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Original Research Article

Antibacterial Activity and Identification of Metabolites from the Semi-Purified Fraction of *Chrysophyllum albidum* Leaf (African Star Apple)Ifeanyi Azih¹, Magnus Ijezie¹, Sylvester N. Ugariogu^{1,2}, Christogonus O. Akalezi^{1,2}¹Department of Chemistry, School of Physical Sciences, Federal University of Technology Owerri (FUTO), PMB 1526 Owerri, Nigeria.²African Center of Excellence in Future Energies and electrochemical Systems, Federal University of Technology Owerri (FUTO), PMB 1526 Owerri, Nigeria

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ABSTRACT

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The medicinal uses of natural products especially medicinal plants have increased tremendously due to the multidrug resistance of some microorganisms and due to the availability and affordability of these plants. This study was aimed at the identification of metabolites from the fractions of *Chrysophyllum albidum* and their antibacterial activity. The phytochemical analysis and antibacterial analysis were done using standard methods; Harborne and Agar-disc diffusion methods, respectively. The metabolite identification was done using infra-red (IR) and gas chromatography – mass spectrometry (GC-MS). The preliminary result of the antibacterial screening showed that the neutral metabolite possessed the highest antibacterial activity and was then chosen for the IR and GC-MS study and purified using preparative thin layer chromatography. Various fractions were obtained from the chromatographic purification and in particular N3 fraction demonstrated the highest antibacterial activity against the test microbes with the zones of inhibition indicated against the test organism as follows: *Streptococcus spp* (18 mm), *Staphylococcus aureus* (20 mm), *Salmonella typhi* (25 mm), *Escherichia coli* (20 mm) and no inhibition zone against *Enterobacter cloacae*. The results confirm that the plant has some antibacterial activity.

Keywords: *Chrysophyllum albidum*, Metabolites identification, Phytochemicals, Antibacterial.

Introduction

Traditional medicine which rely on plant resources to treat various diseases and ailment remains an important (in some case, the only) form of treatment in many developing countries and its use is increasing worldwide.¹ According to the world Health Organization (WHO), 75% of world population still rely on plant based traditional medicines for their primary healthcare of which 85% is based on the use of plant extracts.² Similarly, it is estimated that 39% of 520 new approved drugs (between 1983 and 1994), 50- 80% of antibacterial and anticancer drugs were derived from natural product.³ The increased interest in the use of natural products is for a number of reasons, the perception that such products are safe, ecofriendly, low cost, readily available and of little or no side effects. The use of natural products has led in many drug discoveries as they provided chemical building blocks used in their synthesis or used as new drugs in their unmodified state.⁴ Many natural products are produced as part of chemical defense. Examples of this include synthesis of antimicrobial or antifeedant substances systems to protect the producing organism from attack. This inherent biological activity associated with natural products is the reason for the use of plants in traditional medicines as remedies for various infections.⁵⁻⁷

Chrysophyllum albidum G. known as African star apple, belonging to the family *Sapotaceae* is primarily a forest tree with its natural occurrences in diverse eco-zones of Africa.⁸

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In Nigeria, *C. albidum* is known as “agbalumo” in the South West and “udara” in the South East. The fruit usually available during the dry season (December-April) has been enjoyed over the years by Nigerians. The fleshy pulp of the fruits is eaten especially as snack and its fruit has been found to have higher contents of ascorbic acid than oranges and guava.^{9,10}

Extracts from different parts, including the bark, leaves, roots and seeds of *C. albidum* have been used for the treatment of different ailments, such as yellow fever, malaria, certain skin diseases, stomach ache, and diarrhoea, infertility problems as well as dermatological and urinary related infections.¹¹⁻¹⁴ The extracts have also found use as liniments and in stopping microbial growth in open wounds.¹⁵⁻¹⁷ The split stems and wigs are used as chewing sticks in many parts of Africa and have been commercialized in many cities for years offering natural dental care. The extracts of the leaves and fruits have shown antibacterial and antioxidant properties *in vitro* and *in vivo*.¹⁸⁻²⁵

Despite numerous studies on phytochemical composition, medicinal and antimicrobial activities of plants, reports on African star apple are limited especially report on the particular component responsible for the biological activities. Moreover reports on the phytochemical composition and activity of the metabolite are also scanty. This study therefore focuses on the antibacterial activity of the metabolite from the ethanol extract of *C. albidum* with a view to identify the most active fraction.

Materials and Methods

Sample collection and preparation

Fresh leaves of *C. albidum* were collected within Owerri municipal in Imo State, Nigeria in July 2018. The plant material was identified at the crop science department in the school of Agricultural Technology, Federal University of Technology Owerri. The leaves were dried at room temperature to constant weight, ground to fine powder and then stored in an air-tight container. The powdered sample (80 g) was extracted with 250 mL of ethanol for 12 h using a soxhlet extractor. The ethanol extract after extraction was allowed to evaporate

completely at room temperature to produce a gel. The solid residue obtained was re-dissolved in ethanol/water mixture (4:1) and filtered. The filtrate was used without further purification for the preparation of the basic, acidic and neutral metabolites. Preparation of the Acidic, Neutral, and Basic metabolites was done using the methods described by Ejele and Alinnor, 2010.²⁶

Preparation of basic metabolite

The filtrate of *Chrysophyllum albidum* extract was treated with dilute HCl and extracted with chloroform in a separatory funnel. The lower chloroform layer was removed and reserved for the preparation of the neutral metabolite. The HCl layer was treated with NaHCO₃ until the mixture became basic. The precipitate from the resultant solution was filtered out and washed well with distilled water. The precipitate was dissolved in 40 mL of 95% ethanol and filtered. The filtrate was used for the antibacterial screening without further purification.

Preparation of neutral metabolite

The chloroform layer obtained from basic metabolites was placed in a separatory funnel and treated with NaOH solution (2 M). After equilibrating, the aqueous NaOH solution layer was removed and reserved for preparation of the acidic metabolite. The chloroform layer was removed and allowed to evaporate completely at room temperature to form a gel which was dissolved in 95% ethanol and filtered. The filtrate was used without further purification for antibacterial screening.

Preparation of acidic metabolite

The aqueous alkaline layer obtained from neutral metabolite was treated with dilute HCL until the solution became acidic. The precipitate of the mixture was filtered out and washed well with distilled water. The precipitate was dissolved in 40 mL of 95% ethanol and filtered and the filtrate was used for the antibacterial screening without further purification.

Phytochemical analysis

The phytochemical screenings of the extracts were carried out using the methods described by Harborne²⁷ and Evans.²⁸ The substances that were tested for include tannins, saponins, and flavonoids, cardiac glycosides, alkaloids, anthraquinones and steroids.

Chromatographic purification

Preparative thin layer chromatography was carried out on the neutral metabolite using a chromatographic plate coated with silica gel and cellulose. The sample was spotted on the plate and then developed in a chromatographic tank for 1 hour using ethanol (95%) and chloroform mixture (2:1) as the eluting solvent. After separation, three distinct fractions were obtained and labeled N1, N2 and N3. The fractions were scrapped into different conical flasks re-dissolved with ethanol (95%) and filtered. The filtrates in the different flasks were allowed to evaporate to dryness at room temperature and each fraction was screened to determine their anti-microbial activity against some test microbes.

Antimicrobial Tests

Collection of test organisms

Clinical isolates of *Staphylococcus aureus*, *Streptococcus spp*, *Escherichia coli*, *Salmonella typhi* and the coliform bacilli: *Enterobacter cloacae* were collected from the Department of microbiology Federal Medical Centre, Owerri, Nigeria.

Sterility test of the extracts

Sterility of the extracts was determined by streak inoculation onto freshly prepared Nutrient, Blood and MacConkey agar plates. Plates were incubated at 37°C for 24 hours. At the end of incubation period, plates were examined for growth.

Antibacterial screening

The Disc Diffusion Method was used as earlier described. An inoculating loop or needle was touched on five isolated colonies of the pathogens growing on agar and then used to inoculate a tube of culture

broth. The culture was incubated for a few hours at 35-37°C until it becomes slightly turbid and was diluted to match a turbidity standard. A sterile cotton swab was dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of an agar plate. After the agar surface had dried for about 5 min, the appropriate antibiotic test disc of each fraction was placed on it and the agar plate was immediately placed in the incubator maintained at 35-37°C. After 16-18 h of incubation, the minimum inhibition zone diameters were measured and recorded to the nearest mm.²⁹⁻³¹ The fraction with the highest inhibition zone diameter was selected for spectrometric analyses using Infrared Spectroscopy (IR) and GC-MS.

Spectroscopic analysis

IR spectroscopic analysis of selected antibacterial active fractions was carried out at the Central Research Laboratory University of Ibadan, using the Perkin-Elmer IR spectrophotometer while the GC-MS analysis was carried out at the Obafemi Awolowo University Ile-Ife.

GC-MS analysis

GC-MS analysis was carried out using the method of Dhivya and Manimegalai.³² Identification of the compounds based on their mass-to-charge ratio were determined by the mass spectrometer.³³ Chemical analysis was by triple quadrupole gas chromatography mass spectrometry. Analysis was performed in TIC scan mode. Oven temperature was set at 50°C for 2 min and programmed at 50°C to 230°C at a rate of 4°C/min and at hold 230°C for 2 min resulting in the complete elution of peaks analysed.

Results and Discussion

Table 1 shows the result of the phytochemical screening carried out on the extract and various metabolites. Table 2 compares the antibacterial activity of the crude extract and of the various fractions (metabolites) at same concentration of 100 mg/mL.

Table 3 compares the antibacterial activity of the three fractions (N1, N2, N3) obtained from the chromatographic separation with that of the control drugs at same concentrations.

The result of the phytochemical screening showed the presence of tannins, saponins, flavonoids, phenols, cardio-active glycosides and alkaloids. It has been reported that plant that contained tannins have astringent, hemostatic, antiseptic and toning properties.³³ Tannin containing herbs have been reportedly used in stopping minor hemorrhage, sore mouth, bronchitis, burns, scars of the skin, wound diarrhea and other diseases.³⁴ Saponin have been reported to be active in fighting cancer and also in lowering cholesterol level and blood sugar.³⁵ It has been used in the treatment of hypercalciuria in human and as antidote against lead poisoning.³⁵ Flavonoid on the other hand have been reported to have wide range of biological and pharmacological activities in *in vitro* studies which include anti-allergic, anti-inflammatory, antioxidant, antimicrobial, anticancer and anti-diarrheal activities.³⁶ Natural phenolic compounds have been reported to play an important role in cancer prevention and treatment.³⁷ Phenolic compounds from medicinal herbs and dietary plants include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others. Various bioactivities of phenolic compounds are responsible for their chemopreventive properties (e.g., antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects) and also contribute to their inducing apoptosis by arresting cell cycle, regulating carcinogen metabolism and ontogenesis expression, inhibiting DNA binding and cell adhesion, migration, proliferation or differentiation, and blocking signaling pathways.³⁷ Cardiac active glycoside is valuable in the treatment of congestive heart failure.³⁸ The presence of these phytochemical scientifically proves the leaves of *C. albidum* as a medicinal plant.

The results of the preliminary antibacterial screening of the crude extract and various metabolites of *C. albidum* are shown in Tables 2, from which it was observed that the crude extract, acidic, basic and

Table 1: Phytochemicals Screening of Crude Extract, Acidic, Basic and Neutral Metabolites

Phytochemicals	Crude Extract	Acidic Metabolite	Basic Metabolite	Neutral Metabolite
Tannins	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Phenols	+	+	+	+
Cardio-Active Glycoside	+	+	+	+

Note + = Present, - Absent.

neutral metabolites possessed antibacterial activity against the micro-organism tested to various extents.

The antibacterial activity of the crude extract and metabolites showed that the bioactivities of the extract and metabolites against the test microorganisms were comparable and their inhibition zone diameters ranged from 22 mm to 28 mm (for the crude extract at 100 mg/mL), 25 - 30 mm (for the neutral metabolites at 100 mg/mL), 18 - 25 mm (for the acidic metabolites at 100 mg/mL) and 15 - 25 mm (for the basic metabolites at 100 mg/mL). The data showed that the crude extract exhibited the highest activity against *Staphylococcus aureus* and the least activity was shown against *Salmonella spp.*

The neutral metabolites exhibited its highest activity against *Salmonella spp* with inhibition zone diameter of 30 mm at 100 mg/mL followed by *Streptococcus spp.*, *Staphylococcus aureus* and *Coliform bacilli* with inhibition zone diameter of 25 mm at 100 mg/mL.

The acidic metabolites exhibited its highest activity against *Streptococcus spp*, *Escherichia coli* and *Coliform bacilli* with inhibition zone diameter of 25 mm at 100 mg/mL and the least activity was shown against *Staphylococcus aureus* with inhibition zone diameter of 18 mm at 100 mg/mL. The basic metabolites showed its highest activity against *Streptococcus spp.*, and *Escherichia coli* with inhibition zone diameter of 25 mm and the least activity was shown against *Salmonella spp.* with no inhibition zone diameter.

These differences in bioactivity arose probably from the differences in the phytochemical composition of the various metabolites. The result of the control experiment with the following drugs Cefuroxime, ciprofloxacin and gentamycin against the test micro-organisms are presented in Table 3.

Hence the observed antibacterial activity of the crude extract, acidic, basic and neutral metabolites against the test microorganisms may be due to the presence of tannins or other phenolics, saponins and

flavonoids found to be present in the plant.

The neutral metabolites due to its high activity were chosen for purification and further study. Fraction N₃ obtained from the chromatographic separation of the neutral metabolites exhibited high activity against *Salmonella* and least activity against *Streptococcus* which implies that the neutral metabolite may be used to treat diseases caused by *Salmonella spp.*

The neutral metabolite was purified using preparative thin layer chromatography and three fractions were obtained which were labeled N1, N2 and N3. Antibacterial analysis was carried out on the three fractions and it was observed that fraction N3 was most active against the test microbes because it gave the highest inhibition zone diameter of 18 mm for *Streptococcus spp.*, 20 mm for *S. aureus*, 25 mm for *Salmonella typhi*, 20 mm for *E.coli* and no inhibition for *Enterobacter cloacae*. Due to its high activity against the test organisms, fraction N3 was chosen for IR and GC-MS analyses.

IR Spectral data of Fraction N3

The IR Spectrum of fraction N3 is presented in Figure 1 and showed the following absorptions.

The strong broad absorption at 3367.00 cm⁻¹ suggest the presence of hydrogen bonded OH group of an alcohol.

The strong broad absorption at 2902.85cm⁻¹ suggest the presence of C-H group of benzene.

The strong broad absorption at 2975.57cm⁻¹ suggest the presence of carbon oxygen bond (C-O) of an aliphatic compound.

The weak absorption at 2135.00 cm⁻¹ and 1920.20 cm⁻¹ suggest the presence of a benzene ring.

The strong absorption at 1648.32cm⁻¹ suggest the presence of carbon oxygen double bond (C=O) of a H - bonded ketone.

The strong absorption peak at 1396.56 cm⁻¹ suggest the presence of the CH₃ of an alkane.

The weak absorption at 1327.46 cm⁻¹ suggest the presence of carbon hydrogen bond of an aliphatic compound.

The weak absorption at 1279.01 cm⁻¹ suggest the presence of an ester.

The strong absorption at 1050.80 cm⁻¹ suggest the presence of carbon oxygen bond of an alcohol or ether.

The strong absorption at 878.20 cm⁻¹ suggests the presence of di-substituted benzene ring.

The GC spectrum presented in Figure 2 showed that fraction N3 was impure and contained three major sharp peaks at elution times of 28.999 and 39.721 and 39.878 mins.

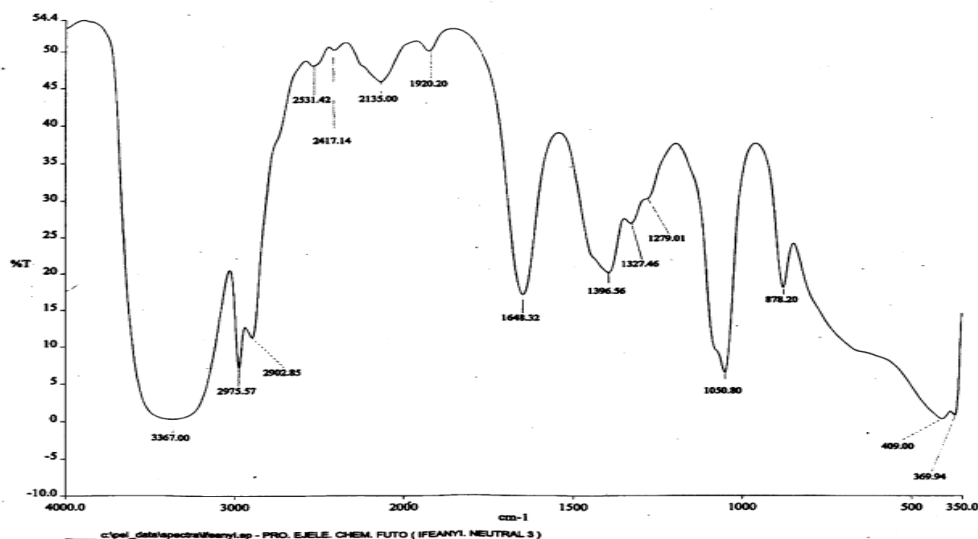
From the result of the GC-MS analysis, it was observed that most of the possible phytochemicals present in the N3 fraction of the neutral metabolites were terpenes, esters, alkaloids, hydrocarbons, carboxylic acids, phenol and tannins.

Table 2: Antibacterial activity of the crude ethanol extract with the various fractions (acidic, basic and neutral metabolites) obtained from the leaves of *C. albidum* at 100 mg/mL

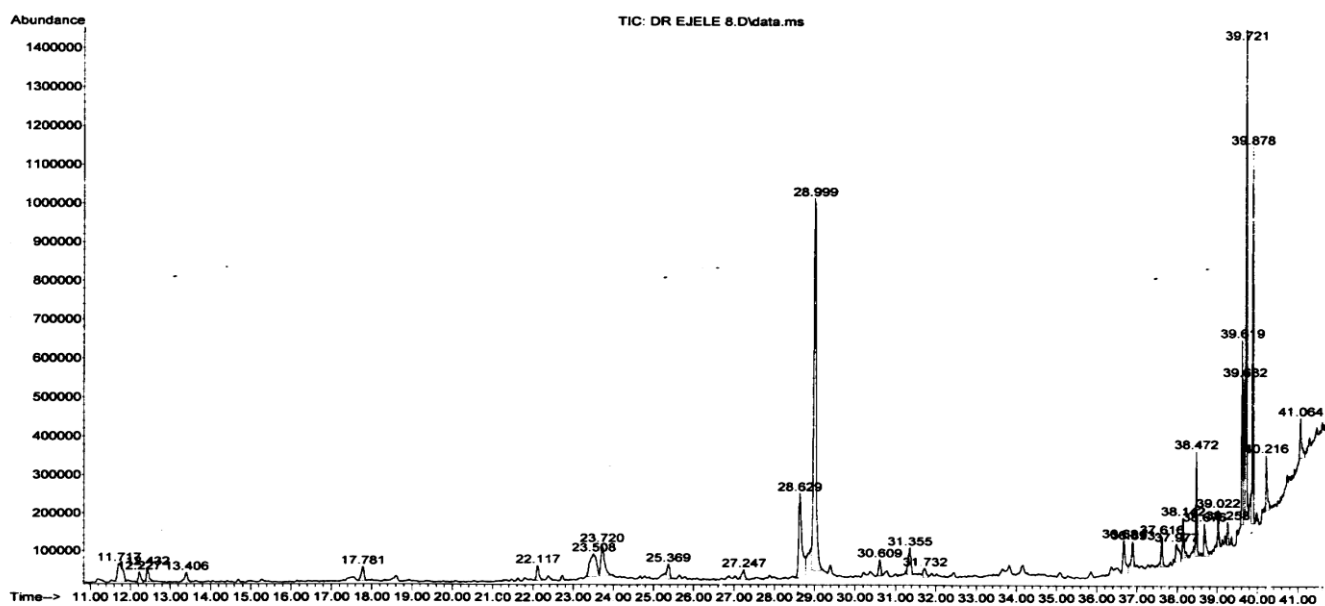
Microorganisms	Zones of Inhibition (mm)			
	Crude extract	Acidic metabolite	Basic metabolite	Neutral metabolite
<i>Strep.spp</i>	22	25	25	25
<i>Staph. Aureus</i>	22	18	20	25
<i>Salmonella spp</i>	18	20	0	30
<i>Escherichia coli</i>	20	25	25	20
<i>Coli form bacilli</i>	20	25	15	25

Table 3: Antibacterial activity of the standard drugs and that of the fractions obtained from the neutral metabolites of *C. albidum* leaf

Test Samples /Standard	Zones of Inhibition (mm)				
	<i>Strep.spp</i>	<i>Staph. Aureus</i>	<i>Salmonella spp</i>	<i>Escherichia coli</i>	<i>Coli form Bacilli</i>
N1 (100 mg/mL)	18	18	20	12	0
N2 (100 mg/mL)	20	22	20	22	0
N3 (100 mg/mL)	18	20	25	20	0
Ciprofloxacin (100 mg/mL)	25	20	27	28	24
Gentamycin (100 mg/mL)	15	14	23	25	15
Cefuroxime (100 mg/mL)	12	15	10	14	13

Figure 1: IR Spectrum of Fraction N₃

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 Acquired : 6 Jan 2016 19:37 using AcqMethod ESSENTIAL OILS_SCAN.M
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Figure 2: GC Chromatogram of Fraction N₃

Conclusion

This study has demonstrated the antibacterial activity of the metabolites of the leaves of *Chrysophyllum albidum* against some human pathogens, and it has been established scientifically that the leaves is medicinal as shown by the antibacterial activity and the presence of potentially bioactive phytochemicals.

Conflict of interest

The authors declare no conflicting interest

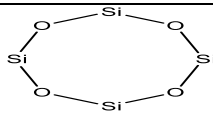
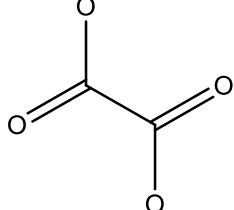
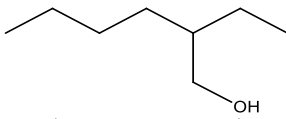
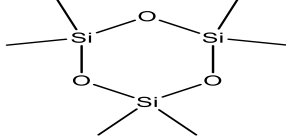
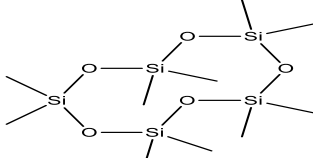
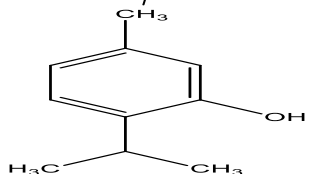
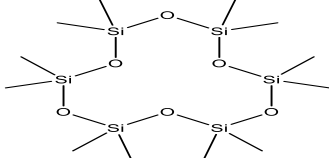
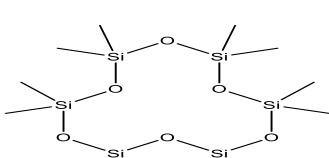
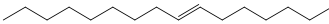
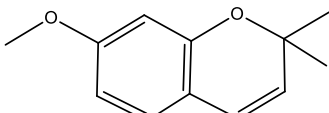
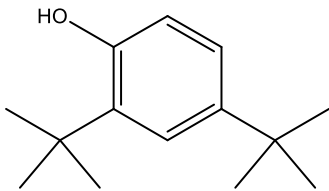
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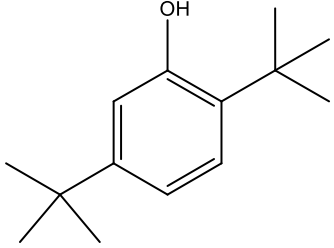
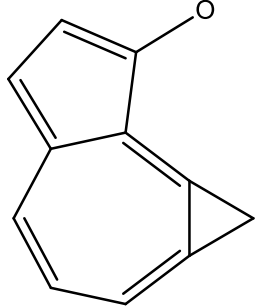

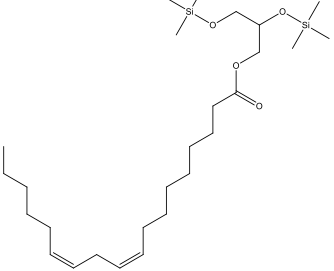
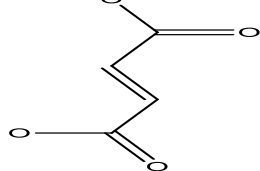
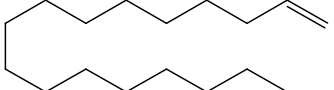
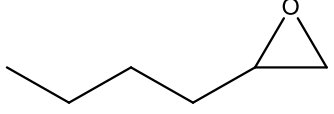
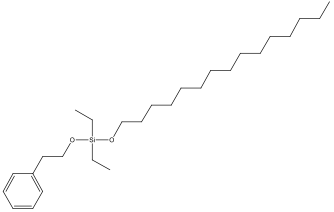
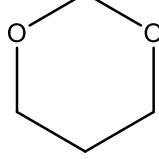
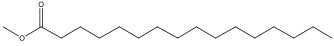
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 4: Some of the possible Phytocompounds present in the N3 fraction of the metabolite from GCMS result.

No	RT (Min)	Molecular weight	Compound Name	Formular	Structure
1	11.717	184.403	Cyclotetrasiloxane	$H_8O_4Si_4$	
2	12.227	90.035	Oxalic acid	$C_2H_2O_4$	
3	12.432	130.228	1-Hexanol, 2-ethyl	$C_8H_{18}O$	
4	13.406	222.462	Hexamethylcyclotrisiloxane	$C_6H_{18}O_3Si_3$	
5	17.781	370.770	Decamethylcyclopentasiloxane	$C_{10}H_{30}O_5Si_5$	
6	22.117	150.2176	Thymol	$C_{10}H_{14}O$	
7	23.508	444.924	Cyclohexasiloxane, dedecamethyl	$C_{12}H_{36}O_6Si_6$	
8	23.720	444.924	Cyclohexasiloxane, dedecamethyl	$C_{12}H_{36}O_6Si_6$	
9	25.369	224.425	7-Hexadecene	$C_{16}H_{32}$	
10	27.247	190.238	Precocene	$C_{12}H_{14}O_2$	
11	28.629	206.106	Phenol, 2,4-bis (1, 1-dimethylethyl)	$C_{14}H_{22}O$	

12	28.999	206.306	Phenol, 2,5-bis (1,1-dimethylethyl)	$C_{14}H_{22}O$	
13	30.609	156.181	1H-Cyclopropa[e]azulen-7-ol	$C_{11}H_8O$	
14	31.355	196.32	5-Tetradecene, (E)-	$C_{14}H_{28}$	
15	31.732	498.886	1-monolinoleoylglycerol trimethylsilyl ether or 2,3-Bis[(trimethylsilyl)oxy]propyl (9Z,12Z)-9,12 octadecadienoate.	$C_{27}H_{54}O_4Si_2$	
16	36.681	116.072	Fumaric acid	$C_4H_4O_4$	
17	36.893	238.452	1-Heptadecene	$C_{17}H_{34}$	
18	37.616	100.159	Oxirane, butyl	$C_6H_{12}O$	
19	37.977	434.770	Diethyl(pentadecyloxy)(2-phenylethoxy) silane	$C_{27}H_{50}O_2Si$	
20	38.142	88.105	1,3-Dioxane	$C_4H_8O_2$	
21	38.472	270.451	Methyl Palmitate	$C_{17}H_{34}O_2$	

22	38.676	182.221	9H-Pyrido[3,4-b]indole, 1-methyl- or Harmane	$C_{12}H_{10}N_2$	
23	39.022	256.467	2-Heptadecanol	$C_{17}H_{36}O$	
24	39.258	149.233	Benzenebutanamine or 4-Phenylbutylamine	$C_{10}H_{15}N$	
25	39.619	196.372	Cyclotetradecene	$C_{14}H_{28}$	
26	39.682	280.445	9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	
27	39.721	282.461	7-Octadecenoic acid	$C_{18}H_{34}O_2$	
28	39.878	298.504	Methyl Stearate	$C_{19}H_{38}O_2$	
29	40.216	179.259	Adamantane-1-carboxamide	$C_{11}H_{17}NO$	
30	41.064	405.443	3,5-Dipiperonylidene-1-propyl-4-piperidone	$C_{24}H_{23}NO_5$	

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