentage Yield of extract} = \frac{9.4\text{ g}}{50\text{ g}} \times 100\%$$
$$= 18.8\ \%$$

Table 1: Phytochemical composition of *Uvaria ovata*

Phytochemicals	**Quantities (mg/100g)**
Alkaloids	14.37±0.48
Tannins	11.88±0.27
Saponins	5.91±0.07
Flavonoids	9.76±0.10
Terpenes	3.24±0.12
Cardiacglycoside	2.06±0.09
Steroids	1.08±0.05
Phenols	1.88±0.09

Values are presented as mean ± standard deviation

GCMS chromatogram of the ethanolic extract of *Uvaria ovata* roots showed eleven peaks which indicated the presence of eleven phytochemical constituents. Table 2 shows the mass spectra of the phytocomponents in the extract as were compared with that in the NIST Library database, leading to the identification and characterization of eleven phytocomponents. The identified components and quantities found include gentamycin A (0.094%), O-2-amino-2-deoxy-α-D-glucopyranosyl-(1-4)-O-(3-deoxy-4-C-methyl-3-methylamino)-β-larabinopyranosyl- (1-6) -2-deoxy-D-streptamine (59.215%), 10,13-octadecadiynoic acid (0.094%), acrolein-2-chloro (0.098%), boranamine (0.098%), di-allo-cystathione (0.118%), S-(2-Aminothyl)-dl-cysteine (0.097%), dl-homocystine (0.096%), mannosamine (0.125%), and cystine (39.84%).

Table 2: GC-MS composition of *Uvaria ovata* root extract

S/N	Name of Compound	Peak Areas (%)	Mol. Weight	Molecular Formula	RT Min	Bioactivity
1	Gentamycin A	0.116	468	$C_{18}H_{36}N_4O_{10}$	1.064	Antibacterial agent (Kushner *et al*., 2016)
2	D-Streptamine,O-2-amino-2-deoxy-α-D-glucopyranosyl-(1-4)-O-(3-deoxy-4-C-methyl-3-methylamino)-β-L-arabinopyranosyl-(1-6)-2-deoxy-	59.215	482	$C_{19}H_{38}N_4O_{10}$	1.665	Possesses antimicrobial properties (Serio *et al*., 2018)
3	Benzene (3-octylundecyl)	0.094	344	$C_{25}H_{44}$	2.926	Treatment of blood disorders, formulation of drugs and pesticides (Verschueren, 2001)
4	10,13-octadecadiynoic acid methylester	0.094	290	$C_{19}H_{30}O_2$	3.758	Anti-cancer (Yu *et al*., 2005)
5	2-Chloroacrolein	0.098	90	C_3H_3ClO	5.652	Immunosuppressant (Roth-Walter, 2017)
6	Boranine, 1-chloro-N, N-dimethyl-1-(1-methyl-2-propenyl)-	0.098	145	$C_6H_{13}BCIN$	6.375	Catalyst (Johnson *et al*., 2014)
7	Di-Allo-Cystathionine	0.118	222	$C_7H_{14}N_2O_4S$	7.144	Proton donor, cysteine intermediate (Ripps and Shen, 2012)
8	S-(2-Aminoethyl)-DL-Cysteine	0.097	164	$C_5H_{12}N_2O_2S$	7.966	Antioxidant (Sekhar and Patel, 2011)
9	DL-Homocystine	0.096	268	$C_8H_{16}N_2O_4S_2$	9.243	Allosteric inhibitor of dopamine receptors, prevents oxidative stress (Agnati *et al*., 2006)
10	Mannosamine	0.125	179	$C_6H_{13}NO_5$	10.422	Anti-inflammatory, signal transducer, cellular adhesive, cellular communicator, breaks obesity-hypertension link (Galeano *et al*., 2007; Peng *et al*., 2019)
11	Cystine	39.848	240	$C_6H_{12}N_2O_4S_2$	12.237	Anti-cancer, natural flavoring substance, antioxidant (Martis *et al*., 2020)

Figure 1 shows the structural formulas of the phytoconstituents of *Uvaria ovata* root extract, while Figure 2 indicates the peak values of the root samples.

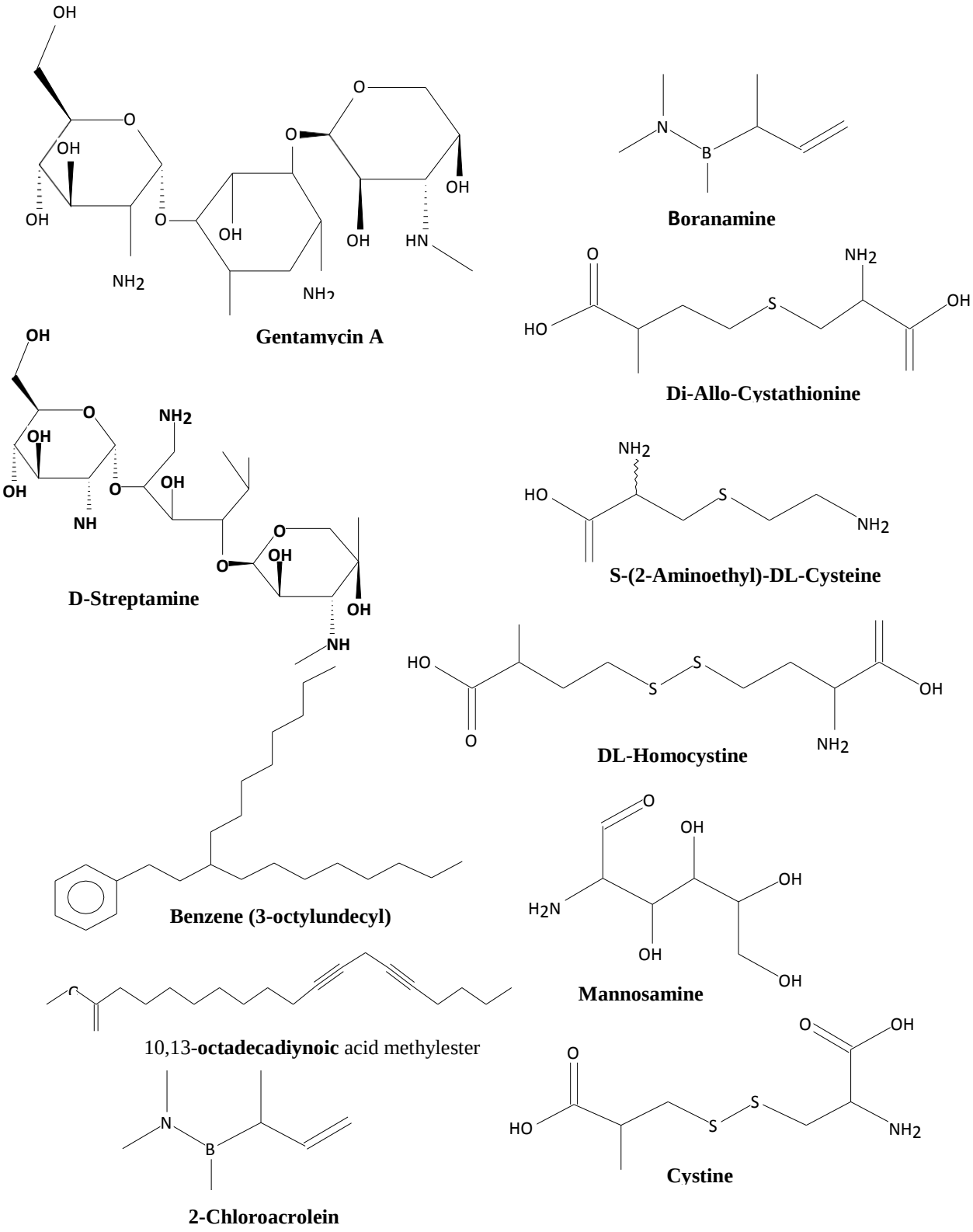

Fig. 1: Structural formulas of GC-MS phytoconstituents of *Uvaria ovata* root extract

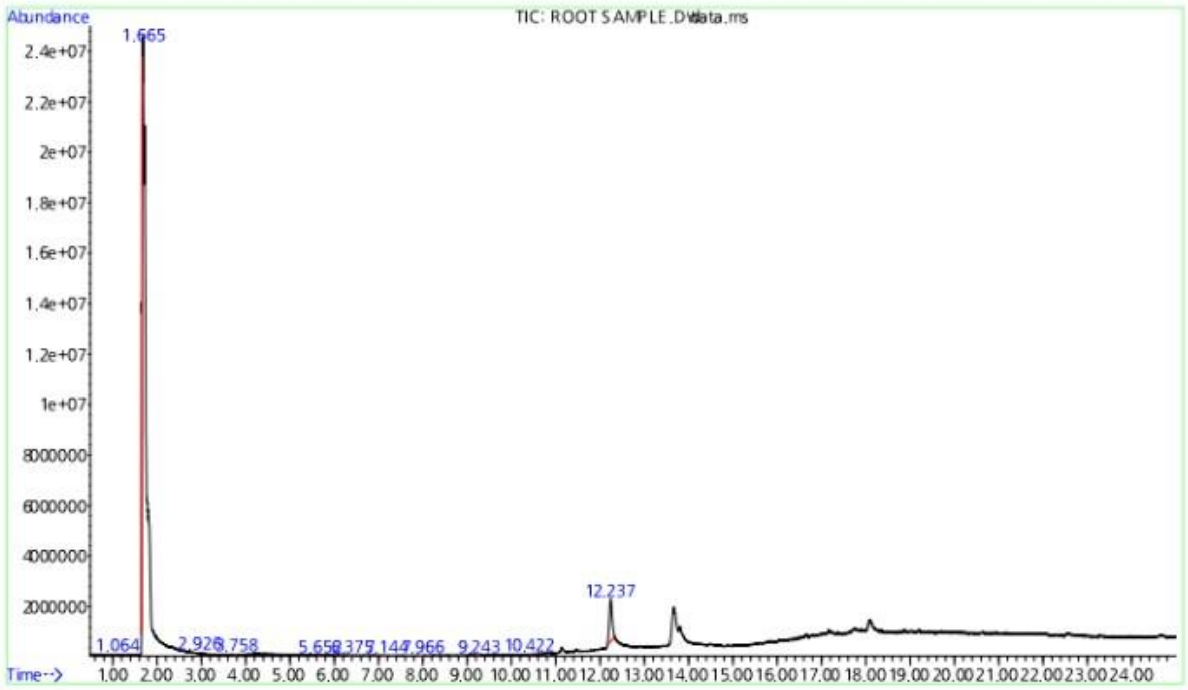

Fig 2: Peaks obtained following GC-MS analysis of *Uvaria ovata* root extract

As represented in Figure 2, the GC-MS chromatogram of the ethanolic extract of *Uvaria ovata* roots showed eleven (11) peaks which indicated the presence of 11 phytochemical constituents.

Discussion

The preliminary phytochemical screening of the root extract of *Uvaria ovata* showed no major differences in the phytochemical constituents from the results of other previous investigations in the same genus (Abu *et al*., 2018; Okwuosa *et al*., 2012). Alkaloids, tannins and flavonoids were found in high amounts, while saponins, terpenes, cardiac glycosides, sterols and phenols were found in moderate amounts. The presence of the aforementioned phytochemicals suggests that *Uvaria ovata* root extract may possess high therapeutic properties, explaining the reason the plant has been used for medicinal purposes over the years. Saliu *et al*. (2012), reports that most leafy vegetables are rich sources of phytochemicals, and *Uvaria ovata* is certainly not excluded, with their therapeutic values attributed to their known phytocomponents. Natural products that are evolutionarily-designed and chemically differentiated from synthesized molecules have played a crucial part in the discovery and development of new drugs owing to their wide structural diversity and wide range of biological activities.

Flavonoids exhibit a wide range of positive effects by interacting with other dietary components in the gastrointestinal tract to effect systemic changes in the body, including protection of the intestinal epithelium against pharmacological insults and food toxins, modulation of enzymatic activities involved in nutrient absorption, modulation of gut secretions and immune response, etc. (Oteiza and Taft, 2018). Falcao *et al*. (2008) showed that in isolation, several alkaloids possess gastroprotective and antiulcer activities by their interaction with various macromolecules, although stating that the compounds were not viable for development as gastroprotective drugs because of their toxicity, arguing that to be used as drugs, their functional groups need

modification before they can serve this purpose. Tannic acid extract, a unique hydrolysable tannin, has been shown to inhibit parasite growth, thereby reduce intestinal lesions. Tannins have also been shown to positively modulate gut microflora and strengthen mucosal immunity by increasing IgA and mucin concentrations within the gut. The astringency of tannins confers them the ability to form strong complexes with other macromolecules such as proteins. This tannin-protein complex layer protects the stomach by promoting greater resistance to mechanical and chemical insults. Tannins have also shown antioxidant activity, and promotion of tissue repair. They are also taken orally to arrest diarrhea. Tannins have been reported to be responsible for decreasing food intake, food efficiency and growth rate (de Jesus *et al*., 2012). All the phytoconstituents of *Uvaria ovata* confirmed in this study are known to influence biological system activities.

GCMS analysis of *Uvaria ovata* showed the presence of Gentamycin A, O-2-amino-2-deoxy-α-D-glucopyranosyl-(1-4)-O-(3-deoxy-4-C-methyl-3-methylamino)-β-L-arabinopyranosyl-(1-6)-2-deoxy-D-Streptamine, 10,13-octadecadiynoic acid, acrolein-2-chloro, boranamine, di-allo-cystathione, S-(2-Aminothyl)-DL-cysteine, DL-homocystine, mannosamine, and cystine. Various reports on the medicinal values of these phytosubstances exist. Of the aforementioned substances, the most abundant were D-streptamine and cystine, and they are reported to be beneficial as antimicrobials and antioxidants respectively (Sekhar and Patel, 2011)

Conclusion

The result obtained from the preliminary phytochemical study and GC-MS analysis of *Uvaria ovata* root extract revealed the presence of several therapeutic bioactive compounds that have proven to be of value in the management of various human ailments, thereby justifying its use by traditional medicine practitioners. Based on this established pharmacological potency, further research is recommended on this plant.

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