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Original article

Garcinia Kola Extracts Improve Biochemical Markers Associated with Erectile Function: Possible Applications in Clinical Treatment?

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SUMMARY

This work investigates the antiradical capacity and potential of *Garcinia kola* extracts to improve the biochemical markers associated with erectile function and its possible application in clinical treatment. Inhibitory properties of aqueous extract of *G. kola* on phosphodiesterase-5 (PDE-5), arginase, angiotensin I –converting enzyme (ACE), and acetylcholinesterase (AChE) in rat's genitals tissues were evaluated. Likewise, *G. kola* extract was explored for reducing power (FRAP) and 1.1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging abilities. *G. kola* extract exhibited greater PDE-5 (IC_{50} = 47.52 ± 0.01 μg/mL), ACE (IC_{50} = 46.90 ± 0.87 μg/mL), AChE (IC_{50} = 44.85 ± 1.33 μg/mL) and arginase (IC_{50} = 47.71 ± 0.25 μg/mL) inhibitory activity in the corpus cavernosum tissue than PDE-5 (IC_{50} = 75.58 ± 2.04 μg/mL), ACE (IC_{50} = 53.00 ± 0.30 μg/mL), AChE (IC_{50} = 54.57 ± 1.50 μg/mL) and arginase (IC_{50} = 69.68 ± 2.42 μg/mL) inhibitory activity in the testicular tissue homogenate. Furthermore, *G. kola* exhibited radical scavenging abilities against FRAP and DPPH* radicals. Hence, our results revealed that *G. kola* extracts are good sources for the development of nutraceuticals and pharmacological properties with attributes to enhance erectile performance.

Key words: Garcinia kola, **erectile function, antioxidants, enzyme inhibition**

List of abbreviations

AChE: acetylcholinesterase; ACE: Angiotensin-I-Converting Enzyme; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; DPPH: 1,1-diphenyl-2-picryl-hydrazil; eNOS: endothelial tissue nitric oxide synthase; ED: erectile dysfunction; NO: nitric oxide; nNOS: neuronal nitric oxide synthase; $ONOO_2$: peroxynitrite; *G. kola: Garcinia kola;* PDE-5: phosphodiesterase-5.

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INTRODUCTION

Erectile dysfunction (ED) could be described as the problem in attaining or the sustaining erection adequate for sexual performance (1). Penile erection is defined as a process consisting of a series of reactions, and alteration at any stage of the reactions might damage the whole process (2). Studies revealed that acetylcholine (ACh) and nitric oxide (NO) perform an important role in the stimulation process (3, 4). Moreover, elevated activity of arginase decreases the generation of NO, since it breakdowns arginine to urea and ornithine during urea cycle thus decreasing the amount of arginine by NO synthase (5). Inhibition of AChE is a means in the management of erectile impairment thus controlling the amount of ACh (6). Oral orthodox medications for managing erectile disfunction are used to facilitate and uphold erection through improving the effect of nitric oxide (7, 8). Nevertheless, the expensive nature of these orthodox medications for ED has led to the use of traditional herbs by patients in developing countries. Medicinal herbs are employed to enhance sexual performance and treat ED. Medicinal plants such as *Massularia acuminata* (9), *Tribulus alatus* (10), *Spondias mombim* (11), *Cnidoscolus aconitefolius* (12) have been documented to exhibit aphrodisiac potential.

There are reports that *G. kola*, a plant cultivated in Africa and distributed globally, exhibited aphrodisiac properties. Generally recognized as bitter kola, this nut is taken as nourishment and are utilized therapeutically for the management of stomach pain, cough, liver disease and erectile impairments (13, 14) in West Africa, Asia, and South America. Because of their bitter flavor, the seeds are employed as a stimulant (15). They are utilized in the management of hepatic diseases and diarrhea (16), type 2 diabetes, bronchitis and esophagus infections (17, 18), and as a natural antimicrobial (19). *G. kola* has also been documented to possess ameliorative and aphrodisiac properties (20, 22). It has also been described to be efficient in the management of skin ailments linked to melanin pigmentation (23). The objective of this research work was designed to examine potentials of aqueous extract from *G. kola* to improve the biochemical markers associated with erectile dysfunction and its possible application in clinical treatment.

MATERIALS AND METHODS

G. kola was procured from a King's market, Ado-Ekiti in October, 2017. It was authenticated by a taxonomist; voucher number was deposited at Ekiti State University, Nigeria. *G. kola* was dried and pulverized in a blender. The fine particles obtained weighing 88 g were macerated in 880 mL distilled water for 24 h as described by Ojo et al. (24) The extract was filtered, and the subsequent filtrate was concentrated to yield 45.25 g of the residue.

CHEMICALS AND REAGENTS

Unless otherwise stated, all chemicals and reagents used were of analytical grades. A UV-visible spectrophotometer was used to measure absorbance.

EXPERIMENTAL ANIMALS

Twenty male Wistar rats (weighing between 270 and 290 g) were obtained from Animal holding house of Department of Biochemistry and were handled in agreement to the guide for Maintenance and Usage of Experimental Animals formulated by the National Academy of Science, issued by the National Institutes of Health (USA). Animals were made to acclimatize for two weeks and under standard conditions. The ethical guidelines were followed with approval number (18/ABUAD/067).

Preparation of penile and testicular homogenate

Experiment rats were placed under ether anesthesia and euthanized by cervical dislocation, then tissue samples were harvested. The erectile organ (penis and testes) were removed, washed and weighed. Tissues were homogenized in normal saline (1/10 w/v) at approximately 1200 rev/min for 10 min at 3000 ×g to produce a residue that was removed and the supernatant was reserved for inhibitory enzyme assay.

Phosphodiesterase-5 (PDE-5) inhibitory activity

Inhibition PDE-5 activity was estimated by Oboh et al. (25). The enzymatic solution comprising 5 mM of the substrate (p-nitrophenyl phenyl phosphonate), (penis and testes) supernatant, Tris buffer (pH 8.0) and the aqueous extracts of G. kola were placed on a steam bath at 37 °C for 15 min. The amount of p-nitro phenol generation was read as a variation in absorbance after 5 min at a wavelength of 400 nm. Control was achieved minus the extracts/Sildenafil. PDE-5 inhibitory enzyme activity was calculated as:

PDE – 5 inhibition (%) = [(Abs control – Abs samples) / Abs control] x 100

Arginase inhibitory activity

Erectile organ homogenates (penis and testes) were made by homogenizing 10 g (w/v) in cold sodium phosphate buffer (pH 7.2). The homogenized tissues were centrifuged for 20 min at 4000 r.p.m and the supernatant was used as enzyme source. Arginase activity was evaluated via the amount of urea generated via Ehrlich's reagent. The solution comprised the totalvolume of 1.0 mM Tris-HCl buffer pH 9.5, MnCl, arginine solution, and G. kola extracts /L-2-amino-[4-(20-hydroxyguanidino)] butyric acid (L-NOHA). The reaction process was placed on a steam bath for 15 min at 37 °C and stopped via the addition of 2.5 mL Ehrlich reagent. Absorbance was read after 20 min at 450 nm wavelength and inhibitory activity was expressed as percent inhibition (26).

Acetylcholinesterase activity assay

Erectile organ (penis and testes) was homogenized in cold sodium phosphate buffer (pH 7.2) and homogenate was employed as the enzyme source. The inhibitory potentials of the aqueous extracts of *G. kola* and standard drug prostigmine on AChE activity were estimated as described by Tel et al. (27) with little adjustments. Acetylcholinesterase activity was estimated in the solution comprising tissue homogenate as the enzyme source, 5,5-dithiol-bis (2-nitrobenzoic) acid (DTNB) and G. kola extracts. This was placed on a steam bathtub for 15 min at 25 °C, and the substrate was added. Absorbance was read at 412 nm. AChE activity was calculated as percentage inhibition.

Angiotensin-I-Converting Enzyme (ACE) inhibitory activity

Inhibition of the ACE activity of aqueous extracts of *G. kola* was estimated following the protocol of Cushman and Cheung (28). Concentrations of the extracts, reference drug lisinopril and organ homogenate (penis and testes) as a source of the ACE enzyme (4 mU/mL) were pre-incubated at 37 °C for 10 min. Afterwards, the process was started via the addition of 200 μL of 8.33mM ACE substrate in 125 mM of Tris-HCl buffer (pH 8.3) to the solution and steam bath at 37 °C for 20 min. The process was terminated with the addition of 300 μL of 1M hydrochloric acid. The hippuric acid (Bz-Gly) generated through the process was removed by adding 2 mL ethyl acetate and further centrifuged to decant the ethyl acetate layer and transferred to a volumetric flask for evaporating to dryness. Absorbance was read at 228 nm. Control was done without the aqueous extract or standard drug lisinopril. ACE percentage inhibition was then calculated in equation.

DETERMINATION OF ANTIOXIDANT ACTIVITIES

Ferric reducing antioxidant ability

Reducing power of the aqueous extract of *G. kola* was estimated via the reduction of the $FeCl_3$ solution by Pulido et al. (29). Three mL of 200 mM phosphate buffer (pH 6.6) and 3 mL of 1% potassium ferricyanide were added to the extract. The resulting solution was placed on a steam bath for 20 min at 50 °C and reaction was stopped by adding 3 mL of 10% TCA. The obtained sample was centrifuged at 650 g for 10 min and 5 mL of the supernatant was added to an equivalent volume of water and 1.0 mL, 0.1% $FeCl_3$. Absorbance was read at 700 nm. Reducing power was expressed in ascorbic acid equivalent (AAE).

Free radical scavenging ability

Free radical scavenging ability of the aqueous extract against DPPH* was measured via the protocol described by Ojo et al. (30). Concisely, a suitable solution of the aqueous extract (2 mL) was added to 1 mL 0.4 mM DPPH solution in methanol. The solution was kept in the dark for 30 min, and the absorbance was read at 516 nm. DPPH* scavenging ability was estimated with deference to the control (sample without extract) and the standard drug used for assessing DPPH activity was vitamin C.

DPPH* scavenging ability (%) = [(Abs control – Abs sapmles)/Abs control] × 100

Data analysis

Data are presented as the mean ± SD (n = 3). The data were analyzed by one-way analysis of variance via a statistical software package (SPSS, Version 20.0, IBM Corporation, NY, USA) one-way ANOVA using Duncan multiple range post-hoc test (DMRT). These analyses were performed with Graph-Pad Prism for Windows version 5.0. Values were considered to be significantly different at $p < 0.05$.

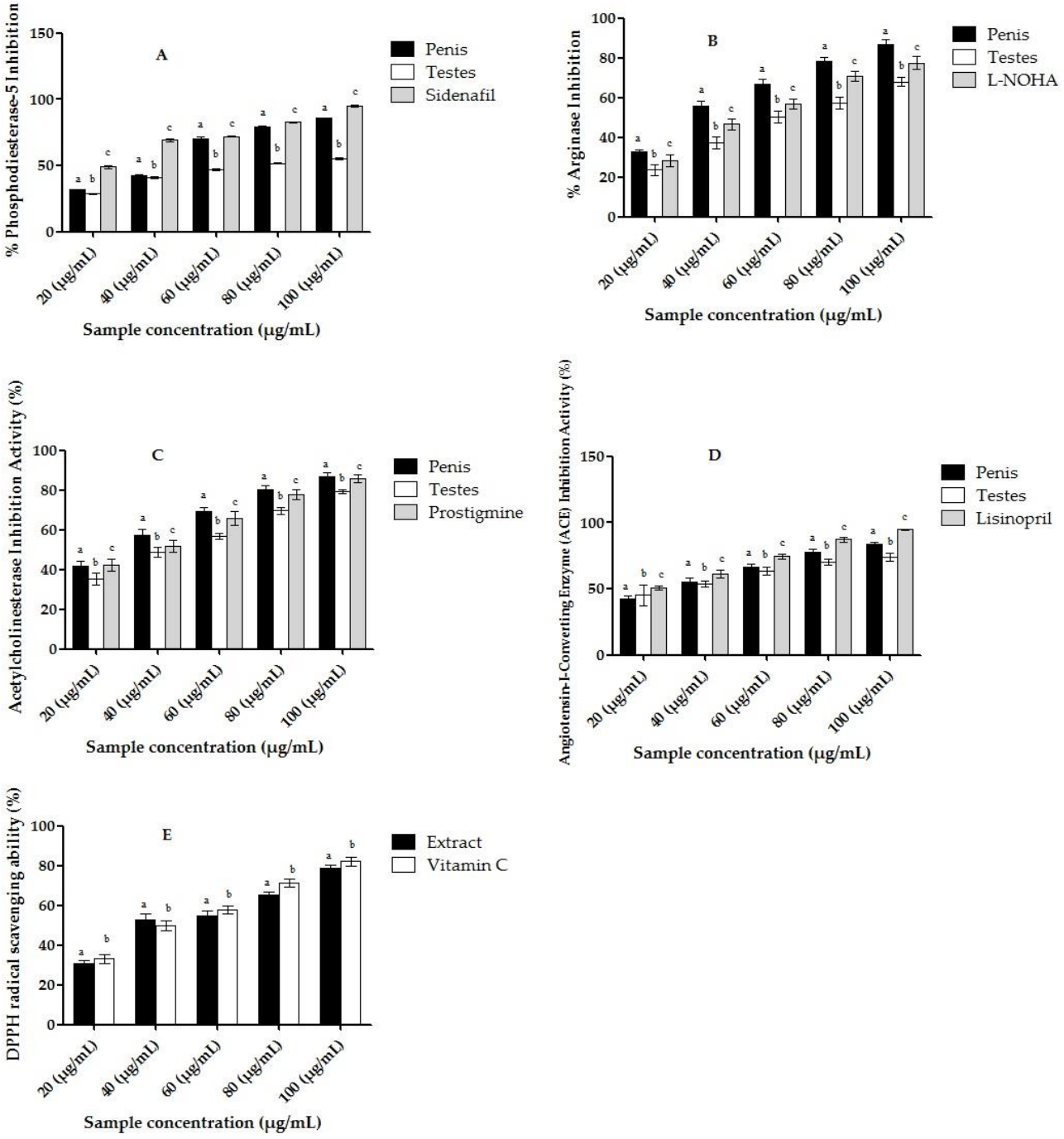

Figure 1. **(A): Inhibition of phosphodiesterase-5 activity, (B) Inhibition of arginase activity, (C) Inhibition of acetylcholinesterase activity, (D) Inhibition of Angiotensin-I-converting enzyme activity in rats' genitals by aqueous extract from *Garcina kola* and (E) DPPH radical scavenging ability of aqueous extract of *Garcina kola* and vitamin C. Data expressed as mean ± SD, n = 3.**

RESULTS

Phosphodiesterase-5 activities (PDE-5) of aqueous extract of *G. kola* in rats' genitals were estimated and presented in Figure 1A.

The result exhibited by the aqueous extract of *G. kola* inhibited PDE-5 enzymes. With regards to the IC_{50} presented in Table 1, it demonstrated that extracts exhibited greater inhibitory activity on corpus cavernosum phosphodiesterase-5 (IC_{50} = 47.52 ± 0.01μg/mL) than testicular phosphodiesterase-5 (IC_{50} = 75.58 ± 2.04 μg/mL). Nevertheless, sildenafil had the utmost activity as shown by the IC_{50} (26.65 ± 0.10 μg/mL).

Table 1. **Ferric reducing properties (FRAP) value of aqueous extract of *Garcinia kola* extract and IC_{50} values (μg/mL) of DPPH, PDE-5, arginase, ACE and AChE inhibitory activities in rat's penile and testicular tissue by *Garcinia kola* extracts and standard drugs/inhibitors**

Sample	PDE-5 (μg/mL)	Arginase (μg/mL)	ACE (μg/mL)
Effect of *G. kola* on penis tissue preparation	47.52 ± 0.01[c]	47.71 ± 0.25[c]	46.90 ± 0.87[a]
Effect of *G. kola* on testis tissue preparation	75.58 ± 0.04[b]	69.68 ± 2.42[b]	53.99 ± 0.85[a]
Sildenafil (μg/mL)	26.65 ± 0.10[a]	-	-
L-NOHA (μg/mL)	-	42.48 ± 0.21[a]	-
Lisinopril (μg/mL)	-	-	38.34 ± 0.01[a]
Prostigmine (μg/mL)	-	-	-
Vitamin C (μg/mL)	-	-	-
G. kola extract	-	-	-
G. kola extract			

Sample	AChE (μg/mL)	DPPH (μg/mL)	FRAP (AAE mg/100g)
Effect of *G. kola* on penis tissue preparation	44.85 ± 1.33[a]	-	-
Effect of *G. kola* on testis tissue preparation	53.00 ± 0.30[a]	-	-
Sildenafil (μg/mL)	-	-	-
L-NOHA (μg/mL)	-	-	-
Lisinopril (μg/mL)	-	-	-
Prostigmine (μg/mL)	48.01 ± 1.02[a]	-	-
Vitamin C (μg/mL)	-	53.81 ± 0.02[a]	-
G. kola extract	-	56.20 ± 1.01[a]	-
G. kola extract			58.65 ± 1.45

Values represent mean ± standard deviation (n=3). Values with the same superscript along the column are not significantly ($p < 0.05$) different. Sildenafil*: standard drug for phosphodiesterase-5 (PDE-5); L-NOHA*: standard inhibitor for arginase; prostigmine*: standard drugs for AChE; Lisinopril*: standard drug for ACE; Vitamin C*: standard drug for DPPH; AAE=Ascorbic Acid Equivalent

Arginase inhibitory activity by the aqueous extract of *G. kola* is given in Figure 1B. The extracts inhibited arginase activity in rats' genitals in all tested concentrations, although, IC_{50} suggested that *G. kola* extracts had a greater inhibitory activity on corpus cavernosum arginase (47.71 ± 0.25 μg/mL) than testicular arginase (69.68 ± 2.42 μg/mL). Nevertheless, L-NOHA had the highest inhibitory activity as shown by IC50 (42.48 ± 0.21 μg/mL).

Figure 1C displayed the inhibitory activity of *G. kola* extracts on acetylcholinesterase (AChE) enzyme. AChE activity was inhibited by aqueous extract of *G. kola* in all tested concentrations. Therefore, *G. kola* extracts had a better inhibitory activity on corpus cavernosum AChE activity (IC_{50} = 44.85 ± 1.33 μg/mL) than testicular AChE activity (IC_{50} = 54.57 ± 1.50 μg/mL).

Figure 1D displayed the inhibitory ACE activity by *G. kola* extracts in rats' genitals. ACE activity was inhibited by both *G. kola* extracts and lisinopril, though *G. kola* extracts had a better inhibitory activity on corpus cavernosum (IC_{50} = 46.90 ± 0.87 μg/mL) than testicular ACE (IC_{50} = 53.00 ± 0.30 μg/mL) activity as shown by their IC_{50} values.

Table 1 revealed the ferric reducing ability of *G. kola* extracts. The result revealed that the extracts had a reducing property of (58.65 ± 1.45 mg/g AAE).

Figure 1E revealed the ability of the leaf aqueous extract of *G. kola* to scavenge DPPH* in rats' genitals. *G. kola* extracts were able to scavenge radicals generated by DPPH*, though, vitamin C scavenge DPPH* better than the *G. kola* extracts, as revealed by their IC_{50} values.

DISCUSSION

Phosphodiesterase-5′ is an enzyme contained in rats' genitals and performs a key function in corpus cavernosum weakness, inhibition of the platelets and dilation of blood vessels (31). The observed inhibitory activities of PDE-5 by *G. kola* indicate that *G. kola* extracts contain active principles with therapeutic attributes for managing ED, resulting in the elevation of cellular concentrations of cyclic-guanosine monophosphate (cGMP) in rats' genitals thus improving blood vessels dilation and smooth muscle relaxation.

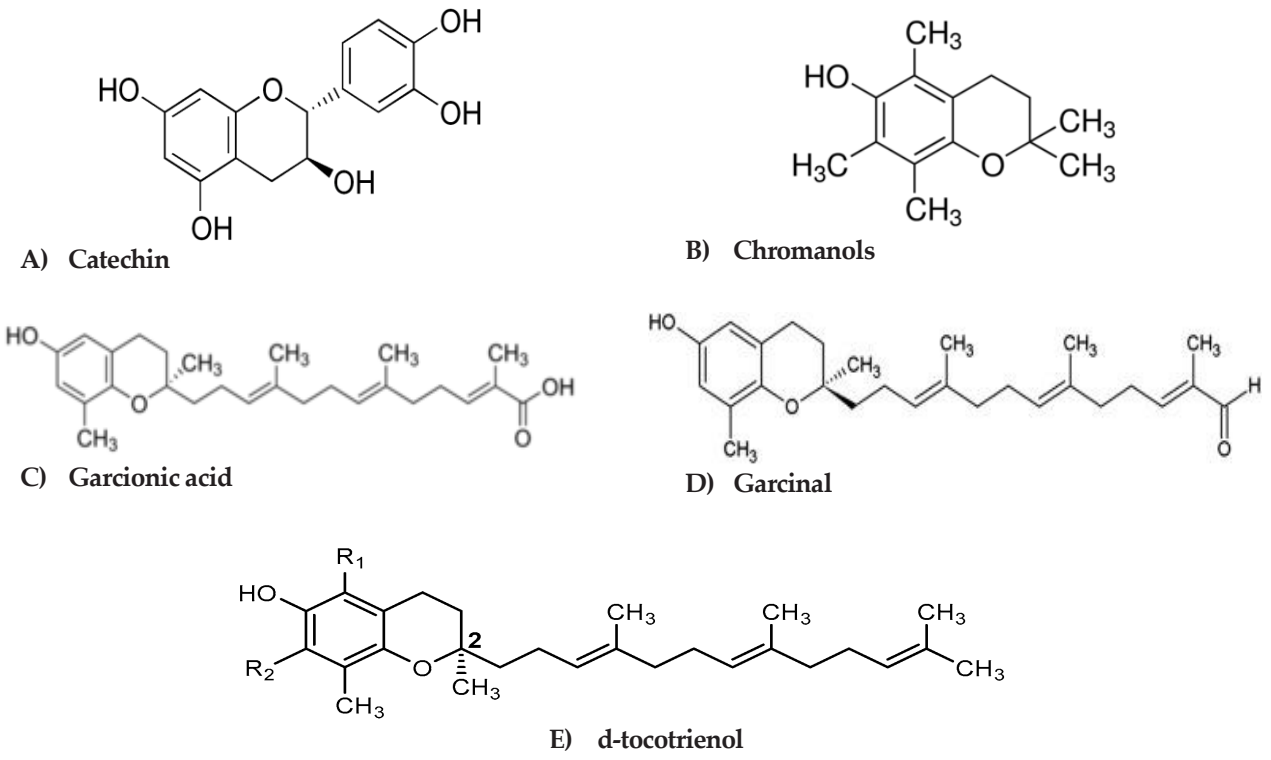

Figure **2. Chemical structures of compounds present in *G. kola* extracts**

The biosynthesis of NO and its availability may reduce due to the increased activity or overexpression of arginase enzyme (32). Current examinations have acknowledged arginase as a marker in managing sexual disorders (33, 34) leading to upregulation of arginase activity and reduced nitric oxide levels in the penile tissues. In ED, reduced NO are documented owing to improved arginase activities, changing the expression of endothelial NO synthase (eNOS) (35). Nevertheless, it is important to note that arginase inhibition by the *G. kola* seed extracts may possibly be an alternative route of increasing the appearance and activities of eNOS, thus raising the bioavailability of nitric oxide. Hence, inhibitory properties of the *G. kola* extracts on arginase might be ascribed to their phenolic and flavonoids components such as catechin, chromanols, garcinoic acid, garcinial and d-tocotrienol (Figure 2) isolated by Farombi (36). This is in correlation with the report of (11, 12) that phenolics and flavonoids compounds of a plant are responsible for the improvement of erectile function.

Studies have documented that cholinergic enzymes in rat's genitals discharge ACh for the activation of nitric oxide production from L-arginine via NO synthase for sexual performance to occur through powering of erectile function (6, 37, 38). The inhibition of the enzyme in rat's genital tissue suggests that *G. kola* seed extracts might be a leading plant with beneficial attributes in the management of ED. Thus, the inhibitory activity of AChE by the *G. kola* extracts might be linked to the occurrence of flavonoids and phenolic compounds, which corroborates with previous reports that herbs reach in phenols might inhibit AChE (12, 39, 40, 41).

Hypertension, a key causative agent of erectile disfunction has been associated with angiotensin (12, 42). ACE inhibition results in the prevention of angiotensin synthesis, thus remaining a remedial point in managing hypertensive-induced ED, thus, hindering the production of angiotensin II in rat's genitals which could possibly be helpful in the treatment of ED. Moreover, flavonoids and phenols are documented to be strong inhibitors of ACE activity (43, 44), and may possibly be accountable for the greater inhibitory effect displayed by *G. kola* extracts. Therefore, this study showed that *G. kola* extracts inhibited ACE activity, subsequently decreasing angiotensin II levels in rat's genitals which could be due to the interactions between the phenolic and flavonoids present in the leaves and disulphidebridge of the enzyme (36, 42, 45, 46).

Total reducing property of a plant serves as an important pointer of its antiradical properties. Free radicals are known to be an important factor of cellular damage in the biological system, and DPPH are utilized to evaluate the radical scavenging ability of natural antioxidants (46, 47). All these radicals are involved in the pathogenesis of several disorders such as erectile dysfunction. Hence, findings from this investigation have shown that *G. kola* extracts possessed antioxidant activity having reasonable scavenging activity towards the various forms of radicals (48, 49).

CONCLUSION

This study revealed that *G. kola* extracts exhibited inhibitory properties on enzyme activities involved in erectile function and displayed radical scavenging abilities which might be connected to their phenolic and flavornoids components. Enzyme inhibitory potentials and antiradical properties of *G. kola* extracts indicate their attributes as nutraceuticals and/or pharmaceuticals in managing ED.

Declaration of Conflict of Interest

Authors have declared no conflict of interest.

Acknowledgments

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References

1. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the United States: Prevalence and predictors. JAMA 1999; 281: 537-44. https://doi.org/10.1001/jama.281.6.537

2. Gratzke C, Angulo J, Chitaley K, et al. Anatomy, physiology, and pathophysiology of erectile dysfunction. J Sex Med 2010; 7(1 Pt 2): 445-75. https://doi.org/10.1111/j.1743-6109.2009.01624.x

3. Kandeel FR, Koussa VK, Swerdloff RS. Male sexual function and its disorders: physiology, pathophysiology, clinical investigation and treatment. Endocr Rev 2001; 22: 342-88. https://doi.org/10.1210/edrv.22.3.0430

4. Corbin JD. Mechanisms of action of PDE5 inhibition in erectile dysfunction. Int J Impot Res 2004; 16: 4-7. https://doi.org/10.1038/sj.ijir.3901205

5. Sakai Y, Masuda H, Kihara K, et al. Involvement of increased arginase activity in impaired cavernous relaxation with aging in the rabbit. J Urol 2004; 172: 369-73. https://doi.org/10.1097/01.ju.0000121691.06417.40

6. Vargas VM, Torres D, Corona F, et al. Cholinergic facilitation of erection and ejaculation in spinal cord-transected rats, Int J Impot Res 2004; 16: 86-90. https://doi.org/10.1038/sj.ijir.3901169

7. Lipschultz LI, Kim ED. Treatment of erectile dysfunction in men with diabetes. JAMA 1999; 281: 465-6. https://doi.org/10.1001/jama.281.5.465

8. Rendell MS, Rafjfer J, Wicker JP, et al. Sildenafil for treatment of erectile dysfunction in men with diabetes. A randomized controlled study. JAMA 1999; 281: 421-6. https://doi.org/10.1001/jama.281.5.421

9. Yakubu MT, Akanji MA, Oladiji AT, et al. Androgenic potentials of aqueous extract of Massullaria acuminate Bullock ex holy stem in male Wistar rats. J Ethnopharmacol 2008; 118: 508-13. https://doi.org/10.1016/j.jep.2008.05.020

10. El-Tantawy WH, Temraz A, E-Gindi OD, et al. Free serum testosterone level in male rats treated with Tribulus alatus extract. Int Braz J Urol 2007; 33: 554-9. https://doi.org/10.1590/S1677-55382007000400015

11. Ojo OA, Afon AA, Ojo AB, et al. *Spondias mombim* L. (Anacardiaceae): Chemical fingerprints, inhibitory activities and molecular docking on key enzymes relevant to erectile dysfunction and Alzheimer's diseases. J Food Biochemistry 2019; e12772 https://doi.org/10.1111/jfbc.12772

12. Ajiboye BO, Ojo OA, Okesola MA, et al. Ethyl acetate leaf fraction of Cnidoscolus aconitifolius (Mill.) I. M. Johnst: antioxidant potential, inhibitory activities of key enzymes on carbohydrate metabolism, cholinergic, monoaminergic, purinergic and chemical finger-printing. Int J Food Properties 2018; 21: 1697-715. https://doi.org/10.1080/10942912.2018.1504787

13. Irvine FR. Woody plants of Ghana. Oxford University Press, Oxford, 1961: 146.

14. Odebunmi EO, Oluwaniyi OO, Awolola GV, et al. Proximate and nutritional composition of kola nut (Colanitida), bitter cola (Garcinia cola) and alligator pepper (Afromomum melegueta). Afr.J Biotechnol 2009; 8: 308-10.

15. Atawodi S, Mende P, Pfundstein B, et al. Nitrosatable amines and nitrosomide formation in natural stimulants, Cola acuminata, Cola nitida and Garcinia kola. Food Chem Toxicol 1995; 33: 625-30. https://doi.org/10.1016/0278-6915(95)00035-Z

16. Blaide VD. Pharmacology effects of Chronic Ingestion of Garcinia kola seeds in the Rats. Phtother Res 1991; 4: 39-41.

17. Orie NN, Ekon EU. The Bronchodilator effects of Garcinia kola. Afr J Pharm Sci. 1993; 22: 172-201.

18. Tita RK, Odeigah PG, Agomo PU, et al. Some properties of medicinal plants used by the Igbos in

Nigeria. In: Kreis Wolfgang., editor. trials, tracts and traces. Germany: 2001: 209-10.

19. Ositelu AO, Adefule AK, Oosa BO, et al. Antifungal activity of Garcinia kola nut extract as an ocular bacterial isolates in Lagos. Nig Qt J Hosp Med 2004; 14: 112-4.

20. Ajibola AO, Satake M. Contributions to the phytochemistry of medicinal plants growing in Nigeria as reported in the 1979–1990 literature-A preview. Afr J Pharm Sci 1992; 22: 172-201.

21. Ralebona N, Sewani-Rusike CR, Nkeh-Chungag BN. Effects of ethanolic extract of Garcinia kola on sexual behaviour and sperm parameters in male Wistar rats. Afr J Pharm Pharmacol 2012; 6: 1077-82. https://doi.org/10.5897/AJPP11.652

22. Yakubu MT, Quadri AL. Garcinia Kola Seeds: Is the Aqueous Extract a True Aphrodisiac in Male Wistar Rats Afr J Tradit Compl Altern Med 2012; 9(4): 530-5. https://doi.org/10.4314/ajtcam.v9i4.9

23. Okunji C, Komarnytsky S, Feara G, et al. Preparative isolation and identification of tyrosinase inhibitors from the seeds of Garcinia kola by high-speed counter-current chromatography. J Chromatography A 2007; 1151: 45-50. https://doi.org/10.1016/j.chroma.2007.02.085

24. Ojo OA, Oloyede OI, Olarewaju OI, et al. Toxicity Studies of the Crude Aqueous Leaves Extracts of Ocimum gratissimum in Albino Rats. IOSR J, Environ. Sci Toxicol Food Technol 2013a; 6: 34-9.

25. Oboh G, Adebayo AA, Ademosun AO, et al. In vitro inhibition of phosphodiesterase-5 and arginase activities from rat penile tissue by two Nigerian herbs (Hunteria umbellata and Anogeissus leiocarpus) J Basic Clin Physiol Pharmacol 2017;28(4): 393-402. http://doi.org/10.1515/jbcpp-2016-0143

26. Kaysen G, Strecker HJ. Purification and properties of arginase of rat kidney, Biochem J 1973; 133: 779-88. https://doi.org/10.1042/bj1330779

27. Tel G, Apaydın M, Duru ME, et al. Antioxidant and Cholinesterase Inhibition Activities of Three Tricholoma Species with Total Phenolic and Flavonoid Contents: The Edible Mushrooms from Anatolia. Food Analytical Methods 2012; 5: 495-504. https://doi.org/10.1007/s12161-011-9275-4

28. Cushman DW, Cheung SH. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem Pharmacol 1971; 20: 1637-48. https://doi.org/10.1016/0006-2952(71)90292-9

29. Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J Agric Food Chem 2000; 48: 396-402. https://doi.org/10.1021/jf9913458

30. Ojo OA, Oloyede OI, Tugbobo OS, et al. Antioxidant and inhibitory effect of scent leaf (Ocimum gratissimum) on Fe2+ and sodium nitroprusside induced lipid peroxidation in rat brain in vitro. Adv Biol Res 2014; 8: 8-17.

31. Rosen RC, Kostis JB. Overview of phosphodiesterase 5 inhibition in erectile Dysfunction. The Am J Cardiol 2003; 92: 9-18. https://doi.org/10.1016/S0002-9149(03)00824-5

32. Oboh G, Nwanna EE, Oyeleye SI, et al. In vitro neuroprotective potentials of aqueous and methanol extracts from Heinsia crinite leaves, Food Sci Human wellness 2016; 5: 95-102 https://doi.org/10.1016/j.fshw.2016.03.001

33. Kim SW, Cuong TD, Hung TM, et al. Arginase II inhibitory activity of flavonoid compounds from Scutellaria indica Archives Pharmacal Res 2013; 36: 922-9. https://doi.org/10.1007/s12272-013-0125-3

34. Segal R, Hannan JL, Liu X, et al. Chronic Oral Administration of the Arginase Inhibitor 2(S)-amino-6-boronohexanoic Acid (ABH) Improves Erectile Function in Aged Rats. J Androl 2012; 33: 1169-75. https://doi.org/10.2164/jandrol.111.015834

35. Cassidy A, Franz M, Rimm EB. Dietary flavonoid intake and incidence of erectile Dysfunction. Am J Clin Nutr 2016; 106: 534-41. https://doi.org/10.3945/ajcn.115.122010

36. Farombi EO. African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. Afr J Biotechnol 2003; 2: 662-71. https://doi.org/10.5897/AJB2003.000-1122

37. Hedlund P, Ny L, Alm P, et al. Cholinergic nerves in human corpus cavernosum and spongiosum contain nitric oxide synthase and heme oxygenase. J Urol 2000; 64: 868-75. https://doi.org/10.1097/00005392-200009010-00064

38. Jung J, Jo HW, Kwon H, et al. Clinical Neuroanatomy and Neurotransmitter-Mediated Regulation of Penile Erection. Int Neurourol J 2014; 18:58-62. https://doi.org/10.5213/inj.2014.18.2.58

39. Ojo OA, Ojo AB, Ajiboye BO, et al. Chromatographic fingerprint analysis, antioxidant properties, and inhibition of cholinergic enzymes (acetylcholinesterase and butyrylcholinesterase) of phenolic extracts from Irvingia gabonensis (Aubry-Lecomte ex O'Rorke) Baill bark. J Basic Clin Physiol Pharmacol 2018a; 29: 217-24. https://doi.org/10.1515/jbcpp-2017-0063

40. Nwanna EE, Oyeleye SI, Ogunsuyi OB, et al. In vitro neuroprotective properties of some commonly consumed green leafy vegetables in Southern Nigeria. NFS J 2016; 2: 19-24. https://doi.org/10.1016/j.nfs.2015.12.002

41. Ojo OA, Ajiboye BO, Ojo AB, et al. HPLC-DAD fingerprinting analysis, antioxidant activity of phenolic extracts from Blighia sapida bark and its inhibition of cholinergic enzymes linked to Alzheimer's disease. Jordan J Biol Sci 2017; 10: 257-64.

42. John S, Schmieder RE. Potential mechanisms of impaired endothelial function in arterial hypertension and hypercholesterolemia. Curr Sci Inc Rep 2003; 5: 199-207. https://doi.org/10.1007/s11906-003-0021-1

43. Guerrero L, Castillo J, Qui~nones M, et al. Inhibition of angiotensin-converting enzyme activity by flavonoids: structure–activity relationship studies, PLoS One 2012; 7: 49493. https://doi.org/10.1371/journal.pone.0049493

44. Shodehinde SA, Adefegha SA, Oboh G, et al. Phenolic composition and evaluation of methanol and aqueous extracts of bitter gourd (Momordica charantia L) leaves on angiotensin-I-converting enzyme and some pro-oxidant induced lipid peroxidation in vitro. J Evid-Based Compl Alternat Med 2016; 21: 67-76. https://doi.org/10.1177/2156587216636505

45. Park JK, Kim SZ, Kim SH, et al. Renin angiotensin system in rabbit corpus cavernosum: Functional characterization of angiotensin II receptors. J Urol 1997; 158: 653-8 https://doi.org/10.1016/S0022-5347(01)64577-4

46. Ojo OA, Ojo AB, Oyinloye BE, et al. *Ocimum gratissimum* Linn. leaves reduce the key enzymes activities relevant to erectile dysfunction in isolated penile and testicular tissues of rats. BMC Complementary and Alternative Medicine 2019b; 19-71. https://doi.org/10.1186/s12906-019-2481-0

47. Ojo OA, Ojo AB, Ajiboye BO, et al. HPLC-DAD Fingerprinting analysis, antioxidant activities of Tithonia diversifolia (Hemsl.) A. Gray Leaves and its inhibition of Key enzymes linked to Alzheimer's disease. Toxicology Reports 2018b; 5: 585-92 https://doi.org/10.1016/j.toxrep.2018.05.003.

48. Ojo OA, Ajiboye BO, Imiere OD, et al. Antioxidative Properties of Blighia sapida K.D. Koenig Stem Bark Extract and Inhibitory Effects on Carbohydrate Hydrolyzing Enzymes Associated with Non-Insulin Dependent Diabetes Mellitus. Pharmacogn J 2018c; 10: 376-83. https://doi.org/10.5530/pj.2018.2.63

49. Ojo OA, Afon AA, Ojo AB, et al. Inhibitory Effects of Solvent-Partitioned Fractions of Two Nigerian Herbs (Spondias mombin Linn. and Mangifera indica L.) on α-Amylase and α-Glucosidase. Antioxidants 2018d; 7: 73. https://doi.org/10.3390/antiox7060073

Ekstrakti biljke Garcinia Kola pozitivno utiču na markere erektilne funkcije: da li je moguća primena u kliničkom lečenju?

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SAŽETAK

Ovaj rad ispituje antioksidativni kapacitet i potencijal ekstrakata biljke *Garcinia kola* u cilju poboljšanja biohemijskih markera koji su u vezi sa erektilnom funkcijom kao i mogućnost primene u kliničkom lečenju. Ispitivana su inhibitorna svojstva vodenih ekstrakata biljke *Garcinia kola* na fosfodiesterazu-5 (PDE-5), arginazu, angiotensin konvertujući enzim (ACE) i acetilholinesterazu (AChE) na genitalijama miševa. Kod ekstrakata je ispitivana redukciona sposobnost (eng. FRAP) kao i oksidativni kapacitet (eng. DPPH). Ekstrakti su pokazali povećanu inhibitornu aktivnost PDE-5 (IC_{50} = 47,52 ± 0,01 μg/mL), ACE (IC_{50} = 46,90 ± 0,87 μg/mL), AChE (IC_{50} = 44,85 ± 1,33 μg/mL) u tkivu corpus cavernosum, nego što je bila inhibitorna aktivnost PDE-5 (IC_{50} = 75,58 ± 2,04 μg/mL), ACE (IC_{50} = 53,00 ± 0,30 μg/mL), AChE (IC_{50} = 54,57 ± 1,50 μg/mL) i arginaze (IC_{50} = 69,68 ± 2,42 μg/mL) u homogenatu testikularnog tkiva. Štaviše, ekstrakti biljke *Garcinia kola* pokazali su se kao potencijalni izvor funkcionalne hrane i farmakoloških supstanci, ali su takođe pokazali i farmakološka svojstva sa ciljem poboljšanja erektilne funkcije.

***Ključne reči: Garcinia kola*, erektilna funkcija, antioksidansi, inhibicija enzima**