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FULL ARTICLE

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Alkaloid extracts from Bitter leaf (*Vernonia amygdalina*) and Black nightshade (*Solanum nigrum*) inhibit phosphodiesterase-5, arginase activities and oxidative stress in rats penile tissue

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Abstract

The erectogenic potential of alkaloids extracted from Bitter leaf (*Vernonia amygdalina*) and Black nightshade (*Solanum nigrum*) was investigated in this study. Fresh leaves obtained from Bitter leaf and Black night shade were air-dried, pulverized, and extracted for alkaloids. The inhibitory potential of the alkaloid extracts on arginase and phosphodiesterase-5 (PDE-5) activities in rats penile tissue was determined in vitro. The antioxidant properties were also evaluated and the constituent alkaloids quantified using GC-MS. The alkaloid extracts inhibited arginase (0–30.51 μg/ml) and PDE-5 (0–133.69 μg/ml) activities in a concentration-dependent pattern. Similarly, the alkaloid extracts inhibited Fe^{2+}-induced lipid peroxidation in rats penile tissues, scavenged DPPH, OH, and NO radicals as a function of concentration. GC-MS characterization revealed over 20 alkaloid compounds. The inhibition of PDE-5-, arginase-, pro-oxidant-induced lipid peroxidative-, and free radicals-scavenging activities by the alkaloids is suggestive of putative mechanisms underlying their therapeutic use for managing erectile dysfunction in folklore medicine.

Practical applications

Alkaloids extracted from Black nightshade (*Solanum nigrum*) and Bitter leaf (*Vernonia amygdalina*) were characterized and investigated by standard procedures for inhibitory action against key erectile dysfunction-linked enzymes and antioxidant activity. The alkaloids inhibited erectile dysfunction-linked enzymes (arginase and PDE-5) and showed considerable antioxidant activity in a concentration-dependent manner. In view of this, we suggest the application of these results in the development of erectile dysfunction drugs in the pharmaceutical industry, with probable minimal or no adverse effect.

KEYWORDS

alkaloids, arginase, erectile dysfunction, phosphodiesterase-5, *Solanum nigrum*, *Vernonia amygdalina*

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1 | INTRODUCTION

Erectile dysfunction (ED) or male sexual impotence is a condition whereby men experience repeated inability to achieve or sustain firm penile erection for a gratifying sexual activity (Montorsi et al., 2010). Erectile capacity decreases as men age, some men experience chronic, severe, or mild ED at some particular stages of their lives, usually at age 40 (Van-Wyk, 2015). Aside of age, other causative factors have been reported in the literature. Impairment to and/or insufficient arterial blood flow within the hypogastric penile bed and into the corpora cavernosa, damages to penile endothelial tissue, perturbations in the nervous system modulating erection or reduced libido are some of the causes of ED (Lue, 2000). Also, oxidative stress-related reactive oxygen and nitrogen species (ROS and RNS) are risk factors for ED. These species attack important cellular macromolecules, such as lipids and proteins, thereby resulting in endothelial tissue dysfunction (Nchegang et al., 2016). The reactive species are implicated in reduction of the bioavailability of NO, which is critically required for penile erection (Nchegang et al., 2016). Living an unhealthy lifestyle, obesity, smoking, alcoholism, abuse of drugs/medications, stress, fatigue, anxiety, fear, sadness, relationship problems, physical/psychological disorders, and unbeneficial enzyme activities are additional factors responsible for the incidence of ED (Cleland & Cotton, 2011).

Arginase which is implicated in ED is a metal containing enzyme that acts on L-arginine and convert it into urea and ornithine while Nitric Oxide Synthase (NOS) converts the same L-arginine to nitric oxide and citrulline during sexual stimulation, which brings about reduction in bioavailability of NO. Both enzymes compete for the substrate, L-arginine (Boucher, Moali, & Tenu, 1999) and are also linked with ED (Oboh et al., 2015). Similarly, PDE-5 is a vital enzyme in the NO/cGMP signal transduction pathway that controls smooth muscle cells twitch and erectile system (Leite et al., 2007). Leite et al. (2007) stated further that PDE-5 enzymes have been implicated in ED because they prevent the continuous expression of cGMP, which helps to maintain and prolong erection by degrading it to 5′GMP, an inactive metabolite. PDE-5 inhibitors (Sildenafil, Tadalafil, and Avanafil) have been used to treat ED, but are reported to provoke some side effects and aftermath experiences (Kloner, Mitchell, & Emmick, 2003; Shabsigh, Kaufman, Steidle, & Padma-Nathan, 2004).

Recourse to therapeutic solutions from plant sources has always been an attractive option with little or no side effects for managing diseased conditions such as ED. Several plants are known to have diverse pharmacological and medicinal properties, most of which are edible and have no side effects (Gratz, Flurer, & Wolnik, 2004; Omojokun, Oboh, & Ademiluyi, 2018). Bitter edible plants (e.g., *Vernonia amygdalina* and *Solanum nigrum*) have the reputation of being aphrodisiacs (Kim, Woo, Lee, & Kim, 1998). The sexual activity enhancing potential of these plants is ascribed to phytochemical constituents such as sterols, phenols, alkaloids, amino acids, and saponin (Eleazu, Eleazu, Awa, & Chukwuma, 2012). These compounds are responsible for improving sexual function through the regulation of neurotransmitters and relaxation of the corpora cavernosa smooth muscles (Kumar, Subramoniam, & Pushpangadan, 2000). It is noteworthy that the bitter-tasting *Solanum nigrum* and *Vernonia amygdalina* are affirmed to have considerable therapeutic effects, which are attributed to their alkaloid contents (Jamil, Shahid, Khan, & Ashraf, 2007; Manoko, van den Berg, Feron, van der Weerden, & Mariai, 2007). Other phytoconstituents reportedly present in the vegetables include sesquiterpenes, flavonoids, phenolic acids, and alkaloids among others (Eleazu et al., 2012). Alkaloids are one of the readily available antioxidants found in bitter plants (Nchegang et al., 2016). In view of the foregoing, this study sought to provide information on possible erectogenic potential of alkaloids extracted from Black nightshade (*Solanum nigrum*) and Bitter leaf (*Vernonia amygdalina*) as alternative therapeutic agents for managing erectile dysfunction.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Leaves of Black nightshade and Bitter leaf were harvested from a vegetable garden at Elizade University, Ilara-mokin with General Packet Radio Service (GPRS) of 7.3497° N, 5.1067° E, Ondo State, Nigeria. Samples of the plants obtained from the farm were authenticated at Crop Soil and Pest Management Department of the Federal University of Technology Akure, Ondo State, Nigeria.

2.2 | Chemicals and reagents

Chemicals used were obtained from Sigma Co. (St Louis MO, USA). Except where otherwise stated, all reagents and chemicals used are of analytical grade. Distilled water used was obtained from the laboratory water distiller.

2.3 | Extraction of plant alkaloids

Leaves collected from the two plant samples were separately extracted for alkaloids by following the same procedure described by Ademiluyi, Ogunsuyi, and Oboh (2016). The leaves were thoroughly rinsed under running tap, air-dried at temperature between 25 and 27°C and pulverized to powder form. Powdered samples were defatted for 24 hr using N-hexane and subsequently soaked in 10% acetic acid in ethanol for 24 hr at 37°C. The mixtures of the different samples were first filtered with muslin cloth and subsequently through Whatman No.2 filter paper. Filtrates were later concentrated at 45°C in a rotary evaporator and precipitated with concentrated ammonium hydroxide (NH_4OH). The precipitates were then collected and washed with more diluted ammonium hydroxide (NH_4OH) so as to obtain the crude alkaloid extracts of the two plant materials.

2.4 | Preparation of penile tissue homogenate

All animals used were Wistar albino rats strain obtained from University of Ibadan Animal House, Ibadan, Oyo state. The animals were kept in battery cages at room temperature (21–25°C), all other appropriate conditions for animal experiments were carefully observed. All rats were given access to water and animal feed ad libitum. The international guidelines as well as the stipulated guidelines of Elizade University Faculty of Basic and Applied Sciences Animal and Research Ethics Committee (in line with European Union Directive 2010/63/EU) were followed for all animals used.

For the lipid peroxidation assays, five adult male Wistar rats strain weighing between 240 and 280 g were sacrificed by cervical dislocation after which the penile tissues were quickly isolated, weighed, and temporarily stored at approximately −4°C, and subsequently homogenized in cold saline. Isolated tissue was centrifuged for about 10 min at 3,000 × g to yield the supernatant and then the pellet. The pellet was discarded while the supernatant was put in firm glassware, placed on ice crystals obtained from laboratory freezer [Angelantoni platilab Model no PLATILAB 340V-3-STD with temperature set at −20°C] shortly prior to use (Belle, Duret, Galtier, & Eyre-Walker, 2004).

2.5 | Assays

2.5.1 | Phosphodiesterase-5 (PDE-5) inhibition

Three male Wistar albino rats weighing between 200 and 250 g were sacrificed by cervical dislocation. The penile tissues were carefully removed and washed twice in 0.1 M Tris-HCl buffer containing 1 mM of $CaCl_2$ and 50 mM of NaCl (pH 8.0) and stored in firm glassware and placed on ice crystals. The tissues were then homogenized and centrifuged at 15,000 g, 4°C for 15 min. The supernatant was decanted and used as enzyme source. The PDE-5 inhibitory activity of the alkaloid extracts was assayed in vitro by the method of Kelly and Butler (1977) with slight modification by Oboh, Adebayo, Ademosun, and Boligon (2017). The reaction mixture containing 5 mM of the substrate (p-nitrophenyl phenylephosphate), 100 μl of enzyme, 20 mM of Tris buffer (pH 8.0), and the extracts, was incubated at 37°C for 10 min. The intensity of p-nitrophenol produced was measured as change in absorbance after 5 min at 400 nm using a UV Visible spectrophotometer (Jenway 6315 model). The control experiment was performed without the test sample, and the effective alkaloid extract concentration eliciting PDE-5 inhibitory activity was expressed as percentage inhibition.

2.5.2 | Arginase inhibition

Four male Wistar albino rats strain weighing between 240 and 280 g were sacrificed by cervical dislocation. The penile tissues were carefully removed, washed in 0.01 M of Tris-HCl buffer containing 0.05 M of $MnCl_2$ (pH 9.5) and stored in firm glassware placed on ice crystals. The tissues were later homogenized in three volumes of ice-cold buffer. The homogenate was centrifuged at 15,000 g, 4°C for 15 min. The supernatant was decanted and used as enzyme source. Arginase activity was determined by the measurement of urea produced by reaction with Ehrlich's reagent. The reaction mixture comprised in final concentration of 1.0 mM of Tris-HCl buffer (pH 9.5) containing 1.0 mM of $MnCl_2$, 0.1 M of arginine solution, and 50 mM of the enzyme preparation in a final volume of 1.0 ml. The mixture was incubated for 10 min at 37°C. Reaction was terminated by adding 2.5 ml of Ehrlich reagent which contain 2.0 g of p-dimethylaminobenzaldehyde in 20 ml of concentrated hydrochloric acid and made up to 100 ml with distilled water. The optical density reading was taken after 20 min at 450 nm using a UV Visible spectrophotometer (Jenway 6315 model). The control experiment was performed without the test sample, and the arginase inhibitory activity expressed as % inhibition (Kaysen & Strecker, 1973).

2.5.3 | Lipid peroxidation

The method of Ohkawa, Ohishi, and Yagi (1979) which was slightly modified was used for the lipid peroxidation and thiobarbituric acid reactions. Hundred microliter of homogenate supernatant (SI fraction) was mixed with 30 μl of Tris-HCl buffer, extracts (0–100 μl) and 30 μl of $FeSO_4$ or sodium nitroprusside (as pro-oxidant). Volume was made up to 300 μl with water and incubated at 37°C for 60 min. Subsequently, 300 μl of 8.1% Sodium dodecyl sulfate (SDS) was added to develop color, followed by addition of 600 μl of acetic acid/HCl and finally 600 μl of 0.8% of TBA. Incubation of mixture was done for 60 min at 100°C and TBARS produced was measured at 532 nm.

2.5.4 | 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging ability

Gyamfi, Yonamine, and Aniya (1999) method was used for the DPPH (1,1-diphenyl-2 picrylhdrazyl) radical scavenging activity. An appropriate dilution of the extracts in the ratio 1:1 (10 ml of extract solution plus 10 ml of distilled water) was mixed with 1,000 μl of 0.4 mM methanol containing DPPH radicals, mixture was incubated in dark cupboard for 30 min and absorbance was measured at 516 nm.

2.5.5 | Inhibition of Fenton reaction (degradation of deoxyribose)

Halliwell, Gutteridge, and Cross (1992) method was used to determine the extracts' OH radical scavenging ability. 50–200 μl of the extract was added to a reaction medium containing deoxyribose, phosphate buffer, $FeSO_4$, and distilled water. Mixture was incubated for 30 min at 37°C and the reaction was then halted by adding trichloroacetic acid (TCA). Subsequently, TBA solution was added and tubes were then incubated for 20 min in water at 100°C. Absorbance was measured using a spectrophotometer at 532 nm.

2.5.6 | Nitric oxide (NO) radical scavenging

Marcocci, Maguire, Droylefaix, and Packer (1994) method was used to assay for the NO radical scavenging potential of the alkaloid extracts. 100–400 μl of alkaloid extract was added to 1,000 μl of Sodium nitroprusside solution and incubated for 120 min at 37°C. Five hundred microliter of the incubation mixture was extracted and then mixed with 300 μl of Griess reagent (containing sulfanilamide in H_3PO_4 and napththlyethylenediamine dihydrochloride). Chromophore absorbance was instantly read at 570 nm against distilled water as blank.

2.5.7 | GC-MS quantification of alkaloid constituents

The aliquot used for the GC-MS qualitative characterization analysis was prepared by dissolving ~500 mg of alkaloid extracted fraction in 5 ml of Methanol.

A qualitative characterization analysis of possible alkaloids present in the plant extracts was carried out using GC-MS. This analysis was performed using 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple axis detector) and electron impact source (Agilent Technologies). The stationary phase of separation of the compounds was carried out on HP-5 capillary column coated with 5% of Phenyl Methyl Siloxane (30 m length × 0.32 mm diameter × 0.25 μm film thickness) (Agilent Technologies). The carrier gas was helium used at a constant flow rate of 1.6 ml/min, an initial nominal pressure of 2.84 psi and at an average velocity of 46 cm/s. One microliter of the samples were injected in splitless mode at an injection temperature of 260°C. Purge flow was 21.5 ml/min at 0.50 min with a total gas flow rate of 25.8 ml/min; gas saver mode was switched on. The oven was initially programmed at 60°C (1 min), then ramped at 4°C/min to 110°C (3 min), followed by temperature programme rates of 8°C/min to 260°C (5 min) and 10°C/min to 300°C (12 min). Run time was 56.25 min with a 3 min solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230°C, quadrupole temperature of 150°C and transfer line temperature of 280°C. Scanning of possible alkaloid compounds was from *m/z* 30 to 550 amu at 2.62s/scan scan rate and were identified by comparing measured mass spectral data with those in *NIST 14 Mass Spectral Library* and literature.

Prior to analysis, the MS was auto-tuned to perfluorotributylamine (PFTBA) using already established criteria to check the abundance of *m/z* 69, 219, 502 and other mechanism optimal and sensitivity conditions.

Analysis validation was conducted by running replicate samples in order to see the consistency of the constituent compound name, respective retention time, molecular weight (amu), Quality ion (Q-Ion), and % Total.

$$\%\,\text{Total} = \frac{\text{Abundance of individual constituents}}{\text{Total abundance of all constituents in extract}} \times 100$$

Each compound identified via the NIST 14 Library Search report has a corresponding mass spectrum showing the abundance of the possible numerous *m/z* peaks per compound (Ademiluyi et al., 2016; Duong, Kadokami, Pan, Matsuura, & Nguyen, 2014).

2.6 | Data analysis

The mean ± standard deviation (*SD*) was estimated after pooling out results of replicate experiments as described by Zar, 1984. EC_{50} which is the concentration of alkaloid extract causing 50% antioxidant activity or enzyme inhibition was calculated using nonlinear regression analysis.

3 | RESULTS

PDE-5 inhibitory potential of the alkaloid extracts from Bitter leaf and Black nightshade in rats' penile tissue in vitro was assessed. As presented in Figure 1, the result showed that both extracts inhibited PDE-5 in a concentration-dependent pattern. It is noteworthy that the alkaloid extract from Bitter leaf (107.99 μg/ml) significantly ($p > 0.05$) exhibited higher PDE-5 inhibitory potential than Black nightshade (133.69 μg/ml) as indicated in Table 1. Furthermore, the ability of the alkaloid extracts from Bitter leaf and Black nightshade to inhibit arginase activities in rats' penile tissues in vitro is presented in Figure 2 with Black nightshade (26.92 μg/ml) significantly ($p > 0.05$) showing higher inhibitory activity than Bitter leaf (30.51 μg/ml).

Assaulting rats penile tissue homogenate with 250 μM of Fe^{2+} significantly increased ($p < 0.05$) the malondialdehyde (MDA) content (Figure 3). However, incubation of the challenged penile tissue homogenate with the crude alkaloid extracts showed inhibition of MDA produced in concentration-dependent (0–210.08 μg/ml) manner (Table 1). In contrast, the alkaloid extract from Bitter leaf (165.01 μg/ml) showed significantly ($p < 0.05$) higher inhibition of Fe^{2+}-induced MDA generation in the penile tissues than Black nightshade (210.08 μg/ml).

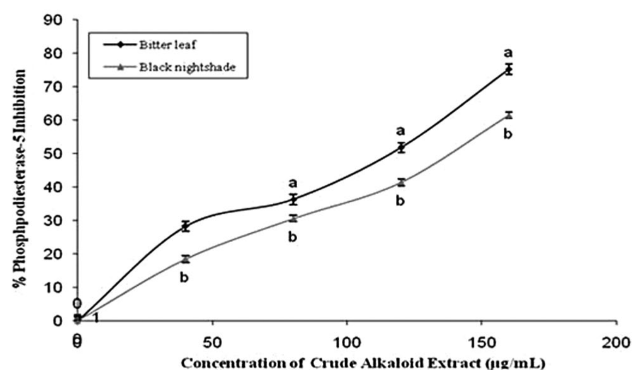

FIGURE 1 Phosphodiesterase-5 Inhibitory activity of crude alkaloid extracts from Bitter leaf and Black nightshade in rat penile tissue

TABLE 1 EC_{50} values of radical scavenging and enzymes inhibitory ability of crude alkaloid extracts from Bitter leaf (*Vernonia amygdalina*) and Black nightshade (*Solanum nigrum*)

	EC_{50} of radical scavenging and enzyme inhibitory ability (µg/ml)	
	Bitter leaf	Black nightshade
DPPH	55.77 ± 1.35^{a}	62.38 ± 2.48^{b}
OH	242.72 ± 3.3^{a}	241.55 ± 5.4^{a}
NO	735.29 ± 4.5^{b}	625.09 ± 2.8^{a}
Fe^{2+}-induced lipid peroxidation	165.01 ± 2.7^{a}	210.08 ± 5.5^{b}
Phosphodiesterase-5 Inhibitory activity	107.99 ± 4.2^{a}	133.69 ± 2.8^{b}
Arginase Inhibitory activity	30.51 ± 1.2^{b}	26.92 ± 1.1^{a}

Note: Values represent mean ± standard deviation ($n = 3$). Values with the same superscript number on the same row are not significantly ($p < 0.05$) different.

Figure 4 presents the DPPH radical scavenging ability of the crude alkaloid extracts from Bitter leaf (*Vernonia amygdalina*) and Black nightshade (*Solanum nigrum*). The result revealed that they both scavenged DPPH radicals in a concentration-dependent pattern. However, as shown by the EC_{50} values in Table 1, Bitter leaf (55.77 µg/ml) significantly exhibited higher scavenging ability than Black nightshade (62.38 µg/ml). The hydroxyl radical (OH) scavenging ability of Bitter leaf and Black nightshade as presented in Figure 5 showed that the alkaloid extracts scavenged OH as typified by the induced breakdown of deoxyribose in concentration-dependent pattern. As indicated in Table 1, EC_{50} values show no significant difference in the OH radical scavenging ability of Bitter leaf (242.72 µg/ml) and Black nightshade (241.55 µg/ml). The alkaloid extracts of Bitter leaf and Black nightshade scavenged NO radical in a concentration-dependent pattern (Figure 6) with Black nightshade (625.09 µg/ml) significantly ($p > 0.05$) exhibiting higher NO radical scavenging ability than Bitter leaf (735.29 µg/ml). The GC-MS characterization and chromatogram of constituent alkaloid compounds in Bitter leaf are shown in Table 2 and Figure 7, respectively. Similarly, Figure 8 shows the chromatogram of the alkaloid compounds in Black nightshade with the constituent alkaloids detailed in Table 3.

4 | DISCUSSION

Plant-derived foods and herbs with medicinal properties have been used in folklore medicines of many nations of the world from time immemorial to manage and treat various health challenges such as, hypertension, diabetes mellitus, cancer, erectile dysfunction, and others (Oboh & Rocha, 2007). *V. amygdalina* and *S. nigrum* have traditionally been used as pain relieving, antispasmodic, germ-free, antinarcotic, diuretic, balmy, purgative, anticancer agents, and for the management of neuronal disorders (Ayesha, Zaheer-Ud-Din, Mushtaq, & Muhammad, 2010; Manoko et al., 2007). The therapeutic attributes have been predicated on the occurrence of abundant base phytonutrients such as phenols, alkaloids, glycosides, and flavonoid. These phytonutrients have considerable antioxidative properties (Oboh et al., 2015; Omojokun et al., 2018). The antioxidant properties and ED-ameliorating potential of alkaloids extracts from *V. amygdalina* and *S. nigrum* were investigated in this study using standard protocols.

Penile erection is dependent on smooth muscle relaxation in the corpus cavernosum. The key mediator of the relaxation process is NO, which acts by increasing the cellular level of cGMP, the key second messenger mediating penile erection (Lugnier, 2006). An increase in cGMP levels can be induced by inhibition of PDE-5,

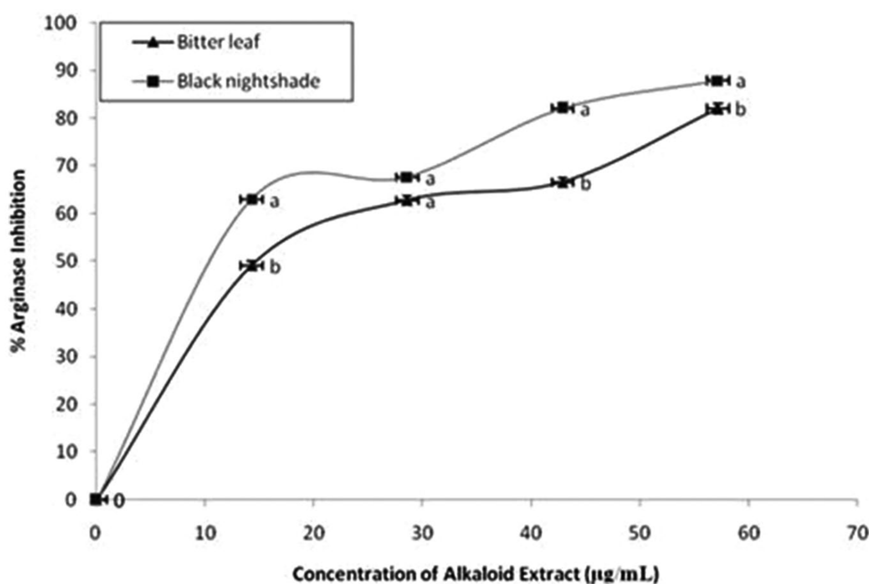

FIGURE 2 Arginase Inhibitory activity of crude alkaloid extracts from Bitter leaf and Black nightshade in rat penile tissue

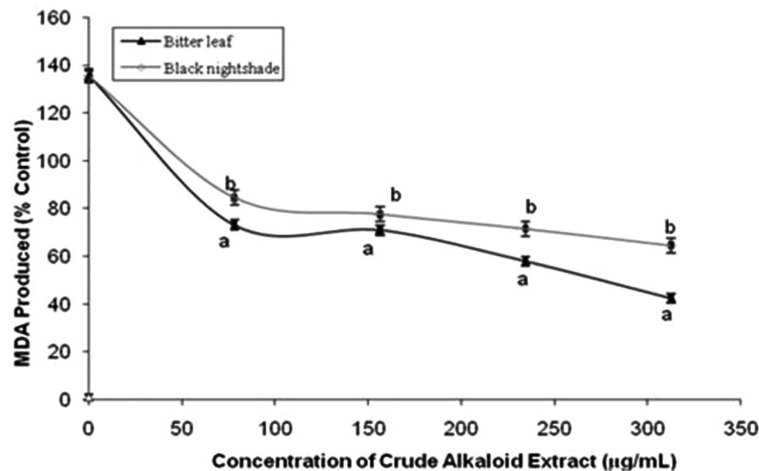

FIGURE 3 Inhibition of Fe^{2+}-induced lipid peroxidation in rat penile tissue by crude alkaloid extracts from Bitter leaf and Black nightshade

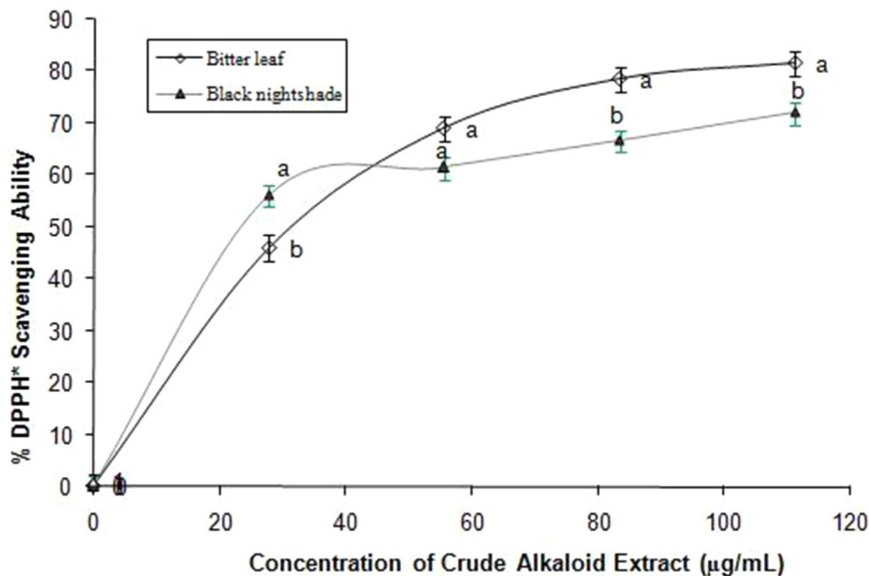

FIGURE 4 DPPH radical scavenging ability of alkaloid extracts from Bitter leaf and Black nightshade leaves

the predominant isoenzyme metabolizing cGMP in the cells of the corpus cavernosum. PDE-5 is a pharmacologic target in erectile dysfunction (Corbin, Francis, & Webb, 2002). Any inhibitors of PDE-5 activity is a potential candidate for managing or treating ED. Previous studies have recognized plant alkaloids as PDE-5 inhibitors (Carson & Lue, 2005; Drewes, George, & Khan, 2003). PDE-5 is a basic protein of the NO/cGMP signaling pathway, which inhibits NO-activated cGMP-mediated tissue relaxation, establishment of basal smooth muscle effect, and penile erection. Lugnier (2006) showed that PDE-5 reduces the activities of cGMP in the corpus cavernosum of penile tissues. Cyclic GMP is required to activate a protein kinase that reduces intracellular calcium level, upgrades muscle relaxation, and thus triggers penile erection (Lue, 2000). The PDE-5 inhibitory activity observed in *V. amygdalina* and *S. nigrum* varied directly with the concentration of alkaloids up to a maximum of (108 μg/ml) and (133 μg/ml) for both plants, respectively. *V. amygdalina* showed significantly ($p > 0.05$) higher inhibitory activity than *S. nigrum*. These results are noteworthy and justify their continuous use in the management of ED in trado-medical practices. This corroborates a recent report that *Hunteria umbellata* and *Anogeissus leiocarpus* inhibited PDE-5 activity in rats' penis (Oboh et al., 2017). These finding is also in agreement with previous reports which described the mechanism of action of therapeutics used in the management of erectile dysfunction (Bivalacqua, Champion, Hellstrom, & Kadowitz, 2000;

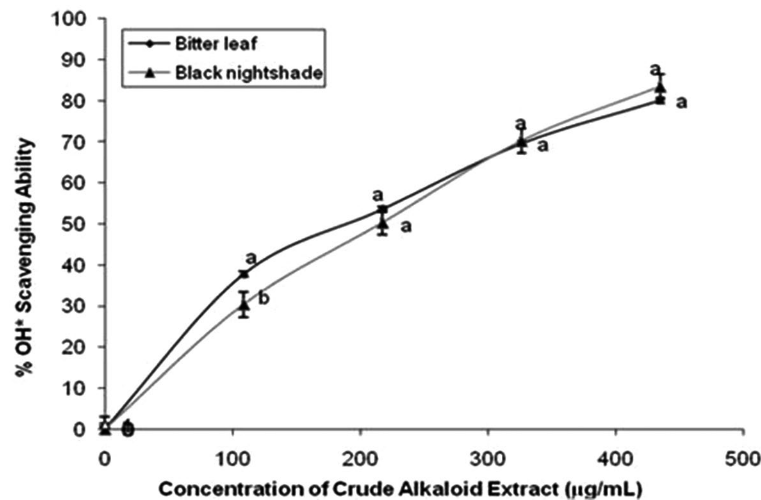

FIGURE 5 OH* scavenging ability of alkaloid extracts from Bitter leaf and Black nightshade leaves

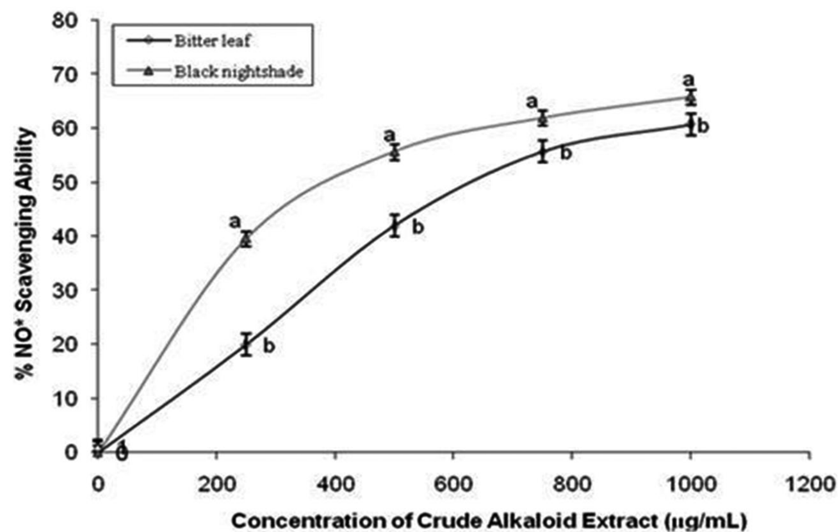

FIGURE 6 NO radical scavenging ability of crude alkaloid extracts from Bitter leaf and Black nightshade

Hatzichristou & Pescatori, 2001). We therefore suggest that these plants are viable sources of remedies or potent anti-PDE-5 factors from which valuable ED-effective drugs with minimal or no adverse effects can be developed. Inhibition of PDE-5 by these plants is expected to enhance penile erection by preventing cGMP degradation. This possibility should give an advantage over branded drugs, such as sildenafil, which is a selective inhibitor of PDE-5 and an effective drug for ED treatment, but with reported side effects in retinal, vascular, and visceral tissues (Corbin et al., 2002).

The inhibition of arginase activity is adjudged to play a key function in the biochemistry of ED (Toque et al., 2011). Bivalacqua, Burnett, Hellstrom, and Champion (2007) and Goswami, Inamdar, Jamwal, and Dethe (2014) recommended that food sources with arginase inhibition potentials are useful for the management of ED.

The inhibition of arginase in penile NOS movement, re-establishes endothelial-determined NO vasodilatation, and erectile capacity. Importantly, the arginase inhibitory potential displayed by the alkaloid extracts from the edible vegetables used in this study could render it a unique nutraceutical in ameliorating ED since it could expand the availability of NO in the body system (Berkowitz et al., 2003; Durante, Johnson, & Johnson, 2007) through arginase inhibition. Polyphenols are reportedly affirmed to aid the generation of hydrogen bond and its interactions at the arginase active site and thus inhibit the enzyme's activity (Dos-Reis, Manjolin, do CarmoMaquiaveli, Santos-Filho, & da Silva, 2013). Hence, we suggest alkaloid extracts from Black nightshade and Bitter leaf could be used for the management of ED having observed a strong inhibition of arginase activity in this study.

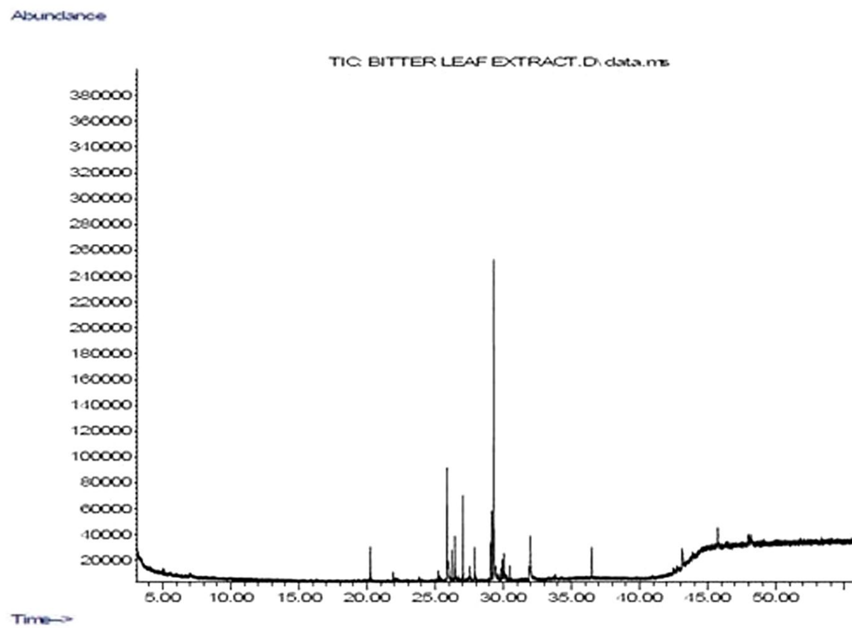

FIGURE 7 Bitter leaf (*Vernonia amygdalina*) GC-MS chromatogram

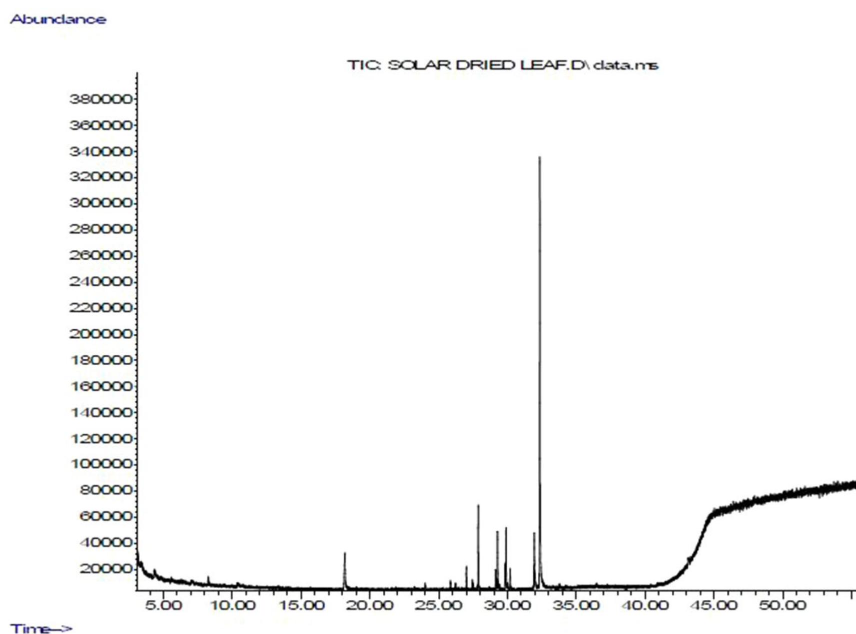

FIGURE 8 Black nightshade (*Solanun nigrum*) GC-MS chromatogram

The result gathered from this study revealed that crude alkaloid obtained from the two edible bitter vegetables exhibited DPPH, nitric oxide (NO), and hydroxyl (OH) radicals scavenging properties. In living system, hydroxyl radical (OH) are produced from hydrogen peroxide and/or superoxide anion (Tushar, Mas, & Kaneez, 2015). Hydroxyl radical is believed to play a significant function in ED (Gazzaruso et al., 2008) and it is known to be profoundly receptive, vivacious, short lived, and very dangerous to living cells (Sikka & Hellstrom, 2002). In addition, nitric oxide radical is implicated in numerous processes toxic to the cells and neurons which could in turn contribute to plaque formation in penile tissue. This radical which is majorly generated as a result of distress or inflammation (Kang et al., 2003) may thus lead to endothelial dysfunction. In the same vein, NO which plays vital function in the commencement of penile erection could as well consolidate

TABLE 2 Constituent alkaloid compounds in Bitter leaf (*Vernonia amygdalina*)

SN	Retention time (min)	Compound name/hit name	Quality ion (relative intensity, %)	% Total of all compound (% total)	CAS number	Entry number in NIST14 library
1	3.306	Oxirane, (propoxymethyl)-	50	0.2520	003126-95-2	8273
2	3.425	Hydroxyacetic acid, hydrazide	50	1.9770	003530-14-1	2253
3	3.847	Carbohydrazide	9	0.1610	000497-18-7	2399
4	4.066	Methanimidamide, N,N-dimethyl-N′-(3-nitrophenyl)-	35	0.3870	002103-47-1	58907
5	4.149	Acetic acid hydrazide	38	0.1620	001068-57-1	788
6	4.298	2-Amino-8-[3-d-ribofuranosyl] imidazo[1,2-a]-s-triazin-4-one	17	0.2510	067410-65-5	142414
7	5.034	1-Hexanamine	22	0.5240	000111-26-2	4205
8	5.200	1,3-Bis(2-chloroethyl)urea	9	0.2130	002214-72-4	51564
9	5.989	p-Dioxane, methylene-	9	0.2050	003984-19-8	3741
10	6.126	MDMA methylene homolog	4	0.2890	1000386-32-2	71431
11	6.327	Dimethylamine	9	2.0550	000124-40-3	88
12	6.844	Alpha methyl 3,6-anhydro, D-Mannopyranoside	9	0.0950	015814-56-9	44787
13	10.880	5,6-Dihydro-2-(4-nitrophenyl)-4H-1,3-oxazin-5-one	4	0.1220	077580-72-4	82808
14	19.023	Propanamide, N,N-dimethyl-	5	0.1210	000758-96-3	4168
15	19.759	Furan, 2,5-dihydro-3-methyl-	7	0.1110	001708-31-2	1460
16	19.884	Pyrrolidine, 3-methyl-	4	0.1910	034375-89-8	1596
17	21.540	Acetamide, 2-fluoro-	4	0.2150	000640-19-7	987
18	22.228	N-Methylallylamine	46	0.6270	000627-37-2	618
19	24.276	Borane carbonyl	4	0.1580	013205-44-2	65
20	25.012	Aminopyrazine	5	0.1920	005049-61-6	2676
21	25.326	Oxalic acid, isobutyl pentyl ester	33	0.1030	1000309-37-0	79373
22	25.445	Azetidine, 1-methyl-	5	0.0970	004923-79-9	622
23	25.872	Neophytadiene	93	5.6850	000504-96-1	138502
24	26.442	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	58	1.7950	102608-53-7	155865
25	26.686	1-Fluorononane	12	0.3200	000463-18-3	22849
26	26.846	3(2H)-Thiophenone, dihydro-, oxime, 1,1-dioxide	4	0.0940	1000327-57-0	24070
27	27.297	Azetidine, 1-methyl-	5	0.1240	004923-79-9	622
28	29.903	1,3-Cyclooctadiene	76	1.4180	001700-10-3	5507
29	30.039	Hexadecanamide	93	1.6450	000629-54-9	116301
30	30.205	Ethyl hydroden pimelate	47	0.6010	1000342-35-7	54902
31	31.452	5-(Prop-2-enoyloxy) pentadecane	25	0.2610	1000245-67-6	142102
32	31.624	5-Butyl-1,3-oxathiolan-2-one	32	0.2920	1000254-54-5	32188
33	31.962	9-Octadecenamide, (Z)-	55	3.7050	000301-02-0	141030
34	33.072	Cyclohexanooxazin-2(1H)-one, 3,9-dihydro-6,8-isopropylideno-9-methyl-	9	0.1010	1000260-31-8	71560

(Continues)

TABLE 2 (Continued)

SN	Retention time (min)	Compound name/hit name	Quality ion (relative intensity, %)	% Total of all compound (% total)	CAS number	Entry number in NIST14 library
35	33.309	Carbamic acid, ethylnitroso-, ethyl ester	9	0.2740	000614-95-9	22338
36	33.636	2-Trifluoroacetoxytridecane	10	0.2550	116465-18-0	155165
37	33.802	1,2-Benzisothiazole, 3-methyl-	9	0.2660	006187-89-9	24231
38	33.998	Propanal,3-hexylimino-2-nitro-	9	0.3130	339192-83-5	65617
39	35.185	1-(2-Adamantylidene) semicarbazide	9	0.3830	065814-27-9	71395
40	35.363	2-Carbamyl-9-[.beta.-d-ribo-furanosyl]hypoxanthine	17	0.1760	121358-21-2	154058
41	35.743	1,3,5-Triazine, 2-chloro-4,6-bis(methylthio)-	9	0.8770	004407-40-3	71684
42	36.141	Formamide, N,N-dimethyl-	9	0.4140	000068-12-2	751
43	36.485	Squalene	90	1.5360	000111-02-4	243220
44	36.948	Carbamic acid, (cyanoacetyl)-, ethyl ester	9	0.0990	006629-04-5	29595
45	38.989	Acetohydroxamic acid	5	0.0890	000546-88-3	883
46	39.257	14.alpha.-Morphinan, 7,8-didehydro-3-methoxy-17-methyl-6-methylene-	9	0.1600	017939-34-3	141077
47	39.577	2-quinoxalinamine, 3-chloro-N-ethyl-	5	0.2090	1000400-56-1	71161
48	40.479	(9-Oxo-9,10-dihydroacridin-4-yl)acetic acid	5	0.1320	145447-90-1	114253
49	40.628	5H-dibenzo[a,d] cyclohepten-5-amine	5	0.1290	1000400-55-2	71669
50	40.734	N-Isopropyl-3-phenylpropanamide	9	0.0870	056146-87-3	56865
51	41.340	Benzenemethanamine, 4-methoxy-N,N-dimethyl-.alpha.-(dimethylamino)-	9	0.7630	122687-84-7	72391
52	42.076	Purine-2,6-dione, 8-(3-ethoxypropylamino)-1,3-dimethyl-3,9-dihydro-	25	0.2080	1000304-55-6	140491
53	42.248	2-(4-Chlorophenyl)-5,7-dimethylimidazo[1,2-a] pyridine-8-carbonitrile	22	0.1980	1000305-50-9	140840
54	42.331	Propanamide, 3-bromo-N-(4-bromo-2-chlorophenyl)-	38	0.1920	1000267-91-3	196417
55	43.109	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine	35	2.3990	1000111-66-9	157264
56	44.165	3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester	35	0.4380	1000362-34-6	114062
57	44.284	N-Methyl-1-adamantaneacetamide	46	2.3830	031897-93-5	71587
58	44.563	6-Chloro-1-ethyl-4-oxo-N-(pyridin-4-ylmethyl) quinoline-3-carboxamide	38	0.2650	1000409-38-7	197998

(Continues)

TABLE 2 (Continued)

SN	Retention time (min)	Compound name/hit name	Quality ion (relative intensity, %)	% Total of all compound (% total)	CAS number	Entry number in NIST14 library
59	44.865	acetic acid, 2-[bis(methylthio) methylene]-1-phenylhydrazide	43	0.4870	1000401-44-4	114619
60	45.720	2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-	41	1.2720	054965-43-4	85540
61	46.860	2′-Hydroxypropiophenone, TMS derivative	46	0.9960	033342-87-9	85144
62	47.061	2-Methyl-7-phenylindole	38	0.2440	001140-08-5	71646
63	47.631	2-Ethylacridine	46	6.1830	055751-83-2	71643
64	48.878	Cyclotrisiloxane, hexamethyl-	43	1.6110	000541-05-9	85992
65	49.804	Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy]benzoate	38	0.3150	1000378-73-2	155034
66	49.904	Benzo[h]quinoline, 2,4-dimethyl-	43	1.9650	000605-67-4	71668
67	50.029	1,1,1,3,5,5,5-Heptamethyltrisiloxane	38	0.8640	001873-88-7	86044
68	50.468	Silicic acid, diethyl bis(trimethylsilyl) ester	59	2.8060	003555-45-1	154747
69	51.317	1,4-Bis(trimethylsilyl)benzene	43	1.1740	013183-70-5	85161
70	52.047	Tris(tert-butyldimethylsilyloxy) arsane	43	0.6450	1000366-57-5	260810
71	53.151	1H-Indole, 1-methyl-2-phenyl-	43	0.5050	003558-24-5	71659
72	53.383	Arsenous acid, tris(trimethylsilyl) ester	43	0.2580	055429-29-3	199618
73	55.033	1,2-Bis(trimethylsilyl)benzene	43	0.7560	017151-09-6	85160
74	55.602	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	46	2.3050	1000129-52-1	71369

with superoxide to produce another free radical and toxic compound known as peroxynitrite. This can promote the development of lipid peroxidation and prompt the arrangement of malondialdehyde and hydroxides which are factors in ED pathophysiology (Agarwal, Nandipati, Sharma, Zippe, & Raina, 2006). Thus, as a result of the endothelial dysfunction caused by NO, reducing its bioavailability will be a good approach to ED treatment (Akomolafe, Oboh, Oyeleye, & Boligon, 2016). Also, inhibition of lipid peroxidation induced by Fe^{2+} in rats' penile tissue homogenate by the vegetables crude alkaloid extracts is an expression of strong antioxidant magnitude. Bio-membrane and biomolecules peroxidation is reported to be associated with the advancement of various health challenges. Also, iron is reported to be involved in inducing lipid peroxidation in living system as its disruption could lead to iron overload which is implicated in oxidative stress-induced ED (Agarwal et al., 2006). Therefore, OH and Fe^{2+} radical scavenging ability is acknowledged efficient antioxidant mechanisms. Hence, the abilities of the alkaloid extracts to scavenge these free radicals as shown in this study could be valuable in erectile dysfunction management (Maggi, Filippi, Ledda, Magini, & Forti, 2000).

Medicinal values of herbs and vegetables are mainly attributed to their phytoconstituents, for example, flavonoids, hydroxycinnamic acids (Omojokun et al., 2018) terpenes, and alkaloids. Various researchers have reported auspicious health boosting potentials (Dinchev et al., 2008; Jamil et al., 2007) of alkaloids. The inhibitory potential of alkaloid extracts from Black nightshade and Bitter leaf on the activities of enzymes linked with erectile dysfunction as well as their antioxidative properties exhibited could be attributed to the synergistic activity of many alkaloid compounds which was characterized in the two vegetables using GC-MS.

TABLE 3 Constituent alkaloid compounds in Black nightshade (*Solanum nigrum*)

SN	Retention time (min)	Compound name/hit name	Quality ion (relative intensity, %)	Percentage total of all compound (% total)	CAS number	Entry number in NIST14 library
1	3.473	9H-Purine-6-thiol, 2-amino-9-butyl-	9	1.588	1000305-19-5	86875
2	4.298	4-Amino-6-hydroxypyrazolo[3,4-d] pyrimidine	16	0.325	005472-41-3	25390
3	5.752	1,1′-(4-Methyl-1,3-phenylene) bis[3-(5-benzyl-1,3,4-thiadiazol-2-yl)urea]	9	0.131	1000224-58-6	271216
4	6.084	benzonitrile, 2-[(1-methyl-2(1H)-pyridinylidene) methyl]-5-nitro-	9	0.242	1000396-68-1	114173
5	6.998	Carbamic acid, (cyanoacetyl)-, ethyl ester	28	1.116	006629-04-5	29595
6	11.218	Sarcocapnidine	7	0.300	087069-33-8	185196
7	11.515	Quinazolin-4(3H)-one, 2,3-dimethyl-	2	0.141	002161-22-0	44122
8	17.415	2,2,2-Trifluoro-N-(7-methoxy-2-oxidoisoquinolin-8-yl) acetamide	2	0.100	1000404-26-8	145424
9	19.035	Propanamide, N-ethyl-	5	0.168	005129-72-6	4146
10	19.367	Propanamide, 3-phenyl-N-ethyl-	25	0.448	1000407-14-7	45319
11	20.917	2H-Azepin-2-one, hexahydro-1-methyl-	5	0.106	002556-73-2	12116
12	22.875	4-Pyridinecarboxylic acid, N′-[[(1-methylethyl)amino] carbonyl]hydrazide	9	0.310	1000327-64-4	84758
13	24.780	Oxazole, trimethyl-	7	0.292	020662-84-4	6219
14	25.677	Acetamide, 2-amino-	3	0.120	000598-41-4	784
15	27.303	2-Propenamide	9	0.707	000079-06-1	597
16	28.751	2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin	10	0.160	108533-24-0	184055
17	30.039	Dodecanamide	64	1.267	001120-16-7	64236
18	30.478	Neophytadiene	53	0.519	000504-96-1	138502
19	30.811	MDMA methylene homolog	4	0.137	1000386-32-2	71431
20	30.888	Dimethylamine	4	0.166	000124-40-3	88
21	31.339	3-O-Acetyl-exo-1,2-O-ethylidene-.alpha.-d-erythrofuranose	25	0.170	077489-44-2	54780
22	31.962	8-Methyl-6-nonenamide	50	2.508	1000293-20-9	39039
23	32.502	1-(2-Adamantylidene) semicarbazide	4	0.166	065814-27-9	71395
24	32.775	2-(1-Piperidino)-5-nitropyridine	5	0.129	026820-61-1	71172
25	33.102	Dodecahydropyrido[1,2-b] isoquinolin-6-one	5	0.202	108873-36-5	71593
26	33.304	Acetamide, 2-cyano-	4	0.446	000107-91-5	1352

(Continues)

TABLE 3 (Continued)

SN	Retention time (min)	Compound name/hit name	Quality ion (relative intensity, %)	Percentage total of all compound (% total)	CAS number	Entry number in NIST14 library
27	34.390	Formamide, N,N-dimethyl-	9	0.099	000068-12-2	751
28	34.758	o-Veratramide	9	0.097	001521-39-7	49053
29	35.221	5-Aminoisoxazole	9	0.164	014678-05-8	1351
30	35.523	4-Allyl-5-furan-2-yl-2,4-dihydro-[1,2,4]triazole-3-thione	5	0.226	1000300-01-3	71831
31	35.618	7-Chloro-4-methoxy-3-methylquinoline	5	0.094	1000213-52-2	71274
32	35.779	Benzenemethanamine, 4-methoxy-N,N-dimethyl-.alpha.-(dimethylamino)-	9	0.153	122687-84-7	72391
33	36.046	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	5	0.224	1000147-64-6	71757
34	36.336	2-Pyridinemethanol 3,5-dichloro-4-hydroxy-6-methyl-	5	0.127	055042-78-9	71721
35	36.485	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	47	0.556	075581-03-2	199592
36	36.645	Purine-2,6-dione, 8-(3-ethoxypropylamino)-1,3-dimethyl-3,9-dihydro-	7	0.147	1000304-55-6	140491
37	37.209	Thieno[2,3-b]pyridine-2-carboxamide, 3-amino-6-methyl-	5	0.610	1000351-59-9	71830
38	37.642	2-quinoxalinamine, 3-chloro-N-ethyl-	9	0.142	1000400-56-1	71161
39	37.797	5H-dibenzo[a,d] cyclohepten-5-amine	5	0.228	1000400-55-2	71669
40	38.058	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-	9	0.374	094427-47-1	71537
41	39.001	9-Borabicyclo[3.3.1]nonane, 9-[3-(dimethylamino)propyl]-	5	0.216	1000160-35-2	71620
42	39.197	1,3,5-Triazine, 2-chloro-4,6-bis(methylthio)-	9	0.686	004407-40-3	71684
43	39.470	11H-Dibenzo[b,e][1,4] diazepin-11-one, 5,10-dihydro-5-[3-(methylamino) propyl]-	5	0.289	013450-70-9	140962
44	40.200	4-Sulfamoyl-thiophene-2-carboxylic acid	5	0.148	1000300-36-6	71683
45	40.919	s-Triazolo[4,3-a]pyridine-3-thiol, 5-methyl-	5	0.232	004926-22-1	35861
46	41.251	2(1H)-Pyrimidinone, 5-chloro-4,6-diphenyl-	10	0.135	028567-83-1	141700
47	41.613	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester	9	0.641	1000351-62-2	71780
48	41.833	1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester	25	0.160	1000197-90-8	125534

(Continues)

TABLE 3 (Continued)

SN	Retention time (min)	Compound name/hit name	Quality ion (relative intensity, %)	Percentage total of all compound (% total)	CAS number	Entry number in NIST14 library
49	42.189	2,6-Dihydroxyacetophenone, 2TMS derivative	16	0.186	1000352-81-3	155019
50	42.349	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	32	0.427	018030-67-6	196432
51	42.480	4-Phenyl-3,4-dihydroisoquinoline	27	0.478	006187-58-2	71666
52	42.693	2-Chloro-4,6-di-piperidin-1-yl-[1,3,5]triazine	25	0.224	007710-36-3	140588
53	43.002	Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy]benzoate	23	0.212	1000378-73-2	155034
54	43.204	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	46	1.286	1000129-52-1	71369
55	43.732	6-Chloro-1-ethyl-4-oxo-N-(pyridin-4-ylmethyl)quinoline-3-carboxamide	38	1.334	1000409-38-7	197998
56	44.290	acetic acid, 2-[bis(methylthio)methylene]-1-phenylhydrazide	43	5.278	1000401-44-4	114619
57	44.776	1,2-Bis(trimethylsilyl)benzene	43	0.463	017151-09-6	85160
58	44.907	Cyclotrisiloxane, hexamethyl-	43	1.108	000541-05-9	85992
59	45.495	Tris(tert-butyldimethylsilyloxy)arsane	43	0.284	1000366-57-5	260810
60	45.732	Silicic acid, diethyl bis(trimethylsilyl) ester	47	4.928	003555-45-1	154747
61	46.112	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	43	0.344	1000161-21-8	111265
62	46.783	2′-Hydroxypropiophenone, TMS derivative	46	0.497	033342-87-9	85144
63	48.498	1,1,1,3,5,5,5-Heptamethyltrisiloxane	38	0.276	001873-88-7	86044
64	48.599	N-Methyl-1-adamantaneacetamide	38	0.786	031897-93-5	71587
65	49.299	1,2,4-Benzenetricarboxylic acid, 4-dodecyl dimethyl ester	38	0.441	033975-29-0	241375
66	49.922	2-Ethylacridine	50	5.174	055751-83-2	71643
67	51.857	2-Methyl-7-phenylindole	43	0.694	001140-08-5	71646
68	53.804	Methyltris(trimethylsiloxy)silane	43	0.833	017928-28-8	168286
69	55.169	Benzo[h]quinoline, 2,4-dimethyl-	46	1.672	000605-67-4	71668
70	55.264	2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-	43	0.333	054965-43-4	85540
71	55.899	Carvacrol, TMS derivative	43	0.384	1000367-02-2	85400

5 | CONCLUSION

Vernonia amygdalina and *Solanum nigrum* alkaloid extracts exhibited free radicals scavenging properties and erectogenic potential as typified by their arginase and phosphodiesterase-5 enzyme inhibitory activities in rats' penile tissues. These properties apparently provide a biochemical basis for their therapeutic efficacy and justify their use in folklore medicine. However, further in-vivo and sexual

behavioral studies as well as clinical trials is advised to fully substantiate this claim.

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CONFLICT OF INTEREST

We declare no conflict of interest as far as this manuscript is concerned.

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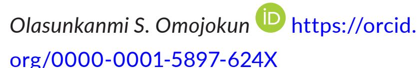
Olasunkanmi S. Omojokun  https://orcid.org/0000-0001-5897-624X

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