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Chemical Composition and Biological Properties of the Petroleum Ether Extract of *Solanum macrocarpum* L. (Local Name: Gorongo)

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Authors' contributions

This work was carried out in collaboration between all authors. OAS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. FIA and TEA managed the analyses and literature searches of the study. IAG performed the antimicrobial work. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To investigate the petroleum ether extract of the unripe fruit of *Solanum macrocarpum* L. (Solanaceae) for its chemical components and its antimicrobial properties against Gram positive, Gram negative bacteria and fungi.
Study Design: Experimental study.
Place and Duration of Study: Department of Chemistry, Faculty of Science, Department of Veterinary Medicine, University of Maiduguri and Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Science, University of Jos, Jos, Nigeria, between October 2007 and July 2009.
Methodology: After authentication of the plant, extracts were prepared from the fruits of *S. macrocarpum* using Soxhlet apparatus. The petroleum ether extract was investigated

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for its chemical composition using GC-MS after it had been fractionated earlier using AGC. The extract was also evaluated for its antimicrobial activities by disc diffusion antimicrobial selectivity test using gentamicin (10µg/disc), ciprofloxacin (5µg/disc) and tetracycline ($2.5x10^5$µg/disc) as standards.

Results: All the microorganisms used were resistant to the effect of the petroleum ether extract. However, a total of sixty eight (68) compounds were identified in the extract and its four (4) fractions, whilst only thirty eight (38) chemical structures of the identified compounds were shown because some of the compounds were common to all the fractions.

Conclusion: The detection of 2-methoxy furan, 4-0-methyl-mannose, 2-hydroxy-Ɣ-butyrolactone, 2, 3-dihydroxypropyl 9-octadecanoate, 3-hydroxyisovaleric acid and butanoic acid validates the antihyperlipidaemic property of the fruit of *S. macrocarpum,* so consumption of this fruit is recommended as a nutraceutical.

Keywords: Solanum macrocarpum GC-MS; petroleum ether extract; antibacterial; mycostatic.

1. INTRODUCTION

In the last two decades, phytotherapy has remained the principal source of treatment in developed countries whilst in the developing countries; there was a decline of phytotherapy during the first half of the 20th century (Abdulrahman, 2004). In the developed nations, the new trend of applying phytotherapy has led to an upsurge in the consumption of medicinal plants. This demand for medicinal plants is steadily increasing not only in developing countries but also in the industrialized nations (Srivastera et al., 1996). The World Health Organization (WHO) estimates that approximately 80% of the developing world's population meets their Primary Health Care needs through traditional medicine (LMPTK, 2006). This is the status of phytotherapy in developing countries. About 25% of prescription drugs dispensed in the United States of America contain at least one active ingredient derived from plant material. Some are made from plant extracts, others are synthesized to mimic a natural plant compound (ANON, 2006). With plant extract however, efficacy, risk factors such as toxicity and side effects are unknown and reasonable safety measures are often established (Garriot and Ramberg, 2006).

The bombardment of the human body by synthetic drugs for the chemotherapeutic control of disease coupled with its long term harmful effects, could be what triggered the search and use of therapeutic agents with less toxic effects wherever possible (Abdulrahman, 2004). The outcome of this development is the association of plant medicine with specific therapies and sometimes completely synthetic medicines.

Herbal medicines are gaining more ground in the developing countries because of the shift from synthetic drugs to medicinal plants. A herbal medicine is one that contains materials as a finished product and may include whole plant parts or other plant materials (Agada et al., 2005).

It is estimated that less than 10% of the world's genetic resources has been studied seriously as sources of medicines (Iwu, 1996; Williamson et al., 1996). Yet from this small fraction, humanity has reaped enormous benefits.

It takes about 15 years to develop a single pharmaceutical entity from discovery to market and at an estimated cost of between $150 million to $300 million. Today, it is possible to convert a crude drug into a standardized modern phytomedicine or personal care product in less than 2 years. The WHO has however emphasized that safety should be the overriding criterion in the selection of herbal medicine for use in health care (Iwu, 1996).

A noteworthy feature in many phytochemical systems is the use of food plants as ingredients for drug preparation (Nutraceuticals). *Nutraceuticals* is a term coined from "nutrition" and "pharmaceutical" in 1989 by Stephen DeFelice and can be defined as, "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease" (Brower, 1998). Such foods also commonly are referred to as medical foods, nutritional supplements and *functional foods* thus signifying they and/or their components may provide a health benefit beyond basic nutrition. Nutraceuticals range from isolated nutrients, dietary supplements, genetically engineered foods, herbal products and processed products such as cereals and soups (Lee et al., 2004).

The genus *Solanum* is well known in traditional medicine (Burkhill, 2000; Grubben and Denton, 2004; Sodipo, 2009). *Solanum* species are about 1,500 in the world (Grubben and Denton, 2004; ANON, 2007). In Africa and adjacent islands, it is represented by at least 1500 indigenous species; about 20 of these are recent introduction (Grubben and Denton, 2004). *Solanum macrocarpum* L. "Gorongo" in Kanuri has been used extensively for its fruits, leaves and vegetables (food) and also for medicinal purposes in the North East Arid zone of Nigeria by the natives. In Sierra Leone, heated leaves are chewed to treat throat troubles (Grubben and Denton, 2004). In Nigeria, fruits are taken as laxatives and to treat cardiac diseases, while flowers and fruits are chewed to clean the teeth (Grubben and Denton, 2004); in Kenya, the juice of boiled roots is drunk to get rid of hookworms, while crushed leaves are taken to treat stomach troubles (Grubben and Denton, 2004). *Solanum macrocarpum* is occasionally grown as an ornamental herb (Grubben and Denton, 2004).

The young leaves and young fruits of *S. macrocarpum* are cooked and consumed as a vegetable. The leaves are eaten as a separate dish or in sauces. The taste is more or less bitter and very much liked. The leaves can either be steamed (as practiced in Uganda) or fried in oil with onions. In West Africa, both leaves and fruits are eaten. In Uganda (East Africa, it is mostly the leaves that are eaten, but the fruits are added to sauce (Grubben and Denton, 2004).

Solanum macrocarpum Linn. (Synonymns: *Solanum daysphyllum* L. and *Solanum macrocarpon* L.) has been reported to exhibit laxative and hypotensive properties (Sodipo et al*.,* 2008a), the powdered fruit showed the presence of alkaloids, cardiac glycosides, tannins, phlobatannins, flavonoids, saponins, combined reducing sugars, reducing sugars and ketoses, whilst extracts contained alkaloids and saponins in both the aqueous and ethanol extracts, flavonoids in all the extracts (aqueous, ethanol, ethyl acetate and petroleum ether), flavone glycoside in only the ethyl acetate extract, steroidal glycosides (in all the extracts) and tannins in the ethyl acetate, ethanolic and aqueous extracts (Sodipo et al*.,* 2008b). The aqueous extract has been shown to exhibit lipid lowering activities (Sodipo et al*.,* 2009c) and at the same time has renal and hepato-protective effects (Sodipo et al*.,* 2009a,b). The fruit in addition is not toxic as the intra peritoneal LD_{50} was 1,280mg/kg

(Sodipo et al., 2009d) and heavy metals like lead (Pb), cadmium, (Cd) and Selenium (Se) were not detected in the fruit (Sodipo et al., 2008b). Thus the fruit is safe if consumed. The reported attributes of this plant and the fact that there is no documented antimicrobial effect except that of the ethyl acetate extract (Sodipo et al., 2010) necessitated the need to separate, purify, isolate, identify and also investigate the antimicrobial activity of the crude petroleum ether extract (CPEE) of the fruit using gas chromatography-mass spectrometry (GC-MS) as little work has been done on the CPEE.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The plant material (*Solanum macrocarpum* Linn.) used in this study was obtained from Alau in Konduga Local Government Area, Borno State, Nigeria, between October and November, 2007. The plant was identified and authenticated by Prof. S.S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Maiduguri, Nigeria. Specimen voucher No. 548 was deposited at the Research Laboratory of the Department of Chemistry.

2.2 Extraction

The fruit (40kg) was air-dried in the laboratory for seven (7) days and extracted according to the methods of Lin et al., (1999). The 2.2kg of the ground fruit was subjected to successive Soxhilet extraction in petroleum ether (60-80ºC), ethyl acetate (76.7-77.7ºC), ethanol (95%) to give the petroleum ether extract (CPEE), ethyl acetate extract and (CEE) respectively. The marc was then soaked in distilled water to give an aqueous extract (CAE). The extracts were concentrated to dryness in *vacuo* and stored at room temperature in a dessicator until when required.

2.3 Gas Chromatography-mass Spectrometry of the CPEE

CPEE 2.3g was fractionated using accelerated gradient chromatography, (AGC) with the solvent mixture: n-hexane, dichloromethane ($CHCl_2$) and methanol (Baeckstrom Separo AB) [Libikas et al., 2005] into five (5) single fractions A, B, C, D and E. However, D was too small to be run on the GC-MS. The CPEE and the other four fractions (A, B, C and E) were then subjected to GC-MS.

A SHIMADZU, QP-2010 plus GC-MS were used. The GC-MS was equipped with a split injector and an ion-trap mass spectrometer detector, together with a fused-silica capillary column having a thickness of 1.00µm, dimensions of 30m x 0.25mm and temperature limits of 60ºC to 325ºC. The column temperature was programmed between 60ºC and 250ºC at a rate of 3.0ml/min. The temperature of the injector and detector were at 250ºC and 200ºC respectively. Helium gas was used as a carrier gas at a flow rate of 46.3cm/sec. Components were identified by computer-aided matching of their spectra with spectra of known compounds from the Library of spectra from the National Institute of Standards and Technology (NIST), formerly National Bureau of Standards, Washington, USA (NIST, 2009). The fragmentation patterns of the identified compounds were then examined for consistency with known data from literature (Williams and Fleming, 1989). In addition, the hit quality which indicated how closely matched, the compound is with the Library data was used to further verify the identity of the compounds in the sample.

2.4 Antimicrobial Studies

2.4.1 Test microorganisms

A total of eleven (11) microorganisms were used in this study: four Gram negative bacteria (*Escherichia coli, Salmonella typhii, Pseudomonas auriginosa* and *Klebsiella pneumoniae*); four Gram positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium spp* and *Bacillus subtilis*) and three fungal strains (*Candida albicans* which is a yeast and both *Penicillium spp*. and *Aspergillus niger* which are filamentous fungi and moulds as well). These organisms were obtained from the Department of Medical Microbiology, University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria. The microorganisms were supplied as pure cultures on agar plates. The bacteria were confirmed for their identity using biochemical tests with 24hr-broth culture (Bello, 2002). The fungi were identified using the germ tube tests with or without lactophenol cotton blue stain (Cheesbrough, 2004). Standard susceptibility antibiotics discs used were ciprofloxacin (5mg/disc) and gentamicin (10mg/disc) [Poly-Test Med. Laboratories, Enugu, Nigeria] while tetracycline (2.5 x 10^5µg/disc) was prepared in the laboratory from 250mg tetracycline capsule (Me Cure Industries Ltd, Debo Industries, Oshodi, Industrial Estate, Lagos under Licene from Renaissance, Pharmaceuticals, Ltd).

2.4.2 Sterilization of materials

Pipettes and other glasswares were sterilized by dry heat in a hot air oven (Memmer, Germany) at 160ºC for 1hr while the media were sterilized in a portable autoclave at 121ºC for 15min. The discs used for the extract preparation and tetracycline were sterilized in a hot air oven at 60ºC for 30min.

2.4.3 Preparation of various concentrations and dilutions of the CPEE extract

The stock solution of the CPEE was 200mg/ml prepared by adding 2g CPEE to 10ml distilled water. This was diluted to give 100mg/ml (by adding 5ml of the 250mg/ml extracts to 5ml distilled water). 50mg/ml and 25mg/ml were also prepared from 100mg/ml and 50mg/ml respectively. The procedure was repeated but this time using ethyl acetate (analar grade) as solvent instead of distilled water.

2.4.4 Preparation of test organisms

One ml each of the 24hr pure broth culture of all the bacteria and *Candida albicans* was added to 9ml sterile sodium chloride (NaCl) solution (prepared by dissolving 4.25g NaCl analar grade, BDH Lab. Poole, England in 500ml distilled water and sterilized in a portable autoclave at 121ºC for 15 minutes. One ml of this was added to another 9ml NaCl solution and from this another one ml of the suspension was added to 9ml NaCl solution to give a final dilution of C x 10^3 organisms. (i.e. serial dilution was carried out to make a tenfold suspension) It was this that was used for the antibacterial work and that of the *Candida albicans*.

The *Aspergillus niger* and the *Penicillium spp*. were used straight from their pure cultures.

2.4.5 Preparation of discs containing graded concentrations of the CPEE and the tetracycline discs

Whatman filter paper No. 1 was punched into circular discs (each 6mm in diameter), with the aid of an office punch. The discs were then put in a glass petri dish and sterilized in a hot air oven at 60ºC for 30min. One ml of each of the different concentrations of the extract were put in sterile glass plates and thirteen (13) sterile discs were put in their using sterile forceps to soak the extract, then they were allowed to dry. The discs were checked to be sure that they were not sticking together (Lamikanra, 1999). These CPEE discs were used for the antibacterial tests and that of *Candida albicans*

One capsule tetracycline 250mg powder was dissolved in one ml distilled water in a sterile, glass Petri dish to give 250mg/ml. Thirteen sterile discs were then put inside it so as to be soaked with the tetracycline and then left to dry. This gave tetracycline discs of 250mg/ml which is equivalent to $2.5x10^5$µg/ml. This concentration of tetracycline disc was prepared because the pilot study revealed that the commercially available tetracycline disc, 50mg/ml is too low to be effective on both the bacterial and fungal species under test.

2.4.6 Preparation of culture media

The culture media used in this study were nutrient agar (Biotec Medical Market, UK) for bacteria and *Candida albicans* and sabouraud-2% glucose agar (Merck, Darmstadt, Germany) for *Penicillum spp*. and *Aspergillus niger*.

The nutrient agar was prepared according to the manufacturer's specifications (by dissolving 18.5g powder in 500ml distilled water) and sterilized at 121ºC for 15min. After autoclaving, the pH was 7.2-7.4 (Bello, 2002). This was poured into 90mm diameter sterile, disposable plastic petri dishes to a depth of 4mm (about 25ml per plate). Care was taken to pour the plates on a level surface so that the depth of the medium would be uniform. The plates were dried upside down in an incubator at 37ºC with their lids opened and inverted so that water would not condense back into the agar.

The sabouraud-2%-glucose agar was prepared according to the manufacturer's specification (by dissolving 18.8g in 400ml distilled water) and sterilizing at 121ºC for 15min. 1ml each of the different concentrations of the CPEE (25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml) was pipette into eight (8) sterile, disposable Petri dishes i.e. 2 plates for each CPEE concentration 25ml of the sabouraud-2%-dextrose agar was poured into the plate, swirled round to mix very well with the CPEE then allowed to set at low temperature. Two other plates were also prepared, but without the CPEE, to act and the control. All the (10) plates were then incubated upside down, with their lids opened at 37ºC in an incubator to dry.

2.4.7 Disc diffusion antibacterial selectivity test and disc diffusion selectively test for *Candida albicans, Penicillium spp* and *Aspergillus niger*

One ml each of the C x 10^3 test organisms (bacteria and *Candida albicans*) was pipetted into the solidified nutrient agar plates and the excess was removed after allowing it to go round the surface of the medium. The antibiotic discs, gentamicin (10µg/disc), ciprofloxacin (5µg/disc) and tetracycline ($2.5x10^5$µg/disc) were placed on the plate that had been uniformly inoculated with the test organism using sterile forceps. The disc of blotting paper that had been previously impregnated with graded concentrations of the CPEE was then placed on each of the plates. The plates were incubated at 37ºC for 24hrs for bacteria and 1-

5 days for *Candida* and examined for antimicrobial diffusion from the discs into the medium to see if the growth of the test organism will be inhibited at a distance from the disc that is related to the sensitivity of the organism (Cheesbrough, 2004).

The antibiotic discs: ciprofloxacin (5µg/disc) gentamicin (10µg/disc) and tetracycline (2.5 x 10^5 µg/disc) were placed on the already prepared sabouraud-2% dextrose agar containing graded concentrations of the CPEE (8 in all) and the control (2 plates). The *Penicillium spp* and the the *Aspergillus niger* were then removed from their pure cultures with a pair of sterile forceps and placed on the plates so that the organisms could spread on the antibiotic discs and the extract in the plates. The plates were incubated at 25ºC-30ºC and examined every 2-3 days and kept for four weeks before being considered negative for the fungi (Bello, 2002).

3. RESULTS

3.1 GC-MS of the CPEE

The results of the GC-MS of the CPEE of *Solanum macrocarpum* fruit are shown in Figs. 1, 2, 3, 4, 5 and Tables 1, 2, 3, 4, 5. Fig 1 shows the chromatogram of the CPEE i.e. SSCR. Eleven (11) clear peaks (levels) were marked out for analysis by the MS. Other peaks were still mixed up or could not be identified from Library data. Table 1 gives a summary of identified peaks, the corresponding compounds, approximate composition in the mixture and the hit quality.

Fig. 2 shows the chromatogram of fraction A of the CPEE i.e. SSAP. Four (4) clear peaks (levels) were marked out for analysis by the MS. Other peaks were still mixed up or could not be identified from Library data. Table 2 gives a summary of identified peaks, the corresponding compounds, approximate composition in the mixture and the hit quality.

Fig. 3 shows the chromatogram of fraction B of the CPEE, i.e. SSBP. Nineteen (19) clear peaks (levels) were marked out for analysis by the MS. Other peaks were still mixed up or could not be identified from libarary data. Table 3 gives a summary of identified peaks, the corresponding compounds, approximate composition in the mixture and the hit quantity.

Fig. 4 shows the chromatogram of fraction C of the CPEE, i.e., SSCP. Twenty one (21) clear peaks (levels) were marked out for analysis by the MS. Other peaks were still mixed up or could not be identified from Library data. Table 4 gives a summary of identified peaks, the corresponding compounds, approximate composition in the mixture and the hit quality.

Fig. 5 shows the chromatogram of fraction E of the CPEE, i.e. SSEP. Thirteen (13) clear peaks (levels) were marked out for analysis by the MS. Other peaks were still mixed up or could not be identified from Library data. Table 5 gives a summary of identified peaks, the corresponding compounds, approximate composition in the mixture and the hit quality.

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GCMS ANALYSIS
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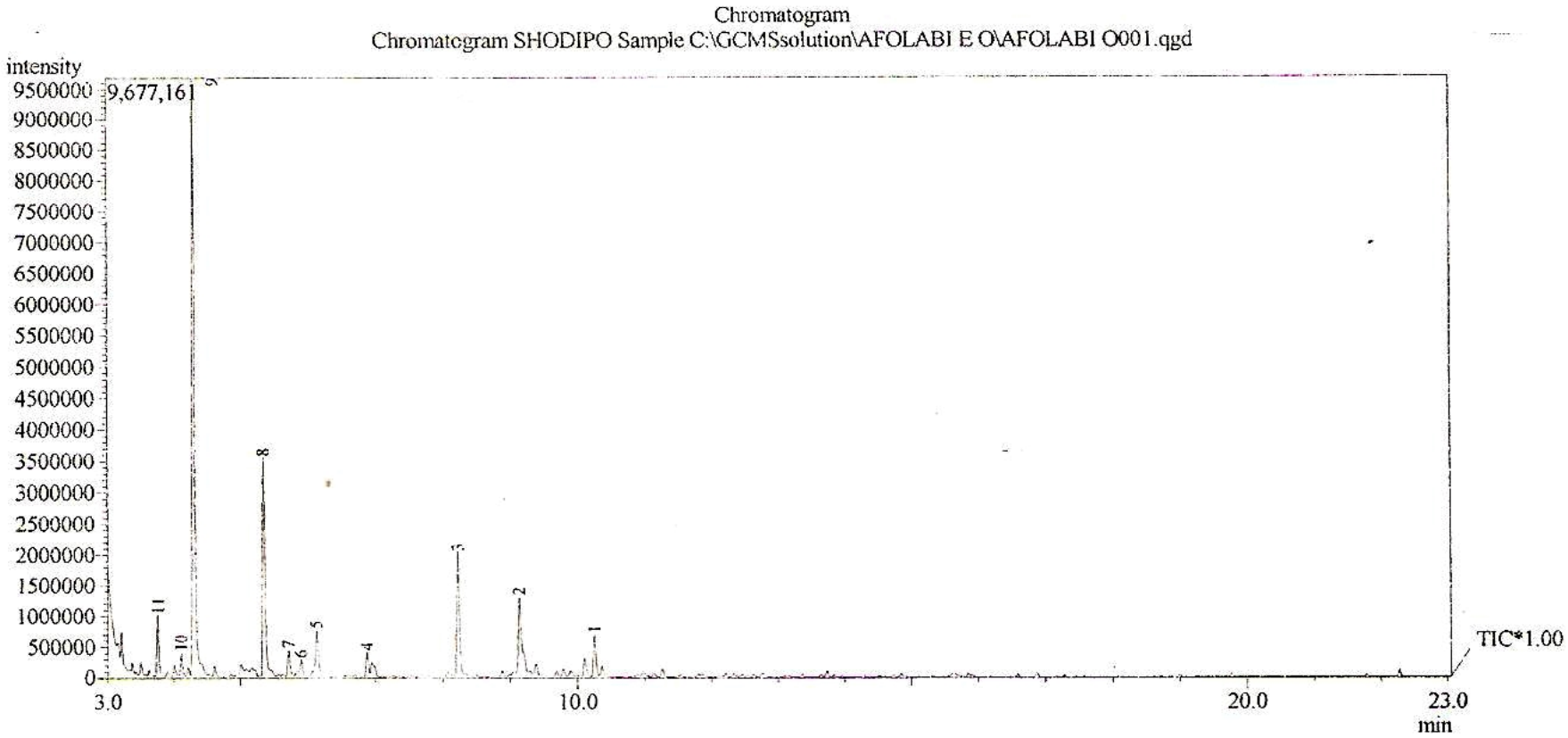

Fig. 1. Gas chromatogram of the crude petroleum ether extract (CPEE), SSCR of the fruit of *Solanum macrocarpum*

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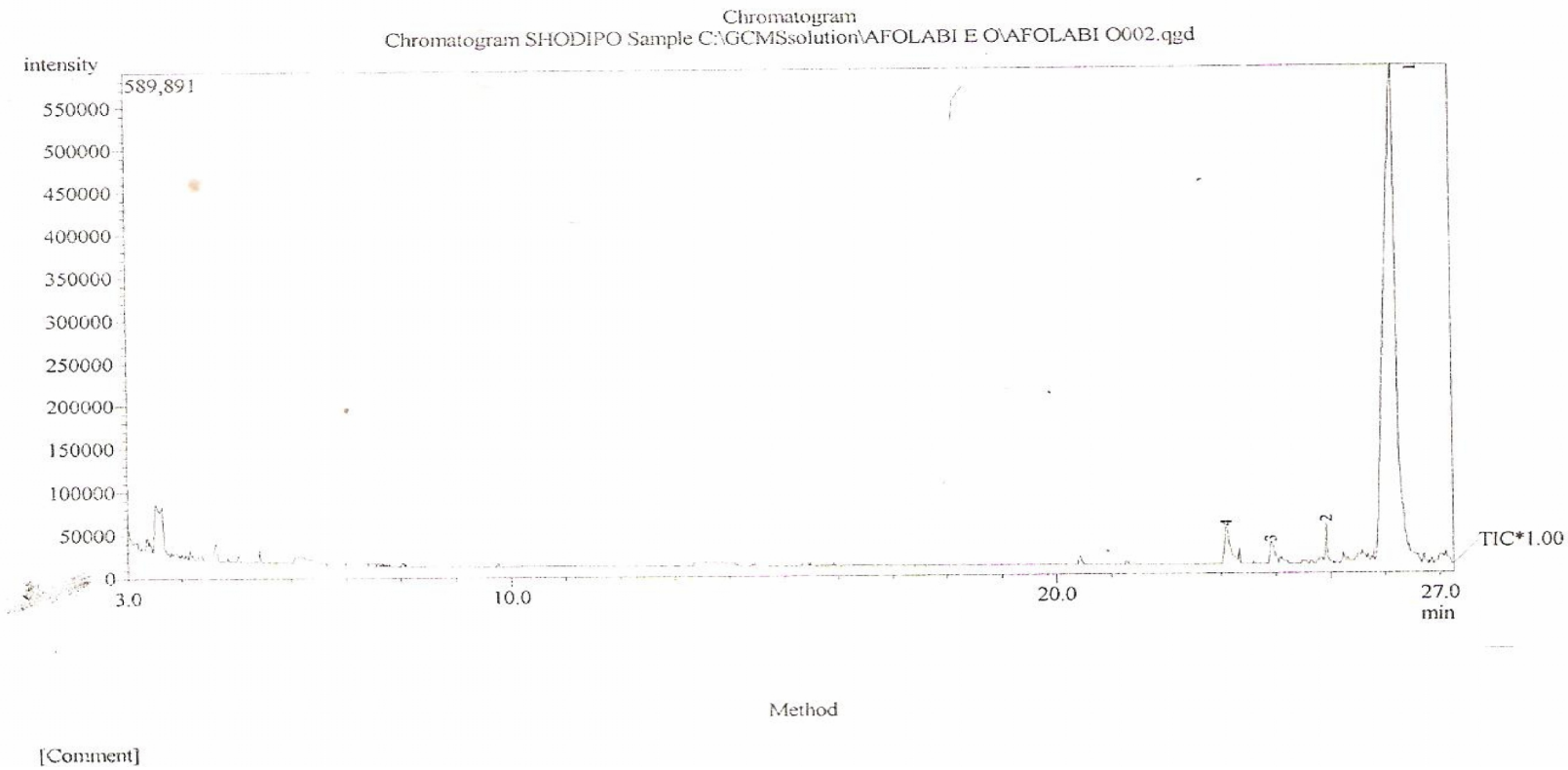

Fig. 2. Gas chromatogram of the A fraction (by AGC) of the petroleum ether extract (SSAP) of the fruit of *Solanum macrocarpum*

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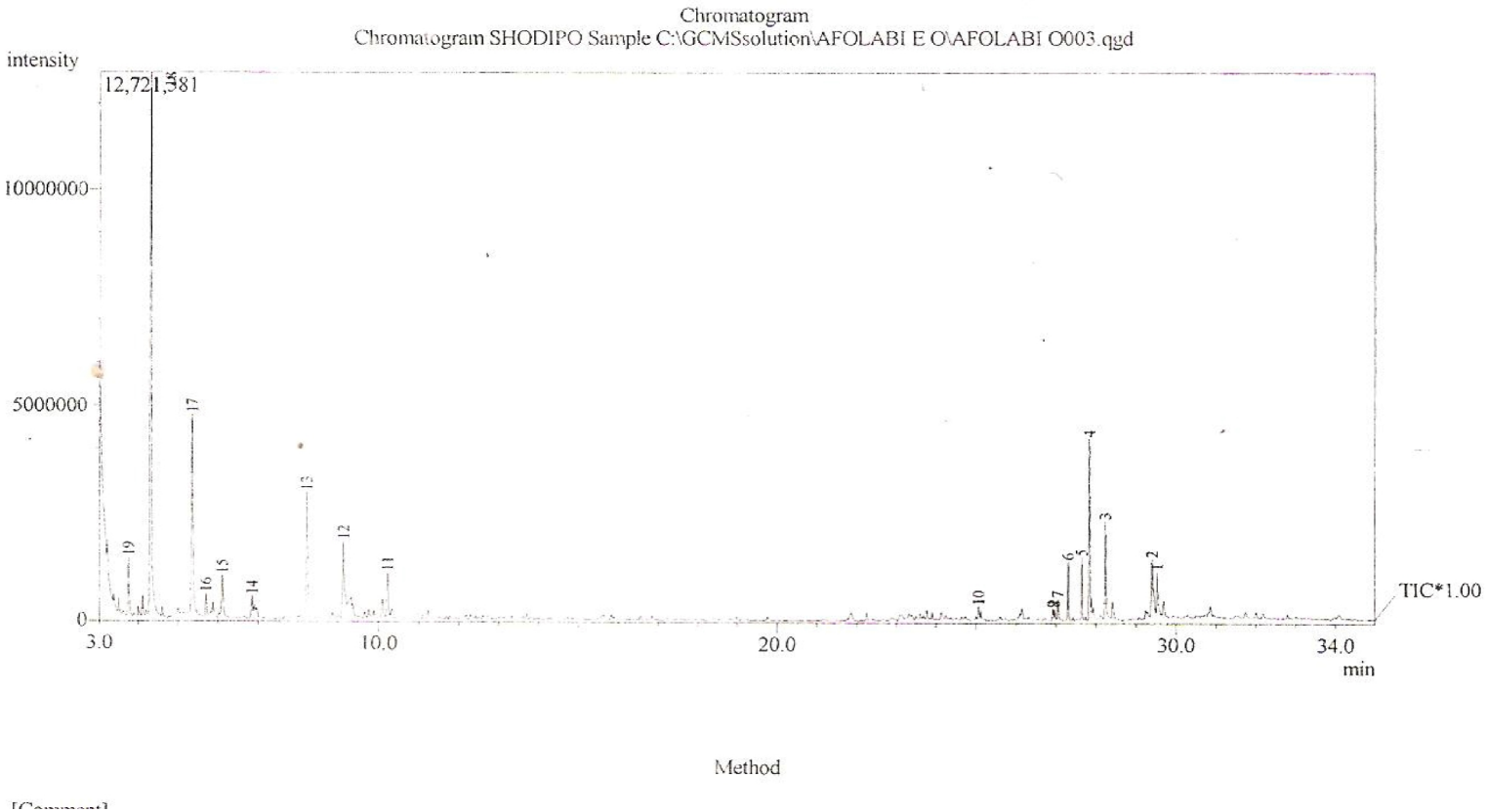

Fig. 3. Gas chromatogram of the B fraction (by AGC) of the petroleum ether extract (SSBP) of the fruit of *Solanum macrocarpum*

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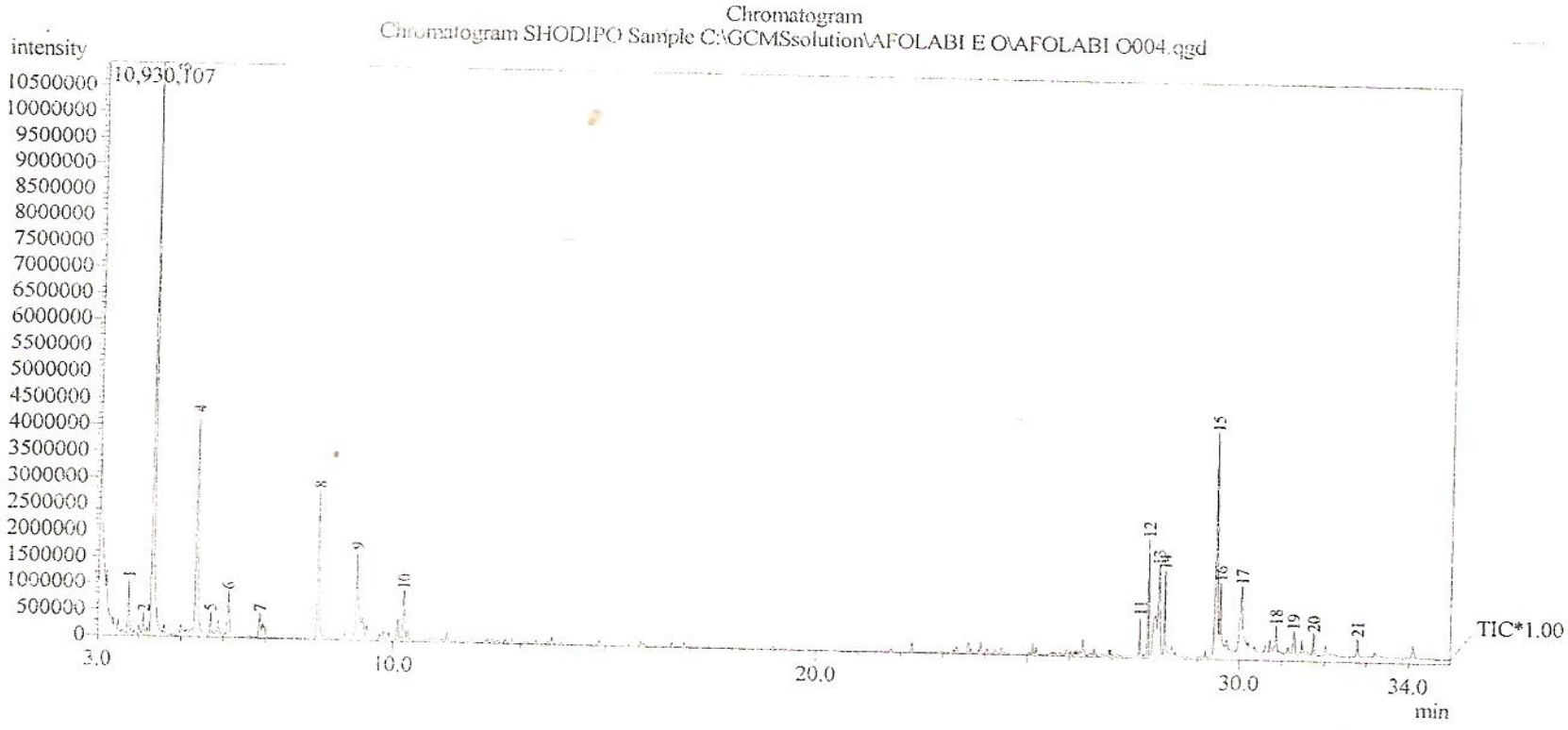

Fig 4. Gas Chromatogram of the C fraction (by AGC) of the petroleum ether extract (SSCP) of the fruit of *Solanum macrocarpum*

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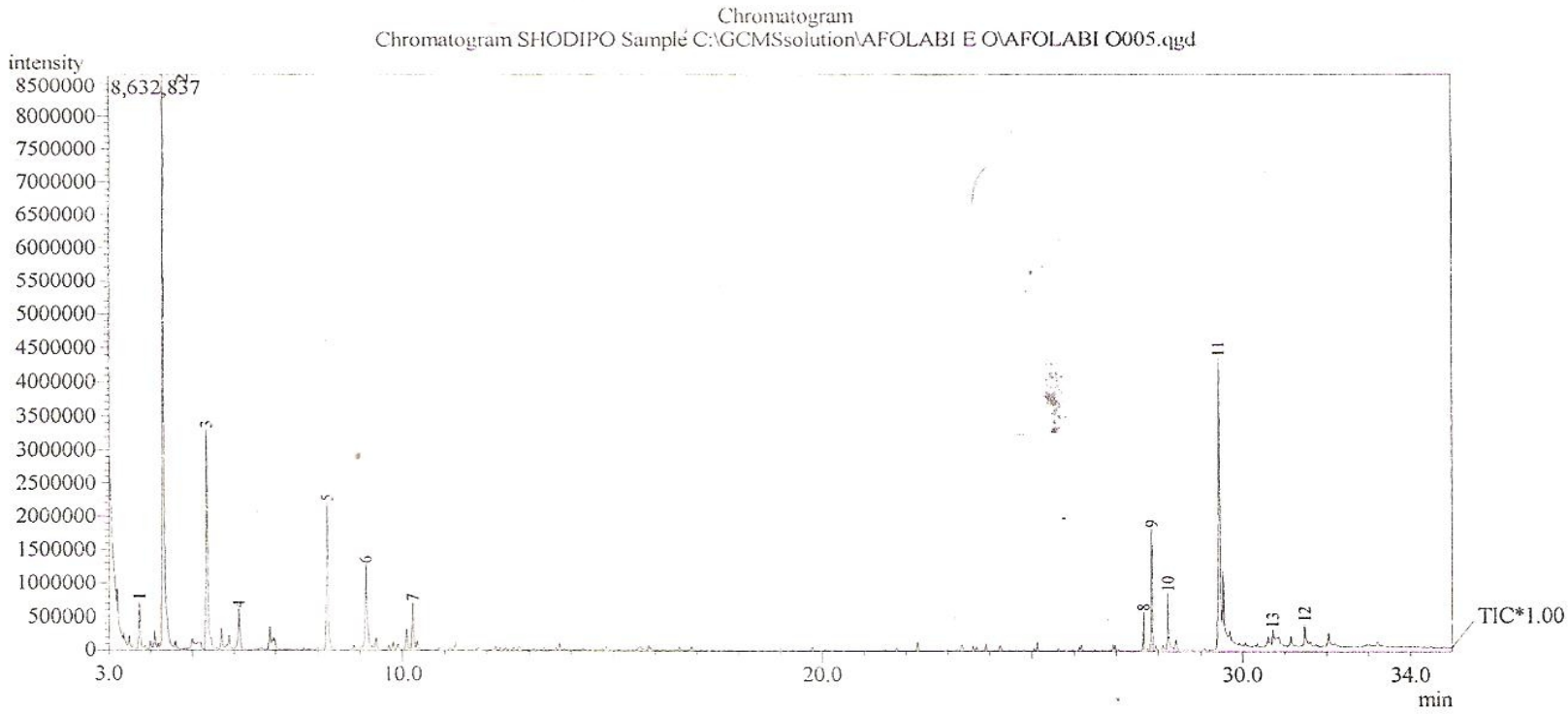

Fig. 5. Gas chromatogram of the E fraction (by AGC) of the petroleum ether extract (SSEP) of the fruit of *Solanum macrocarpum*

Table 1. GC-MS Data on the crude petroleum ether extract, SSCR

Peak #	Retention time t_R (min)	Compound	Hit quality (%)	% composition
1	10.3	1, 2, 3-trimethylbenzene	86	0.54
2	9.1	Hexylene glycol	76	0.86
3	8.2	Butanoic acid-3-hydroxy-ethyl ester	97	1.62
4	6.9	0-Dimethyl benzene (xylene)	95	0.43
5	6.1	0-Dimethylbenzene (xylene)	97	0.65
6	5.9	Ethylbenzene	95	0.22
7	5.7	Guanidine	92	0.32
8	5.4	Acetone dimethylcarbinol	92	2.71
9	4.3	Furan (2-methoxy furan)	95	7.23
10	4.1	2, 4-dimethyl-1, 3-dioxane	96	0.27
11	3.8	Toluene (methylbenzene)	83	0.76
		Unidentified		9.95
Total			9.68	100

Table 2. GC-MS data on the A fraction (by AGC), SSAP of the petroleum ether extract

Peak #	Retention time t_R (min)	Compound	Hit quality (%)	Composition %
1	26.2	4-0-Methyl mannose	81	7.18
2	24.9	1.4-cyclohexadiene	69	0.73
3	23.9	Butanoic acid	77	0.52
4	23.1	2-hydroxy-γ-butyrolactone	78	0.84
		Unidentified		0.84
Total			9.26	100

Table 3. GC-MS data on the B fraction (by AGC), SSBP of the petroleum ether

Peak #	Retention time t_R (min)	Compound	Hit quality (%)	% composition
1	29.5	Octadecanoic acid (palmitic acid)	3.49	84
2	29.4	1, 11, 13-octadecatriene	3.93	85
3	28.2	n-hexadecanoic acid (palmitic acid)	6.11	86
4	27.8	2-ethoxyethyl butyrate	10.49	77
5	27.6	1-(isopropylsulfonyl) butane	3.06	67
6	27.3	2-ethyoxyethylbutyrate	3.28	78
7	27.1	1-(isoprophyl sulfonyl) butane	1.31	70
8	27.0	2, 6, 10,15,-tetramethylheptadecane	0.87	91
9	26.9	9-eicosene	0.43	91
10	25.1	Pentadecane	2.62	92
11	10.2	1,2,4-trimethylbenzene	4.37	87
12	9.1	1,3-dioxane	6.99	75

Table 3 continues…….

13	8.2	Butanoic acid	1.31	97
14	6.9	Xylene (1-2-dimethylbenzene)	2.62	95
15	6.1	1,4-xylene	1.31	96
16	5.7	Guanidine	11.79	91
17	5.4	Acetonyldimethylcarbinol	30.13	92
18	4.3	Isobutenymethylketone	3.06	95
19	3.7	Toluene (methylbenzene)	2.40	81
		Unidentified	2.40	
Total			100	94.94

Table 4. GC-MS data on the C fraction (by AGC), SSCP of the petroleum ether extract

Peak #	Retention time t_R (min)	Compound	Hit quality (%)	% Composition
1	3.7	Toluene (methylbenzene)	82	1.92
2	4.1	2,4-dimethyl-1,3-dioxane	95	0.82
3	4.3	Isobutenyl methyl ketone	94	18.63
4	5.4	Acetonyldimethyl carbinol	92	7.13
5	5.7	Guanidine	91	0.82
6	6.1	Xylene	97	1.64
7	6.9	1, 4-Xylene	95	1.08
8	8.2	Butanoic acid	97	4.93
9	9.1	5-ethyl, 2-2-dimethyl-1,3-dioxane	73	3.01
10	10.2	1,2,3-trimethyl benzene	86	1.64
11	27.7	1-(isopylsufonyl) butane	71	1.37
12	27.8	2-ethoxylethyl butyrate	78	5.20
13	28.0	Ethyl stearate (Ethyl octadecanoate)	82	3.01
14	28.2	n-hexadecanoic acid (palmitic acid)	85	2.74
15	29.4	2-hydroxycyclopentadecone	88	7.67
16	29.5	Octadecanoic acid (stearic acid)	83	2.74
17	30.0	2, 3-dihydroxypropyl-9 octadecanoate	74	2.47
18	30.9	Dodecanoic acid, 2,3,-bis(acetyloxy)propylester	58	1.37
19	21.3	Ethyl docosanoate (bethyl behenate)	85	1.08
20	31.7	Benzoic acid,-2-amino-3-hydroxy,-tris (trimethylsilily) derivative	67	0.82
21	32.8	Benzoic acid, 4-amino-2-hydroxy-,tris (trimethylsilily) derivative	62	0.82
		Unidentified		0.81
Total			59.41	100

Table 5. GC-MS data on the E fraction (by AGC), SSEP, of the petroleum ether extract

Peak #	Retention time t_R (min)	Compound	Hit quality (%)	% Composition
1	3.7	Toluene (methyl benzene)	81	0.31
2	4.3	Isobutenyl methylketone	94	3.47
3	5.3	Acetonyldimethyl carbinol	92	1.38
4	6.1	Xylene (1, 2-dimethyl benzene)	96	0.26
5	8.2	Ethyl-β-hydroxylbutyrate	96	0.92
6	9.1	3-hydroxylsovaleric acid	76	0.56
7	10.2	1,2,3-trimethylbenzene	86	0.31
8	27.7	1-(isoprophylsulfonyl) butane	71	0.25
9	27.8	2-ethyoxyethyl butyrate	79	0.77
10	28.2	Octadecanoic acid (stearic acid)	85	0.36
11	29.4	2-hydroxycyclopentadecanone	88	1.84
12	31.5	7-tetradecenal	84	0.15
13	30.7	n-eicosanoic acid (arachidonic acid)	81	0.10
		Unidentified		0.35
Total			10.73	100

3.2 Disc Diffusion Antimicrobial Selectivity Test

All the bacteria (Gram + and Gram -) and the *Candida albicans* were not sensitive to the effect of the CPEE under the condition of the experiment as the bacteria and the *Candida albicans* grew up to the edge of the discs. *Penicillium spp*. and *Aspergillus niger* were not inhibited in their growth (Table 6). It was only the antibiotic discs that the organisms were sensitive to.

Chemical structures of identified compounds have been presented in the Supplementary materials.

4. DISCUSSION

The identification of eleven (11) possible different organic compounds from the CPEE (i.e. SSCR), four (4) from the SSAP (fraction A), nineteen (19) from SSBP (fraction B), twenty one (21) from the SSCP (fraction C) and thirteen (13) from the SSEP (fraction E) with the GC-MS confirms that complex mixtures can be identified in detail with little or no prior chemical information about the sample (Ayim et al., 2000).

The detection and identification of 1-(isopropylsulfonyl)-butane confirms the presence of sulphur earlier reported in the elemental analysis of the fruit (Sodipo et al., 2008b; Sodipo et al., 2010).

Important aromatic compounds identified include toluene (methylbenzene) and xylene (dimethylbenzene). From toluene, through sulphonation, butylated hydroxytoluene, a standard antioxidant, saccharin (an artificial sweetener) and chloramine (an antiseptic), can be synthesized (Olaniyi et al., 1998). Antioxidants prevent peroxidation of lipids (Olaniyi et al., 1998; Khan, 2008). Xylene and toluene are also used as solvents and industrial chemicals (Olaniyi et al., 1998).

Table 6. *In-vitro* antimicrobial activity of CPEE of the fruit of *S. macrocarpum*

S/N	Microorganism	Diameter of zones of inhibition (mm)							
		Concentration of CPEE (mg/ml)					Antibiotic discs (µg/disc)		
		250	200	150	100	50	Ciprofloxacin 5	Gentamicin 10	Tetracycline $2.5x10^5$
1	*Staphylococcus aureus* (+)	R	R	R	R	R	28	16	20
2	*Streptococcus pyogenes* (+)	R	R	R	R	R	30	17	22
3	*Corynebacteria spp.* (+)	R	R	R	R	R	32	17	24
4	*Bacillus subtilis* (+)	R	R	R	R	R	29	18	24
5	*Escherichia coli* (-)	R	R	R	R	R	35	15	28
6	*Salmonella typhii* (-)	R	R	R	R	R	32	14	29
7	*Pseudomonas aeriginosa* (-)	R	R	R	R	R	26	18	20
8	*Klebsiella pseumoniae* (-)	R	R	R	R	R	27	13	26
9	*Candida albicans* (Y)	R	R	R	R	R	24	14	22
10	*Aspergillus niger* (FF)	R	R	R	R	R	R	R	R
11	*Penicillium spp* (FF)	R	R	R	R	R	R	R	R

Key: CPEE = Crude petroleum ether extract ; Y = Yeast
R = Resistance (i.e. not sensitive); FF = Filamentus fungus
+ = Gram +; - = Gram –

The identification of benzoic acid-2-amino-3-hydroxy-tris(trimethylsilily) derivative and benzoic acid-4-amino-2-hydroxy-tris(methylsililyl) derivative confirm the presence of a silicone polymer, which may probably be used like simethicone (or dimethicone), an antifoaming agent that is used in ointments and creams for application to the skin as a water-repellant and protective against contact irritation (Olaniyi et al., 1998).

5-ethyl, 2-2-dimethyl-1,3-dioxane and 2,4-dimethyl-1,3-dioxane identified may have useful pharmaceutical applications as dioxanes and their sulphur analogoues are widely used as industrial solvents.

4-0-methyl mannose, a 6-membered sugar identified confirms the presence of sugar and carbohydrate earlier detected in the phytochemistry of the plant (Sodipo et al., 2008b). All sugars are hexoses. In the free state, glucose exists entirely as the pyranose form (6-membered) and many of its derivatives retain this ring structure (Olaniyi et al., 1998). Thus, it is not surprising that the 4-0-methyl mannose existed in the pyranose form.

Identification of 2-methoxy furan is also significant. Furan, a five-membered heterocyclic, is a volatile, fairly stable compound which arises from the decomposition of sugars (Olaniyi et al., 1998). Thus, the 2-methoxy furan could have been produced from the decomposition of the sugar, 4-0-methyl mannose. A naturally occurring dihydrofuran derivative is ascorbic acid (vitamin C) [Olaniyi et al., 1998], a powerful antioxidant used for stability of pharmaceutical products and also in treating vitamin C deficiency. It may be possible to exploit the possibility of producing antioxidants on a commercial basis from this plant, using either 2-methoxy furan or 4-0-methyl mannose, as antioxidants according to Khan (2008) may prevent peroxidation of lipids, thus lowering hyperlipidaemia. Also, since furans can be obtained from sugars, and furan derivatives may act as antioxidants, then the plant itself may probably lower hyperlipidaemia on its own due to the presence of the sugar 4-0-methyl mannose, thus buttressing the use of the plant as an antihyperlipidaemic agent as earlier reported by Sodipo et al., (2010).

Hexylene glycol and acetonyldimethyl carbinol are some of the alcohols identified. Alcohols are used as industrial and pharmaceutical solvents, reaction media and chemical intermediate. Glycols (dihydric alcohols) like ethylene glycol, are used as an antifreeze and for making polyester and terylene (Olaniyi et al., 1998). It is probable that hexylene glycol identified may possibly find use in future for the above mentioned purposes.

2, 3-dihydroxypropyl-9-octadecanoate (an ester of a hydroxyl acid) and 3-hydroxy isovaleric acid (a hydroxyl acid) were identified. Hydroxyl acid esters can be hydrolyzed to produce hydroxy acids, which decompose on heating with dilute acids. γ-hydroxy acids yield γ-lactones, with a 5-membered ring (Olaniyi et al., 1998). A γ-lactone identified is 2-hydroxy-butyro-lactone (a compound with two functional groups). It is thus possible that the 2-hydroxy- γ-butyro-lactone could have been produced from the earlier mentioned hydroxy acids and their esters. A major function of hydroxyacids and their esters is that of sequestering agent's e.g. tartaric acid and citric acid (Olaniyi et al., 1998). Probably the hydroxy acids identified in the plant may act like saponins, sequestering bile acids, preventing absorption of cholesterol, thus lowering hypercholesterolaemia (MacDonald et al., 2005). This confirms the hypolipidaemic effect of the plant earlier mentioned (Sodipo et al., 2010).

A short chain fatty acid, butanoic acid was also identified. Short chain fatty acids (acetic acid, propionic acid and butyric acid) as found in oat, (*Avenia sativa*) may modify cholesterol

synthesis and also influence the production of glucose and its utilization by peripheral tissues (i.e. it is beneficial to those with diabetes mellitus) (Welch, 1995; Bridges et al., 1992), reduce the risk of coronary heart disease, tumor incidence, cancer, lower blood pressure and reduce the rate of cholesterol and fat absorption (Chaturvedi et al., 2011). Thus, the butanoic acid identified may be responsible for some of the documented attributes of the plant i.e. that of lipid lowering activity (Sodipo et al., 2009 a, b) and lowering of blood pressure (Sodipo et al., 2008a).

The long chain saturated fatty acids identified include palmitic acid (hexadecanoic acid), stearic acid (octadecanoic acid) and arachidonic acid (eicosanoic acid). Flaxseed (*Linum usitatissimum*) contains α-linolenic acid (unsaturated 18-carbon acid) which is converted to long chain omega-3 fatty acids (Chaturvedi et al., 2011) in the nutraceutical and functional food area. α-Linolenic acid is a precursor of Omega-3-fatty acids such as eicosapentaenoic acid (unsaturated 20-carbon acid), which have been associated with improved cardiovascular outcomes (Oomen et al., 2001; Chaturvedi et al., 2011) and a lipid lowering effect (Chaturvedi et al., 2011). Probably the identified long chain saturated fatty acids may be reduced to their unsaturated forms to produce the drug, Omega-3-marine triglyceride (Maxepa), which is used in lowering hyperlipidaemia (Graham Smith and Aranson, 2002; Katzung, 2004; Sodipo, 2009). Esters of long chain fatty acids were also identified: ethylstearate, ethyl docosanoate and dodecanoic acid-2-3bis (acetoxyl) propyl ester. They can be hydrolyzed to their respective corresponding acids: stearic acid (18 C atoms), docosanoic acid (22 C atoms) and dodecanoic acid (12 C atoms). These acids in turn may then be used for the production of Omega-3-marine fatty acid.

The bacteria and fungi were not sensitive to the CPEE even though many bioactive compounds were identified. These compounds might probably have been synthesized by the plant for different defensive duties. Further biological testing for such compounds against different test systems could lead to solutions to some relevant health problems.

5. CONCLUSION

The petroleum ether extract of the fruit of *Solanum macrocarpum* does not have antibacterial and antifungal activities. The detection of 2-methoxy furan, 4-0-methyl-mannose, 2-hydroxy-γ-butyrolactone, 2, 3-dihydroxypropyl 9-octadecanoate, 3-hydroxy isovaleric acid and butanoic acid validates its antihyperlipidaemic property. Consumption of plant - based foods like “Gorongo”, is recommended as a nutraceutical.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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