Aqueous extract from *Ficus capensis* leaves inhibits key enzymes linked to erectile dysfunction and prevent oxidative stress in rats' penile tissue

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**A B S T R A C T**

Context: *Ficus capensis* Thunb (Moraceae) is a medicinal plant widely grown in tropical and subtropical regions with the leaf decoction commonly taken in traditional folklore as fertility agent in men for ages.

Aim: This study investigated the effects of aqueous extract from *Ficus capensis* leaves on angiotensin-I-converting enzyme (ACE), acetylcholinesterase (AChE) and arginase activities in vitro. The antioxidant properties of the extract as typified by the abilities to scavenge radicals [nitric oxide (NO), hydroxyl (OH)], chelate Fe2+ and inhibit Fe2+ -induced lipid peroxidation were also assessed.

Methods: The aqueous extract (1:10 w/v) of *Ficus capensis* leaves was prepared and the ability of the extract to inhibit arginase, angiotensin-I-converting enzyme (ACE), acetylcholinesterase (AChE) and antioxidant properties of the extract in rat’s penile tissue in vitro was investigated using various spectrophotometric methods. Phenolic constituent was carried-out using high performance liquid chromatography coupled with diode array detection (HPLC - DAD).

Results: The extract inhibited ACE (IC50 = 52.17), AChE (IC50 = 172.60 μg/mL) and arginase (IC50 = 112.50 μg/mL) activities in a dose-dependent pattern. Gallic acid, quercetin, caffeic acid, ellagic acid, rutin and chlorogenic acid were the most abundant phenolic compounds identified in the sample. Furthermore, extract scavenged NO (IC50 = 0.12 μg/mL) and OH (IC50 = 0.53 μg/mL) radicals, chelated Fe2+ (IC50 = 0.16 μg/mL) and inhibited Fe2+ -lipid peroxidation (IC50 = 435.17 μg/mL) dose-dependently.

Conclusion: Inhibition of ACE, AChE, arginase, Fe2+ -induced lipid peroxidation as well as radical scavenging and Fe2+ -chelating abilities could be some of the possible mechanisms by which *F. capensis* leaves could be used in the treatment/management of erectile dysfunction (ED).

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1. Introduction

Erectile dysfunction (ED), which is defined as the inability to achieve and/or maintain penile erection sufficient for satisfactory sexual performance, is a widespread problem affecting many sexually active men across all age groups [1]. Previous report has revealed that ED is prevalent in over 150 million men all over the world and has been predicted to affect about 250 million men by 2025 [1]. Normal penile erection is a function of neurovascular event, which depends on neural integrity, a functional vascular system and healthy cavernosal tissue [2]. These systems are mediated by nitric oxide (NO) via the activation of NO-cyclic guanosine monophosphate (cGMP) dilator pathway. The impairment of this pathway by different factors could lead to ED [2].

Increased activity of arginase enzyme, a metalloenzyme that converts L-arginine to urea and ornithine in a number of cells, has been implicated in the pathophysiology of ED [3]. Recent trends in the management of ED involve increase in NO levels with the use of arginase inhibitors. This is because in ED increase arginase activity limits NO synthase activity and thereby reduces NO biosynthesis [4–6]. Report has also shown that high blood pressure via inveterate change in blood pressure which can alter the flow of blood in penile vessels is another causative factor ED [3]. Moreover, angiotensin-II, which is produced from the conversion of angiotensin-I in a reaction catalysed by angiotensin-I-converting enzyme (ACE), is a potent vasoconstrictor capable of inducing vascular hypertrophy and endothelial dysfunction via decrease in NO production [7]. Also, ACE activity deactivates the physiological role of bradykinin, a vasodilator which has been implicated in erectile function via the release of NO and relaxation of the corpus cavernosum and consequently impair erectile function [8]. Likewise, the enzyme acetylcholinesterase (AChE) is found in penile tissue [9]. The enzyme causes hydrolysis of acetylcholine (ACh), a neurotransmitter reported to be involved in erectile function via the activation of endothelial NO synthase and consequently release/produce NO enzyme

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that causes endothelium-derived NO production [10–12]. The decrease in bioavailability of ACh restrains vascular relaxation and blood flow in erectile process [13]. Therefore, enhancement of ACh-producing system could contribute to penile erection [8] and sexuality by stimulating the penile cavernous nerves to evoke acetylcholine release [10–12], and inhibitor of AChE has been proven effective [9,13].

Oxidative stress has also been linked with ED due to excessive generation of free radicals in the penile (cavernosal) tissue [14]. Superoxide combines with NO to form highly toxic peroxynitrite (NOO−2); a culprit in the initiation/formation of lipid peroxidation and oxidative stress [15]. Oxidative stress in ED reduces the availability of NO which is required for penile erection [16]. Antioxidants are capable of reducing oxidative stress by scavenging free radicals and, phenolics are the most abundant antioxidants in plant based human diet such as fruits and vegetables [16]. Several studies have shown various relationships between the consumption of polyphenol or polyphenol-rich foods and diseases such as ED, diabetes, and cardiovascular and neurodegenerative diseases [17].

Ficus capensis Thumb (Moraceae) also known as Ficus sur Forssk is a medicinal plant widely grown in tropical and subtropical regions with the leaf decoction commonly taken as fertility agent in men [18] and for the treatment of dysentery, oedema, leprosy, epilepsy, rickets, gonorrhoea, respiratory disorders and emollient [19]. The leaves of F. capensis possess various pharmacological properties such as antioxidant, anti-inflammation, and antimicrobial effects [20]. However, there is dearth of information on the possible use of these plant leaves or its extract in any form for the treatment/management of ED. Hence, this study was designed to investigate the inhibitory effects of aqueous extract from F. capensis leaves on key enzymes (ACE, AChE and arginase) relevant to ED and its antioxidant potential.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals such as acetylthiocholine iodide, 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), trichloroacetic acid (TCA), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were sourced from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany). Acetic acid was procured from BDH Chemical Ltd., (Poole, England). Methanol, gallic, chlorogenic, caffeic and ellagic acids, and catechin were purchased from Merck (Darmstadt, Germany). Rutin, quercetin, quercitrin and isoquercitrin, rutin, and kaempferol. Stock solutions of standard references were prepared in the HPLC mobile phase at a concentration range of 0.030–0.250 mg/mL for kaempferol, quercetin, isoquercitrin, rutin, catechin, and epicatechin and 0.050–0.450 mg/mL for ellagic, gallic, and chlorogenic acids. Chromatography peaks were confirmed by comparing their retention time with those of reference standards and by DAD spectra (200–500 nm). All chromatography operations were carried out at ambient temperature and in triplicate [22].

2.2. Sample collection and preparation of aqueous extract

Fresh leaves of F. capensis were collected from a farm land around Akure metropolis, Nigeria. The leaves were collected in May, 2015 and authenticated at the Department of Plant Science, Ekiti State University by Mr F.O Omotayo with voucher number UHAE 2015/31 which was deposited in the university herbarium. The leaves were air dried at room temperature and pulverized which was sieved in Willey 60 mesh size and stored in a refrigerator. The powder sample was used for HPLC–DAD analysis. Five grammes of the sample was soaked in 100 mL of distilled water for about 24 h at 37 °C. The mixture was filtered using Whatman no. 1 and further centrifuged at 357.80 g for 10 min to obtain a clear supernatant which was used for subsequent analysis.

2.3. High performance liquid chromatography–diode array detector (HPLC) analysis

Chromatographic analyses were carried out under gradient conditions using C18 column (4.6 mm × 150 mm) packed with 5 μm diameter particles in C18 (Phenomenex, Torrance, California); the mobile phase was water containing 1% formic acid (A) and acetonitrile (B), and the composition gradient was 13% of B until 10 min and changed to obtain 20, 30, 50, 60, 70, and 20, and 10% B at 20, 30, 40, 50, 60, 70, and 80 min, respectively [21]. F. capensis leaf extract and mobile phase were filtered through 0.45 μm membrane filter (Millipore, Billerica, Massachusetts, USA) and then degassed by ultrasonic bath prior to use; the extract was analysed at a concentration of 20 mg/mL. The flow rate was 0.7 mL/min, the injection volume was 40 μL, and the wavelength in a photo-diode array detector – SPD-M204 was 254 nm for gallic acid, 280 nm for catechin and epicatechin, 325 nm for chlorogenic and ellagic acids, and 365 nm for quercetin, quercetin, isoquercitrin, rutin, and kaempferol. Stock solutions of standard references were prepared in the HPLC mobile phase at a concentration range of 0.030–0.250 mg/mL for kaempferol, quercetin, isoquercitrin, rutin, catechin, and epicatechin and 0.050–0.450 mg/mL for ellagic, gallic, and chlorogenic acids. Chromatography peaks were confirmed by comparing their retention time with those of reference standards and by DAD spectra (200–500 nm). All chromatography operations were carried out at ambient temperature and in triplicate [22].

2.4. Determination of total phenolic contents

The total phenol content of the extract was determined as described by Singleton et al. [23]. Briefly, appropriate dilution of the extract was oxidized with 2.5 mL 10% Folin–Ciocalteu’s reagent (v/v) and neutralized by 2.0 mL of 7.5% NaCO3. The reaction mixture was incubated for 40 min at 45 °C and the absorbance was measured at 765 nm in the spectrophotometer. Gallic acid was used as standard and the total phenol content was subsequently calculated as gallic acid equivalent. The total flavonoid content was determined using a slightly modified method [24]. Briefly, 0.5 mL of appropriate diluted extract was mixed with 0.5 mL of methanol, 50 μL of 10% AlCl3, 50 μL of 1 M potassium acetate, and 1.4 mL H2O. The mixture was incubated at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm. Quercetin was used as standard and the total flavonoid content was calculated as quercetin equivalent.

2.5. Nitric oxide (NO) radical scavenging activity

Nitric oxide radical scavenging assay was performed using Griess reagent method [25]. Briefly, 0.3 mL of sodium nitroprusside (5 mM) was added to 1 mL of each of various volumes (0–400 μL) of the extract and/or Vitamin C (Standard). The tubes were then incubated at 25 °C for 150 min. Thereafter, 0.5 mL of Griess reagent (prepared by equal volume of 1% sulphanilamide on 5% orthophosphoric acid and 0.01% naphthyl ethylenediamine in distilled water, used after 12 h of preparation) was added. The absorbance was measured at 546 nm. NO radical scavenging ability of the extract was calculated and expressed as percentage inhibition using the formula:

\[
\% \text{ Inhibition} = \left( \frac{\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}}}{\text{Abs}_{\text{ref}}} \right) \times 100
\]

where Abs_{sam} is the absorbance without the extract and Abs_{ref} is the absorbance of the extracts.
2.6. Hydroxyl radical (OH) scavenging ability

The method of Halliwell and Gutteridge [26] was used to determine the ability of the extract to scavenge hydroxyl radical produced from Fe(II)/H₂O₂-induced decomposition of deoxyribose. The extract/Vitamin C (standard) of various volumes (0–200 μL) was added to a reaction mixture containing 120 μL of 20 mM deoxyribose, 400 μL of 0.1 M phosphate buffer, and 40 μL of 500 μM of Fe₂SO₄, and the volume was made up to 800 μL with distilled water. The reaction mixture was incubated at 37 °C for 30 min and the reaction was then stopped by the addition of 0.5 mL of 28% trichloroacetic acid. This was followed by the addition of 0.4 mL of 0.8% thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in a spectrophotometer. OH radical scavenging ability was calculated using Eq. (1).

2.7. Fe²⁺ chelation assay

The Fe²⁺-chelating ability of the extracts was determined using a slightly modified method [27,28]. Freshly prepared 500 μM FeSO₄ (150 μL) was added to a reaction mixture containing 168 μL of 0.1 M Tris–HCl (pH 7.4), 218 μL of saline (0.9%), and extracts and/or EDTA (Standard) (0–100 μL). The reaction mixture was incubated for 5 min, before the addition of 13 μL 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe(II)-chelating ability was subsequently calculated (Eq. (1)).

2.8. Handling of experimental animals

Thirty adult male Wistar strain albino rats (weighing between 205 and 210 g) of about 6–7 weeks old were purchased from the animal breeding colony of the Animal Production and Health, Federal University of Technology, Akure. Their handling was in accordance with the Guide for Care and Use of Laboratory Animals prepared by the National Academy of Science, published by the National Institute of Health (USA) [29]. The rats were allowed to acclimatize for 14 days and maintained at room temperature under laboratory conditions with access to standard animal feed and water ad libitum.

2.9. Preparation of penile tissue homogenate and lipid peroxidation assay

The rats were decapitated under mild diethyl ether anaesthesia and rapidly dissected. The penile tissue was rapidly removed and placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1/10 w/v) with about 10-up-and-down strokes at approximately 107.34 °C. The homogenate was subsequently centrifuged for 10 min at 3000 × g. The supernatant was used as the source of enzyme. The high-speed supernatant (S1) that was kept for lipid peroxidation assay [30]. Hundred microliters of penile homogenate supernatant was mixed with a mixture containing 30 μL of 0.1 M Tris–HCl buffer (pH 7.4), extract and/or EDTA (standard), and 30 μL of the pro-oxidant (250 μM iron (II) sulphate). The volume was made up with 300 μL of distilled water before incubation at 37 °C for 2 h. The colour reaction was developed by adding 300 μL of 8.1% SDS (sodium dodecyl sulphate) to the reaction mixture containing the homogenate, followed by the addition of 600 μL of acetic acid/HCl (pH 3.4) and 600 μL of 0.8% thiobarbituric acid (TBA). This mixture was incubated at 100 °C for 1 h. The absorbance of thiobarbituric acid reactive species (TBARS) produced was measured at 532 nm. MDA (malondialdehyde) produced was expressed as % control [31].

2.10. Angiotensin-I converting enzyme (ACE) inhibition assay

The inhibition of ACE activity of the extract was determined according to the described method of Cushman and Cheung [32]. Different volumes (5–25 μL) of the extract or Captopril (Standard drug) and 50 μL of penile homogenate as a source for ACE (EC 3.4.15.1) were pre-incubated at 37 °C for 15 min. Thereafter, enzymatic reaction was initiated by adding 150 μL of 8.33 mM ACE substrate [ hippuryl-l-histidyl-l-leucine (HHL)] in 125 mM of Tris–HCl buffer (pH 8.3) to the reaction mixture and incubated at 37 °C for 30 min. The reaction was stopped by adding 250 μL of 1 M HCl. The hippuric acid (Bz-Gly) produced by the reaction was extracted with 1.5 mL ethylacetate. The mixture was then centrifuged to separate the ethyl acetate layer, after which the 1 mL of the ethyl acetate layer was transferred to a clean test tube and evaporated to dryness. The residue was re-dissolved with distilled water and its absorbance was measured at 228 nm. The control experiment was performed without the test sample. The percentage ACE inhibition was subsequently calculated (Eq. (1)).

2.11. Arginase inhibition assay

Penile homogenates were prepared by homogenizing 10 g (w/v) of penile tissue in three volumes of homogenization cold buffer (phosphate buffer, pH 7.2). The homogenate was centrifuged for 20 min at 357.80 g and the supernatant was used as the source of enzyme. Arginase activity was determined by the measurement of urea produced by the reaction of Ehrlich’s reagent. The reaction mixture contained in final concentration 1.0 mM Tris–HCl buffer, pH 9.5, containing 1.0 mM MnCl₂ 0.1 M arginine solution and 50 mM of the enzyme preparation in a final volume of 1.0 mL. The mixture was incubated for 10 min at 37 °C. The reaction was terminated by the addition of 2.5 mL Ehrlich reagent (2.0 g of p-dimethylaminobenzaldehyde in 20 mL of absolute hydrochloric acid (37% purity) and made up to 100 mL with distilled water). The optical density reading was taken after 20 min at 450 nm. The control experiment was performed without the test sample or standard and the arginase inhibitory activity was calculated (Eq. (1)) and expressed as percentage inhibition [33].

2.12. AChE inhibition assay

Penile homogenate was prepared by homogenizing 10 g (w/v) of penile tissue in three volumes of homogenization cold buffer (phosphate buffer, pH 7.2). The homogenate was centrifuged for 20 min at 357.80 g and the supernatant was used as the source of enzyme. The AChE activity was determined in a reaction mixture containing 200 μL of the homogenate in 0.1 M phosphate buffer, pH 8.0, 100 μL of a solution of 5.5'-dithio-bis (2-nitrobenzonic) acid (DTNB 3.3 mM in 0.1 M phosphate buffered solution, pH 7.0, containing NaHCO₃ 6 mM), extract/Pretigmine [standard AChE inhibitor (0–100 μL)] and 500 μL of phosphate buffer, pH 8.0. After incubation for 20 min at 25 °C, acetylthiocholine iodide (100 μL of 0.05 mM water solution) was added as the substrate, and AChE activity was determined by UV spectrophotometry from the absorbance changes at 412 nm for 3 min at 25 °C. The AChE activity was expressed as percentage AChE inhibition [34] following Eq. (1).

2.13. Data analysis

The results of three replicate experiments were pooled and expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to analyse the mean and the post hoc treatment was performed using Duncan multiple test [35]. Significance was accepted at P < 0.05. IC₅₀ (extract concentration causing 50% effectiveness) was calculated using non-linear regression with GraphPad Prism version 5.00 for windows. Differences between groups of HPLC were assessed by an analysis of variance model and Tukey’s test. The level of significance for the analyses was set to P < 0.05. These analyses were performed by using the free software R version 3.1.1.
3. Results

3.1. Phenolic constituents and total phenolic contents

The HPLC–DAD analysis as presented in Table 1 revealed the presence of phenolic acids such as gallic acid (51.19 mg/g), catechin (12.75 mg/g), chlorogenic acid (23.98 mg/g), caffeic acid (30.67 mg/g), ellagic acid (31.45 mg/g), caffeic acid (30.67 mg/g) and epicatechin (13.26 mg/g), and flavonoids such as quercetin (49.07 mg/g), quercitrin (18.54 mg/g), kaempferol (6.15 mg/g), and rutin (25.03 mg/g). The results of the total phenol and flavonoid contents of the F. capensis leaf extract are presented in Table 2. The total phenol content reported as gallic acid equivalent was 61.18 mg GAE/100 g, while the total flavonoid content reported as quercetin equivalent was 32.14 mg QUE/100 g.

3.2. Radical scavenging and Fe$^{2+}$ chelating abilities

The extract scavenged OH and NO radicals in a dose-dependent manner as shown in Fig. 1a and b respectively with IC$_{50}$ values of 0.53 mg/mL (OH radical) and 0.12 mg/mL (NO radical) (Table 2). Fig. 1c shows the Fe$^{2+}$–chelating ability of the extract from F. capensis. The extract was able to chelate Fe$^{2+}$ in a dose-dependent manner with IC$_{50}$ = 0.16 mg/mL (Table 2).

3.3. Inhibition of malondialdehyde production

The incubation of penile homogenate in the presence of Fe$^{2+}$ caused a significant increase (P < 0.05) in the malondialdehyde (MDA) content (153.08%) as shown in Fig. 1d. However, the addition of extract from F. capensis leaves in a dose-dependent manner inhibited MDA levels with IC$_{50}$ = 435.17 µg/mL (Table 2).

4. Discussion

F. capensis is known with various medicinal benefits which have been attributed to its phytochemicals such as phenolic compounds [20]. Study has revealed that phenolics of medicinal plants are related to their antioxidant capacity [36]. The result obtained in this study shows that the studied extract exhibited hydroxyl (OH) and nitric oxide (NO) radical scavenging abilities. In biological system, hydroxyl radical (OH) could be generated from superoxide anion and hydrogen peroxide by the Haber–Weiss reaction or from hydrogen peroxide via the Fenton reaction [37]. OH radical is highly reactive, energetic, short live and very toxic to cells [38], and played a significant role in diabetes-associated ED [39,40]. Nitric oxide (NO) radical is capable of mediating many cytotoxic and pathological processes and could contribute in part to the formation of plaque in penile tissues. This radical is usually generated in response to inflammation [41]. Although NO is required for the initiation of penile erection but can also combine with superoxide (O$_2^-$) to form peroxynitrite: a cytotoxic compound that is capable of inducing the formation of lipid peroxidation and nitration, which leads to the formation of malondialdehyde and hydroperoxides; a risk factor in pathophysiology of ED [5,42,43]. Therefore, radical scavenging abilities of the studied extract as revealed in this study could be beneficial in the management of ED and could be attributed to the presence of polyphenols [44].

The disruption of iron balance in the body system can bring about iron overload: another culprit in oxidative stress-induced ED [42]. Iron overload increases the formation of reactive oxygen species (ROS) which could induce initiation of lipid peroxidation [45]. Iron II (Fe$^{2+}$) reacts with H$_2$O$_2$ in the Fenton reaction to produce the highly reactive hydroxyl radical, which can damage proteins, lipids, and nucleic acids. Our findings in this study revealed that extract from F. capensis leaves chelated Fe$^{2+}$ in a dose-dependent pattern. This chelating ability might be due to the presence of polyphenols. Phenolic compounds can form a complex with iron thereby aiding its excretion from the body. Fe$^{2+}$–chelating ability of extract could therefore be beneficial in the management/prevention of ED [46]. Our previous research has showed that phenolic compounds such as gallic acid, chlorogenic acid, catechin, kaempferol, quercetin, and quercitrin are capable of chelating metallic ion [47].

Superoxide anions and other reactive oxygen species (ROS) have been shown to be a major contributor to the pathogenesis of ED via the initiation of lipid peroxidation [48,49]. Increase in malondialdehyde (MDA) content when rat penile homogenate was incubated with Fe$^{2+}$ could be through the breakdown of hydrogen peroxide to generate OH radical [50]. Fe$^{2+}$-induced lipid peroxidation and oxidative stress have been reported to cause injuries to the penile tissue [51–53]. However, the inhibition of MDA production caused by the extract from F. capensis leaves could be attributed to the ability of the extract to chelate Fe$^{2+}$ and scavenged radicals thus preventing the initiation of oxidative damage [54].

Some experimental investigations have revealed that increase in blood pressure could be linked with ED [55]. Therefore inhibition of ACE which catalyses the conversion of angiotensin I to angiotensin II has marked therapeutic effects in the management of high blood pressure and consequently activating the release of NO which improves erectile function. Furthermore, the inhibition of ACE activates bradykinin which has been implicated in erectile function [5,54]. The observed

### Table 1

<table>
<thead>
<tr>
<th>Components</th>
<th>mg/g</th>
<th>%</th>
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<tbody>
<tr>
<td>Gallic acid</td>
<td>51.19 ± 0.03</td>
<td>5.11</td>
</tr>
<tr>
<td>Catechin</td>
<td>12.75 ± 0.01</td>
<td>1.27</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>23.98 ± 0.02</td>
<td>2.39</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>30.67 ± 0.01</td>
<td>3.06</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>31.45 ± 0.03</td>
<td>3.14</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>13.26 ± 0.02</td>
<td>1.32</td>
</tr>
<tr>
<td>Rutin</td>
<td>25.03 ± 0.01</td>
<td>2.5</td>
</tr>
<tr>
<td>Quercitrin</td>
<td>18.54 ± 0.02</td>
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<td>Quercetin</td>
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<td>4.9</td>
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<tr>
<td>Kaempferol</td>
<td>6.15 ± 0.03</td>
<td>0.61</td>
</tr>
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</table>

Values represent means ± standard deviation of triplicate readings.

### Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenol (gallic acid equivalent)</td>
<td>61.18 ± 0.30</td>
</tr>
<tr>
<td>Total flavonoid content (quercetin equivalent)</td>
<td>32.14 ± 0.16</td>
</tr>
<tr>
<td>NO radical scavenging ability (mg/mL)</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>OH radical scavenging ability (mg/mL)</td>
<td>0.53 ± 0.00</td>
</tr>
<tr>
<td>Fe$^{2+}$ chelating ability (mg/mL)</td>
<td>0.16 ± 0.00</td>
</tr>
<tr>
<td>Inhibition of Fe$^{2+}$-induced lipid peroxidation (µg/mL)</td>
<td>435.17 ± 4.18</td>
</tr>
<tr>
<td>Inhibition of ACE activity (µg/mL)</td>
<td>52.17 ± 6.46</td>
</tr>
<tr>
<td>Inhibition of AChE activity (µg/mL)</td>
<td>172.60 ± 8.79</td>
</tr>
<tr>
<td>Inhibition of arginase activity (µg/mL)</td>
<td>112.50 ± 7.03</td>
</tr>
</tbody>
</table>

Values represent means ± standard deviation of triplicate readings.

### 3.4. Effects of the extract on ACE, AChE and arginase activities

The interaction of the extract with ACE as shown in Fig. 1e revealed that the extract inhibited ACE (IC$_{50}$ = 52.17 µg/mL) activity in a dose-dependent manner. Also, the result in Fig. 1f revealed that the extract inhibited AChE activity in a dose-dependent manner with IC$_{50}$ = 579.63 µg/mL (Table 2). Furthermore, extract inhibited arginase activity in a dose-dependent manner with IC$_{50}$ = 112.50 µg/mL (Table 2).
Fig. 1. (a) Nitric oxide (NO) radical scavenging ability; (b) hydroxyl (OH) radical scavenging ability; (c) Fe^{2+}-chelating ability; (d) Fe^{2+}-induced MDA inhibition in rat penile tissue homogenate; (e) ACE inhibitory ability, (f) AChE inhibitory ability, (g) arginase inhibitory ability of extract from F. capensis leaves. Values represent means ± standard deviation of triplicate readings.
inhibitory effects of the extract on ACE activity could contribute to erectile function. Previous report has shown that phenolics can interact with disulfide bridges of the ACE enzyme thereby modifying the structure and reducing its activity [55]. Moreover, Oboh and Rocha [16] also reported that phenolic extract from *Moringa oleifera* leaves inhibited ACE activity.

The penile physiological states of flaccidity or erection result from the contraction or relaxation, respectively, of smooth muscle cells in the penile corpora cavernosa. On sexual stimulation the cavernous nerves release ACh that could stimulate the release of NO via the NO–cGMP pathway [56]. Thus, dose-dependent inhibition of arginase activity by the studied extract could be of great importance in the management of ED as this could increase genital blood flow during sexual arousal. The inhibitory properties of the extract could be attributed to the phenolic components. Polyphenols have been reported to possess inhibitory effects on arginase activity [4,57]. More so, flavonoids such as catechin, epicatechin, and quercetin and its derivatives (quercitrin and isoorientin) have shown to be strong inhibitors of arginase activity and could be linked to the formation of hydrogen bond and hydrophobic interactions between these polyphenolic compounds and the hydrophobic active site of the enzyme [4,57].

5. Conclusion

The antioxidant properties and ACE, AChE and arginase inhibitory effects of extract from *F. capensis* leaves could be therapeutic points in the management of ED. These findings revealed the possible mechanism of action of the *F. capensis* leaf in the management/treatment of ED. However, this health promoting effect is suggested to be a function of its phenolic compounds.

Conflict of interests

The authors declare no conflict of interests regarding this paper.

References


