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COMPARATIVE EVALUATION OF ANTIOXIDATIVE PROPERTIES AND EFFECTS AQUEOUS EXTRACTS OF *COLA NITIDA* AND *VITEX DONIANA* ON Fe^{2+} - GENERAT OXIDATIVE STRESS IN RAT TESTES *IN VITRO*

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ABSTRACT

Male sexual dysfunction (MSD) might be produced by multifactorial determinants which include psychological disorders, androgen deficiencies, chronic medical conditions, vascular insufficiency, penile disease, pelvic surgery, neurological disorders, drugs, life style, aging and systemic diseases. This study sought to assess the antioxidant properties of the water-extractable component of the leaves of Black plum (Vitex doniana) and bark of Kola nut (Cola nitida),evaluate their effect on pro-oxidant generated lipid peroxidation in rat's testes; and examine the effect on arginase which is among the major enzymes associated with Erectile dysfunction. The results of the total phenol, total flavonoid of aqueous extracts of Cola nitida and Vitex doniana revealed that Cola nitida (10.64 mgGAE/g) had significantly (P<0.05) more total phenol content than Vitex doniana (4.68 mgGAE/g). The result also revealed that Vitex doniana (2.1 mgQE/g) had significantly (P<0.05) higher total flavonoid content than Cola nitida (1.3 mgQE/g). Also, Vitex doniana (20.24 mgAEE/g) had significantly (p<0.05) higher reducing property than Cola nitida (17.43 mgAEE/g). The results of the 2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid (ABTS) radical mopping up capacity of the water extractable component of Vitex doniana and Cola nitida also showed that the extracts are able to scavenge ABTS* radicals, however, Vitex doniana (1.8 Mmol TEAC/100g) had significantly (P<0.05) higher ABTS* mopping up capability than Cola nitida (1.2 Mmol TEAC/100g). Furthermore, the DPPH* result revealed that Cola nitida and Vitex doniana extracts scavenged DPPH* radicals in a concentration-dependent pattern. However, Vitex doniana (IC_{50} = 1.28mg/ml) had a significantly (P<0.05) higher DPPH* mopping up capability than Cola nitida (IC_{50} = 0.83 mg/ml). Both extracts were able to inhibit $FeSO_4$-generated lipid peroxidation at a dose-dependent manner; however, Vitex doniana (IC_{50} = 1.07 mg/ml) had a higher inhibitory action of Fe^{2+} induced lipid peroxidation than Cola nitida (IC_{50} = 1.01 mg/ml). Vitex doniana (IC_{50} = 0.38mg/ml) has the higher arginase inhibitory activity than Cola nitida (IC_{50} = 0.34mg/ml). High phenolic content and strong antioxidant properties could be part of the mechanisms through which the water extractable phytochemicals of Cola nitida (bark) and Vitex doniana (leaves) exhibits its preventive measure of erectile function. However, Vitex doniana displayed a stronger effect on Male reproductive function than Cola nitida.*

Keywords: Sexual dysfunction, *Vitex doniana*, *Cola nitida*, Antioxidant, Arginase.

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Contribution/ Originality

This study contributes in the existing literature that water extractable phytochemical of Cola nitida and Vitex doniana are rich in phenolic compounds and exhibited both anti-arginase and antioxidant activity with Vitex doniana displaying a stronger male sexual function than Cola nitida. These herbs showed potential as a functional food/nutraceutical in the managing of male reproductive malfunction such as Erectile dysfunction as it exhibited inhibitory activity on the key enzyme (arginase) linked to this dysfunction. Therefore, this could be part of the possible mechanisms through which the extracts exert their aphrodisiac properties by inhibiting arginase activities and preventing oxidative stress generated erectile malfunctioning.

1. INTRODUCTION

Purposes of marriage include procreation (reproduction) and for sexual satisfaction of the couples. For continuity, an organism must reproduce itself before it dies. In humans, sexual intercourse Olfneewteb male and female bring about the fusion of sperm and egg during fertilization (Fullick, 1994). Efficient sexual stimulation, arousal and sexual satisfaction in males requires the proper erection of penis. However, the non-performance of this normal physiological process result into a condition known as sexual dysfunction (Guay *et al*., 2003). This condition which is of various types may be controlled by aphrodisiacs. An aphrodisiac can be defined as any substance that potentiates sex drive and/ or sexual pleasure. Aphrodisiac can also be seen as any food, drug, scent or material that can stimulate or increase sexual drive or libido (Rosen and Ashton, 1993). Some plants like *Terminalia catappa* seeds (almond fruit), *Syzygium aromaticum* flower bud (Clove), *Fadogia agrestis* stem (Black aphrodisiac) have been discovered to possess aphrodisiac activities in male rats (Tajuddin *et al*., 2004).

Sexual arousal of male Homo sapiens leads to a sets of psychological, neuronal, vascular, and local reproductive morphologies. Not less than three different types of these changes have been identified. A psychosexual response cycle that comprise four stages which are : stimulation, peak, orgasm, and resolution. Kolodny *et al.* (1979) generally, various factors must work in tandem to regulate apropriate sexual function. Such factors include neural activity, vascular events, intracavernosal nitric oxide system and androgens (Guay *et al*., 2003). Therefore, inappropriate functioning of any of these might result into sexual abnormality.Furthermore,erectile dysfunction is more common in males than females therefore, it is expedient to concentrate more on male sexual difficulties. Research has shown that men of age bracket 17 and 96 years old might have impaired sexual function due to emotional or physical health challenges (Guay *et al*., 2003). Sexual impairment, a consequence of unavoidable aging process affects over 50% of men at age range of 50 and 70 years (Rendell *et al*., 1999; Johannes *et al*., 2000). The Leydig cells decrease in number by about 40% as men advance in years, also, the vitality of vibrating lutenizing hormone secretion is dampened. In addition to these, free testosterone level also decreases by about 1.2% per anum. All these have accounted for the frequence of sexual impairment in the elderly (Guay *et al*., 2003). Several factors could invoke Male sexual impairment (Feldman *et al*., 1994). These are: psychological disorders (performance anxiety, strained relationship, depression, stress, condemnation and phobia of sexual inadequacy), androgen deficiencies (testosterone deficiency, hyperprolactinemia), chronic clinical conditions (diabetes mellitus, high blood pressure, vascular inadequacy (atherosclerosis, venous leakage), penile disease (Peyronie's, priapism fear, phinosis, smooth muscle dysfunction), pelvic surgery (to correct arterial or inflow disorder), neurological disorders (Parkinson's disease, stroke, cerebral trauma, Alzhemier's spinal cord or nerve injury), drugs (side effects) (anti-hypertensives, central agents, psychiatric medications, antiulcer, antidepressants, and anti-androgens), life style (chronic alcohol abuse, cigarette smoking), aging (decrease in hormonal level with age) and systemic diseases (cardiac, hepatic, renal pulmonary, cancer, metabolic, post-organ transplant) (Feldman *et al*., 1994). Sexual impairment in men occurs in various ways. An impairment may be protracted and often results, developed,

conditional, or systemic. Medicinal plants are very ancient and solely herbal medications had proved to be effective in many instances. They may be administered as decorction, infusion or indirectly in form of extracts for the treatment of several diseases due to the availability of plethora of phytoconstituents. They could serve as raw materials in pharmaceutical industries. There is a widespread consumption of herbs in both developing and developed countries in recent time (Gill, 1992). Plants are widely employed to manage sexual impairment. For instance, Ginseng, is an active component in Chinese folklore medicine (Choi *et al*., 1999; Jang *et al*., 2008) and at least 6 million Americans use the root of this slow-growing perennial (Jang *et al*., 2008). Moreover, Maca (*Lepidium meyenii*), has locally been utilized by Peruvian dwellers living at high elevations as a nutrient, an energizer, as sexual stimulants and/or fertility-elicitor. It was discovered to possess sexual enhancing drive (libido) in men (Córdova *et al*., 2001) and sexual response in male rats and mice (Zheng *et al*., 2000). Similarly, other authors (Yakubu *et al*., 2005) have lend scientific credence to the application of *Fadogia agrestis* (English: native aphrodisiac, Hausa: Baakin gagai) stem as an sexual stimulants by accentuating the level of serum testosterone made possible by its saponin content. Traditional herbs have also been a radical advancement in the treatment of sexual insufficiencies (sexual dysfunction) and have been regarded as an "instant remedy globally (Adimoelja, 2000). Some of these herbs include *Terminalia cattapa* seeds (Almond fruit), root of *Garcina kola* Heckel (Yoruba: orogbo), stem bark and twig of *Carpolobia albe* (Yoruba: osunsun, osun), complete plant of *Euphorbia hirta* L (Yoruba: egele) and vegetative parts of *Musa parasidiaca* L (plantain) (Gill, 1992).

In Chinese culture, much of the aphrodisiac power of ginseng and rhinoceros horn comes from their appearance rather than their chemical composition (Choi *et al*., 1999). In folklore medicine in Nigeria, the consumption of (*Vitex doniana)* leaves and Kola tree (*Cola nitida)* bark has been in practice but the mechanism by which they do this is unknown. It is therefore expedient to assess the antioxidant properties of the water extractable fraction of both leaves of Black plum (*Vitex doniana)* and bark of Kola nut (*Cola nitida),*evaluate the impact of the water extractable fraction of both leaves of Black plum (*Vitex doniana)* and bark of Kola nut (*Cola nitida)* pro-oxidant generated lipid peroxidation in rat's testes; and examine the influence of water extractable fraction of the leaves of Black plum (*Vitex doniana)* and bark of Kola nut (*Cola nitida)* on arginase, a principal enzyme associated with Erectile dysfunction.

2. MATERIALS

2.1. Sample Collection

Fresh sample of the bark of tree of the Kola nut (*Cola nitida)* and the leaves of Black plum (Vitex doniana) were obtained from Adekunle ajasin university farm in Akungba Akoko, Ondo state. The samples were dried, blended and kept in the refrigerator for subsequent analysis. Blended samples of 0.5g each (barks and leaves) were weighed into a separate containers and 200ml of distilled water was added and kept covered for a minimum of 24 hours. The solution was filtered into a container using Watman filter paper (size 2), after the 24 hours extraction period, the filtrate was refrigerated for analysis.

2.2. Chemicals and Reagents

Chemicals/ reagents include thiobarbituric acid (TBA), 1,10-phenantroline, gallic acid, Folin-Ciocalteau's reagent were procured from Sigma-Aldrich, Inc, (St Louis, MO), trichloroacetic acid (TCA) was purchased from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany), methanol, hydrogen peroxide, acetic acid, hydrochloric acid, sodium carbonate, aluminium chloride, potassium acetate, sodium dodecyl sulphate, iron (II) sulfate, ferric chloride and potassium ferricyanide were procured from BDH Chemicals Ltd, (Poole, England). All other reagents and chemicals were of standard grades and the distilled water.

3. METHODS

3.1. Total Flavonoid Content Determination

The total flavonoid content was assessed by a little modification of method employed by Meda *et al.* (2005). Briefly 0.5ml of appropriately mixed sample which contained 500µl methanol, 50µl 10% $AlCl_3$, 50µl 1M Potassium acetate and 1.4ml water, and incubated at room temperature for 30min. The absorbance of the reaction mixture was then read at 415 nm in the JENWAY UV-Visible spectrophotometer; the total flavonoid content was calculated accordingly using quercetin as standard.

3.2. Total Phenol Content Detection

The total phenol content was evaluated by the procedure of Singleton *et al.* (1999). Briefly, appropriate dilutions of the water extractable fractions were reacted with 2500µl 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2000µl of 7.5% Na_2CO_3. The reacting mixture was incubated at 45°C for 40minutes and the absorbance read at 765nm using JENWAY UV-Visible spectrophotometer. The total phenol content was then calculated as gallic acid equivalent.

2,2-AZINO-BIS(3-ETHYLBENZTHIAZOLINE-6-SULPHONIC ACID) ABTS* RADICAL SCAVENGING ABILITY

This assay was quantified using the method designed by Re *et al.* (1999). This radical was produced by the reaction of 7 mmol/l ABTS solution plus $K_2S_2O_8$ (2.45 mmol/l) placed away from light for 16 hours and then adjusted the absorbance to 0.700 at 734nm using ethanol. Required dilution of the fraction of 0.2ml was introduced to 2.0ml of ABTS radical solution and later measured at 734nm (after 15mins in incubation period in the dark) on the JENWAY UV-Visible spectrophotometer. The trolox equivalent antioxidantcapacity (TEAC) was subsequently calculated.

1,1-DIPHENYL-2 PICRYHYDRAZYL FREE RADICAL (DPPH) SCAVENGING ABILITY

DPPH radical scavenging capacity was determined using the method of Gyamfi *et al.* (1999). Precisely, required dilution of the sample (1 ml) was diluted with 1 ml, 0.4 mM DPPH radicals in methanol. The reaction mixture was incubated in the dark for 30 minutes and the colour intensity was measured at 516 nm .

IRON (Fe) CHELATION ABILITY

The Fe^{2+} complexing capacity of the plants was evaluated applying a modification method of Minotti and Aust (1987) with a slight modification by Puntel *et al.* (2005). A new preparation of 500µmol L^{-1} FeSO4 (150µl) was introduced to a mixture containing 168µl of 0.1M Tris-HCl at pH 7.4, 218µl of 0.9% NaCl and appropriate volume of the extracts (0 – 100µl). Incubation was then carried out for 5 mins, thereafter the introduction of 13µl of 0.25% 1, 10-phenanthroline (w/v).The measurement of the absorbance at 510nm using a spectrophotometer then followed. Calculation of the Fe^{2+} complexing capacity was finally done.

3.3. Determination of Reducing Potential

The reducing potential was evaluated by examining the capacity of the plants to reduce $FeCl_3$ following the method of Oyaizu (1986). Aliquot of 2.5ml was diluted with 2.5 ml 200 mM sodium phosphate buffer at pH 6.6.and 1% of 2.5ml potassium ferricyanide. Incubation was done for 20 mins at 50 °C and subsequently the addition of 10 % 2.5 ml trichloroacetic acid. Centrifugation of the mixture for 10 mins at 650 rpm was carried out. The mixture of a 5 ml of the supernatant with the same volume of water plus 0.1% of 1 ml ferric chloride then followed. Measurement

of the absorbance at 700 nm with JENWAY UV-Visible spectrophotometer and finally the calculation of the ferric reducing antioxidant potential was performed.

4. LIPID PEROXIDATION ASSAY

4.1. Preparation of Testes Tissue Homogenates

The decapitation of the rats was done using a mild diethyl ether as anaesthesic and the testes was rapidly isolated and put on ice after weighing. The tissue was then homogenized in chilled saline (1/10 w/v) with about 10-up-and –down strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenates were centrifuged for 5 - 10 minutes at 4000 x g to yield a pellet that was discarded, and a low-speed supernatant (S1) was kept for lipid peroxidation assay (Belle *et al*., 2004).

4.2. Lipid Peroxidation and Thiobarbibutric Acid Reaction

The lipid peroxidation assay was carried out using the modified method of Ohkawa *et al.* (1979) briefly 50µl testes tissue homogenate fraction was mixed with a reaction mixture containing 30µl of 0.1M pH 7.4 Tris-HCl buffer, extract (0 - 100µl) and 30µl of 250µM freshly prepared $FeSO_4$. The volume was made up to 300µl by water before incubation at 37°C for 1h. The colour reaction was developed by adding 300µl 8% SDS (Sodium dodecyl sulphate) to the reaction mixture containing S1, this was subsequently followed by the addition of 500µl of acetic acid/HCl (pH 3.4) mixture and 500µl 0.8% TBA (Thiobarbituric acid). This mixture was incubated at 100°C for 1h. TBARS (Thiobarbituric acid reactive species) generated were measured at 532nm in the JENWAY UV-Visible spectrophotometer and the absorbance was compared with that of standard curve using MDA (Malondialdehyde).

5. ENZYME INHIBITION ASSAY

5.1. Arginase Assay

Arginase assay in tissue homogenates is hinged upon the colorimetric detection of generated urea nitrogen, using 2,3-butanedione reagent (Geyer and Dabich, 1971). The reaction contains 0.05M maleic acid, pH 7.0 with 0.05M manganous sulfate 0.713M L-arginine, reagent distilled water, 20mg NaOH per litre, 0.075%, 2,3-butanedione in buffered arsenic sulfuric acid. Incubate a one mg/ml solution of the enzyme in maleic-manganous sulfate buffer at 37c for 4hours. Dilute to 1-2 microgram/ml in reagent distilled water pH 9.5.incubate in 37c water bath for 5minutes to achieve temperature equilibration. At timed intervals start reaction by adding: then incubate for another 30minutes at 37°c. stop reaction at timed intervals by adding B.U.N. reagent. Cap tubes and develop color by boiling in water bath for 12minutes. Chill tubes for 3minutes in an ice bath and the colour was read at 490nm against the blank.

5.2. Data Analysis

The result of triplicate experiments were pooled and expressed as mean ± standard deviation (SD) (Zar, 1984). A one-way analysis of variance (ANOVA) and the least significance difference (LSD) were carried out. Significance was accepted at $P \leq 0.05$.

6. RESULTS

The results of the total phenol, total flavonoid of aqueous extracts of *Cola nitida* and *Vitex doniana* is shown in Table 1. The result revealed that *Cola nitida* (10.64 mgGAE/g) had significantly ($P<0.05$) higher total phenol content than *Vitex doniana* (4.68 mgGAE/g). The result also revealed that *Vitex doniana* (2.1 mgQE/g) had significantly ($P<0.05$) higher total flavonoid content than *Cola nitida* (1.3 mgQE/g). As revealed by the results,

Vitex doniana (20.24 mgAEE/g) had significantly ($p<0.05$) higher reducing property than *Cola nitida* (17.43 mgAEE/g). The results of the 2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid (ABTS*) radical scavenging ability of the aqueous extracts of *Vitex doniana* and *Cola nitida* are presented in Table 1. The result showed that the extracts are able to scavenge ABTS* radicals, however, *Vitex doniana* (1.8 Mmol TEAC/100g) had significantly ($P<0.05$) higher ABTS* scavenging ability than *Cola nitida* (1.2 Mmol TEAC/100g).

Table-1. The total phenol (mgGAE/100g), total flavonoid (mgQE /100g) and ABTS* scavenging ability of the aqueous extracts of the *Vitex doniana* (Leaves) and *Cola nitida* (Bark).

Samples	Total Phenol	Total Flavonol	Reducing Power	ABTS
(mg/ml)	(mgGAE/100g)	(mgQE/100g)	(mgAEE/g)	(MmolTEAC/100g)
V. doniana	10.64±0.66	2.10±0.63	20.24±0.07	1.8±0.04
C. nitida	2.68±0.66	1.30±0.37	17.43±0.44	1.2± 0.03

The total phenol (mgGAE/100g), total flavonoid (mgQE /100g) and ABTS* scavenging ability of the aqueous extracts of the *Vitex doniana* (Leaves) and *Cola nitida* (Bark).

Table-2. IC50 of, Fe^{2+} chelating ability, DPPH* radical scavenging ability and inhibition of Cu2+/Fe2+ induced lipid peroxidation of the aqueous extracts of the bark of Cola nitida and leaves of Vitex doniana.

IC_{50}(mg/ml)	V.doniana	C.nitida
Fe^{2+} Chelation	0.25±0.03	0.53±0.04
DPPH	1.28±0.02	0.83±0.04
Inhibition of Fe^{2+} induced L.P	1.07± 0.07	1.01±0.06
Inhibition of C u^{2+} induced L.P	0.59±0.04	0.72±0.02

IC50 of, Fe^{2+} chelating ability, DPPH* radical scavenging ability and inhibition of Cu2+/Fe^{2+} induced lipid peroxidation of the aqueous extracts of the bark of Cola nitida and leaves of Vitex doniana.

Furthermore, the DPPH* free radical scavenging ability of the aqueous extracts of *Cola nitida* and *Vitex doniana* is presented in Figure 1, and their IC_{50} values in Table 2. The result revealed that the aqueous extracts of *Cola nitida* and *Vitex doniana* scavenged DPPH* radicals in a concentration-dependent pattern. However, *Vitex doniana* (IC_{50} = 1.28mg/ml) had a significantly ($P<0.05$) higher DPPH* scavenging ability than *Cola nitida* (IC_{50} = 0.83 mg/ml). Also, the Fe^{2+} chelating ability of both extracts is presented in Figure 2, and their IC_{50} values in Table 2. The result revealed that the aqueous extracts of *Cola nitida* (IC_{50} = 0.53mg/ml) had a significantly ($P<0.05$) higher Fe^{2+} chelating ability than *Vitex doniana* (IC_{50} = 0.25 mg/ml). The Inhibition of $FeSO_4$ and $CuSO_4$ induced lipid peroxidation of aqueous extracts of *Cola nitida* and *Vitex doniana* are presented in Figures 3 and 4 with their IC_{50} values in Table 2. The result revealed that both extracts were able to inhibit $FeSO_4$ induced lipid peroxidation in a dose-dependent manner; however, *Vitex doniana* (IC_{50} = 1.07 mg/ml) had a higher inhibition of Fe^{2+} induced lipid peroxidation than *Cola nitida* (IC_{50} = 1.01 mg/ml). In like manner, *Cola nitida* (IC_{50} = 0.72 mg/ml) had a higher inhibition of $CuSO_4$ induced lipid peroxidation than *Vitex doniana* (IC_{50} = 0.59 mg/ml).

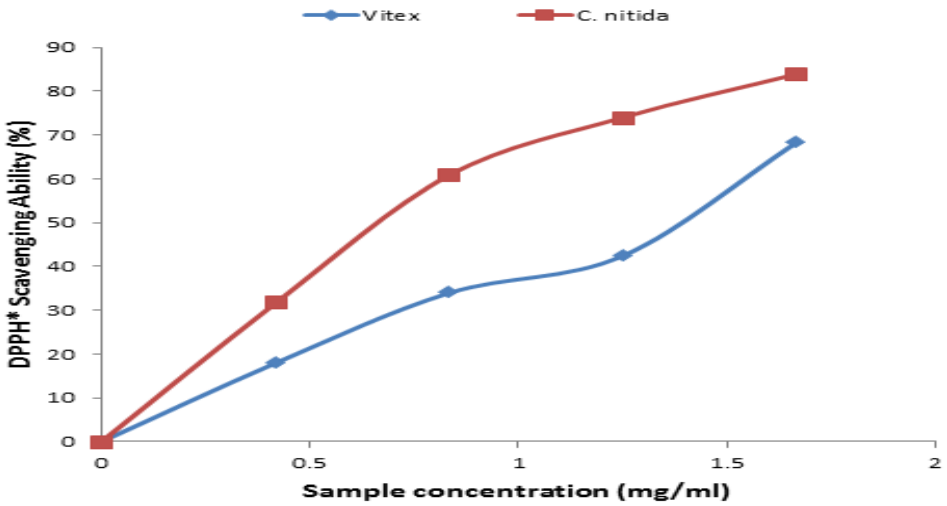

Figure-1. DPPH radical scavenging abilities of aqueous extracts of the bark of *Cola nitida* and *Vitex doniana*

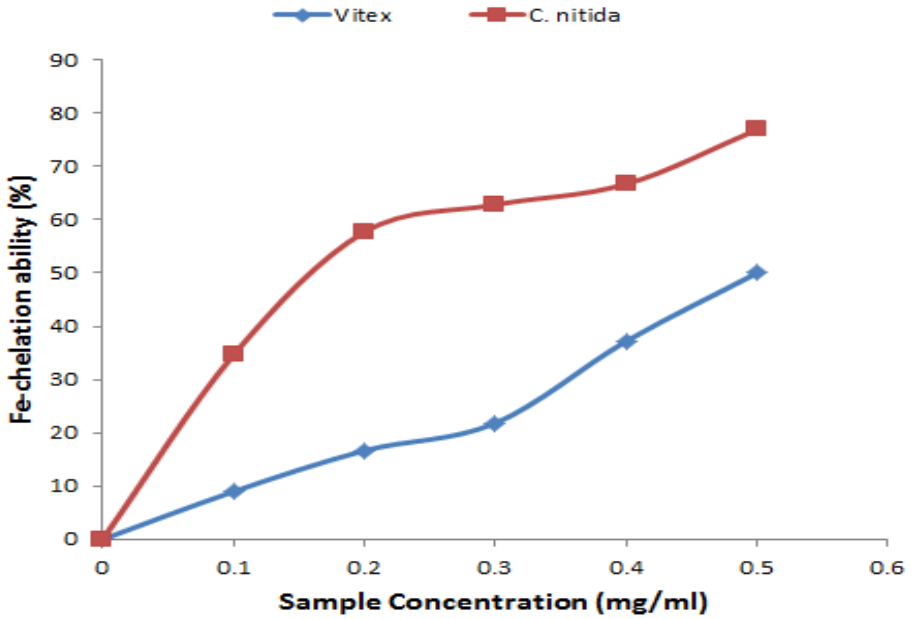

Figure-2. Fe^{2+} Chelating ability of aqueous extracts of the bark of *Cola nitida* and *Vitex doniana*

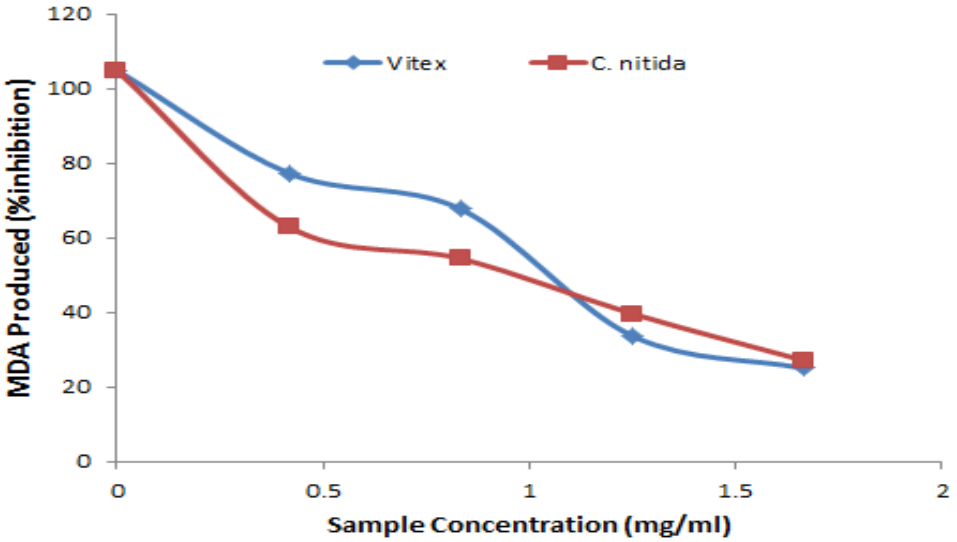

Figure-3. Inhibition of Fe^{2+} induced lipid peroxidation of aqueous extracts of the bark of *Cola nitida* and leaves of *Vitex doniana*

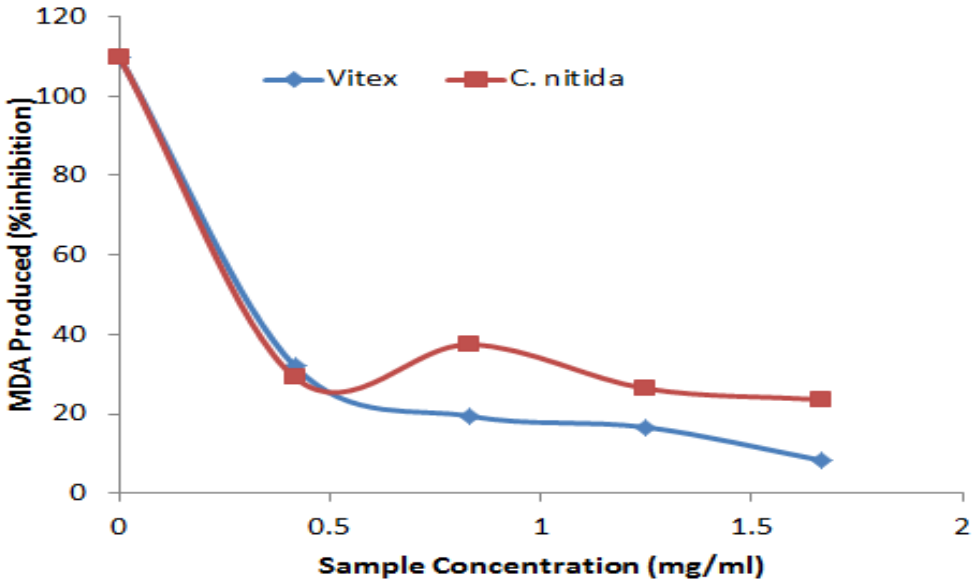

Figure-4. Inhibition of Cu^{2+} induced lipid peroxidation of the aqueous extracts of the bark of *Cola nitida* and leaves of *Vitex doniana*.

The ability of the aqueous extracts of *Cola nitida* and *Vitex doniana* to inhibit arginase activity *in vitro* was investigated and the result is presented in Figure 5 with its IC_{50} in Table 3. The result revealed that both extracts inhibited arginase activity in a dose dependent manner. However, *Vitex doniana* (IC_{50} = 0.38mg/ml) has the higher arginase inhibitory activity than *Cola nitida* (IC_{50} = 0.34mg/ml).

Table-3. IC50 of arginase inhibitory activity of the aqueous extracts of Cola nitida and Vitex doniana

Samples	IC50 (mg/ml)
Vitex doniana	0.38±0.01
Cola nitida	0.34±0.01

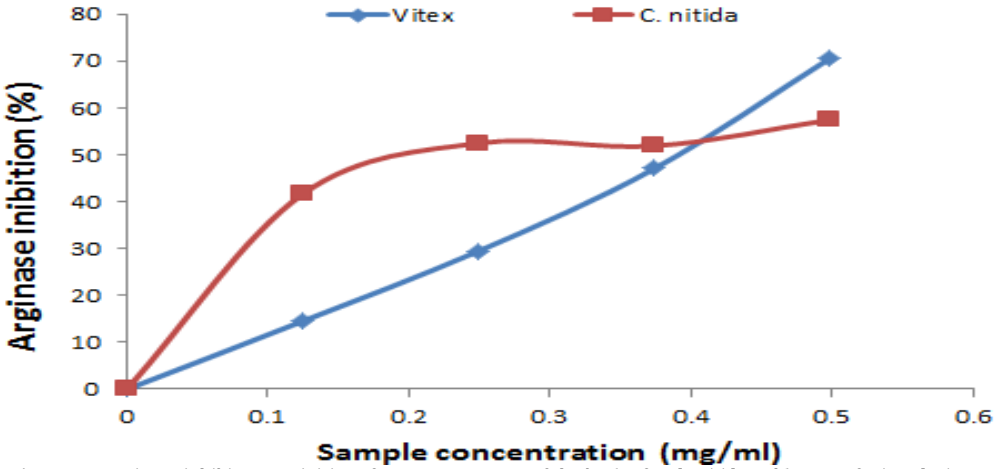

Figure-5. Arginase inhibitory activities of aqueous extracts of the bark of *Cola nitida* and leaves of *Vitex doniana*

7. DISCUSSION

Over the past decades, herbal medicine is now a thing of global significance, having economic and medicinal implications. Oboh *et al.* (2007) had earlier delineated that numerous plants are repository of phytoconstituents which their consumption confer protective potential against degenerative diseases. Herbs are widely used throughout the world and its use had generated some treats on its value, safety, and efficacy. This makes it expedient for exact scientific assessment as a precondition for acceptance of herbal health claims. The total phenol

content shows that the water extractable component of *Cola nitida* has greater aggregate phenol constituent than *Vitex doniana*. Compounds of phenol had been shown to shield man's body from reactive species generated from the natural cell's metabolism. They are potent antioxidants capable of removing free radicals, they may chelate metallic catalysts, activate antioxidant enzymes, decrease α-tocopherol species and impede oxidases (Amic *et al*., 2003). Phenolic constituents of plants are considered as antioxidants and they perform preservative properties by either checking the synthesis of reactive species or by counterbalancing/ mopping up reactive species generated in human body (Oboh, 2006). Also, the mopping up capacity of *Cola nitida* (root) and *Vitex doniana* (leaves) extracts were studied using a moderately stable nitrogen-centred radical species, DPPH (Gyamfi *et al*., 1999) and ABTS* coupled with the Iron (II) complexing capacity of the extracts. The result showed and *Cola nitida* (root) and *Vitex doniana* (leaves) that the extracts are capable of mopping up ABTS* radicals, however, *Vitex doniana* had a higher ABTS* radical mopping up capability than *Cola nitida*. The extract of *Cola nitida* showed a higher DPPH scavenging ability than *Vitex doniana* while *Cola nitida* extract displayed a stronger Fe^{2+} complexing capacity than *Vitex doniana*. The antioxidant activities of plant phytochemicals occur by inhibiting the synthesis of reactive species or by truncating/ mopping up reactive species synthesized in the body or decreasing/ complexing transition meal constituents of foodstuffs (Amic *et al*., 2003; Oboh *et al*., 2007). This is an important antioxidant mechanism demonstrated by these plants and could play some part in the prevention of erectile malfunction caused by oxidative stress. Exposure of rat testes tissues to 250 μM $FeSO_4$ resulted into a remarkable increase in the malodialdehyde level in testes. This observation was in consonance with that of Oboh *et al*. (2012) where a notable rise in the generation of malodialdehyde in the brain of rat was found after being exposed to Fe^{2+}. Elevated Fe^{2+} level in the testes was connected with a host of male sexual problem and high Fe^{2+} contents have been localized to degenerate regions of male sexual function from Erectile dysfunction's patients, a finding also investigated in animal types of the disease. In this experiment, both extracts inhibited MDA synthesis in testes of rat in a concentration dependent fashion. However, *Vitex doniana* had a higher reduction of Iron (II) generated lipid peroxidation than *Cola nitida*. This can be compared with previous work whereby extracts from plants truncated iron(II) generated lipid peroxidation in the brain of rat (Zago *et al*., 2000). In addition, water extractable part of *Cola nitida* and *Vitex doniana* inhibited arginase. The result revealed that extracts inhibited arginase actvities in a concentration based fashion. However, *Vitex doniana* had higher arginase inhibitory activities than *Cola nitida*. Inhibition of arginase is considered a promising approach in the therapy of Male sexual malfunction especially Erectile dysfunction.

8. CONCLUSION

Water extractable phytochemical of *Cola nitida* and *Vitex doniana* are rich in phenolic compounds and exhibited both anti-arginase and antioxidant activity with *Vitex doniana* displaying a stronger Male sexual function than *Cola nitida*. These herbs showed potential as a functional food/nutraceutical in the management of male sexual malfunction such as Erectile dysfunction as it exhibited inhibitory activity on the key enzyme (arginase) linked to this dysfunction. Therefore, this might be the possible mechanism through which the extracts exert their aphrodisiac properties by inhibiting arginase activities and preventing oxidative stress produced erectile malfunction.

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