Effect of Ethanolic Extract from Seeds or Pods of *Xylopia Aethiopica* (Dunal) A. Rich (Annonaceae) on the Testicular Function of Adult Male Rats

Sunny O. Abarikwu¹ · Aemere Ogunlaja² · Chiagoziem A. Otuechere³ · Olatunbosun Gideon²

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Abstract *Xylopia aethiopica* (Annonaceae) is used in some folk medicines and widely consumed as a spice in some parts of Nigeria. Its efficacy as an anti-androgenic substance has warranted the attention of African scholars. This study evaluated the enzymatic activity of lactate dehydrogenase (LDH), γ -glutamyl transferase (γ -GT), sperm quality (motility, count, morphology), testosterone level and histo-pathological changes of the testis of rats chronically treated with ethanolic extract of the pods (without seeds), seeds, and fruits (pods + seeds) of *Xylopia aethiopica*. Male Wistar (224–246 g) rats were treated with the extract of the pods, seeds, and fruits of *Xylopia aethiopica* at the dose of 0, 50, 100, and 200 mg/kg body wt. for 60 days. Serum biochemistry, sperm quality and histo-pathological examination of the testis were assessed for any treatment-related adverse effects. After treatment with *Xylopia aethiopica*, testosterone level was decreased dose-dependently in the animals treated with the seed extract compared to all other groups. The enzymatic activities of LDH and γ -GT were higher in rats treated with the seed and fruit extracts compared with those treated with the pods. The numbers of motile sperm, and counts were decreased while the numbers of sperm with morphological defects were higher in rats treated with the seed and fruit extracts compared to the control.

Histopathological changes of the testis were also more severe in rats treated with the highest dose of the seed extract. We conclude that the compounds related to the anti-infertility effects of *Xylopia aethiopica* are present in the seeds.

Keywords *Xylopia aethiopica* · Testosterone · Testis · Lactate dehydrogenase · γ -glutamyl transferase

Introduction

Xylopia aethiopica (Dunal) A. Rich (Annonaceae family) commonly called the African guinea pepper” or “Ethiopian pepper” is a tropical evergreen tree that is widely distributed in West Africa. The Plant List [1] reports nine synonyms for *Xylopia aethiopica* (Dunal) A. Rich: *Annona aethiopica* (Dunal) Steud., *Habzelia aethiopica* (Dunal) A.DC., *Habzelia aethiopica* DC., *Unona aethiopica* Dunal., *Uvaria aethiopica* (Dunal) A. Rich., *Xylopia dekeyzeriana* De Wild., *Xylopia eminii* Engl., *Xylopia gillettii* De Wild., *Xylopicrum aethiopicum* (Dunal) Kuntze. It has been traditionally used to induce placental discharge postpartum and for the treatment of rheumatism, headache, neuralgia, bronchitis, asthma and colic pain [2]. The Fruit is used in combination with *Piper nigrum*, *Tetrapleura tetraptera*, *Capsicum frutescens* to prepare concoction for woman who newly delivered, believed to help in uterus contraction [3]. The tree belongs to the Annonaceae family and grows particularly in tropical Africa [4]. In Nigeria, it is found all over the lowland rain forest and most fringe forest in the Savanna zones of Nigeria. After drying, the green coloured matured pods takes a brown-black coloration and are commonly used as spices (often instead of pepper) in the preparation of two special local soups named

✉ Sunny O. Abarikwu
sunny.abarikwu@uniport.edu.ng; abarikwus@gmail.com

¹ Department of Biochemistry, University of Port Harcourt, Choba, Nigeria

² Department of Biological Sciences, Redeemer's University, Ede, Nigeria

³ Department of Chemical Sciences, Redeemer's University, Ede, Nigeria

“Obe ata” and Isi-ewu taken widely in the Southern parts of Nigeria [5].

The fruit has been shown to have antibacterial, anti-plasmodial, antifungal, and anti-gonadotropic properties, as well as possess haematopoietic activity, and immune boosting effect [6–8]. Several studies regarding the adverse effects of *Xylopia aethiopica* on the male gonadal functions have been demonstrated by several researchers [8–10]. The findings of Woode et al. [2], were however contrary to those of the above studies [8–10], and reported that ethanolic fruit extracts of *Xylopia aethiopica* have positive effects on spermatogenesis, sperm counts, testosterone and luteinising hormone levels as well as weight of the reproductive organs (e.g. testis and epididymis) of rats. So far the effects of *Xylopia aethiopica* on the testicular function of male rats are still inconclusive and controversial, perhaps because most of the previous studies have been carried out with experimental designs that have limited physiological significance. For example, studies for 28–30 days duration that focus on spermatogenesis in rats [8–10] would be less biologically significant since one complete spermatogenic cycle for rats requires at least 54 days [11]. As a result of the reported contrasting effects of ethanolic extract of *Xylopia aethiopica* on the testicular function of adult male rat, we aim to determine the effect of different doses of the ethanolic extract of the pods (without seeds) and seeds (without pods) of *Xylopia aethiopica* alone or in combination (seeds + pods) on sperm quality (count, motility, morphology, viability) testosterone level, histopathology of the testis, and on the activities of spermatogenesis marker enzymes including LDH and γ -GT of adult male rats after 60 days of administration. The study therefore aimed to contribute in the ongoing efforts by several African researchers on the effects of dietary food spices on male reproductive health.

Materials and Methods

Plant Materials and Chemicals

The dried fruits of *Xylopia aethiopica* were purchased from a local vendor in Oyingbo market Lagos State, Nigeria. Their botanical identification and authentication were confirmed by Dr Ernest Durugbo of the Department of Biological Sciences, Redeemers University, Nigeria, and Mr. Benjamin Daramola and Mr. Thomas Edewo of the University of Lagos Herbarium, and the voucher specimen was deposited at the Herbarium of the Department of Biological Sciences, Redeemer's University, Ede, Nigeria under reference number RH019. All reagents were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK).

Preparation of Extract

The powdered seeds, pods and fruits were extracted in ethanol using maceration extraction method for 24–36 h in an air tight container. Using a vacuum rotary evaporator, the ethanol filtrate was concentrated at a low temperature, under reduced pressure. This resulted in a brown solid (jelly-like) extract of seed, a dark greenish solid (jelly-like) extract of pod, and a green solid (jelly-like) extract of the fruit. This was then preserved in a cool, dry place. The residue was suspended in olive oil to prepare stock solutions, from which different doses (mg) were prepared and administered to the animals according to their weights.

Animals and Treatments

Fifty adult male Wistar rats with average weight of 234 ± 8.6 g were provided by the Animal House of the College of Natural Sciences, Redeemer's University, and housed in 10 groups each containing 5 animals. The animals were maintained under 12-h light: 12-h dark cycles and were supplied with water and feed ad libitum. The rats were housed for a minimum of 1 week to acclimatise before being dosed with the test substances. Corn Oil was used as vehicle and was administered to the control group (2 mL/kg body weight). The S50, S100, and S200 groups were treated with 50, 100, and 200 mg/kg body weight respectively of the ethanolic extract from the seed. The P50, P100, and P200 groups were treated with 50, 100, and 200 mg/kg body weight respectively of the ethanolic extract from the pod (without seeds). The SP50, SP100, and SP200 groups were treated with 50 mg/kg (25 mg/kg seed extract and 25 mg/kg pod extract mixed together), 100 mg/kg (50 mg/kg seed extract and 50 mg/kg pod extract mixed together), and 200 mg/kg (100 mg/kg seed extract and 100 mg/kg pod extract mixed together) b. wt of the ethanolic extract of both the pod and the seed respectively. The treatments were administered by oral gavage and continue for 60 days. This duration was chosen on the basis of literature information, which reported that one spermatogenesis cycle in rats takes at least 54 days to reach completion [11]. Rats were weighed and killed 24 h after the final treatment by cervical dislocation and blood was collected by heart puncture. The testes, epididymides, prostate and seminal vesicles (intact) were removed, cleaned of adhering connective tissues and weighed. The ethic regulations on animal care and handling of the National Academy of Science were followed during experiments.

Determination of Sperm Quality

The methodology for determining the number of homogenisation-resistant sperms in the cauda epididymides

was as previously described by us with some modifications [12]. Briefly, epididymides were dissected out, weighed, and immediately minced in a Petri dish containing 5 mL of physiological saline, and then incubated at 37 °C for 30 min to allow spermatozoa to leave the epididymal tubules. The resulting suspension was filtered into a clean test tube through a nylon mesh to remove fibrous tissue and other debris from the sperm suspension. Each sample was mixed gently before counting the number of homogenization resistant sperm cells on a Neubauer haemocytometer. To estimate the percentage of motile sperms, the epididymal sperm obtained from the cauda epididymis was placed on a slide and diluted with buffered 2.9 % sodium citrate, pre-warmed to 37 °C. The motility of epididymal sperm was evaluated visually at 400× magnification within 2–4 min of their isolation from the cauda [12]. Motility estimations were performed from three different fields in each sample. The mean was used as the final motility score and data were expressed as percentages. To determine the percentage of live spermatozoa and sperm morphology, a portion of the sperm suspension placed on a slide glass was smeared out with another slide and stained with Wells and Awa's stain (0.2 g of eosin and 0.6 g of fast green dissolved in distilled water and ethanol in ratio 2:1) for morphological examination and 1 % eosin and 5 % nigrosine in 3 % sodium citrate dehydrate solution for viability evaluation [13]. At least 200 sperms from each rat were examined for abnormalities in different regions of spermatozoa following the methodology of Wyrobek and Bruce [14].

Estimation of the Activities of γ -GT and LDH and Testosterone Level

The enzyme activities of γ -GT and LDH were estimated in the testis supernatant using Randox commercial kits (Crumlin, United Kingdom) according to the manufacturer's instructions. Serum-free testosterone was determined using an enzyme immunoassay kit (Immunometrics Ltd, London, UK). Briefly, standards, specimens, and controls were dispensed into appropriate wells, followed by testosterone horseradish peroxidase reagent and anti-testosterone reagent, before mixing thoroughly and incubating at 37 °C for 90 min. Wells were then rinsed with deionized water and substrate solution was dispensed into each well, gently mixed, and incubated for 20 min. The reaction was stopped with the "stop reagent" and the absorbance recorded at 450 nm. The minimum detection limit in the testosterone assay was 0.1 ng mL⁻¹. The cross-reactivity assay with other steroids tested showed 0.01 %.

Histopathological Examination

At necropsy, one testis of each rat was initially fixed with Bouin's solution for 6 h, and then transferred to 10 % buffered formalin. The formalin-fixed tissues were embedded in paraffin wax, and 5- μ m-thick sections were cut with a rotary microtome. The sections were stained with hematoxylin and eosin for microscopy.

Statistical Analysis

Data were expressed as mean $\pm$ SD, and the statistical analysis was performed with the SPSS Statistics 17.0 (SPSS Inc., Chicago, IL). All the statistical analyses were analyzed by ANOVA followed by Dunnett's test as a post hoc test. *p* values less than 0.05 were considered to indicate statistical significance.

Results

Table 1 shows the effect of the different extracts of *Xylopia aethiopica* on body weight. At the end of each treatment, the final body weight increased in all groups as follows: 12.82, 13.54, 14.29, 10.48, 14.91, 12.09, 13.67, 9.76, 14.89, 15 % (or rats treated with vehicle (control), S50, S100, S200, P50, P100, P200, SP50, SP100, and SP200 ethanolic extracts respectively). There was no statistical change in the absolute weights as well as the relative weights of the testes, epididymides, seminal vesicles and prostate at the end of the experiment (Data not shown).

Figure 1 shows the effect on sperm motility, sperm count in the epididymis, live dead ratio, and abnormal sperm morphology in adult rats of the 60 days of treatment with ethanolic extract of the seeds, pods (without seeds) and fruits (pods + seeds) of *Xylopia aethiopica*. A decrease in sperm motility was observed in rats treated with the ethanolic extract of the seeds, pods, and fruits of *Xylopia aethiopica*. The sperm motility was decreased by about 30 % in all the rats treated with the extract of *Xylopia aethiopica* and was not dependent on dose. In addition, rats treated with ethanolic extract of the seeds, pods (without seeds) and fruits (pods + seeds) of *Xylopia aethiopica* showed lower levels in sperm number in epididymis compared with the control group, and these decreases were also not dependent on dose. These effects were mild as the sperm number was decreased by 7 % in the S50, 12 % in the S100, 8 % in the S200, 4 % in the P50, 6 % in the P100, 10 % in the P200, 5 % in the SP50, 9 % in the SP100, and 7 % in the SP200 compared to the control value. No statistically significant differences were

Table 1 Effect of ethanolic extract from seeds or pods of *Xylopia aethiopica* on body weights (g) of male Wistar rats

Groups	Control	S50	S100	S200	P50	P100	P200	SP50	SP100	SP200
Initial (g)	234 ± 35.78	229 ± 17.89	224 ± 53.67	248 ± 33.47	228 ± 33.47	248 ± 33.47	234 ± 28.28	246 ± 35.78	235 ± 30.23	240 ± 56.57
Final (g)	264 ± 35.80	260 ± 45.60	256 ± 28.28	274 ± 48.99	262 ± 43.81	278 ± 43.82	266 ± 40.46	270 ± 38.29	270 ± 23.09	276 ± 53.67

Data are presented as the mean ± SD (n = 5)

found in the live/dead ratio of all the experimental groups when compared with the control group not treated with the ethanolic extract of the seeds, pods (without seeds) and fruits (pods + seeds) of extract of *Xylopia aethiopica*. Abnormal sperm morphology was not statistically changed in the S50 (11.24 %), P50 (10.64 %) and SP50 (10.54 %) groups when compared with the control values (10.7 %). An increase in the abnormal sperm morphology by 13 % was observed in the S100 compared to the control values and by 27 % in the S200 group compared to the control values. Similarly, the sperm morphology was increased by 17 % in the P200 group compared to the P50 group. In rats treated with the ethanolic extract of the fruits (pods + seeds) of *Xylopia aethiopica*, the percent abnormal sperm morphology was increased from 10.54 % in the SP50 group to 12.1 and 12.5 % in the SP100 and SP200 groups respectively.

LDH activity showed a significant increase in the S100 and S200 groups and the effect was dose-dependent, whereas the lower dose (S50) did not show any effect on testicular LDH activity (Fig. 2). Similarly the P50 animals did not show statistically significant differences in LDH activity when compared with control values. In the P100 groups, the LDH activity was increased by 42 % compared to the control values. The LDH activity was increased by 30.1 % when the P200 group was compared with P50 groups and by 78 % when compared to the control values. In the SP50 animals, the LDH activity increased significantly by 75 %. This was further increased by 119 and 151 % in the SP100 and SP200 groups compared to the control values. γ -GT activities were dose-dependently increased from 55 % in the S50 group of animals to 138 % in the S200 group of animals. γ -GT activity was increased by 41 % in the P50 animals compared to the control values and then increased by 100 % in the P200 animals when compared to the control animals, and by 35 % in the P200 animals when compared to P50 animals. In the SP50 animals, the γ -GT activity was increased by 39.5 % when compared to the control values and then increased to 59.5 % in the SP100 and 104.5 % in the SP200 groups of animals. The γ -GT activity in the SP200 group of animals was higher by 28 % than those of the SP100 group ($p < 0.05$).

Testosterone level in the control rats was 14.23 ± 0.83 mmol/L (Fig. 2). This was decreased by 86.54 % in the S50 groups of animals compared to the control, and the effect was dose-dependent in the group of animals treated with the ethanolic seed extract of *Xylopia aethiopica*. In the animals treated with the ethanolic extract of the pods (without seeds), the testosterone level was higher compared with those treated with the seed extract. The testosterone level in the P50 animals was decreased by 34 % and then further decreased by 53 % in the P200 group of animals when compared to the control value. When the

Fig. 1 Effect of ethanolic extract of the seeds, pods (without seeds) and fruits of *Xylopiæ aethiopica* on the sperm quality **1** Sperm motility **2** Sperm number in the cauda epididymis **3** % Live sperm **4** Abnormal sperm morphology of adult male Wistar rats after 60 days of

administration. Data are presented as the mean $\pm$ SD ($n = 5$). *Significantly different from the control group, **Significantly different from the P200 group, ***Significantly different from the P50 group, #Significantly different from the SP50 group ($p < 0.05$)

animals were treated with the ethanolic extract of the fruits (pods + and seeds), the testosterone level was decreased by 70.3 % in the SP50 group of animals, by 84 % in the SP100 group of animals and by 94.38 % in the SP200 group of animals when compared to the control value (Fig. 2).

There were no visible lesions seen in the testes of the S50, S100, P50, P100, P200, SP50, SP100 groups of animals. In the S200 group of rats, there was a large focally extensive area of severe germinal and basement cell necrosis (Fig. 3). The seminiferous and interstitial architecture was also distorted. Many of the tubular lumina have clumps of immature germinal cells in the SP200 group of animals consistent with the decreased level of testosterone.

Discussion

The lack of change on organ weights observed in the present study could be that organ weights were changed in a manner proportional to body growth [15]. This seems

reasonable to assume because the body weight gain for all animals were not statistically different. Several studies on the male reproductive health effects of extracts of *Xylopiæ aethiopica* have been reported with contrasting findings. For example, some studies reported that the ethanolic extract of the fruit of *Xylopiæ aethiopica* decreased the sperm quality, induced testicular degenerative changes and decreased serum testosterone levels in rats after 28 days [9, 10]. To the best of our knowledge, there is only one study in literature that reported induced spermatogenesis, improved sperm quality, increased testosterone level as well as increased weight of testis and epididymides in rats after 60 days of administration of alcoholic fruit extract of *Xylopiæ aethiopica* [2]. It is worthy to note that studies that focus on testicular function in rats would require at least 54 days for any testicular effect to be translated to epididymal events [11, 16]. Our results are different from those of Woode et al. [2] even though the duration of both experiments was for 60 days. One possible explanation for this disagreement is that in our study we used Wistar rats

Fig. 2 Effect of ethanolic extract of the seeds, pods (without seeds) and fruits of *Xylopiæ aethiopica* on testicular lactate dehydrogenase activity (1), testicular γ -glutamyl transferase activity (2), serum testosterone level (3) of adult male Wistar rats after 60 days of

administration. Data are presented as the mean $\pm$ SD ($n = 5$). *Significantly different from the control group, **Significantly different from the P200 group, ***Significantly different from the P50 group, #Significantly different from the SP50 group ($p < 0.05$)

whereas Woode et al. [2] used Sprague–Dawley (SD) rats. The reproductive capacity of SD rats would be different from those of Wistar rats especially if there are weight differences between the animals [17]. The highest weight of the animals used in our study at the end of 60 days treatment was 278 g while the least weight was 260 g. In the work reported by Woode et al. [2], the highest weight of the animals used was 185 g and the least weight was 161 g, hence the weight gain of the animals were lower at the end of the 60 days duration. It seems likely that weight differences at the start of experiments done by different authors usually also reflect different age of the rats which in turn may influence spermatogenesis. Furthermore, other reports confirm that body weight reduction contributes to lower testosterone level [18].

Further findings of our study also indicates that the ethanolic extract of the seeds, pods (without seeds) and fruits (seeds + pods) of *Xylopiæ aethiopica* caused a decrease in the sperm motility, sperm number in the

epididymis without any change in the percentage of Live spermatozoa. The abnormal sperm morphology was dose-dependently increased in the groups of rats treated with the ethanolic extracts of the seeds, pods or fruits (pods + seeds) of *Xylopiæ aethiopica*. Because these effects were mild or at most modest in all the rats treated with the ethanolic extracts of *Xylopiæ aethiopica*, we did not consider sperm variables as sensitive indices for studying the testicular effects of *Xylopiæ aethiopica* in rats. One study [9] reported a dramatic change in these variables study as opposed to our findings. The reasons for this could be that the study used doses of the fruits extracts different from what we used in our study. We used doses of the extracts from 50 to 200 mg/kg (0.3–0.4 mL per rat) for 60 days whereas Nwangwa [9] administered doses as high as 1 mL for rats weighing up to 208 g for 28 days, and for a solution whose stock concentration was 5 g/mL. This concentration may not be realistic in physiological terms, and thus whatever the outcome including sperm count decrease,

Fig. 3 Representative histopathology of the testis of rats treated with the ethanolic extract of the seeds, pods (without seeds) and fruits (seeds + pods) of *Xylopiya aethiopica* after 60 days of administration (1) control (2) 50 mg seed extract (3) 100 mg seed extract (4) 200 mg seed extract (5) 50 mg pod extract (6) 100 mg pod extract (7) 200 mg pod extract (8) 50 mg fruit extract (seed + pod) (9) 100 mg fruit

extract (seed + pod extract (10) 200 mg fruit extract (seed + pod extract). Long arrows shows distorted seminiferous and interstitial architecture and focally extensive severe germinal and basement cell necrosis and short arrows shows clumps of immature germinal cells in the tubular lumina. H&E, mag $\times 100$

increased sperm morphology e.t.c. may be unconnected to *Xylopiya aethiopica* effect, and therefore limits the biological significance of the author's observation in both physiological and toxicological terms.

The elevated activity of testicular γ -GT by the ethanolic extract of *Xylopiya aethiopica* suggests impairment of the function of Sertoli cells. LDH is widely distributed in the testes and is important in the physiology of germ cells [19]. The elevated testicular LDH activity might be due to increase in functional activity of the testes as a result of extensive damage to germ cells [19]. Because the observed effects on testosterone level were dramatic especially in rats treated with the seed extracts, we think that the seed extracts have more inhibitory effects than the pods on testosterone level, and therefore testosterone level is most responsive to the testicular effects of *Xylopiya aethiopica* in the rats. It is known that both testicular lactate level and γ -GT activity are under hormonal control [20, 21]. Consequently, the hormonal control on LDH and γ -GT activities were perturbed by the diminished testosterone level, leading to the increased activities of LDH and γ -GT in the testes. Because testicular LDH and γ -GT enzymatic activities were affected similarly tend to suggest that the

same regulatory mechanism and testicular factors could be involve in the catalytic activities of γ -GT and LDH in response to *Xylopiya aethiopica* treatment.

Threshold levels of testosterone are also required for the initiation and maintenance of spermatogenesis and for the stimulation of growth and function of the accessory sex glands e.g. prostate and seminal vesicles [22]. We don't know the actual duration of decreased testosterone level in our study after the 60 days treatment regimen, so the accessory organs may not have experienced testosterone deprivation long enough for weight deficit to be evident. There are studies were different levels of testosterone are required for the different accessory organ functions. For example, some researchers observed decrease in testosterone level with decrease in accessory organ weights while others observed anti-fertility effects with no changes in testosterone level after long term treatment of mice with extracts of anti-fertility agents [16]. Based on our findings, it is proposed that the seed extract of *Xylopiya aethiopica* has the following effects on the male gonadal function of adult rats: (1) potent inhibitory effect on testosterone level, (2) mild effect on the variables of sperm function, and (3) no inhibitory effect

on the weight of accessory sex organs (prostate, seminal vesicles and epididymides).

The animals treated with the high dose seed extract (S200) have a large focally extensive area of severe germinal and basement cell necrosis and a distorted seminiferous and interstitial architecture. Furthermore, many of the tubular lumina in the SP200 animals have clumps of immature germinal cells consistent with the decreased level of testosterone. The extract of the pods therefore have less effect on the testosterone level and on the histoarchitecture of the tubular lumina. Consequently, the anti-androgenic effects of the fruit preparations are attributed to the anti-androgenic effects of the seeds. The structural analysis of extract of *Xylopia aethiopica* by thin layer chromatography, high-performance liquid chromatography, and nuclear magnetic resonance have shown that it contains different kaurene derivatives and diterpenoids including xylopic acid, kaurenoic, acetylgrandifloric acid, trachylobane and kolavane diterpenes as well as several chemical constituents including volatile oils (monoterpenoids: cineole, cuminic aldehyde, terpinone, β -pinene); essential oil, resin, rutheroside fat, bitter principles, alkaloids, glycosides, saponins, tannins, sterols, carbohydrate, protein, free fatty acid, and mucilage which may contribute to the pharmacological effects of the ethanolic extract of *Xylopia aethiopica* as reported in several laboratories [4, 23–26]. Furthermore, the kaurene diterpene, xylopic acid, was reported to be a major constituent in the fruits of *Xylopia aethiopica* and possess anti-spermatogenic and anti-androgenic effects in rats [25, 26]. For example, xylopic acid isolated from the fruits of *Xylopia aethiopica* was reported to decrease serum testosterone and luteinising hormone

levels as well as epididymal sperm count, motility and viability in rats [4, 27]. Similarly, kaurane derivatives isolated from other plants e.g. *Stevia rebaudiana* were also reported to possess anti-androgenic and anti-spermatogenic properties in rats [27, 28]. Therefore, we infer that the anti-fertility activities exhibited by ethanolic extract of *Xylopia aethiopica* could be associated with the kaurane derivatives present in the seeds (Fig. 4).

In conclusion, the extract of the seeds but not the pods of *Xylopia aethiopica* have more potent inhibitory actions on testosterone level than on sperm quality variables. Thus, the active principles associated with the anti-androgenic actions of *Xylopia aethiopica* are found in the seeds. Hence, sub-fertile men or men with marginal fertility status should abstain from consuming high doses *Xylopia aethiopica*-based diet when trying to make their female partners to achieve pregnancy.

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Compliance with Ethical Standards

Conflict of interest SOA declares that he has no conflict of interest. AO declares that she has no conflict of interest. CAO declares that he has no conflict of interest. OG declares that she has no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Fig. 4 Chemical structures of kaurene derivatives of *Xylopia aethiopica* [29, 30]

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