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Fatty acids composition, α-glucosidase inhibitory potential and cytotoxicity activity of *Oncoba spinosa* Forssk

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ABSTRACT

Oncoba spinosa is traditionally reputed for its medicinal potential particularly in southwest of Nigeria for the treatment of diabetes and cancer. 500 g of the leaves were extracted with 70 % aqueous ethanol and sequentially partitioned with hexane and chloroform. The extracts were separately tested for the presence of secondary metabolites and subsequently screened for α-glucosidase inhibitory, radical scavenging and cytotoxicity activities. Alkaliods, flavonoids, tannins, sterols, and anthraquinones were detected in the extracts while saponin was found absent. The crude and aqueous extracts showed better activities than the standard antidiabetic drug (acarbose) in concentration dependent manner. The aqueous and chloroform extracts had antioxidant activities comparables to ascorbic acid at concentrations 125 and 250 µg/mL. The brine shrimps lethality assay indicated the cytotoxicity of the crude, chloroform and hexane extracts, this was further established using cervical (HeLa) and Lung (A549) cancer cell lines. The results support the folkloric use of the plant as antihyperglycemic and antineoplastic agent.

Introduction

Oncoba spinosa is a spiny shrub that belongs to family of Flacourtiaceae which has about 95 genera and 800-1000 species. The leaves are dark, glossy green in colour and somewhat leathery and hairless. The fruits is grow up to 60 mm in diameter, it consists of hard shell that becomes dark -reddish brown when mature with shiny seeds embedded in a dry yellowish pulp [1]. Traditionally, the plant is used for the treatment of diabetes and tumour. Diabetes mellitus is a serious chronic disease resulting from a metabolic disorder in which the body does not produce or properly use insulin. It causes disturbance in carbohydrate, protein and lipid metabolism which consequently lead to other medical complications [2]. Although oral anti hyperglycemic agents and insulin are often successful in diabetes treatment, they have prominent side effects and fail to significantly alter the course of diabetic complications [3]. Thus, plant kingdom remains a veritable source for a new and efficacious antidiabetic drug. Also, plants have a long history of use in the treatment of cancer. Currently, 60% of anti-cancer agents used are derived in one way or another from natural sources, including plants, marine organisms and micro-organisms. Indeed, molecules derived from natural sources (natural products), including plants, marine organisms and micro-organisms, have played, and continue to play a dominant role in the discovery of leads for the development of conventional drugs for the treatment of most human diseases [4]. The research aimed at establishing the secondary metabolites constituents of the plant and investigating the folkloric claim of using the plant for the treatment of diabetes and cancer.

Plant materials

Oncoba spinosa was collected by Mr. A.A. Ekundayo of the Herbarium unit Forestry Research Institute of Nigeria (FRIN), Ibadan in the month of August, 2009. The plant was authenticated at the institute and a voucher copy with herbarium number FHI 108806 was deposited.

Extraction of plant material

The air-dried plant materials (500 g) were pulverized, exhaustively percolated separately with aqueous ethanol (70%) and intermittently placed in ultrasonic water bath for 72 h at room temperature. The extract was decanted, double filtered using cotton wool and whatmann NO 1 filter paper, concentrated on rotatory evaporator at 37^{o} C to about 50 mL and freeze dried. The powdery extract was weighed, re-suspended in distilled water and sequentially partitioned with hexane and chloroform.

Phytochemical analysis

The extracts were screened for secondary metabolites using methods described by Trease and Evan, and Harbone [5-6]. The presence of anthraquinone, alkaloids, phenol, sterol, flavonoids, saponin, tannin and carbohydrate were determined.

DPPH radical scavenging activity

The ability of the plant extract to scavenge 1, 1-dyphenyl-2-picrylhydrazyl (DPPH) free radicals was evaluated using method described by Stankovic *et al.* [7]. Briefly, DPPH (20 mg) was dissolved in methanol (250 mL) to give a concentration of 80 µg/mL. The stock solutions (1 mg/mL) of the plant extracts were prepared in methanol. Dilutions were made to obtain concentrations of 250, 125, 62.5 and 31.25 µg/mL. 1 mL of each concentration was mixed with DPPH (1 mL). The mixture was shaken and incubated in darkness at 23^{o}C for 30 min, after which the absorbance was recorded at 517 nm.

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The control samples contained all the reagents except the extract.

α-Glucosidase Inhibition Assay

The enzyme inhibition study was carried out using method described by Li *et al*. [8]. A total 60 µL reaction mixture containing 20 µL of 100 mM phosphate buffer (pH 6.8), 20 µL of 2.5 mM *p*-nitrophenyl α-D-glucopyranoside (PNP-G) (Biochem) in the buffer, and 20 µL of plant extract in methanol at various concentrations of 200, 100, 50, 25 and 12.5 µg/mL were added to each well of the 96 micro plate, followed by 20 µL of 0.2 U/mL α – glucosidase in phosphate buffer (10 mM, pH 6.8). The plate was incubated at 37 °C for 15 min after which the reaction was terminated by adding 80 µL of 0.2 mol/L Na_2CO_3 and absorbance was recorded at 405 nm with a Tecan GENios multifunctional microplate reader (Männedorf, Switzerland). Controls contained the same reaction mixture except the same volume of phosphate buffer was added instead of a plant extracts. Acarbose (Bayer), at the same concentrations as plant extracts were used as a positive control. The inhibition (%) was calculated as: (%) = $\{(OD_{control}-OD_{sample})/OD_{control}\}$ ×100.

Cytotoxicity Assay

Brine shrimps eggs were added to sea water in a small tank with perforated dividing dam and allowed to hatch within 48 h at room temperature. 10 shrimps were placed in test tubes containing solutions of concentration 10, 100 and 100 µg/mL of test extracts. Each test solution was prepared in triplicates

The count of the number of surviving shrimps was taken after 24 h and analysis was done using Finney Computer Programme to determine LC_{50} value at 95% confidence limit [9-10]

Cell line and culture

Human lung adenocarcinoma cells (A549) and human cervical epithelioid carcinoma cell line (HeLa) were cultured in RPMI-1640 medium supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin and 10% fetal bovine serum (FBS). The cells were maintained at 37^{o}C in a humidified air containing 5% CO_2 prior to use.

MTT Assay

This assay is dependent on the reduction of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) by the mitochondrial dehydrogenase of viable cells to a blue formazan product that can be measured spectrophotometrically [11]. Tumour cell suspensions were obtained by trypsinization of monolayer cultures using 0.25% trypsin-EDTA (Gibco). Cells were harvested, counted, and 100 µL of 1.4 x 10^5 cell/mL of A549 and 6.6 x 10^4 cell/mL of HeLa respectively were separately transferred into a 96-well plate and incubated for 24 h prior to the addition of test extracts. The test extracts were serially diluted from their stock solutions to give concentrations of 50, 100, 200, 400, and 600 µg/mL. DMSO of total concentration less than 1 % was used to solubilise non polar extract while ultra pure Milli-Q water was used for polar extracts. 100 µL of various concentrations of the test extracts were added to each well and incubated for 72 h. Each well was supplemented with 50 µL of a 2 mg/mL solution of MTT in phosphate buffer solution (PBS). The plates were incubated in 37 ^{o}C and 5% CO_2 for another 4 h. The media was carefully removed from each well and 150 µL of DMSO was added. The plates were gently agitated until the colour reaction was uniform and the OD_{570} was determined using a microplate reader Tecan GENios multifunctional microplate reader (Männedorf, Switzerland) [12-14]. PBS and 5-Fluorouracile (5-Fu) at concentrations of 100, 50, 25 and 12.5 µg/mL in PBS were used in place of test extracts as negative and positive controls respectively. All the experiments were performed in triplicates and done twice.

Inhibition (%) = [1-(Average $OD_{570\ nm}$ value of treated culture/Average $OD_{570\ nm}$ value of negative control culture)] ×100 %.

Results and discussion

Table 1. Extraction Yield

Extracts	Weight (g)	% yield
Crude	23.90	4.78
Aqueous	1.20	0.24
Chloroform	5.70	1.14
Hexane	6.00	1.20

Table 2. Secondary Metabolites

Metabolites	Crude	Aqueous	Chloroform	Hexane
Anthraquinone	-	+	+	-
Alkaloids	+	+	+	-
Sterol	-	-	+	+
Flavonoids	+	+	-	-
Tanins	+	+	+	-
Saponins	-	-	-	-
Carbohydrates	+	+	-	-

Present (+) Absent (-)

Compound	Retention time (min)	% Composition
Ethanol,2-(1,1-dimethylethoxy)	5.02	1.63
Benzene,1,2,4-timethyl	9.83	0.74
Tetradecanal	23.07	0.36
Methyltetradecanoate	23.16	0.59
Tetradecanoic acid, ethylester	23.91	0.22
Pentadecanoic, methylester	24.24	0.48
6,10,14-trimethyl-2-pentadecanone	24.47	0.59
Pentadecanoic acid,ethylester	24.95	0.45
Hexadecanoic acid, methylester	25.28	8.66
Heptadecanoic acid, methylester	26.26	0.45
Heptadecanoic acid,ethylester	26.90	0.69
9,12-octadecadienoic acid(z,z),methylester	26.93	4.12
11-octadecenoic acid,methylester	27.00	6.21
Octadecanoic acid,methylester	27.21	17.23
9,12-octadecadienoic,methylester	27.54	1.95
Ethyloleate	27.59	2.99
Octadecanoic acid,ethylester	27.80	2.64
Eicosanic acid, methylester	28.96	0.18
4,8,12,16-tetramethylheptadecan-4-olide	29.25	0.44
19-methyleicosanoate	29.49	0.35
Testololactone	29.78	0.35
Octacosane	30.33	0.32
20-Methylheneicosanoate	30.58	0.28
Docosanoic acid,ethylester	31.07	0.83
Ethyltetracosanoate	32.82	1.57
Tetracosanoic acid, 2,9-dimethyl,methylester	33.92	2.09
Hexacosanoic acid, 2-methylester	35.24	0.65
β- Tocopherol	36.52	0.98
α- Tocopherolquinone	37.92	10.13
4,4,6a,8a,11,11,14b-octamethyl-1,4,4a,5,6,6b,7,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-pic	42.32	1.57
Lup-20(29)-en-3-one	43.61	2.20
Ergosta-4,6,8(14),22-tetraen-3-one	43.84	1.40
Stigmast-4-en-3-one	46.16	11.42
Stigmasta-3,5-dien-7-one	47.12	1.47
Total		86.23

Fig.1 Inhibition of α-glucosidase

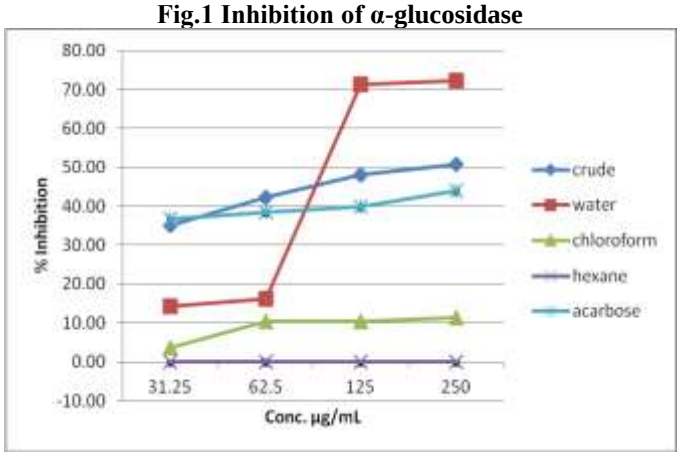


Fig. 2 Radical scavenging activity

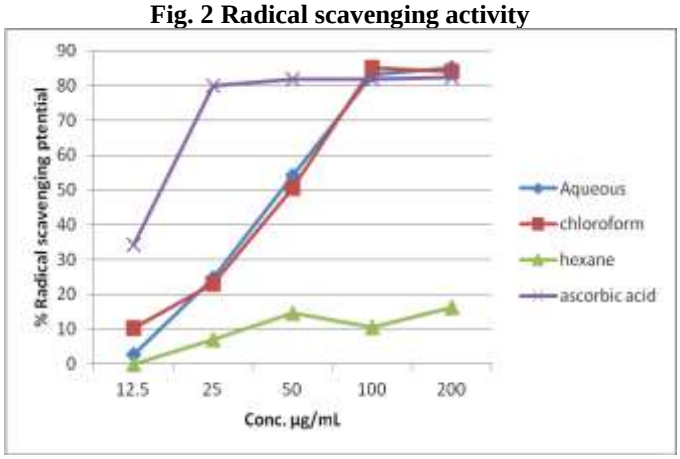
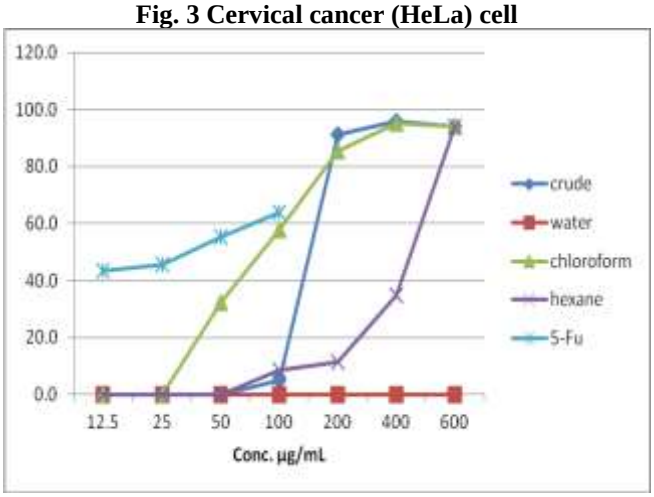


Table 3. Brine shrimps Cytotoxicity

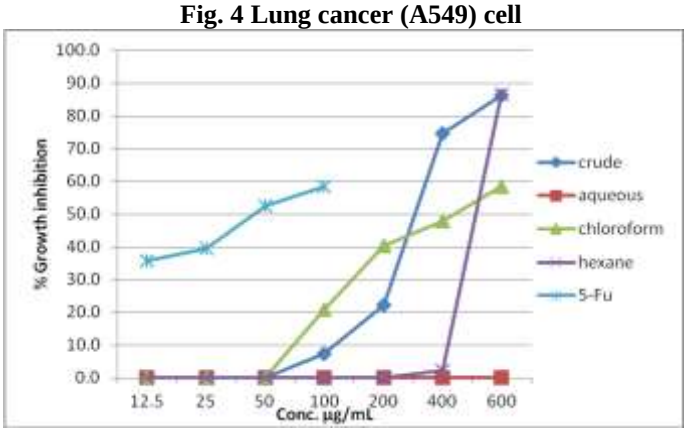
Extract	LD_{50} (µg/mL)
Crude	13.02
Aqueous	467.67
Chloroform	8.50
Hexane	21.03

Fig. 3 Cervical cancer (HeLa) cell



The phytochemical screening of the plants extracts showed rich secondary metabolites content. Except for hexane extract, other extracts contained alkaloids, phenol and tannin. Flavonoids and carbohydrate were found present only in crude and aqueous extracts. Anthraquinone was detected in aqueous and chloroform extracts. The presence of saponin was not found in any of the extracts (Table 2). These secondary metabolites are reputed for having good pharmacological and physiological activities, and could largely responsible for the biological activities of the plant [15 -16].

Fig. 4 Lung cancer (A549) cell



The GC-MS analysis of the fatty acids identified a total of 34 compounds representing 86.23 % composition. There were preponderances of octadecanoic acid, methylester, stigmast-4-en-3-one and α- tocopherolquinone with percentage compositions of 17.23, 11.42 and 10.23 respectively. Stigmast-4-en-3-one has been shown to exhibit good *in vivo* hypoglycaemic activity, thus making it a potent oral hypoglycaemic agent occurring naturally in food [17]. Alpha-tocopherolquinone was found to inhibit lipid peroxidation induced by ascorbate/Fe^{2+} in liposomes and bycumene hydroperoxide in submitochondrial particles of rat liver which is indicative of good antioxidant activity [18]. Also, Yang *et al*., 2010, showed that alpha-tocopherolquinone inhibited beta-amyloid aggregation and cytotoxicity, disaggregated preformed fibrils and decreased the production of reactive oxygen species, NO and inflammatory cytokines [19].

The inhibition of α-glucosidase assay showed that the crude extract demonstrated better activity than acarbose while the aqueous extracts had a much better inhibition of 71.27 and 72.28 % respectively on α-glucosidase enzyme. On the contrary, hexane and chloroform extracts showed no antihyperglycemic activities at the concentrations used (Fig. 1). This pattern of activities agree with previous works on antidiabetes from natural products in which aqueous extracts and crude performed better [20-24]. According to Labovitz, 2007, the *in vivo* effects of alpha-glucosidase inhibitors can be predicted from their mechanism of action. Administration of alpha-glucosidase inhibitors will slow the digestion of carbohydrate. Instead of complete digestion of carbohydrates and absorption of monosaccharides in the proximal jejunum, the digestion of carbohydrate in the jejunum is incomplete. The rise in postprandial glucose is therefore diminished and delayed. The undigested carbohydrate is slowly digested by alpha-glucosidase enzymes in the distal jejunum and ileum. When enzyme activity in the distal small bowel is insufficient, the carbohydrate spills into the large intestine where bacteria metabolize the carbohydrate to short-chain fatty acids, hydrogen, carbon dioxide, and methane which consequently lead to decrease in the blood glucose [25].

The DPPH scavenging potential of the plant's extracts indicated a concentration dependent pattern in the chloroform and aqueous extracts. At higher concentrations of 50 and 100 g/mL, both extracts exhibited activities comparable to that of ascorbic acid (Fig. 2). These activities could be attributed to high phenolic content of the chloroform and aqueous extracts. The hexane extract showed poor activity. Many anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective [26-28] drugs have recently been shown to have

an antioxidant and/or radical scavenging mechanism as part of their activities.

The brine shrimps lethality studies showed high cytotoxicity potential of the crude, chloroform and hexane extracts (LD_{50}< 100 µg/mL). The aqueous extract had a LD_{50} of 467.67 µg/mL which is indicative of moderate toxicity (Table 3). In the MTT cytotoxicity assay, none of the extracts had better activity than 5-Fu on the cancer cells used. However, chloroform extract at 100 µg/mL exhibited inhibitory activity of 57.8 % which is fairly close to 64.10 % exhibited by 5-Fu. The inhibitory activities of crude and chloroform extracts at 200 µg/mL on cervical (HeLa) cell line were 91.30 and 85.60 % which were better than 22.30 and 40.20 % respectively on lung cancer (A549) cell line (Fig.3 & 4). The aqueous extract had no activity on both cell lines at various concentration used. The result corroborated the brine shrimps lethality test in which the crude and chloroform extracts had the least LD_{50} and aqueous extract had the highest.

In conclusion, the aqueous extracts of *O. spinosa* demonstrated good antihyperglycemic and radical scavenging potentials but had poor cytotoxicity activity. The crude extract exhibited good antihyperglycemic and cytotoxicity activities while chloroform extract showed no antihyperglycemic potential but demonstrated good radical scavenging and cytotoxicity activities. The secondary metabolites profile, antihyperglycemic, radical scavenging and cytotoxicity of the leaves of *O. spinosa* are being reported for the first time.

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