NFS Journal 4 (2016) 15–21

Contents lists available at ScienceDirect

NFS Journal

journal homepage: http://www.journals.elsevier.com/nfs-journal/

Original article

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

Seun F. Akomolafe [a,b,*], Ganiyu Oboh [b], Sunday I. Oyeleye [b], Aline A. Boligon [c]

[a] Department of Biochemistry, Ekiti State University, PMB 5363, Nigeria
[b] Functional Foods and Nutraceuticals Unit, Department of Biochemistry, Federal University of Technology, PMB 704, Akure 340001, Nigeria
[c] Phytochemical Research Laboratory, Department of Industrial Pharmacy, Federal University of Santa Maria, Building 26, Room 1115, 97105-900 Santa Maria, RS, Brazil

ARTICLE INFO

Article history:
Received 5 February 2016
Received in revised form 9 June 2016
Accepted 14 June 2016
Available online 18 June 2016

Keywords:
Ficus capensis
Erectile dysfunction
ACE
Arginase
AChE
Polyphenols

ABSTRACT

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 Fe^{2+} and inhibit Fe^{2+}-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 (IC_{50} = 52.17), AChE (IC_{50} = 172.60 μg/mL) and arginase (IC_{50} = 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 (IC_{50} = 0.12 μg/mL) and OH (IC_{50} = 0.53 μg/mL) radicals, chelated Fe^{2+} (IC_{50} = 0.16 μg/mL) and inhibited Fe^{2+} lipid peroxidation (IC_{50} = 435.17 μg/mL) dose-dependently.
Conclusion: Inhibition of ACE, AChE, arginase, Fe^{2+}-induced lipid peroxidation as well as radical scavenging and Fe^{2+}-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).

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

* Corresponding author.

http://dx.doi.org/10.1016/j.nfs.2016.06.001

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^-); 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 Thunb (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. Chemical and reagent

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 kaempferol were acquired from Sigma Chemical Co. (St. Louis, MO, USA). Sodium carbonate, methanol, aluminium chloride, potassium acetate, potassium ferricyanide, and ferric chloride were of analytical grade while water was glass distilled. HPLC–DAD equipped with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan) and Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software were used in this study. Kenxin refrigerated centrifuge Model KX3400C was used while UV–Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom) was used to measure the absorbance.

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, quercitrin, 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, quercitrin, 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–Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% $NaCO_3$. 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% $AlCl_3$, 50 μL of 1 M potassium acetate, and 1.4 mL H_2O. 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:

$$\% \text{ Inhibition} = [(\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}})/\text{Abs}_{\text{ref}}] \times 100 \quad (1)$$

where Abs_{ref} is the absorbance without the extract and Abs_{sam} is the absorbance of the extracts.