http://dx.doi.org/10.4314/jpb.v7i2.17
Vol. 7 no. 2, pp. 164-172 (September 2010)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Gas Chromatography-Mass Spectrometry (GC-MS) analysis and antimicrobial investigation of the ethyl acetate extract of "*Gorongo*" - *Solanum macrocarpum* L.

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Received 9th August 2010; Accepted 7th September 2010

Abstract
Solanum macrocarpum Linn. (Solanaceae) is used in East and West African Ethnomedicine for treating constipation, cardiac diseases and hyperlipidaemia. The aqueous extract of the fruit had been shown to lower high blood pressure, relieve constipation and lower hyperlipidaemia. The plant was therefore investigated for its chemical constituents and antimicrobial properties. The crude ethyl acetate extract (CEAE) of the unripe fruit was therefore analysed using gas chromatography-mass spectrometry (GC-MS). The microorganisms used included Gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Cornybacterium spp*. and *Bacillus subtilis*); Gram-negative bacteria (*Echerichia coli*, *Salmonella tyhii*, *Pseudomonas aeruginosa* and *Kleibsiella pneumonia*); and three fungal strains (*Candida albicans*, *Penicillium* spp and *Aspergillus niger*). The antimicrobial effect was assayed using the disc diffusion antimicrobial selectivity test. All the microorganisms used were resistant to CEAE. γ-Sitosterol, a tetracylic triterpenoid with a steroidal nucleus, is believed to be the main chemical compound responsible for the hypolipidaemic effect ascribed to the fruit of the plant. Other compounds of CEAE, identified through GC-MS were ethyl palmitate, ethyl stearate, 4-(1-methyl-1-[4-(proprioxy) phenyl] ethyl phenyl propionate, 1-nonadecene, 9-eicosene, glycerol acetate, glycerol diacetate, 5-(1-methyl-1(-imidazol)-2-γ-sulponyl-1-phenyl tetrazole and 1-heptadecene.

Keywords: Solanum macrocarpum; GC-MS; Antimicrobial activity; Ethyl acetate extract

Introduction

Plants have played very important roles all over the world since creation. They are used as medicines, food, shelter, clothing, cosmetics, flavours and spices (Gamaniel, 2000; Cordell, 2006, Tor-Anyiin *et al.*, 2006). The study of natural products is therefore an important area of scientific activity. In a broad sense, the term natural product means 'products of nature' and includes raw or processed natural materials derived from plant, animal, microbial or even mineral resources. Thus, the crude plant material, its extracts, distilled or pressed oils and purified

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ISSN 0189-8442

single substances or secondary metabolites are all natural products. Not included are products that are obtained by synthesis or manufactured by use of chemical process (Dagne, 2008). About 80% of the people in Africa rely on herbal medicine for their primary health care according to the estimate of the World Health Organisation (Bannerman *et al.,* 1983).

The genus *Solanum* is well known in traditional medicine (Burkill, 2000; Grubben and Denton, 2004; Sodipo, 2009). *Solanum* species are about 1,500 in the world (Grubben and Denton; ANNON, 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* 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 necessitated the need to separate, purify, isolate, identify and also investigate the antimicrobial activity of the crude ethyl acetate extract (CEAE) of the fruit using gas chromatography-mass spectrometry (GC-MS) as little work has been done on the CEAE.

The GC-MS is a hyphenated technique. It combines chromatography with spectrometry. This need arose because the identification capability of chromatography alone is generally insufficient for the identification and quantification of every component of a complex mixture, especially for direct assay of pharmaceutical product (Ayim *et al*., 2000). When applied to a complex sample without a separation step, MS spectrometer will not lead to the identification or quantification of even one component. Only when the separation power of chromatographic techniques is combined with the identification power of the sensitive physiochemical-character determining techniques of MS, can its full power as a tool for analysis of mixtures be realised. This hyphenated technique, GC-MS has the important characteristic in that identification is accomplished with unique spectral information rather than the retention time data and does not have the limitations inherent in chromatography or spectrometry, applied alone. Therefore, complex mixtures often can be analysed in detail with little or no prior chemical information about the sample. It can provide confirmation of structure, identify unknown drugs and their metabolites in body fluids and tissues and be of great value in the analysis of therapeutic agents. It is capable of identifying as little as 10^{-10}g (0.1ng) of material and when operated in the selected-ion detector (SID) mode, can detect and provide positive identification of as little as 10^{-12}g(1pg) of material (Ayim *et al.,* 2000).

Experimental

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. Voucher Specimen No. 548 was deposited at the Herbarium of the Department of Biological Sciences.

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 Soxhlet extraction in petroleum ether (60-80^{o}C), ethyl acetate, and ethanol (95%) to give the petroleum ether extract (CPPE), ethyl acetate extract (CEAE), and ethanol extract (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 desiccator until when required.

Gas chromatography-mass spectrometry (GC-MS) of the CEAE. A Shimadzu QP-2010 Plus GC-MS was 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 60^{o}C to 325^{o}C. The column temperature was programmed between 60^{o}C and 250^{o}C at a rate of 3.0ml/min. The temperature of the injector and detector were at 250^{o}C and 200^{o}C 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 indicates how closely matched, the compound is with the Library data) was used to further verify the identity of the compounds in the sample.

Antimicrobial Studies

Test microorganisms. A total of eleven (11) microorganisms were used in this study: Four Gram negative bacteria (*Escherichia coli*, *Salmonella typhii*, *Pseudomonas aurugenosa* 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 which are also moulds). These organisms are clinical isolates 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 24 h 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^{5}mg/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).

Sterilization of materials. Pipettes were sterilized by dry heat in a hot air oven (Memmer, Germany) at 160^{o}C for 1h while the media and the discs used for extract preparation and tetracycline and other glassware were sterilized in a portable autoclave at 121^{o}C for 15min.

Preparation of various concentrations and dilutions of the CEAE extract. The stock solution of the CEAE was 200mg/ml prepared by adding 2g CEAE 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.

Preparation of test organisms. 1ml 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 121^{o}C for 15 minutes. 1ml of this was added to another 9ml NaCl solution and from this another 1ml of the suspension was added to 9ml NaCl solution to give a final dilution of C x 10^3 organisms. 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.

Preparation of discs containing graded concentrations of the CEAE 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 160 ^{o}C for 1hr. 1ml 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 CEAE discs were used for the antibacterial tests and that of *Candida albicans*

One capsule tetracycline 250mg powder was dissolved in 1ml 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 2.5 x 10^5µg/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.

*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 speficiations (by dissolving 18.5g powder in 500ml distilled water) and sterilized at 121^{o}C 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 37 ^{o}C 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 121 ^{o}C for 15min. 1ml each of the different concentrations of the CEAE (25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml) was pipetted into eight (8) sterile, disposable Petri dishes i.e. 2 plates for each CEAE concentration 25ml of the sabouraud-2%-dextrose agar was poured into the plate, swirled round to mix very well with the CEAE then allowed to set at low temperature.

Two other plates were also prepared, but without the CEAE, to act and the control. All the (10) plates were then incubated upside down, with their lids opened at 37^{o}C in an incubator to dry.

Disc diffusion antibacterial selectivity test. 1ml each of the C x 10^3 test organisms (bacteria and *Candida albicans*) was pipetted into the solified nutrient agar plates and the excess was removed after allowing it to go round the surface of the medium. The antibiotic discs, gentamicin (10 µg/disc), ciprofloxacin (5 µg/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 CEAE was then placed on each of the plates. The plates were incubated at 37^{o}C 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 (Cheesebrough, 2004).

Disc diffusion antifungal selectivity test. The antibiotics discs: ciprofloxacin (5µg/disc) gentamicin (10µg/dics) and tetracycline (2.5 x 10^5 µg/disc) were placed on the already prepared sabouraud-2% dextrose agar containing graded concentrations of the CEAE (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 25^{o}C-30 ^{o}C and examined every 2-3 days and kept for four weeks before being considered negative for the fungi (Bello, 2002).

Results

GC-MS of the CEAE. The results of the GC-MS of the CEAE of *Solanum macrocarpum* fruit are shown in Fig. 1 and Table 1. Fig 1 shows the chromatogram of the CEAE. Nine (9) clear peaks 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 compound, approximate composition in the mixture and the hit quality.

Disc diffusion antimicrobial selectivity test. All the bacteria (Gram +ve and Gram -ve) and the *Candida albicans* were not sensitive to the effect of the CEAE under the condition of the experiment as the bacteria and the *Candida* grew up to the edge of the discs. Also, *Penicillium spp*. and *Aspergillus niger* were not inhibited in their growth.

Discussion

The chromatogram of the CEAE showed nine (9) clear peaks which were marked out for MS analysis, other peaks were mixed up and as such could not be separated further. The identification of nine (9) possible different organic compounds from the CEAE with the GC-MS confirms that complex mixtures can be analyzed in detail with little or no prior chemical information about the sample (Ayim *et al.,* 2000).

The γ -sitosterol identified is a lipid (phytosterol) which has been shown by Malini and Vanithakoumar (1990); Lange *et al.,* (2007) to lower hyperlipidaemia in rats administered with it subcutaneously for 60 days. Sitosterol is a tetracyclic triterpene (30 carbon atoms) [Olaniyi *et al.,* 1998] confirming the reported folkloric hypolipidaemic effect of the fruit. Thus the CEAC may probably also be used in lowering hyperlipidaemia. The detection of sitosterol confirms the presence of the steroidal/triterpenoidal nucleus earlier detected in the initial phytochemical

screening of the fruit of the CEAE of *S. macrocarpum* (Sodipo *et al.*, 2008b).

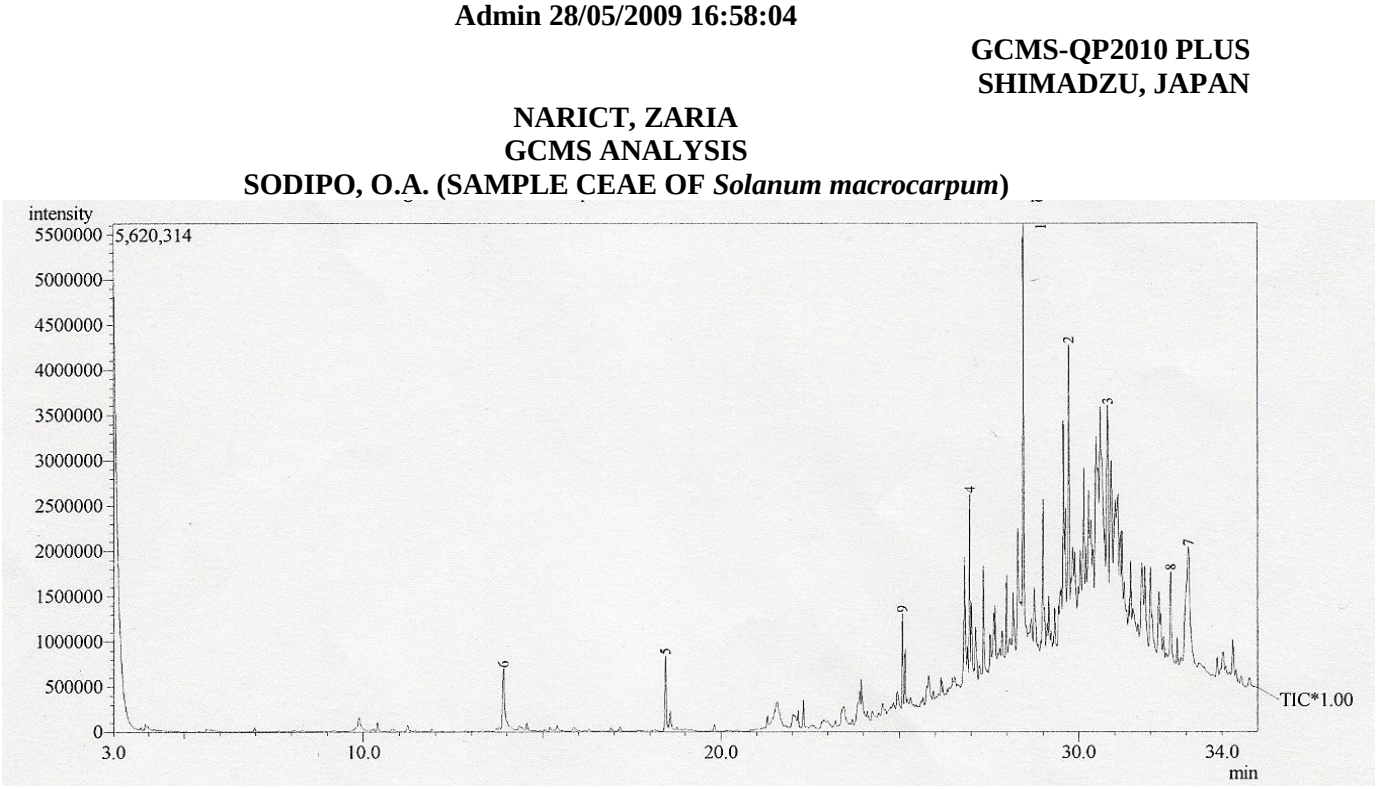
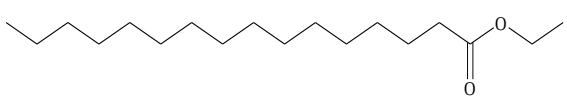


Fig. 1: Gas chromatogram of the crude ethyl acetate extract (CEAE) of the fruit of *Solanum macrocarpum*

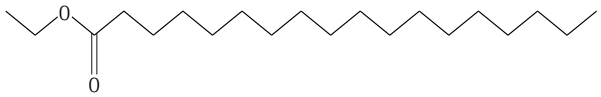
Table 1: GC-MS data on the ethyl acetate extract (CEAE)

Peak #	Retention time t_R (min)	Compound	Hit quality (%)	% Composition
1	28.45	Ethyl palmitate	93	11.0
2	29.71	ethyl stearate	85.0	8.3
3	30.80	4-(1-methyl-1-[(4propioxy)phenyl] ethyl phenyl propionate)	65	7.0
4	26.95	1-nonadecene and 9-eicosene	94	5.1
5	18.48	glycerol diacetate	90	1.6
6	13.90	glycerol acetate	96	1.4
7	33.06	γ–sistosterol (stigmast -5-en-3-ol)	67	3.9
8	32.55	5-(1-methyl-1H-imidazol-2- γ - sufanyl)-1-phenyl-tetrazole	100	3.5
9	25.08	1-Heptadecene (Hexahydroaplotaxene)	100	2.5
		Unidentified		55.8
		Total		100.0

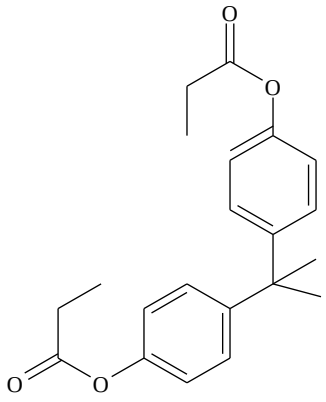
CHEMICAL STRUCTURES OF IDENTIFIED COMPOUNDS



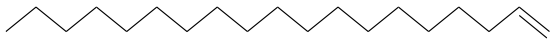
Ethyl palmitate (ethyl hexadecanoate)



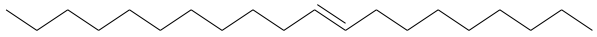
Ethyl stearate (ethyl octadecanoate)



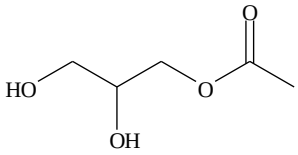
4-(1-methyl-1-[4-(propioxy) phenyl] ethyl phenyl propionate



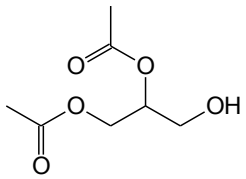
1-nonadecene



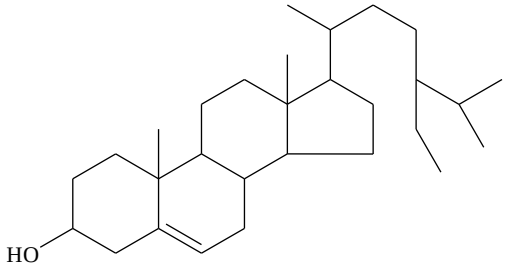
9-eicosene



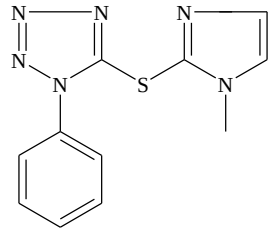
Glycerol acetate



Glycerol diacetate



γ-sitosterol



5-(1-methyl-1H-imidazol-2-γ-sulfanyl)-1-phenyl-tetrazole

1-heptadecene

The bacteria and fungi used were not sensitive to the CEAE. Although there are many bioactive azoles, the azole detected in CEAE appears not to confer any antimicrobial activity. The azole, 5-(1-methyl-1H-imidazol-2- γ -sulfonyl)–1-phenyl-tetrazole, is not like metronidazole (a nitro azole) in which the nitro group undergoes reduction and thus, inhibits deoxyribonucleic acid (DNA) synthesis in the microorganism (Lamikanra, 1999, Lawrence *et al.*, 1997; Katzung, 2004). The azole detected in the CEAE lacks a nitro group, so it could not undergo nitro reduction for antibacterial action to take place. Also, the other types of azoles like the antifungal azoles e.g. clotrimazole, ketoconazole, eiconazole, muconazole, disrupt fungal cell membrane (Katzung 2004) leading to antifungal activity. Since the fungi used in the study were not sensitive to the CEAE, it also shows that the CEAE does not possess this ability. The anthelmintic azoles like albendazole, mebendazole and thiabendazole inhibit microtubule synthesis (Katzung, 2004). Further research may yet reveal a probable anthelmintic activity of CEAE, as it has been reported that the boiled root of *S. macrocarpum* has been used in tradomedicine to treat hookworm infestation (Grubben and Denton, 2004). The detection of 5-(1-methyl-1H-imidazole) -2-γ-sulfanyl)-1-phenyl tetrazole also confirms the presence of sulphur earlier reported in the elemental analysis of the fruit (Sodipo *et al.*, 2008b).

Conclusion

The ethyl acetate extract of the fruit of *Solanum macrocarpum* does not exhibit antibacterial and antifungal activities. The detection of γ-sitosterol by the GC-MS validates its antihyperlidaemic property

Acknowledgments

The authors gratefully acknowledge the technical assistance of Mr. Fine Akawo, Department of Chemistry, University of Maiduguri, Maiduguri, the National Research Institute for Chemical Technology (NARICT), Zaria, for the GC-MS analysis and the University of Maiduguri for the award of a Study Fellowship to the first author.

References

ANNON (2007). Nightshade http://www.library.viuc.edu/vex/toxic /rightsha/nightsh.htm. Access Date 26/5/2007.

Ayim SK, Ogundaini A, Ogungbamila O, Olugbade T and Olaniyi (2000). Spectroscopic methods III. In: *Principles of Drug Quality Assurance and Pharmaceutical Analysis* (ed A.A. Olaniyi). Mosuro Pub. Ibadan, Nigeria. pp.329-332.

Bannerman RB, Buton J and We-Chieh C (1983). Traditional medicine and health coverage. World Health Organisation, pp 9-13.

Bello CSS (2002). *Laboratory Manual for Students of Medical Microbiology*. Satographics Press, Jos, Plateau State, Nigeria. 113pp.

Burkill HM (2000). *The Useful Plants of West Tropical Africa* (Vol. 5) Families S-Z. 2nd ed. Royal Botanic Gardens, Kew, London, U.K. pp. 119, 124-125, 130.

Cheesbrough MC (2004). *District Laboratory Practice in Tropical Countries Part 2*. Cambridge University Press, UK. pp. 135-147.

Cordell GA (2000). Biodiversity and drug discovery – A symbiotic relationship. *Phytochemistry*. **55**: 463-480.

Dagne, E. (2008). Ten outstanding natural products of Ethiopia. In the First Joint International Organisation for Chemistry in Development (IOCD) and the International society for the Development of Natural Products (ISDNP) International Symposium on Natural Products, Botswana, 25th – 29th February. Book of Extended Abstracts (ed M. Bezabih and B. M. Abegaz) pp. 15-16.

Gamaniel KS (2000). Toxicity from medicinal plants and their products. *Nig. J. Nat. Prod. Med.* **4**: 4-7.

Grubben GJH and Denton OA (2004). PROTA 2. *Plant Resources of Tropical Africa 2. Vegetables*. Poen and Looijen hv, Wagening en, Netherlands, 667pp.

Katzung BG (2004). *Basic and Clinical Pharmacology*, 9th ed. A Lange Medical Pub. McGraw Hill Co. Singapore, 1151pp.

Lamikanra AA (1999). *Essential Microbiology*. 2nd ed. Amkra Books 8, Obokun St. Ilupeju Estate, Lagos, Nigeria. pp.125-131, 304.

Lawrence DR, Bennet PN and Brown MJ (1997). *Clinical Pharmacology*. 8th ed. Churchill Livingstone, Singapore. p 213.

Lin J, Opuku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK and Van-Standen J (1999). Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antibacterial activities. *J. Ethnopharmacol.* **8** : 267 – 274.

Long LT, Dzeufiet PDD, Dimo T, Asongalem EA, Sokeng SN, Fleyou J, Callard P and Kamtchouing P (2007). Acute and subchronic toxicity of *Anacardium occidentale* Linn (Anacardiaceae) leaves extract in mice. *Afri. J. Trad. CAM.* **4** : 140-147.

Malini T. and Vanithakoumari G (1990). Rat toxicity studies with β-sitosterol. *J. Ethnopharm*. **28** : 221-234.

NIST (2009). National Institute of Standards and Technology, Washington, USA, http://en./wikipedia.org/wiki/national institute of Standards and Technology Access Date: 11/06/2009.

Olaniyi AA, Ayim JSK, Ogundaini AO and Olugbade TA (1998). *Essential Inorganic and Organic Chemistry*. Omoade Printing Press, Ibadan, Nigeria, pg. 582.

Sodipo OA, Abdulrahman FI, Sandabe UK, and Akinniyi JA (2008a). Effect of aqueous fruit extract on *Solanum macrocarpum* Linn. on cat blood pressure and rat gastrointestinal tract. *J. Pharm. Biores.* **5** (2): 52-59.

Sodipo OA, Abdulrahman FI, Akan JC and Akinniyi JA (2008b). Phytochemical screening and elemental constituents of the fruit of *Solanum macrocarpum* Linn. *Cont. J. Appl. Sci.* **3**: 88-97.

Sodipo OA (2009). Studies on chemical components and some pharmacological activities of *Solanum macrocarpum* Linn. Fruit (Garden egg). Ph.D. Thesis, University of Maiduguri, Maiduguri, Nigeria. 387pp.

Sodipo OA, Abdulrahman FI, Sandabe UK and Akinniyi JA (2009a). Effect of aqueous extract of *Solanum macrocarpum* Linn. on serum creatinine, urea and some electrolytes in rats pre-fed 1% cholesterol and groundout oil. *Sahel J. Vet. Sci.* **8** (1): 19-23.

Sodipo OA, Abdulralhaman FI, Sandabe UK and Akinniyi, JA (2009b). Effect of *Solanum macrocarpum* on biochemical, liver function in diet-induced hypercholesterolaemic rats. *Nig. Vet. J.* **30** (1): 1-8.

Sodipo OA, Abdulrahman FI, Sandabe UK and Akinniyi JA (2009c). Total lipid profile with aqueous fruit extract of *Solanum macrocarpum* Linn. in hypercholesterolaemic albino rats. *J. Pharm. Biores.* **6** (1): 10-15.

Sodipo OA, Abdulrahman FI, Sandabe UK and Akinniyi JA (2009d). Efects of the aqueous fruit extract of *Solanum macrocarpum* Linn. on some haematological indices in albino rats fed with cholesterol-rich diet. *Sahel J. Vet. Sci.* **8** (2): p5-12.

Tor-Anyiin TA, Sha'ato R and Oluma HOA (2006). Phytechemical screening and antibacterial activity of *Cissampelos mucronata* A. Rich. *J. Pharm. Bores.* **3** (2): 103-106.

Williams DH and Fleming I (1989). *Spectroscopic Methods in Organic Chemistry*. 4th ed. McGraw Hill Book Company, London, pp. 150-197.