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In vitro aphrodisiac potentials of some Nigerian herbal drinks

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Abstract

Background and Aim: Despite the widespread use of herbal aphrodisiac drinks in Nigeria, there is a dearth of information about their efficacy; hence, this study. This study investigates the inhibitory effects of selected aphrodisiac drinks on phosphodiesterase-5 (PDE-5) and arginase enzymes from rat penile tissue.

Materials and Methods: Six male Wistar rats were sacrificed humanely, their penile tissues collected, washed, homogenized, and centrifuged. The supernatant served as the enzyme source for PDE-5 and arginase. Appropriate reaction mixtures were added to the supernatant to assay the phosphodiesterase-5 and arginase enzyme inhibition.

Results: All the thirty-one herbal aphrodisiac drinks screened possessed arginase and phosphodiesterase-5 enzyme inhibitory activities. For phosphodiesterase-5 inhibitory activity, the aphrodisiac drink with the nearest inhibitory activity to the standard (IC_{50} = 0.090645) was Zazoozeh, with an IC_{50} of 0.110275 mg/ml. For the arginase inhibitory assay, Ojapa showed the highest activity with an average IC_{50} reading of 0.315191 mg/ml, which was the nearest to the standard (Sildenafil) which had an IC_{50} reading of 0.182577 mg/ml. Following Ojapa was Zazoozeh (IC_{50} = 0.448014 mg/ml).

Conclusion: All the tested aphrodisiac drinks possessed *In vitro* phosphodiesterase-5 and arginase enzymes inhibitory activities, and the Zazoozeh had the best activity vis-à-vis the two assays used.

Keywords: Erectile dysfunction; Herbal drinks; Aphrodisiacs; Phosphodiesterase-5; Arginase; Penile tissue; *In vitro*

1. Introduction

Erectile dysfunction (ED) can be defined as the inability of men to attain or maintain a penile erection sufficient to achieve satisfactory sexual performance. It is a disorder that affects men above 40 years of age, and it has been shown to progressively worsen with age [1]. Erectile dysfunction has been linked to a few conditions such as hypertension, diabetes mellitus, metabolic syndrome, hyperlipidemia, depression, lower urinary tract symptoms and an unhealthy lifestyle [2]. Erection is achieved when males are stimulated by visual, auditory, or olfactory stimuli or sexual thoughts, these signals are passed to the cerebral cortex and then through the parasympathetic pathway from the sacral plexus. This cascade leads to the release of nitric oxide from non-adrenergic non-cholinergic nerve fibers. The release of Nitric oxide causes an increase in the concentration of cGMP, and a decrease in the intracellular calcium level thereby leading to the relaxation of smooth muscle and allowing more blood to be retained in the corpora cavernosa. This results in erectile hardness, and then a rigid erection [3,4]. The release of nitric oxide plays an important role in enhancing penile erection, and quality control of sperm is needed in spermatogenesis [5]. Phosphodiesterase-5 (PDE-5) enzymes are

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greatly expressed in the vascular smooth muscle, and it is the most common PDE subtype in the smooth muscle of the penis [4]. They degrade cyclic guanosine monophosphate (cGMP), thereby leading to the contraction of smooth muscles and the inability of the penis to be erect [6]. The PDE-5 enzyme inhibitors are the recommended first-line therapy for erectile dysfunction. Four PDE-5 inhibitors have been approved by the United States Food and Drug Administration (FDA). They include: Avanafil, Tadalafil, Sildenafil, and Vardenafil [4,7].

L-arginine is the precursor responsible for the formation of nitric oxide by the enzymatic action of nitric oxide synthase (NOS). Arginase enzymes regulate L-arginine's bioavailability, thereby reducing the pool of L-arginine available for nitric oxide biosynthesis. Hence, inhibition of arginase enzymes will enhance nitric oxide biosynthesis [8].

Traditional remedies are considered more acceptable, affordable and accessible than orthodox medicine and believed to have fewer side effects; hence, there is the popularity of usage of these remedies in underdeveloped nations [5,9,10]. Some of the herbal remedies studied for use in the management of erectile dysfunction include *Epimedium* herbs (icariin), Panax ginseng, *Piper guineense, Butea superba, Tribulus terrestris, Pausinystalia johimbe (*yohimbine), *Securidaca longipedunculata* [11], *Lepidium meyenii* [12], etc. The aphrodisiac activity of these plants is due to the presence of xanthones, alkaloids, polyphenols, steroids, [13], flavonoids and amino acids [14]. An aphrodisiac is any substance that boosts sexual arousal, behavior, performance, desire, or pleasure [15]. In Nigeria, there are several herbal drinks marketed with claims of aphrodisiac activities at street corners, car parks, kiosks, and supermarkets [16]. These drinks are typically packaged in sachets and small-volume containers, with alcohol popularly used as the vehicle. The alcoholic content of these drinks typically varies from a minimum of 15% to a maximum of 45% alcohol content [17]. However, despite the prevalence of usage of these drinks, there is little to no biochemical information to support their use. It is therefore necessary to unravel some biochemical mechanisms behind the use of these drinks for the treatment of erectile dysfunction. Hence, this study evaluated the inhibitory effect of some selected aphrodisiac drinks on phosphodiesterase-5 and arginase enzymes from rats' penile tissue.

2. Materials and methods

2.1. Chemicals and Drugs

This study used thirty-one brands of herbal aphrodisiac drinks. The basis of this selection was a previous study, where fifty herbal aphrodisiac drinks in the Nigerian markets were assessed for the suitability of information on their product packs and the effects of the intake of these drinks on some health parameters of commercial bus drivers in Ife-Central Local Government Area, Osun State, Nigeria [18]. The ethics approval for the study was obtained from the Health Research Ethics Committee, Institute of Public Health, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. At the time of this study, only thirty-one brands of these drinks were available in the market. The 31 brands were purchased from Bito wine stores, and Ace Supermarket in Ile-Ife, Osun state, Nigeria. Viagra (sildenafil), used as the control drug, was obtained from Boluwatife Pharmacy, Aworeni junction, Ile-Ife, Osun state, Nigeria. Other chemicals used were obtained from reputable sources and verified to be of analytical grade.

2.2. Enzymes Sources

Phosphodiesterase-5 (PDE-5) inhibition assay: By cervical dislocation, three male rats were sacrificed, the penile tissues were detached and washed twice in Tris-HCl buffer (0.1M), which contained $CaCl_2$ (1 mM) and NaCl (50 mM, pH 8.0). The penile tissue was homogenized in three volumes of ice-cold buffer. The homogenate was centrifuged at 3,000 revolutions per minute for 15 min. The temperature was set at 4 °C, and the enzyme was obtained from the supernatant layer of the homogenate. The final reaction mixture containing 5 mM of p-nitrophenyl phosphate as the substrate, 100 µL of the phosphodiesterase-5 enzyme, 20 mM of Tris buffer (pH 8.0), and the aphrodisiac drinks was incubated at 37 °C for 10 min. The intensity of p-nitrophenol produced was measured as a change in absorbance after 5 min at 400 nm. The inhibitory activity of the aphrodisiac drinks on PDE-5 enzyme was expressed as percentage inhibition; the control experiment was undertaken without the test sample.

Arginase inhibition assay: By cervical dislocation, three male rats were sacrificed, the penile tissue was extracted and washed twice in Tris-HCl buffer (0.01M), which contained $MnCl_2$ (0.05M, pH 9.5). Then the penile tissue was homogenized in three volumes of ice-cold buffer. The homogenate was centrifuged at 3,000 revolutions per minute for 15 min. The temperature was set at 4 °C, and the enzyme was obtained from the supernatant layer of the homogenate. The urea produced by the reaction of Ehrlich's reagent was used as an indicator of the activity. The final reaction mixture contained Tris-HCl buffer (1.0 mM, pH 9.5) containing $MnCl_2$, Arginine solution (0.1M), the enzyme preparation and the aphrodisiac drink in a volume of 1.0ml. This mixture was placed in an incubator at 37^0C for 10 minutes. 2.5ml of Ehrlich's reagent (2.0g of p-dimethylaminobenzaldehyde in 20ml of concentrated hydrochloric acid and made up to 100ml with

distilled water) was used to terminate this reaction. Then the optical reading was taken at 450 nm after 20 minutes. The control experiment was performed without the aphrodisiac drink.

3. Results

As presented in Table 1, all thirty-one aphrodisiac drinks had phosphodiesterase-5 enzyme inhibitory activities. The most active of them is the Zazoozeh brand, with a half-maximal inhibitory concentration (IC_{50}) value of 0.11028 ± 0.01958 mg/ml, followed by Ace Bitters, which had 0.12225 ± 0.02581 mg/ml as its IC_{50}. All the aphrodisiac drinks had a higher IC_{50} value when compared with Sildenafil, which served as the positive control.

Table 1 Half-maximal inhibitory concentrations (IC_{50}) of some Nigerian herbal aphrodisiac drinks on Phosphodiesterase-5 enzyme.

S/N	TEST AGENT	IC_{50} (mg/ml) ± SEM
1	Sildenafil (control)	0.09066 ± 0.00394
2	Zazoozeh	0.11028 ± 0.0195
3	Ace Bitters	0.12225 ± 0.02581
4	Enu ose	0.12631 ± 0.06943
5	Manpower	0.13802 ± 0.06209
6	Ojapa	0.15747 ± 0.00898
7	Bo kaadi	0.17249 ± 0.00721
8	Japata	0.21404 ± 0.00654
9	3D Action	0.21936 ± 0.08119
10	Gbe se e	0.25783 ± 0.00926
11	Origin	0.28581 ± 0.04439
12	Skirt	0.29210 ± 0.01272
13	Action bitters	0.30741 ± 0.00733
14	Sorosoke	0.30845 ± 0.01773
15	Confam bitters	0.31400 ± 0.04211
16	Ewe ati egbo	0.33200 ± 0.02017
17	Alogin	0.34754 ± 0.00688
18	Action bitters	0.36224 ± 0.03733
19	1960 rootz	0.38046 ± 0.05914
20	Agbara	0.43591 ± 0.04561
21	Kolaq alagbo	0.47613 ± 0.02013
22	Jaleke	0.47767 ± 0.03750
23	Chairman brandy	0.51124 ± 0.07876
24	Kick and fire	0.60811 ± 0.12898
25	Jekonmo	0.62903 ± 0.00517
26	Striker	0.64021 ± 0.09648
27	Sapele water	0.68133 ± 0.17050
28	Merry Jedi	0.69402 ± 0.01036
29	4 horses	0.76513 ± 0.00846
30	Jedi Oloyin	0.77290 ± 0.03097
31	Stone	0.87592 ± 0.01511

As presented in Table 2, all thirty-one aphrodisiac drinks had arginase enzyme inhibitory activities. The most active of them is the Ojapa brand, with a half-maximal inhibitory concentration (IC_{50}) value of 0.31519 **±** 0.03310 mg/ml, followed by Zazoozeh, which had 0.44801 **±** 0.10584 mg/ml as its IC_{50}. All the aphrodisiac drinks had a higher IC_{50} value when compared with Sildenafil, which served as the positive control.

Table 2 Half-maximal inhibitory concentrations (IC_{50}) of some Nigerian herbal aphrodisiac drinks on Arginase enzyme.

S/N	TEST AGENT	IC_{50} (mg/ml) ± SEM
1.	Sildenafil	0.18258 ± 0.00740
2.	Ojapa	0.31519 ± 0.03310
3.	Zazoozeh	0.44801 ± 0.10584
4.	3D Action	0.54040 ± 0.01930
5.	4 horses	0.67087 ± 0.03725
6.	Manpower	0.88035 ± 0.02601
7.	Japata	0.88868 ± 0.03333
8.	Agbara	0.89882 ± 0.02523
9.	Merry Jedi plus	1.01196 ± 0.00193
10.	Skirt	1.02303 ± 0.05908
11.	Stone	1.05539 ± 0.03747
12.	Action bitters	1.08621 ± 0.05379
13.	Ace bitters	1.16911 ± 0.01960
14.	Chairman brandy	1.20166 ± 0.10454
15.	Enu ose	1.23187 ± 0.02258
16.	Kick and fire	1.24441 ± 0.05800
17.	Ewe ati egbo	1.30344 ± 0.01543
18.	Sapele wota	1.36622 ± 0.06758
19.	Alogin	1.38931 ± 0.24470
20.	1960 rootz	1.41041 ± 0.09619
21.	Sorosoke	1.46885 ± 0.04709
22.	Gbesee	1.48062 ± 0.12823
23.	Bo kaadi	1.48498 ± 0.02389
24.	Origin	1.52193 ± 0.11060
25.	Action	1.54770 ± 0.25479
26.	Kolaq	1.61443 ± 0.19423
27.	Jaleke	1.62542 ± 0.06522
28.	Jekomo	1.65689 ± 0.08694
29.	Striker	1.76020 ± 0.03292
30.	Jedi oloyin	1.88382 ± 0.12218
31.	Confam bitters	2.43257 ± 0.03069

4. Discussion

The enzyme phosphodiesterase (PDE) -5 is well expressed in the smooth muscles of penile tissues, and it is implicated in erectile dysfunction. The PDE-5 enzyme predisposes to erectile dysfunction by degrading cyclic guanosine monophosphate (cGMP) in the penile tissues. Notably, cGMP facilitates a reduction in intracellular calcium in the blood

vessels innervating the penis, and this leads to a relaxation of the smooth muscles surrounding the blood vessels, which then leads to dilation of the vessels, an increase in volume of blood reaching the vessels and enhanced erection. Hence, the reduction in the amount of cGMP caused by PDE-5 enzyme leads to an inability to achieve and/or maintain an erection [19]. Phosphodiesterase-5 inhibitors work by promoting penile blood flow and relaxing the smooth muscle of the corpus cavernosum (CCSM), or penile tissue [20].

The concentration of L-arginine in the penile tissue directly affects the synthesis of nitric oxide, and nitric oxide has been shown to play a pivotal role in the enhancement of penile erection. Arginase enzymes degrade L-arginine, thereby reducing the concentration of nitric oxide in the penile tissue. Therefore, the inhibition of arginase enzymes enhances the bioavailability of L-arginine and increases the synthesis of nitric oxide, which in turn enhances penile erection.

From this study, all thirty-one aphrodisiac drinks tested possessed inhibitory activities on phosphodiesterase-5 and arginase enzymes, as shown in Tables 1 and 2. The half maximal inhibitory concentration (IC_{50}) was used to assess the potency of test agents; the lower the $IC_{50,}$ the higher the potency of the test agent. The results obtained lend credence to the claims of the manufacturers of these herbal aphrodisiac drinks in treating erectile dysfunction. Most herbal products marketed in Nigeria are listed by the National Agency for Food, Drug, and Control (NAFDAC), and the product packs usually contain disclaimers that the claims of efficacy advertised by the product manufacturers have not been validated, even those that have been marketed for decades. This work provides scientific information to unravel the possible mechanisms of aphrodisiac activities of the analyzed products.

The results obtained clearly revealed that the positive control (sildenafil) possessed better *In vitro* PDE-5 and arginase inhibitory activities when compared with the herbal aphrodisiac products tested in this work (As shown in Tables 1 and 2). This could be a clear departure from the assertion of many users of herbal products who believe that herbal products are usually more effective in the management of health conditions than orthodox drugs. Conversely, because there are other mechanisms responsible for the sex enhancing activities of aphrodisiac agents (inhibition of Adenosine deaminase, Acetylcholinesterase, and angiotensin-I converting enzyme, increased secretion of tropic hormones and sex hormones e.t.c) [21] and this research does not provide information on the activities of the test products using the other mechanisms of action, therefore, it cannot be concluded that sildenafil is more effective than the test agents by depending on only the *In vitro* PDE-5 and arginase inhibitory activities [21]. Additionally, *in vivo* aphrodisiac studies, which involve evaluation of the aphrodisiac effects of test agents in animal models, will provide more reliable scientific information on the aphrodisiac activities of the tested herbal products, and this can be carried out on the most active aphrodisiac products [22].

The Zazoozeh brand had the highest phosphodiesterase-5 inhibitory activity and came second in the arginase inhibition assay. The main constituent of this product is *Musa paradisiaca* (plantain). Studies carried out on the aqueous extract of the unripe fruit of *Musa paradisiaca* revealed the presence of alkaloids, tannins, resins and saponins and validated the enhancing effects of the extract on sperm count and motility [23]. Another study carried out on the unripe and ripe plantain peel extracts showed that the extracts reduced the activity of phosphodiesterase-5 and arginase enzymes as well as acetylcholinesterase (AChE), angiotensin-I converting enzyme (ACE) and adenosine deaminase [24]. These findings corroborate our research work. Furthermore, Ojapa, the herbal aphrodisiac product with the highest arginase inhibitory activity, has been shown to contain *Cinnamomum cassia,* and the methanolic extract of *Cinnamomum cassia* has been found to possess *In vitro* arginase inhibitory activity [20]. These herbal aphrodisiac products can serve as important sources in the discovery of newer chemical compounds with better aphrodisiac activities and a lower side effect profile when compared with the drugs that are currently used in the management of erectile dysfunction.

List of all abbreviations

- ACE - Angiotensin-I Converting Enzyme
- AChE - Acetylcholinesterase
- cGMP - Cyclic Guanosine Monophosphate
- CCSM - Corpus Cavernosum Smooth Muscle
- ED — Erectile Dysfunction
- FDA — Food and Drug Administration
- HCl — Hydrochloric Acid
- IC_{50} — Half-Maximal Inhibitory Concentration
- $MnCl_2$ — Manganese(II) Chloride
- NAFDAC — National Agency for Food and Drug Administration and Control
- NaCl — Sodium Chloride
- NOS — Nitric Oxide Synthase

- PDE-5 — Phosphodiesterase-5

5. Conclusion

All the Nigerian aphrodisiac drinks used in this study had inhibitory activity on phosphodiesterase-5 and arginase enzymes, with the Zazoozeh brand demonstrating the highest activity among the tested brands. Further *In vitro* and *in vivo* research can be done to unravel other possible mechanisms of action of these herbal drinks. Isolation and characterization of the active ingredients in the promising brands of the aphrodisiac drinks is another research area worth pursuing.

Compliance with ethical standards

Disclosure of conflict of interest

The Authors declare no conflict of interest.

Availability of data and materials

All data generated or analysed during this study are included in this published article

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Authors' Contributions

Samuel Folarin Olaniran participated in the conceptualisation, design, definition of intellectual content and supervision of the work. He also played a key role in the preparation of the manuscript and its review. Esther Oyinlola Olatunbosun participated in the literature review, experimental studies, data acquisition and data analysis. Idowu Julius Olawuni participated in the conceptualisation and design of the study alongside the data analysis. Blessing Abraham Ogungbe participated in the literature search, manuscript preparation and manuscript editing. Ayodele John Adeyelure participated in the data analysis and manuscript editing.

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