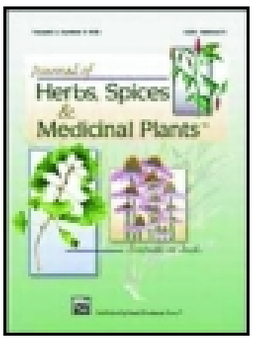

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Chemical Composition, Gastroprotective, and Antioxidant Activities of *Schrebera arborea* Fruits

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ABSTRACT

The chemical composition, gastroprotective, and antioxidant activities of *Schrebera arborea* fruits were investigated. The solvent extracts were analyzed for fatty acid and polyphenolic content using gas chromatography-mass spectrometry (GC–MS) and high performance liquid chromatography (HPLC), respectively. Acute toxicity study was done by oral administration at 2,000 mg kg^{-1}. Gastroprotective effect was evaluated at 50, 100, or 150 mg kg^{-1} in ethanol and indomethacin-induced ulcer models in rats. The antioxidant activities of the extract were also evaluated. Sitosterol (41.55%) was the major component identified by GC–MS. Quantitative HPLC analysis for phenolic compounds indicated the presence of gallic acid (0.19 mg g^{-1}), caffeic acid (0.13 mg g^{-1}), rutin (0.30 mg g^{-1}), apigenin-7-glucoside (1.21 mg g^{-1}), catechin (1.84 mg g^{-1}), and kaempferol (6.53 mg g^{-1}), respectively. The ethanolic extract of the fruit produced 63.5% and 80.8% inhibition of ethanol and indomethacin-induced ulcer at 100 mg kg^{-1}, respectively. The extract at 100 mg kg^{-1} reduced MDA level and increased glutathione, catalase, and superoxide dismutase activities in rat stomach tissue. Significant radical scavenging antioxidant activity was recorded *in vitro*.

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KEYWORDS
HPLC analysis; GC–MS; Schrebera arborea

Introduction

The genus *Schrebera* comprises about eight species, five of which occur in tropical Africa, two in tropical Asia, and one in South America.[1] In Nigeria, *Schrebera arborea* (Oleaceae) fruits are used in healing skin tear on a child's head (head fontanel) and in the treatment of wounds and ulcers.[2] A related species *Schrebera swietenioides* from India has been reported to have healing potential,[3] antidiabetic,[4] antioxidant, anti-inflammatory, and antipyretic activities.[5] In this study, the fruits of *S. arborea* were characterized for chemical composition, gastroprotective, and antioxidant properties.

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Materials and Methods

Plant Material and Extraction

Dried fruits of *S. arborea* were purchased from the Mushin market, Mushin, Lagos, Nigeria (6° 31′ 45″ North, 3° 21′18″ East), identified and a voucher specimen (LUH 6463) deposited at the Herbarium unit of the Department of Botany, University of Lagos, Nigeria. The fruits were cleaned, grounded into powder using a mechanical grinder (Christy and Morris Limited, England), and divided into two portions. One part (200 g) was placed into a Soxhlet apparatus for extraction with hexane for 8 h and evaporated in a rotary evaporator (Buchi Labortechnik, CH-9230, Flavil, Switzerland) at 40°C to obtain a pale-yellow fat. The second portion (500 g) was extracted by maceration in absolute ethanol for 48 h, filtered through Whatman No. 1 paper, and evaporated to yield a dark brown extract. The hexane extract was subjected to GC–MS analysis while the ethanol extract was used for pharmacological assays and HPLC analysis.

Determination of Volatile Components

The volatile components of the extracted lipid were determined by GC–MS. The analysis was performed on Agilent 7890 A GC system furnished with an auto sampler (Agilent G4513A injector series) coupled to a 5975C network mass detector (GC–MS) (based on a quadruple mass separator). A J&W Scientific HP-5MS silica fused capillary column (30 m × 0.320 mm i.d. × 0.25 μm film thickness) was used with helium as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. Split less injection of 2 μL of the sample was automated by an injector on the instrument from a syringe 10 μL. The oven temperature was set at 70°C and ramped at 4°C min^{-1} to 250°C. The injector temperature was 250°C and detector temperature 280°C. Mass spectra were taken at 70 eV with a mass range of m/z 40–500. Identification of the components was by comparison of the retention time and mass spectra of each separated peak with the databank of the instrument, NIST 2014 library, and literature.

Analysis of Phenolic Compounds Using HPLC

The phenolic composition of the ethanolic extract of *S. arborea* was analyzed using HPLC-Agilent Technologies 1100 series liquid chromatograph (RP-HPLC) coupled with an ultraviolet (UV)–vis multiwavelength detector and a reversed phase, prepacked Hypersil BDS C_{18} column (250 × 4.6 mm, 5 μm particle size). The extract (2 g) was dissolved in small amount of water and partitioned with hexane and ethylacetate. The ethylacetate extract was dissolved in HPLC grade methanol to get a concentration of 10 mg mL^{-1}. Before injection into the HPLC system, an aliquot of 10 μL was filtered through a 0.45-μm Millipore filter. The mobile phase consisted of water with 0.1% formic acid (solvent A) and acetonitrile

0.1% formic acid (solvent B). The chromatographic gradient was as follows: 0 min, 94% A; 14 min, 83.5% A; 16 min, 83% A; 18 min, 82.5% A; 20 min, 82.5%; 22 –24 min, 81.5%; and 27–40 min, 80% A.[6] There was a 5-min post run at initial conditions for equilibration of the column. The flow rate was kept constant throughout the analysis at 1.5 mL min^{-1} and the separation monitored at 280 nm. Stock solutions of standards – rutin, kaempferol, quercetin, apigenin-7-glucoside, amentoflavone, catechin, isorhamnetin, caffeic acid, gallic acid, ferulic acid, and *p*-coumaric acid – were prepared in methanol at 1 mg mL^{-1} and further diluted with methanol for the preparation of standard calibration curve. Results were expressed as mg g^{-1} dry weight of sample. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 1996, guideline was followed for the determination of limit of detection (LOD) and limit of quantification (LOQ).[7]

Experimental Animals

Male Albino Wistar rats, weighing between 100 and 140 g, were purchased from a private vendor, Komad farm limited, Sango, Ogun state, Nigeria. The animals were maintained at temperature of 23°C ± 2°C and had free access to pelletized feed (Livestock Feeds PLC, Ibadan, Oyo state, Nigeria) and water *ad libitum*. The experimental procedures were in accordance with the US States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research[8] and approved by the College of Medicine of the University of Lagos Health Research and Ethics Committee (CM/HREC/12/16/085).

Acute Oral Toxicity Test

Acute oral toxicity test of the ethanolic extract of *S. arborea* was determined in mice according to the Organization of Economic Co-operation and Development guidelines for testing of chemicals.[9] Two groups of seven mice were fasted for 12 h prior to the experiment. A single dose of 2,000 mg kg^{-1} of the extract was administered orally to one group of the mice while the control group received the vehicle (distilled water). All the animals were then allowed free access to food and water 2 h after administering the extract. The animals were observed for a period of 48 h for signs of toxicity and monitored daily until the 14th day.

Evaluation of Gastroprotective Activity

Ethanol-Induced Ulcer Model

The experiment was performed as described[10] with slight modifications. The rats were divided into five groups containing six rats each. After 24 h fasting, the animals were orally administered 50, 100, or 150 mg kg^{-1} of the extract, distilled water (10 mL kg^{-1}), or the reference drug (misoprostol, 0.1 mg kg^{-1}). One hour

later, 1 mL of absolute ethanol was orally administered to the rats for the induction of gastric ulcer and the animals were sacrificed 1 h after that. Each rat's stomach was removed, longitudinally excised along the greater curvature and rinsed in normal saline. This was followed by macroscopic examination of the gastric mucosal for ulcer lesions. The length of each gastric lesion was measured in millimeter and the ulcer index calculated.[11]

Indomethacin-Induced Ulcer Model

The animals were deprived of food for 48 h before the experiment but had free access to water. After 48 h of fasting, animals were orally administered 50, 100, or 150 mg kg^{-1} of *S. arborea* extract, distilled water (10 mL kg^{-1}) or reference (omeprazole, 100 mg kg^{-1}). One hour later, indomethacin (80 mg kg^{-1}, p.o.) was administered to induce ulcer in all the groups.[12] The animals were sacrificed 6 h later and the stomachs were removed, longitudinally excised along the greater curvature, rinsed thoroughly in normal saline, and ulcer lesions measured.

Assessment of Tissue Biochemical Studies

The effect of the effective dose (100 mg kg^{-1}) of the extract on oxidative damage was assessed using stomach tissues of rats in ethanol-induced ulcer model. The stomach tissue homogenates (10%, w/v) were prepared in cold 50 mM potassium phosphate buffer (pH 7.4) and centrifuged at 4,000*g* for 10 min. The supernatants were used for the assays. Lipid peroxidation was determined as described[13] and the superoxide dismutase (SOD) assayed.[14] Glutathione (GSH) and catalase (CAT) levels were also determined[15,16]. All assays were carried out at room temperature and in triplicate.

In Vitro *Evaluation of Antioxidant Activity*

The ability of the extract to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was measured at 517 nm[17] and its reducing capacity determined.[18] The capacity of extract to scavenge hydroxyl radical[19] and ferrous ions chelating activity[20] were evaluated.

Statistical Analysis

The data were analyzed by one-way analysis of variance followed by Tukey's posttest ($p< 0.05$) using GraphPad Prisms 5.0 (Graphpad Software, San Diego, USA).

Results

GC–MS Analysis of S. arborea Fruits

The GC–MS analysis of the hexane crude extract from the fruits of *S. arborea* yielded 10 components (Fig. 1 and Table 1). β-Sitosterol was the major component (41.55%), though fatty acids including 9-octadecenoic acid, methyl ester (10.71%), hexadecanoic acid, methyl ester (7. 94%), and 9,12-octadecadienoic acid, methyl ester, (*E,E*)- (6.34%) appeared in moderate concentrations.

HPLC Analysis of Phenolics from S. arborea Fruits

The chromatographic profile (Fig. 2) revealed the presence of phenolic acids (gallic acid and caffeic acid) and flavonoid (catechin, rutin, apigenin-7-glucoside, and kaempferol) as per their retention times and UV spectra compared to reference compounds. The HPLC data showed that the calibration curves were linear ($R^2 \geq 0.993$) in the tested concentration range (1.41–22.50 μg mL^{-1}) (Table 2). The LOD and LOQ for the compounds ranged from 0.16 to 2.74 μg $mL^{-1}$and 0.50 to 8.31 μg mL^{-1}, respectively. The relative standard deviation (RSD) of intra- and inter-day variations for the analytes were all less than 2.2%. The concentrations of phenolic acid, gallic acid and caffeic acid were 0.19 and 0.13 mg g^{-1}, while the content of flavonoids was determined as 0.30 mg g^{-1} of rutin, 1.21 mg g^{-1} of apigenin-7-glucoside, 1.84 mg g^{-1} of catechin, and 6.53 mg g^{-1} of kaempferol, respectively.

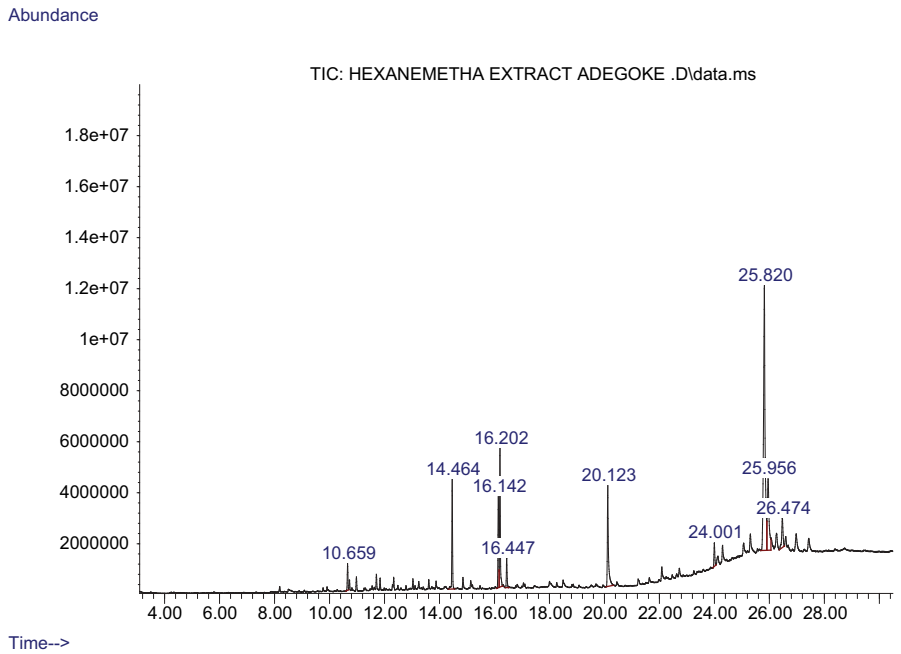

Figure 1. GC–MS chromatogram of the hexane extract of *Schrebera arborea* fruits.

Table 1. Chemical composition of the hexane extract of *Schrebera arborea* fruits by GC–MS.

Peak no.	RT (min)	Name of the compounds	Molecular weight	Area (%)
1	10.66	Caryophyllene oxide	220	1.86
2	14.46	Hexadecanoic acid, methyl ester	270	7.94
3	16.14	9,12-Octadecadienoic acid, methyl ester, (*E*,*E*)-	294	6.34
4	16.20	9-Octadecenoic acid (*Z*)-, methyl ester	296	10.71
5	16.45	Methyl stearate	298	2.29
6	20.12	Bis-(2-ethylhexyl) phthalate	389	10.87
7	24	1,4-Phthalazinedione, 2,3-dihydro-6-nitro	207	1.89
8	25.82	β-Sitosterol	414	41.55
9	25.96	Piperidin-4-one, 3-(2-furanylmethylene)-1,2,5-trimethyl	222	12.61
10	26.47	1, 2-Benzisothiazol-3-amine, TBDMS derivative	264	3.95

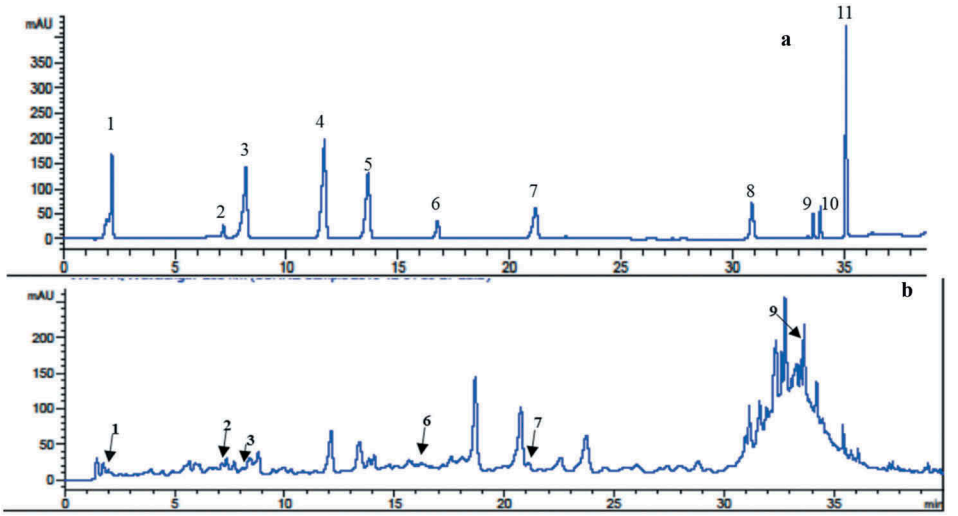

Figure 2. HPLC chromatogram of mixed phenolic standards (a) and *Schrebera arborea* fruit extracts (b). Peaks: 1, gallic acid; 2, (+)-catechin; 3, caffeic acid; 4, *p*-coumaric acid; 5, ferulic acid; 6, rutin; 7, apigenin-7-glucoside; 8, quercetin; 9, kaempferol; 10, isorhamentin; 11, amentoflavone.

Acute Toxicity

No mortality and toxicity symptoms were observed in mice at doses up to 2,000 mg kg^{-1} of the ethanolic extract.

Gastroprotective Property of S. arborea Fruits

The extract (100 and 150 mg kg^{-1}) and misoprostol (0.1 mg kg^{-1}) decreased the mean ulcer scores, in ethanol-induced ulcer model (Fig. 3). The percentage inhibition of ulcerations at doses of 100 and 150 mg kg^{-1} was 63.53% and 49.41%, respectively. The effect of misoprostol was higher than that of the extract. In the indomethacin-induced ulcer model, the extract showed protective effect at 100 mg kg^{-1} (80.8%, inhibition) and 150 mg kg^{-1} (55.1%, inhibition) (Fig. 3).

Table 2. Analytical characteristics of the calibration curves of the standards.

Compound	R_T (min)	Linear regression equation[a]	Linear range (μg mL^{-1})	R^{2b}	LOD (μg mL^{-1})[c]	LOQ (μg mL^{-1})[d]	RSD (%) Intra-day	RSD (%) Inter-day
Gallic acid	2.18	$y = 11.675x - 3.1504$	1.41–22.50	0.9999	0.30	0.92	1.70	1.79
(+)-Catechin	7.18	$y = 5.9883x - 9.137$	1.41–22.50	0.9930	2.74	8.31	2.20	1.83
Caffeic acid	8.18	$y = 22.346x - 8.382$	1.41–22.50	1.0000	0.16	0.50	0.29	1.36
p-Coumaric acid	11.7	$y = 29.498x - 7.7384$	1.41–22.50	0.9999	0.26	0.78	0.14	1.80
Ferulic acid	13.66	$y = 18.685x - 4.5928$	1.41–22.50	0.9999	0.28	0.86	0.16	1.64
Rutin	16.79	$y = 5.0044x - 1.9295$	1.41–22.50	0.9999	0.42	1.28	0.07	1.49
Apigenin-7-glucoside	21.17	$y = 11.675x - 3.1504$	1.41–22.50	0.9999	0.35	1.05	0.35	1.63
Quercetin	30.88	$y = 9.1382x - 5.1469$	1.41–22.50	0.9998	0.48	1.44	0.11	1.72
Kaempferol	33.62	$y = 2.9856x - 0.5818$	1.41–22.50	0.9999	0.40	1.21	0.20	1.82
Isorhamnetin	33.94	$y = 3.456x - 0.825$	1.41–22.50	0.9999	0.35	1.05	0.08	1.79
Amentoflavone	35.10	$y = 22.141x - 2.8155$	1.41–22.50	0.9999	0.30	0.90	0.13	1.30

[a]In the regression equation, y= peak area, x= concentration of standards in (μg mL^{-1}). [b]R^2 = Correlation coefficient for five data points in the calibration curves (n= 3). [c]LOD: Limit of detection. [d]LOQ: limit of quantitation.

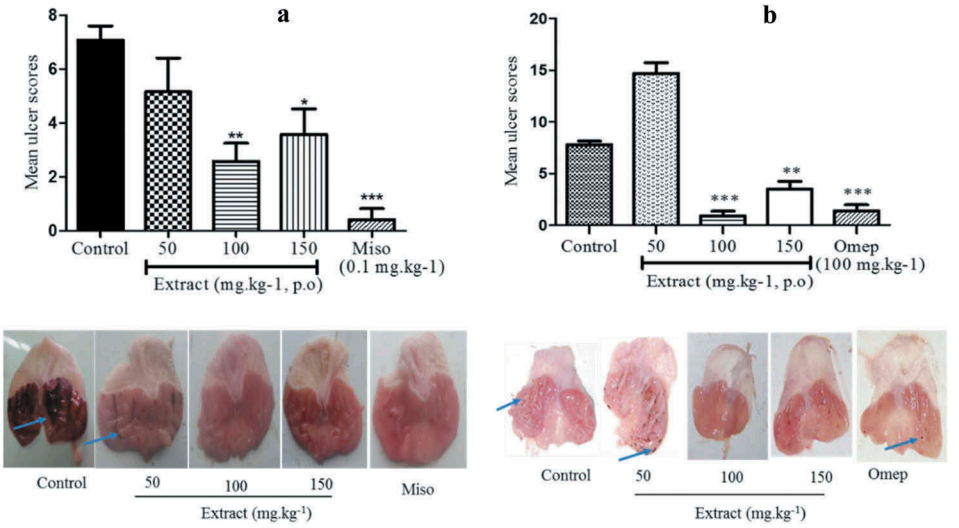

Figure 3. Effect of *Schrebera arborea* fruit extracts on (a) ethanol and (b) indomethacin-induced gastric ulcer. Data are mean ± SEM (n = 5 or 6). Data analyzed by one-way ANOVA, mean separation by Tukey's multiple comparison (*p < 0.05, **p < 0.01, ***p < 0.001). Miso-Misoprostol (0.1 mg kg^{-1}, p.o.), Omep-Omeprazole (100 mg kg^{-1}, p.o.). Arrows indicate lesions.

In Vivo *and* in Vitro *Antioxidant Activity*

Ethanol increased MDA level and decreased the levels of GHS, CAT, and SOD. Pretreatment with ethanolic extract of *S arborea* reduced the level of MDA and increased antioxidant enzymes activities. (Fig. 4). The results of the *in vitro* antioxidant assays (Fig. 5) showed potent DPPH free radical scavenging activity with the IC_{50} value of 33.68 µg mL^{-1}. The reducing power potential of the extract at 100 µg mL^{-1} was 0.42 ± 0.02 compared to butylated hydroxytoluene (BHT) (1.06 ± 0.01) and quercetin (2.50 ± 0.01). The chelating ability of the fruit extract was weak compared to that of ethylenediaminetetraacetic acid (EDTA). The percentage inhibition of hydroxyl radical

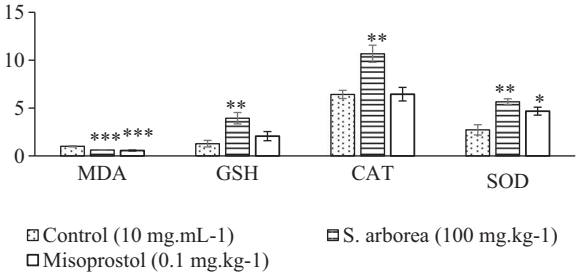

Figure 4. Effect of *Schrebera arborea* fruit extracts on MDA, GSH, CAT, and SOD levels in ethanol-induced rats. The results are expressed as mean ± SEM (n = 5). MDA (µmol mg^{-1} protein), GSH (µmol mg^{-1} protein), CAT (unit mg^{-1} protein), SOD (unit mg^{-1} protein). Data analyzed by one-way ANOVA, mean separation by Tukey's multiple comparison (*p < 0.05, **p < 0.01, ***p < 0.001).

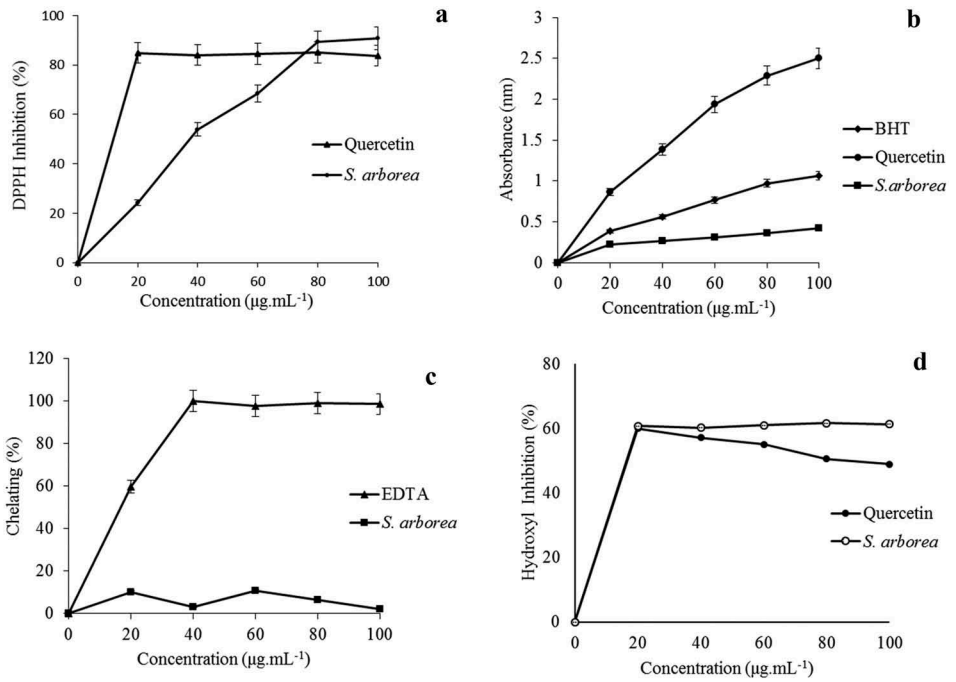

Figure 5. *In vitro* antioxidant activities of the extracts of *Schrebera arborea* fruits. (a) DPPH radical scavenging, (b) reducing power assay, (c) ferrous ion chelating, and (d) hydroxyl radical scavenging.

scavenging activity of the extract and quercetin were 59.96% and 60.75% at the lowest concentration (20 µg mL^{-1}), respectively.

Discussion

The major compound detected in the nonpolar extract of the *S. arborea* fruit was β-sitosterol that has previously been reported to have antiulcer activity, among other biological properties.[21,22] Fatty acids, octadecenoic acid, methyl ester (10.71%) and hexadecanoic acid, methyl ester were also identified. Polyunsaturated fatty acids are reported to reduce the development of dexamethasone and indomethacin-induced gastric ulcers.[23,24]

Several studies have associated the protection of gastric ulcer to the presence of phenolic acid and flavonoids in plant extracts. In the HPLC analysis, the most abundant compound detected was kaempferol which has been reported to have antiulcer effect in ethanol and stress-induced ulcer models.[25] Other identified phenolic compounds in moderate amount included apigenin-7-glucoside and catechin. The antiulcer property of catechin has been reported.[26,27]

Ethanol causes vascular damage and gastric cell necrosis by increasing mucosal permeability and release of vasoactive products, which, in turn, leads to ulcer formation.[28] It has been established that reactive oxygen species and

impaired prostaglandin synthesis contribute to gastric mucosal damage in experimental models of gastric lesions induced by ethanol.[29] The extract protected the gastric mucosa against damage caused by ethanol, suggesting a cytoprotective mechanism.

Ulcer formation induced by indomethacin, a nonsteroidal anti-inflammatory drug, is known to be related to the inhibition of prostaglandin biosynthesis, which are responsible for regulating the secretion of mucus and bicarbonate, blood flow, and proliferation of epithelial cells.[30] The extract exhibited protective effect in this model with maximal protection at 100 mg kg^{-1}. These results suggest the possible involvement of prostaglandin production in the gastroprotective effect of the extract.

Antioxidant properties are important mechanism of beneficial activity of plant-derived compounds and extracts. In the *in vivo* study, ethanol induced-treated rats had elevated MDA level with reduced GSH, CAT, and SOD activity, indicating oxidative stress. However, *S. arborea* extract reduced MDA level and increased GSH, CAT, and SOD activity, suggesting the involvement of antioxidant mechanism in the gastroprotective effect of the extract at the test dose.

The *in vitro* antioxidant activity of the fruit extract was also evaluated by four methods; DPPH free radical scavenging activity, reducing power assay, chelating activity, and hydroxyl radical scavenging. DPPH is one of the most widely used assays to evaluate the ability of compounds to act as free radical scavengers or hydrogen donors and thus evaluate antioxidant activity.[31] The reducing power of a compound may also serve as an indicator of its potential antioxidant activity. In this assay, the presence of reductants or plant extracts would result in the reduction of Fe^{3+} to Fe^{2+}.[32] Hydroxyl radical is a highly reactive free radical formed by the successive monovalent reduction of O_2 in cell metabolism. It can potentially react with all biological molecules such as DNA, proteins, lipids, and almost any constituent of cells.[33] The extract showed high antioxidant activity in DPPH free radical and hydroxyl radical assays and considerable reducing power capability but exhibited weak chelating property of ions. These results indicate the presence of some bioactive compounds in the extract with reducing ability and electron donor properties for neutralizing free radicals and converting them into stable products. These findings reinforced that the reduction in oxidative damage could be correlated with the antioxidant properties of the extract. The oral administration of the extract did not have any sign of acute toxicity in the animals, indicating that the LD_{50} value could be greater than 2,000 mg kg^{-1} and suggests the safety of the extract.[9]

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