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Separation, Purification, Isolation, Identification and Biological Activity of the Aqueous Fruit Extract of “Gorongu”- *Solanum macrocarpum* L.

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The plant was investigated for its chemical constituents and antimicrobial properties. Using gel filtration with Sephadex LH-20 (lipohylic) and thin layer chromatography (TLC) of the fractions with reversed phase, RP-18 plates the aqueous extract (CAE) of the unripe fruit was therefore analysed. The microorganisms used included four Gram positive bacteria and four Gram negative bacteria, and three fungal strains. All the microorganisms used were resistant to the effect of the CAE. The antimicrobial effect was assayed using the disc diffusion antimicrobial selectivity test. The purified saponin from the gel filtration chromatography resulted into five (5) spots with defined outlines (R_f 0.57; 0.65; 0.68; 0.71; 0.75) indicating that the purified saponins consists of a considerable mixture of saponins even in the same part of the plant. The saponins may contribute to the reported antihyperlipidaemic effect attributed to fruit of the plant and supporting the nonmicrobial activity of the fruit of the plant.

Key words: *Solanum macrocarpum*, aqueous extract, antimicrobial activity, gel filtration, sephadex LH-20 (lipohylic), R_f value.

INTRODUCTION

For thousand of years, the product of nature provided the only medicines for human illness and most of these remedies were obtained from higher plants (Winks, 1999, Sodipo, 2006). The number of species of higher plants on the planet earth is estimated between 370,000 and 50,000. All plants, in reaction to stress, infection, danger or environmental changes, produce a wide range of diverse chemicals, *secondary* (species) metabolites (SMs), many of which have therapeutic potential for a number of human ailments and are useful as medicines (Williamson *et al.*, 1996; Winks, 1999; Sodipo, 2006). These chemicals have also been used in traditional medicine. Over the years, medicinal plants have been used as sources of drugs administered in the cure of

diseases. Medicinal plants are plants that are commonly used in treating and preventing specific ailments and diseases, and are generally considered to play a beneficial role in healthcare. Medicinal plants are already important to the global economy. Demand is steadily increasing not only in developing countries but also in the industrialized nations (Srivastara *et al.*, 1996; Sodipo, 2006; Sofowora, 2008). The World Health Organization, (WHO), estimates that approximately 80% of the developing world's population meet their Primary Health Care needs through traditional medicine (LMPTK, 2006, Sodipo *et al.*, 2010). Also, about 25% of prescription drugs disposed in the United States contain at least one active ingredient derived from plant material. Some are made from plant *extract*, others are synthesized to mimic a natural plant compound (ANNON, 2006).

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

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world (Grubben and Denton, 2004, ANNON, 2007). In Africa and adjacent Islands, it is represented by at least 1,500 indigenous species, about 20 of these are recent introduction (Grubben and Denton, 2004). *Solanum macrocarpum* Linn (Synonyms: *Solanum daysphyllum* L. and *Solanum macrocarpon* L.) has been reported to exhibit laxative and hypotensive properties (Sodipo *et al.*, 2008a), the aqueous extract contained alkaloids, saponins flavonoids, steroidal glycosides combined reducing sugars, reducing sugars, ketoses and tannins (Sodipo *et al.*, 2008b). The aqueous extract has been shown to exhibit lipid lowering activities (Sodipo *et al.*, 2009c; 2011) and at the same time has renal and hepatoprotective effects (Sodipo *et al.*, 2009a, b). The fruit in addition is not toxic as the intra peritoneal LD₅₀ 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 subject the identified chemical constituents from the phytochemical analysis to chromatographic technique for separation, purification, isolation, identification and also to investigate the antimicrobial activity of the crude aqueous extract (CAE) using gel filtration with sephadex LH-20 (Lipophylic) and thin layer chromatography (TLC) of the fractions using reversed phase, RP-18 plates as little work has been done on this aspect of the CAE.

Initial thin layer chromatography (TLC) of the crude aqueous extract showed high polarity, so there would be so much tailing i.e. there will be too many overlapping bands if Accelerated Gradient Chromatography (AGC) were to be used for the separation. The solvent system used for this initial TLC was methanol/water (50:50) with Reversed Phase, RP-18 plate (activated at 110°C for 1 hr before use. On spraying with 2 M H₂SO₄, purple to brown spots were observed suspected to be saponins. Based on this preliminary TLC trial the right condition for running the separation was found to be gel filtration chromatography by Sephadex LH-20 (Lipophylic) (Williams and Wilson, 1975; Ayim and Olaniyi, 2000). Gel filtration is used to describe the separation of molecules of varying molecular size utilizing gel materials (Williams and Wilson, 1975). The most commonly used gels are cross-linked dextrans (Sephadex). The dextran gels are obtained by cross-linking the polysaccharide dextran with epichlorhydrin. In this way, the water-soluble dextran is made water insoluble, but it retains its hydrophilic character and swells rapidly in aqueous media, forming gel particles suitable for gel filtration. By varying the degree of cross-linking, several types of Sephadex have been obtained. They differ in porosity and consequently are useful over different molecular size ranges. Due to the random distribution of cross-linking, there is also a wide distribution of pore sizes in each gel type. This means that molecules of a size below the limit where

complete exclusion occurs are either partly or fully able to enter the gel. Each type of Sephadex is characterised by its *water regain* i.e. the amount of water taken up in the completely swollen granules by one gramme of Sephadex (Williams and Wilson, 1975).

MATERIALS AND METHODS

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.

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 ground fruit (2.2kg) was subjected to successive Soxhlet extraction in petroleum ether (60-80°C), ethyl acetate (76.7-77.7°C), 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.

Gel filtration chromatography of the CAE with sephadex LH-20 (Lipophylic)

The crude aqueous extract (CAE) (3.00 g) was subjected to gel filtration by Sephadex LH-20 (lipophylic) to further separate the extract to various fractions and the solvent system used was water. Prior to use, Sephadex LH-20 (Lipophylic) was converted to the swollen form by allowing it to swell in water for 3 hrs until it reached equilibrium (otherwise the final swelling will occur in the column, resulting in too tight packing and a consequent increase in flow resistance). Fifty grams swollen Sephadex LH-20 was then added to 100 µl distilled water and packed in the column to a height of 10 cm for maximum separation and the sample was applied in the standard manner like in the normal column chromatography.

Packing of column is the most critical factor in achieving a successful separation. It was carried out by

gently pouring a slurry of gel in the solvent (water is the mobile phase) into the column which had its outlet closed, whilst the upper part of the slurry in the column was stirred and the column gently tapped to ensure that no air bubbles were trapped and that the packing settled evenly. The slurry was added until the required height (10 cm) was attained. Once this was so, the flow of solvent through the packed column was started by opening the outlet, and continued until the packing had completely settled. To prevent the surface of the column from being disturbed either by addition of solvent to the column or during the application of the sample to the column, a suitable protection device (a filter paper disc) was placed on the surface of the column. Once the column had been prepared, no part of it was allowed to "run dry" i.e. a layer of solvent was always maintained above the glass surface. In gel filtration, a height to diameter ratio of 10:1 of the column was normally suitable as this influences the amount of material which could be separated on the column (Williams and Wilson, 1975).

The actual application of the crude aqueous sample to the top of the prepared column was carried out by using a capillary tubing and a peristaltic pump for passing the sample directly to the column surface. Care was taken to avoid overloading the column with the sample, to avoid irregular separation from occurring. The sample was also desalted before application to the column so as to prevent anomalous adsorption effects (Williams and Wilson, 1975). The various components were permeated through the column by the steady addition of eluant. The effluent which was measured from the moment when the sample was applied was collected in fractions in test tubes, so that the compounds which had been separated on the column remain resolved. Each of these fractions were then analysed.

Once all the components were eluted from the column, a new experiment was launched immediately, as no regeneration of the gel was necessary. The fractions collected with similar bands were then recombined.

Analytical thin layer chromatography (TLC) of the fractions of the aqueous extract of *Solanum macrocarpum* using reversed phase, RP-18 plate

In this experiment, commercially available RP-18 plates (Whatman) were activated 1 hr prior to the use at 110°C for 1 hr and 0.25 µl applied manually using a capillary tube. The RP-18 plate was 5 × 20 cm × 0.25 mm thick. The solvent system used was methanol/water 50:50 (Birk, 1969; Gennaro, 1985; Patrick-Iwuanyawu and Sodipo, 2007). The fractions were spotted on the reversed chromatoplates and the plate put in the solvent system in the Shandon chromatank[®] (micro one) and covered for the solute to travel. The plate was air-dried and sprayed with 2 M H₂SO₄ (Fenwick and Oakenfull, 1981) and observed from time to time to see the

development of the spot, the outline and the colour. If the spots developed were purple to brown, then the compounds present would probably be saponins. The remaining recombined fractions were then dried with a rotary evaporator (R.E-52, B.Bran Sci. & Instrument Co. England) and stored in clean indole bottles for further possible structural elucidation.

Antimicrobial studies

Microorganisms and reagents

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 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 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 × 10⁵µ/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). All the glasswares used in the study were sterile

Preparation of various concentrations and dilutions of the CAE extract

The stock solution of the CAE was 200mg/ml prepared by adding 2g CAE 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. The stock concentration used for the determination of the LD₅₀ (the concentration that killed 50% of the test organism) was arrived at after a pilot study of the LD₅₀ was carried out. The concentration 200 mg/ml was the one that did not kill the animals from the different concentrations used for the pilot study. Based on this, 200 mg/ml was used for determination of LD₅₀. this stock

concentration was used for all pharmacological experiment carried out. For the antimicrobial studies, this concentration was also used and diluted to give other concentrations.

Preparation of microbial suspensions

1ml each of the 24hr pure broth culture of all the bacteria and *Candida albicans* was added to 9ml sterile saline 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°C for 15 minutes. One milliliter of this was added to another 9ml saline solution and from this another 1ml of the suspension was added to 9ml saline solution to give a final dilution of $C \times 10^3$ organisms. (i.e. serial dilution was carried out to make a ten fold 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.

Preparation of discs containing graded concentrations of the CAE 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 60°C for 30min. 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 CAE 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 $2.5 \times 10^5 \mu\text{g/ml}$. 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 *Penicillium 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 121°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°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°C for 15min. 1ml each of the different concentrations of the CAE (25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml) was pipetted into eight (8) sterile, disposable petri dishes i.e. 2 plates for each CAE concentration 25ml of the sabouraud-2%-dextrose agar was poured into the plate, swirled round to mix very well with the CAE then allowed to set at low temperature. Two other plates were also prepared, but without the CAE, to act and the control. All the (10) plates were then incubated upside down, with their lids opened at 37°C in an incubator to dry.

Disc diffusion antibacterial selectivity test and disc diffusion selectively test for *Candida albicans*

1ml each of the $C \times 10^3$ 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 (10 μg /disc), ciprofloxacin (5 μg /disc) and tetracycline ($2.5 \times 10^5 \mu\text{g}$ /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 CAE was then placed on each of the plates. The plates were incubated at 37°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 antibiotic discs: ciprofloxacin (5 μg /disc) gentamicin (10 μg /disc) and tetracycline ($2.5 \times 10^5 \mu\text{g}$ /disc) were placed on the already prepared sabouraud-2% dextrose agar containing graded concentrations of the CAE (8 in all) and the control (2 plates). The *Penicillium spp* and 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

Table 1. Recombined Fractions from Gel Filtration by Sephadex LH-20 (Lipophylic) of the Aqueous Extract

Recombined Fraction	Weight of Recombined Fraction (g)	Dried % (^{w/w}) of Recombined Fraction	Components
A	0.40	13.33	F (1-7)
B	0.17	5.67	F (8-11)
C	0.02	0.67	F (12-14)

Initial weight of crude aqueous extract = 3.00 g

Amount recovered = 0.59 g

Percentage recovered = 19.67% (^{w/w})

Table 2: Analytical TLC of the Gel Filtration Fractions of the Aqueous Extract (CAE) of *S. macrocarpum* Fruit using Reversed Phase, RP-18 Plate

Recombined Fraction	Fraction	Number of Spots	R _f
A	F (1-7)	5	0.57 0.65 0.68 0.71 0.75
B	F (8-11)	NR	
C	F (12-14)	NR	

NR = Not resolved

$$R_f = \text{Retardation factor} = \frac{\text{Distance travelled by the solute from the origin}}{\text{Distance travelled by the solvent from the origin}}$$

Length of run = 5.10 cm

Time of run = 23 mins

Colour of spot = Purple to brown with a distinct outline

antibiotic discs and the extract in the plates. The plates were incubated at 25°C-30 °C and examined every 2-3 days and kept for four weeks before being considered negative for the fungi (Bello, 2002).

RESULTS

Gel filtration chromatography by sephadex LH-20 (lipophylic) of the crude aqueous extract (CAE) of *S. macrocarpum* fruit

The gel filtration chromatography of the aqueous extract resulted in 14 fractions F (1-14) which on recombination resulted in 3 fractions: A = F (1-7), B = F (8-11) and C = F (12-14) (Table 1). On drying, A weighed 0.40 g (13.33% ^{w/w}), B, 0.17 g (5.67% ^{w/w}) and C, 0.02 g (0.67% ^{w/w}).

Reversed phase TLC of the recombined fractions of gel filtration chromatography of the aqueous fractions of *S. macrocarpum* fruit

The TLC on reversed phase RP-18 plate of the recombined fractions yielded five spots on A, F (1-7) which had purple to brown colouration (Table 2) with 2 M H₂SO₄, each with a distinct outline. The spots had R_f values of 0.57, 0.65, 0.68, 0.71 and 0.75 respectively.

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 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 3). It was only the antibiotic discs that the organisms were sensitive to.

Table 3: *In-vitro* antimicrobial activity of CAE of the fruit of *S. macrocarpum*

S/N	Microorganism	CAE diameter of zones of inhibition (mm)							
		Concentration of CPEE (mg/ml)					Antibiotic discs (mg/disc)		
		250	200	150	100	50	Ciprofloxacin 5	Gentamicin 10	Tetracycline 2.5x10 ⁵
1	<i>Staphylococcus aureus</i> (+)	R	R	R	R	R	20	15	12
2	<i>Streptococcus pyogenes</i> (+)	R	R	R	R	R	22	20	11
3	<i>Corynebacteria spp.</i> (+)	R	R	R	R	R	28	18	13
4	<i>Bacillus subtilis</i> (+)	R	R	R	R	R	19	17	14
5	<i>Escherichia coli</i> (-)	R	R	R	R	R	30	16	18
6	<i>Salmonella typhi</i> (-)	R	R	R	R	R	25	22	10
7	<i>Pseudomonas aeruginosa</i> (-)	R	R	R	R	R	21	17	18
8	<i>Klebsiella pneumoniae</i> (-)	R	R	R	R	R	23	14	10
9	<i>Candida albicans</i> (Y)	R	R	R	R	R	21	17	16
10	<i>Aspergillus niger</i> (FF)	R	R	R	R	R	R	R	R
11	<i>Penicillium spp</i> (FF)	R	R	R	R	R	R	R	R

Key: CAE = Crude Aqueous Extract
R = Resistance (i.e. not sensitive)
+ = Gram +ve
- = Gram -ve

Y = Yeast
FF = Filamentous fungus

DISCUSSION

The results of the TLC of the crude aqueous extract of the fruit of *S. macrocarpum* using silica gel, kieselgel 60G, MERCK, Darmstadt as adsorbent and chloroform: n-hexane, 2:3, 100ml as solvent and iodine crystals as the developing agent were not conclusive, and from most of the runs, the components of the fruit could not be separated as they showed tailing effects. In view of this, the CAE separation was carried out by gel filtration chromatography with Sephadex LH-20 (Lipophylic) and the resolution was by TLC using reversed phase, RP-18 plate.

From gel filtration chromatography by Sephadex LH-20 (lipophylic) of the aqueous extract, the amount obtained was small, 0.59g (19.67% ^{w/w}) from the initial 3g. This indicates that the chromatographic technique employed enabled a

partial concentration and separation of saponins and removal of accompanying materials (Pasich, 1961; Dahiru and Sodipo, 2003). The small quantity of purified saponins obtained shows that saponins occur in small quantities as demonstrated by workers such as Birk (1969); Gennaro (1985); Dahiru and Sodipo (2003); Patrick-Iwuanyanwu and Sodipo (2007). This further buttresses the fact that medicinal plants contain small amount of saponins.

On the reversed phase TLC plate, the purified fruit saponin was resolved into five (5) spots with defined outlines (R_f 0.57; 0.65; 0.68; 0.71; 0.75). This indicated that the purified saponins consist of a considerable mixture of saponins even in the same part of the plant (Chandel and Rastogi, 1980). This is in agreement with the results of some previous workers that saponins occur as mixtures in nature (Birk, 1969; Gestetner *et al.*,

1971; Oleszek, 1988; Awe and Sodipo, 2001; Dahiru and Sodipo, 2003; Patrick-Iwuanyanwu and Sodipo, 2007). The efficiency of the octadecylsilane is evident in the number of spots resolved from the analytical TLC using reversed phase RP-18 plate. The positive results with Lieberman-Buchard's, Keller-Killani's and Salkowski's tests in the initial phytochemical screening of the extracts, probably indicated the presence of both steroidal and triterpenoidal saponins (Mahato *et al.*, 1982; Awe and Sodipo, 2001; Dahiru and Sodipo, 2003; Patrick-Iwuanyanwu and Sodipo, 2007). The steroidal saponins have the 27 carbon unit with the perhydrocyclopentenophenanthrene nucleus whilst the triterpenoids with 30 carbons represent the majority of saponins in nature. The third group of saponins are the steroidal glycoalkaloids found in the *Solanaceae*, the family to which *S.*

macrocarpum belongs (Patrick-Iwuanyanwu and Sodipo, 2007). Structural elucidation may probably reveal the type(s) of saponins present in the fruit of *S. macrocarpum*.

The other fractions of the aqueous and ethanol extracts that did not resolve on TLC probably contain the other active principles (tannins, alkaloids and other steroidal glycosides) earlier detected by phytochemical tests (Sodipo et al., 2008b). Structural elucidation with U.V. (Ultraviolet), I.R. (Infrared), CNMR (carbon 13-nuclear magnetic resonance) and ¹HNMR [Proton (nuclear magnetic resonance)] could detect these constituents. The types of sugar (combined and reducing) could also be detected.

The bacteria and fungi were not sensitive to the CAE even though the purified fraction contained five (5) different saponins. These purified saponins might be for lowering hyperlipidaemia as saponins are known to sequester bile acids, thus preventing absorption of cholesterol and thus lowering hyperlipidaemia (MacDonald et al., 2005). This confirms the hypolipidaemic effect of the plant earlier reported by Sodipo et al., (2010) with the ethyl acetate extract of the fruit. Also, from literature no antimicrobial activity has been reported in the fruit of the plant (Grubben and Denton, 2004). Also, the γ -sitosterol in the crude ethyl acetate extract confirmed hypolipidemic effect (Sodipo et al., 2010) but not antimicrobial effect of the plant.

CONCLUSION

The aqueous extract of the fruit of *Solanum macrocarpum* does not have antibacterial and antifungal activities. The results of this study have provided a chemical basis for the pharmacological actions of the fruit of the plant in that the saponins may be responsible for lowering hyperlipidaemia.

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