ANTIOXIDANT PROPERTY AND INHIBITION OF ERECTILE DYSFUNCTION-LINKED ENZYMES BY SOME COLA SPP AND GACINIA KOLA


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Abstract

Erectile dysfunction (ED) is a common problem among men. Kola nut seeds are claimed to possess aphrodisiac effects and have been employed in folklore for the management of ED. This study investigated the possible mechanism of actions behind the aphrodisiac properties of some tropical Cola spp [Cola nitida (white and red) and Cola acuminata] and, Garcinia kola via their antioxidant properties and possible inhibition of some key enzymes (AChE, ACE and Arginase) linked to ED in vitro. The aqueous extract was prepared and the radicals (DPPH* and ABTS*) scavenging and Fe2+ chelating abilities, ferric reducing antioxidant power (FRAP) as well as the effects on the Fe-induced lipid peroxidation in rats' penile homogenate were determined. The total phenolic (phenol and flavonoids) contents were also determined. The extracts scavenged the radicals, chelated Fe2+, and inhibited Fe2+-lipid peroxidation in a dose-dependent pattern. The results revealed that Cola acuminata had the higher arginase and AChE inhibitory activities than Garcinia kola while the later had the highest ACE inhibitory effect. Interestingly, Cola acuminata had the highest phenolic content among the entire Cola sample tested. These antioxidative and enzyme inhibitory effects of the selected kola nut seeds may be due to their phenolic contents and these could also be some possible mechanisms underlying their erectogenic potential in folklore medicines.

Keyword: Cola spp, aphrodisiac, erectile dysfunction, antioxidant, enzyme inhibition

Introduction

Sexual activity is among the important factors in social and biological relationships of human life, and erectile dysfunction (ED) in men constitutes a major hindrance to the sexual relationship. ED is the inability to achieve/maintain penile rigidity enough for sexual performance (Somer et al. 2007). Age and health challenges are the most common risk factor of ED, as it occurs commonly between the age 45 and above (Papaharitou et al. 2006). The normal erectile function is controlled by a series of biochemical actions, leading to increased blood flow into the penile tissue (Gratzke et al. 2010; Andersson, 2011). The principal biomolecule is nitric oxide (NO), which could be activated by NO–cyclic guanosine monophosphate (cGMP) dilator pathway; hence, its reduced level/impaired production could lead to ED (Gratzke et al. 2010; Andersson 2011; Oboh et al. 2015). Several mechanisms have been reported to impair NO level/production; among such is NO–radical species interaction, overexpression of arginase and acetylcholinesterase (AChE) as well as phosphodiesterase type-5 (PDE-5) activities (Butterfield and Lauderback, 2002; Segal et al. 2012; Oboh et al., 2017).

The use of natural aphrodisiac, which is described as any natural substance that enhances penile arousal and rigidity in the treatment of ED, is on the increase nowadays. This is because of the inability to afford modern medical healthcare and several side effects exhibited by the synthetic drugs (Moreira et al. 2000; Kiroglu et al. 2006). Herbal products exhibiting little or no side effects but have beneficial pharmacological and therapeutic uses in a number of illnesses (Weber et al. 1999). Many plant extracts are traditionally used to improve sexual performances (Kametchouing et al. 2002; Kamatenesi-Mugisha and Oryem-Origa, 2005).

In Nigeria, Cola nitida alba, (White), Cola nitida rubra (Red), A. Chev, and Cola acuminata Schott & Endl) and Garcinia kola seeds are claimed to possess aphrodisiac potentials (Kamatenesi-Mugisha and Oryem-Origa 2005; Atawodi et al. 2007; Ndukui et al. 2012). These plants are endemic in Central and Western Africa, and are used primarily as stimulants and natural aphrodisiacs. However, much information has not been provided on the scientific justification and biochemical mechanisms behind their use as natural aphrodisiacs. In view of this, we aimed at investigating and comparing the antioxidant and inhibition of enzymes-linked to ED (arginase, ACE and AChE) the aqueous extracts from white and red Cola nitida (WCN and (RCN), Cola acuminata (CA) and Garcinia kola (GK) seeds.

Materials and Method

Chemical and reagent

All chemicals and reagents used were of analytical...
Angiotensin I Converting Enzyme (ACE) Inhibition Assay

Extract (50 µL) and penile homogenate as source of ACE were incubated at 37 °C for 15 minutes. The enzymatic activity was initiated by adding 8.33 mM of the substrate (150 µL, Bz-Gly-His-Leu in 125 mM Tris-HCl buffer pH 8.3). After incubation for 30 minutes at 37°C, the action was arrested by adding 250 µL of 1 M HCL. The Gly-His bond was then cleaved, and extracted with 1.5 mL ethyl acetate, and centrifuged to separate the ethyl acetate layer, which was transferred to a clean test tube and evaporated to dryness. The residue was re-constituted with DW and its absorbance was measured at 228 nm. The ACE inhibitory activity was calculated and expressed as percentage inhibition using eq. 1 (Ademiluyi et al. 2016).

Acetylcholinesterase (AChE) Inhibition Assay

Acetylcholinesterase (AChE) inhibitory activity of the extracts was assessed (Ellman et al. 1961). The AChE activity was determined in a reaction mixture containing 200 µL of the penile homogenate as source AChE (EC 3.1.1.7), 5–dithio–bis -2-nitrobenzoicacid (DTNB) 3.3 mM in 0.1 M phosphate buffered solution, pH 7.0, containing NaHCO 3 6 mM, extracts (0–100 µL) and 500 µL of phosphate buffered, pH 8.0. After incubation for 20 minutes at 25°C, acetylthiocholine iodide (100 µL, 0.05 mM water solution) was added as the substrate. AChE inhibition was measure at 412 nm for 30 minutes at 25 C. The AChE inhibitory activity of the extracts was expressed as percentage inhibition using eq. 1.

Determination of Radicals (DPPH and ABTS) Scavenging and Fe-chelating Abilities

The free radicals scavenging ability of the sample extracts was determined using two models; the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radicals scavenging abilities according to the method of Oboh et al. (2016) and Ademiluyi et al. (2016) respectively. The DPPH method was based on the strength of the extract to reduce the violet solution of DPPH in ethanol to colorless solution within 10 minutes in the dark. The absorbance of the colourless solution was taken at 516 nm and the radical scavenging ability was subsequently calculated. The ABTS method involved the ability of the extracts to decolourize the ABTS radical into colourless solution, which was measured at 734 nm after 15 minutes of the reaction. The trolox equivalent antioxidant capacity was subsequently calculated.
The method of Ademiluyi et al. (2016) was used for the determination of Fe²⁺ chelating ability of the samples which was based on the ability of the extract to decrease the red colour intensity formed from the complex formation between 1, 10-phenanthroline and Fe²⁺. The resultant colour intensity was subsequently measured at 510 nm.

**Lipid Peroxidation and Thiobarbituric Acid Reactions**

The lipid peroxidation assay was carried out using a modified method of Akomolafe et al. (2017). Briefly, the penile homogenate was incubated with the reagent containing tis-HCL buffer, pro-oxidant (250 µM FeSO₄, sodium lauryl sulphate (SDS) and 10% (w/v) trichloroacetic acid (TCA) and extracts. The MDA produced was reacted with a chromogenic TBA to form a pink thiobarbituric acid reactive substance (TBARS) that was subsequently measured by spectrophotometer at 532 nm.

**Determination of Total Phenol and Total Flavonoid Contents**

The total phenol content of the extract was determined using the method reported by Adefegha et al (2016) and the total phenolic content was subsequently calculated as gallic acid equivalent (GAE) using gallic acid as the standard. The total flavonoid content of the extract was determined using the method used in the report of Adefegha et al. (2016) and calculated using quercetin as standard.

**Data Analysis**

Data obtained in triplicate experiments were pooled and analyzed with descriptive statistic (mean, standard deviation, table and figure). The means were compared using one way Analysis of Variance (ANOVA) followed by Turkey test, for mean separation where significant difference occurred, using statistical package for social science (SPSS) 16.0 for windows. The significance level was taken at p<0.05 and the IC₅₀ (sample concentration causing 50% inhibition/antioxidant activity) was subsequently calculated.

**Results and Discussion**

In this study, the interaction of the kola seeds extracts with arginase was investigated and the result is presented in Figure 1A. The result revealed that the extracts inhibited the arginase activity in a concentration-dependent manner. The IC₅₀ value (Table 1) revealed that there is a marked difference (P < 0.05) in the arginase inhibitory activities of the Cola seed. CA had the highest arginase inhibitory activity, followed by WCN, while GK had the least.

![Figure 1](image)

**Figure 1** Inhibition of arginase activity by Cola seed extract. Values represent mean of standard deviation of triplicate readings.

The use of medicinal plants in traditional medicine for the treatment of human ailments is a long way practice. Several herbal materials, including Cola nitida (white and red), Cola acuminata and Garcinia Kola seeds are commonly used for the treatment of erectile dysfunction (ED) in folklore (Atawodi et al. 2007; Singhet 2012). The potentials of these kola seeds have been associated with their constituent bioactive components, including polyphenols (Atawodi et al. 2007). Penile erection is mediated by the production of nitric oxide (NO), which is synthesized by NO synthase (NOS) from L-arginine (Oubanjo et al. 2017; Oboh et al. 2017). Arginase, which is an enzyme of the urea cycle, involving in the conversion L-arginine to L-ornithine and urea, has been found in the human corpus cavernosum (Cox et al. 1999; Bivalacqua et al. 2001). Given that arginine is a common substrate for both NOS and arginase, hence overexpression of arginase activity could...
The study of the kola seeds on ACE activity (Figure 2) revealed that ACE activity was inhibited. As listed in the IC\textsubscript{50} Table (1), CA had the highest ACE inhibitory activity followed by RCN, while WCN and GK had the least with no significant difference (P > 0.05). According to Becker et al. (2001), experimental reports have shown that high blood pressure, otherwise known as hypertension and ED are interrelated (McCullough 2003; Doumas et al. 2007). This is because over expression of ACE, which is common to both pathologies have been linked. ACE catalyses the conversion of angiotensin I to angiotensin II, and deactivate bradykinin, which has been implicated in erectile function (Teixeira et al., 1997; Doumas et al., 2007). Also the production of angiotensin II induced high blood pressure, a major culprit in the pathophysiology of ED (Oyeleye et al., 2018). ACE inhibitors play a therapeutic role in patients who have hypertension and ED. The observed inhibitory effects of the seed extracts on ACE activity could be responsible for the penile function and rigidity potentials as reported in folklore medicine. This effect could, however, be linked to the phenolic constituents, Previous report has shown that phenolic can interact with disulphide bridges at the active site of ACE, thereby, modifying the enzyme structure, and consequently reducing its activity (Stefan et al. 2003).

Penile tissues from humans and several animal species have been reported to be rich in cholinergic nerves (Hedlund et al. 1999, 2000). Acetylcholine (ACH) in these nerves acts on muscarinic receptors located on corpus cavernosum smooth muscle cells and on the endothelium of sinusoids. This causes a fall in cystolic Ca\textsuperscript{2+} and hence induces smooth muscle relaxation. ACh also acts on vascular endothelium to release NO via eNOS, which in turn could stimulate an increase in the cGMP level of the corpus cavernosum and favour penile erection. However, AChE rapidly degrades ACh; thereby render the potential use of ACh less, hence inhibition of AChE would be beneficial in maintaining ACh-induced NO production. This study revealed that Cola seeds inhibited AChE activity (Figure 3). The IC\textsubscript{50} values (Table 1) revealed that WCN had the highest AChE inhibitory activity compared to CA and GK, while RCN had the least. The observed inhibition of the penile AChE activity by the tropical kola nut seed indicates their erectogenic modulatory property. The penile's AChE inhibitory effect of some phenolic compounds has been reported (Akomolafe et al. 2016; Oboh et al. 2017; Odubanjo et al. 2017), hence, inhibit endothelium-derived NO production by depleting the available substrate needed for NOS for the production of NO (Akomolafe et al. 2016; Adefegha et al. 2017). Therefore, inhibition of arginase activity could be a control point, thereby making L-arginine available for eNOS activity for the production of NO. The arginase inhibitory property of the kola seeds could be attributed to the phenolic components. Polyphenol generally have been reported to possess arginase inhibitory potentials, as a result of their ability to form hydrogen bonds between the polyphenol compounds and the active site of the enzyme (Akomolafe et al. 2016; Oboh et al. 2017; Odubanjo et al. 2017). Hence, the observed arginase inhibitory effect of the kola nut seeds could explain their potential therapeutic use in the folklore, for the management of ED.
Alteration in the integrity of the endothelial layer’s cell of the corpus cavernosum could resulted to ED. Studies have reported the vulnerability of endothelial cells to free radical damage (Youdim et al. 2002), hence, improved antioxidant level could help counteract this free radical mediated damage to the endothelial cells, and consumption of the plant based foods rich in phenolic compounds has been reported to be of help (Youdim et al. 2002). The antioxidant properties of the kola seed extracts were tested using five (5) models of antioxidant assays: radicals (ABTS\(^*\) and DPPH) scavenging, Fe\(^{2+}\) chelating abilities and ferric reducing antioxidant property as well as inhibition of Fe-induced TBARS production models.

### Table 1: IC\(_{50}\) values (µg/ml) of the arginase, angiotensin-1 converting enzyme and acetylcholinesterase inhibitory activities, and DPPH radical scavenging and Fe\(^{2+}\) chelating abilities of some tropical Cola seeds

<table>
<thead>
<tr>
<th>Samples</th>
<th>WCN</th>
<th>RCN</th>
<th>CA</th>
<th>GK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginase</td>
<td>0.51±0.03(^b)</td>
<td>0.58±0.02(^c)</td>
<td>0.40±0.02(^a)</td>
<td>0.66±0.03(^d)</td>
</tr>
<tr>
<td>ACE</td>
<td>0.51±0.02(^a)</td>
<td>0.40±0.02(^b)</td>
<td>0.33±0.02(^a)</td>
<td>0.51±0.03(^c)</td>
</tr>
<tr>
<td>AChE</td>
<td>0.34±0.05(^a)</td>
<td>1.15±0.06(^c)</td>
<td>0.64±0.04(^b)</td>
<td>0.62±0.02(^b)</td>
</tr>
<tr>
<td>DPPH radical</td>
<td>5.19±0.05(^d)</td>
<td>2.39±0.04(^c)</td>
<td>1.90±0.07(^a)</td>
<td>2.04±0.08b</td>
</tr>
<tr>
<td>Fe(^{2+}) Chelation</td>
<td>31.70±0.22(^a)</td>
<td>35.75±0.30(^d)</td>
<td>20.94±0.70(^b)</td>
<td>26.49±0.20b</td>
</tr>
</tbody>
</table>

Values represent means of triplicate. Values with different alphabet along the same row are significantly different (P>0.05). Key- WCN: White *Cola nitida*, RCN: Red *Cola nitida*, CA: *Cola* acuminate, GA: *Garcinia Kola*
The result revealed that the extracts scavenged DPPH radical in dose-dependent manner (Figure 4). CA had higher DPPH radical scavenging ability ($P > 0.05$), followed by GK and RCN while WCN had the least (Table 1). CA had the lowest ABTS radical scavenging ability followed by WCN and RCN, while and GK had the least (Table 2). All the seed extracts chelated Fe$^{2+}$ (Figure 5). However, CA had the highest Fe$^{2+}$ chelating abilities (Table 1). The FRAP result of the Cola nut seeds, reported as ascorbic acid equivalent (AAE) shown that CA seed had the highest reducing power while WCN had the least. Incubation of the rat's penile homogenate with 250 µM Fe$^{2+}$ caused a significant increase ($P<0.05$) in the rat's penile MDA content (Figure 6). All the kola seed inhibited the production of MDA in the tissue homogenates, with CA seed extract having the least MDA produced (Figure 6)
The results of the total phenol and flavonoid contents of the tropical kola nut seeds are presented in Table 2. The total phenol content ranged from 0.98 (WCN) to 1.84 mg/GAE 100 g to (CA), while the total flavonoid content ranged from 0.52 (GA) to 0.80 mg/QE 100 g (CA). It was evident that kola seeds are rich in phenolic compound which validated their antioxidant and inhibition of enzymes associated with ED. The studied two Cola species (Cola nitida and acuminata) have been reported to contain appreciable amounts of polyphenols [(+)-catechin), (-)-epicatechin, apigenin, narigenin, and chlorogenic, quinic and tannic acids] and purine alkaloid (caffeine and theobromine) (Odebode 1996; Atawodi et al. 2007; Oboh et al. 2014a; Oboh et al. 2014b). According to previous findings, phenolic compounds and alkaloid from plant extracts have been reported to possess the antioxidant property and inhibit enzymes related to ED (Akomolafe et al. 2016; Adefgha et al. 2017; Odubanjo et al. 2017; Oboh et al. 2017). Also, various phenolic compounds have been reported to affect smooth muscle contractility in response to various agonists (Xu et al., 2007).

Table 2 Total phenol and flavonoid contents, ABTS radical scavenging ability and the reducing power of the selected tropical kolanut seeds

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total phenol (mg GAE/g)</th>
<th>Total flavonoid (mg QE/g)</th>
<th>ABTS* scavenging (mmol. TEAC/g)</th>
<th>FRAP (mg AAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WCN</td>
<td>0.98 ± 0.02d</td>
<td>0.63±0.02c</td>
<td>0.66±0.03b</td>
<td>32.76±0.12c</td>
</tr>
<tr>
<td>RCN</td>
<td>1.35±0.03b</td>
<td>0.68±0.05b</td>
<td>0.68±0.05b</td>
<td>40.52±0.23b</td>
</tr>
<tr>
<td>CA</td>
<td>1.84±0.11a</td>
<td>0.80±0.02a</td>
<td>0.80±0.02a</td>
<td>87.22±0.02a</td>
</tr>
<tr>
<td>GK</td>
<td>1.21±0.13c</td>
<td>0.52±0.02d</td>
<td>0.52±0.02d</td>
<td>60.61±0.02d</td>
</tr>
</tbody>
</table>

Values represent means of triplicate. Values with different alphabet along the same column are significantly different (P>0.05). Key- WCN: White Cola nitida, RCN: Red Cola nitida, CA: Cola acuminate, GA: Garcinia Kola

Interestingly, Garcinia kola demonstrated the strongest inhibitory effect probably due to its higher phenolic and flavonoid contents (Table 2) which could offer protection to the lipids via its potent iron (II) chelating property and radical scavenging ability, thereby preventing the generation of ROS and inhibiting oxidative assault in the process. This result is supported by previous studies where inhibitory effects of plant extracts against pro-oxidant induced lipid peroxidation in selected animal tissues were established (Oboh and Rocha 2007).
Conclusion
This study has been able to show that the selected tropical Cola spp [Cola nitida (white and red), Cola acuminata] and Garcinia Kola possessed antioxidant properties and inhibited key enzymes (arginase, ACE and AChE) linked to ED. The antioxidant properties and enzyme inhibition by the aqueous extract of these seeds suggest their therapeutic potential in the management of ED. However, this health promoting effect is suggested to be a function of its phenolics and/or in synergy with the alkaloid compound. It is noteworthy that Cola acuminata seed extract appeared to be the most potent among the samples and may serve as the basis for future formulation of functional foods for the treatment of ED.

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