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Evaluation of the Androgenic Properties of Wild and Cultivated *Euadenia trifoliolata* (Schum. & Thonn.) Oliv. (Capparaceae)

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Abstract

This study was part of the program of valorization of medicinal plants. For this purpose, wild *Euadenia trifoliolata* plants from Adzopé, Banco, Ehotilé Islands and Marahoué and plants grown at Nangui Abrogoua University were used. The average yield of the crude extract was calculated as a function of the treatment, origin, and type of organ. Investigations into the androgenic activity of these plants were undertaken and consisted of studying the effect of different extracts on body weight, serum testosterone, LH (luteinizing hormone) and FSH (follicle-stimulating hormone) levels in Wistar rats. The results obtained showed that the administration of wild and cultivated plant extracts had induced a weight growth rate of 100 mg b.w/kg for leaves and 250 mg b.w/kg b.w for roots. Also, plant extracts stimulated the production of testosterone, LH and FSH in rats. The production of these hormones depended on the areas of harvest and the organ of the plant used. It did not function a dose. The best induction dose of hormones was 250 mg/kg b.w. However, the cultivated plants had a better serum level of hormones. These results showed that *E. trifoliolata* has androgenic properties.

Keywords

Euadenia trifoliolata, Wild Plants, Cultivated Plants, Androgenic Properties, Wistar Rats

1. Introduction

Plants have a long history in medicine and are the main source of medicine. They

are really important in the production of drugs [1] [2]. Phytoandrogens are plants that contain androgens or those that stimulate androgenic activity in men [3]-[5]. It's a relatively new concept. In contrast to phytoestrogens, phytoandrogens provide androgens to men instead of estrogens. They increase the level of free testosterone in the body, and shift the body/androgen/estrogen ratio to the androgenic side of the equation [6] [7]. Androgens are steroid hormones necessary for the normal expression of the male phenotype, including the development of secondary sexual characteristics, the initiation and maintenance of spermatogenesis [8] [9]. Thus, hyperactive steroidal sex receptors (androgens and estrogens) are associated with increased risks of hormone-sensitive tumors such as prostate and breast cancers. The availability and binding to the related ligands of steroidal sex receptors are necessary for the proportional expression of specific genes responsible for such hormone-mediated processes [5] [10].

A hormone is a substance released in the extracellular space or in the capillaries of the gland, acting on the metabolism of other cells at a distance. It may be a polypeptide (made up of many water-soluble AAs) or a steroid (formed from fat-soluble cholesterol). Steroids are secreted by the gonads and adrenal glands. Testosterone is a steroid hormone. It plays a vital role in human physiology (male and female), such as skeletal muscle development, bone density, fertility and libido. Testosterone has a metabolic action mainly oriented towards protein anabolism. Protein accumulation is mainly related to skeletal muscle, kidney and bone tissue. [11] [12]. It promotes positive energy balance through food intake. Indeed, the stimulation of the intestinal flora, antidepressants, corticosteroids, insulin (peripheral anabolic action), glucocorticoids (anabolic and stimulate the accretion of lipids to type I receptors) promotes the positive energy balance [2] [13]. Therefore, it is important to know the androgenicity and estrogenicity of medicinal plants to maximize their benefit in clinical applications. The androgenic status of plants is related to the presence of chemical compounds that are among others flavonoids, sterols and polyterpenes, vitamins [14] [15]. *Euadenia trifoliolata* (Schum. & Thonn.) Oliv. is a medicinal plant, family Capparaceae, grows mainly in the tropics and subtropics. Different parts of the tree are used in traditional medicine to cure ear pain, eye pain, anemia, acute renal failure, and erectile dysfunction. The pharmacopoeia revealed that the roots of *E. trifoliolata* are traditionally used as an aphrodisiac [16] [17]. Investigations show that this species contains flavonoids and other phytocompounds with therapeutic and nutritional activity [18] [19]. Therefore, the present work has been undertaken to evaluate the androgenic properties of *E. trifoliolata*. But to our knowledge, there is no scientific information in the literature that has substantiated or refuted the androgenic properties of this plant.

2. Material and Methods

2.1. Collection of Plant Material and Preparation of Extracts

Leaves and roots of *Euadenia trifoliolata* (Capparaceae) were collected at Adzopé,

Banco, Ehotilé Islands, Marahoué and Nangui Abrogoua University from May to September, then identified and authenticated by expert botanists from the Centre National Floristique of Côte d'Ivoire. These organs of various origins were dried at room temperature and then crushed using a grinding machine "Culatti typ MFC" brand. The various powders obtained were macerated in distilled water. 400 g of different organs were macerated 24 hours and then filtered. The mac (residue) was recovered and macerated again for 24 hours twice always in distilled water. The filtrates were dried in an oven at 40˚C. In order to obtain the aqueous extracts [20]. The doses of plant extracts used are respectively 100 mg/Kg b.w, 250 mg /Kg b.w and 500 mg /Kg b.w.

2.2. Supply of Experimental Animals

Healthy albino male Wistar strain (*Rattus norvegicus*) rats, about three months old and weighing between 140 and 175 grams, were taken from the breeding hall of Nangui Abrogoua University. The rats were housed in polypropylene cages and kept in an environment-controlled room with a light and dark cycle of 12:12 hours at 25˚C. They were fed with pellets (Faci) and tap water at will. The rats were acclimated to the laboratory for 15 days before the experiment. The experiment was conducted for 28 days [19] [20]. Animals were maintained and all experiments were conducted following the OECD guidelines 425 as recommended by the ethic group of our university [21]. The different experimental protocols were followed in accordance with the protocols for the protection of experimental animals of the European Council on legislation 2012/707 [22].

2.3. Preparation of the Cramming Solution

Rats were weighed in batches and the mean (P) for each lot was determined. For the preparation of the solution, the formula below has been used [23].

$$C = \frac{DP}{V}$$

P: Body weight;
V: Volume to gavage (ml);
D: Dose to gavage (mg/kg of body weight);
C: Concentration of the solution (mg/ml).

2.4. Cramming Methodologies

13 batches of five male Wistar rats were constituted. Rats were randomly assigned to one of the following groups: Group A receiving distilled water was the negative control. Groups B and C were then treated with sildenafil citrate (Viagra) at a dose of 100 mg/kg b.w and mesterolone (Testosterone) at 25 mg/kg b.w respectively and were served as a positive control. Sildenafil and mesterolone are used as positive controls (reference substances) in this study to validate the sensitivity of the assays by two distinct mechanisms: the improvement of testicular blood flow

(Sildenafil) and the direct androgenic action (Mesterolone) [24] [25]. Groups D, F, H, and J received aqueous extracts of wild leaves from Adzopé, Banco, Ehotilé, and Marahoué, respectively. Groups E, G, I, and K were then treated with aqueous extracts of the roots of wild plants from these same localities. Aqueous extracts of leaves from regenerated plants were administered to group L. Finally, group M received aqueous extracts of the roots of cultivated plants [22] [23]. The doses received by the test groups were 100 mg/kg b.w, 250 mg/kg b.w and 500 mg/kg b.w. The cramming volume was 1 ml per day. The first weighing was done (P0) and then the weighings were done every three days (P0, P1, P4, P8, P12, P16, P20, P24, P28). For presentation purposes, the weights recorded on (J0; J1; J4; J8; J0; J12; J16; J20; J24; J28). Thus each batch and every three days of weighing, the percentage P (see formula below) is calculated where (P1 - P0) represents the weight at the first cramming day and the weight Pn were n cramming days [24] [26]. The experiment was carried out for 28 days. On the 29th day the rats were sacrificed und er isofuran anesthesia between 6 h and 10 h in order to minimize the hormonal variations due to the nycthemeral rhythm. Blood was collected in the heart of the animals using a syringe in special dry tubes and then centrifuged at 3000 rpm for 5 minutes. The collected supernatant was distributed in microtubes and then were stored at −20˚C for the assay. FSH, LH and testosterone were measured using the Magnum 800 device at the Institut Pasteur in Abidjan (Côte d'Ivoire) [14] [22].

$$P = \frac{Pn}{P1 - P0} \times 100$$

P: percentage of variation of weight;
Pn: weight at nth days after experimentation;
P1: weight after 1st cramming;
P0: weight before 1st cramming.

2.5. Statistical Analysis

Statistical analysis was performed using STATISTICA 7 1. and GraphPad Prism V5.01 software (Washington, USA). The data are expressed as mean ± deviation to mean (ESM). Analysis of variance (ANOVA) was performed to first compare the mean yields of aqueous extracts according to origin, group, and organ. Then, the effect of different doses of wild and cultivated extracts on the measured parameters (weight, testosterone, LH, and FSH levels) was determined. In cases of significant differences, post-ANOVA comparisons were performed using Tukey's and Dunnett's methods, respectively. Tukey's test was used for multiple comparisons of the yields of the different extracts. Dunnett's test was used to compare the effect of the different extracts to that of the control. The significance of the difference in means was determined by comparing the probability P associated with the Fisher-Snedecor test statistic at the theoretical threshold of $\alpha = 0.05$.

3. Results

3.1. Average Yield

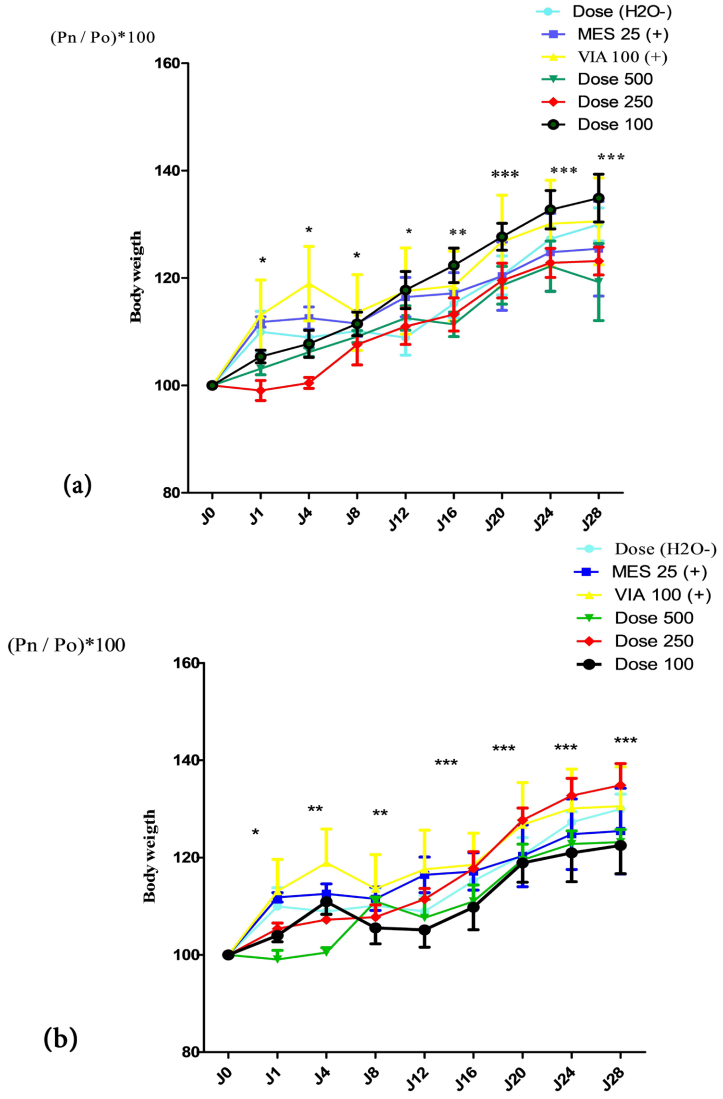
Table 1. Average yield according to the treatments, origins and organs.

Treatments/Origins	Group	Organs	Doses (ml or mg/kg)	Average yield (g)	Statistics	
					P	*F*
Vehicule	A	-	100	-		
Positive control (Sildenafil citrate)	B	-	25	-		
Negative control (Mesteronol)	C	-	100	-		
Adzopé	D	Leaves	100	11.3 ± 0.52^{h}		
		Leaves	250			
		Leaves	500			
	E	Roots	100	15.5 ± 0.06^{f}		
		Roots	250			
		Roots	500			
Banco	F	Leaves	100	14.9 ± 0.13^{gf}		
		Leaves	250			
		Leaves	500			
	G	Roots	100	15.12 ± 0.01^{f}		
		Roots	250			
		Roots	500			
Ehotilé Islands	H	Leaves	100	17.28 ± 0.00^{e}	<0.01	28.42
		Leaves	250			
		Leaves	500			
	I	Roots	100	17.86 ± 0.05^{e}		
		Roots	250			
		Roots	500			
Marahoué	J	Leaves	100	20.65 ± 0.01^{dc}		
		Leaves	250			
		Leaves	500			
	K	Roots	100	22.01 ± 0.04^{c}		
		Roots	250			
		Roots	500			
Nangui Abrogoua University	L	Leaves	100	31.97 ± 0.03^{a}		
		Leaves	250			
		Leaves	500			
	M	Roots	100	27.06 ± 0.01^{b}		
		Roots	250			
		Roots	500			

On the same column, values containing the same letters are statistically equal.

Average yield value according to treatments, origins, and different plant parts is shown in the table below. The analysis shows a significant difference ($p < 0.001$; $F = 28.42$). The average yield of leaves from regenerated plants was highest (31.97 ± 0.03 g), followed by that of roots. Comparison of plant parts from wild and regenerated plants shows that the yield of cultivated plants is consistently high. Among wild plants, the leaves from Marahoué gave the highest yield in terms of quantity (22.01 ± 0.04 g), while the lowest value was recorded for leaves from wild plants from the Éhotilé Islands (11.3 ± 0.52 g) (Table 1).

3.2. Effects of Leaf and Root Extracts of *Euadenia trifoliolata* (Schum. & Thonn.) Oliv. (Capparaceae) on Body Weight

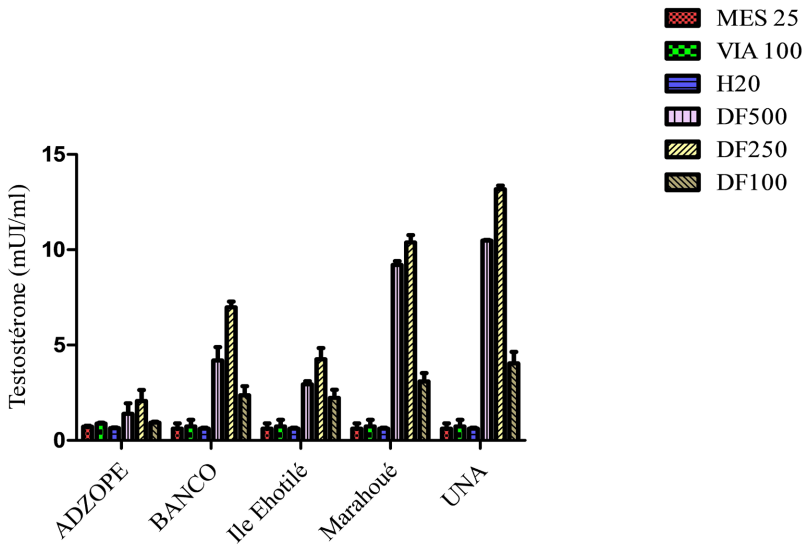


Mes: mesteronone; Via: viagra (sildenafil).

Figure 1. Evolution of Rat body weight as function of organ type and administer dose. (a) Effect of different doses of leaves extracts according to controls. Signification level: * = $p < 0.05$; ** = $p < 0.001$; *** = $p < 0.0001$; n = 5. (b) Effect of different doses of roots extracts according to controls. Signification level: * = $p < 0.05$; ** = $p < 0.001$; *** = $p < 0.0001$; n = 5.

The influence of the aqueous extracts (100, 250 and 500 mg/kg b.w) *E. trifoliolata* on the body weight evolution of the rats were shown in Figure 1. Figure 1(a) showed the impact of the aqueous leaf extracts while Figure 1(b) showed the effect of the roots. Treatment with aqueous organ extracts has been a significant effect ($p < 0.05$ - 0.0001) on body weight. Rats receiving leaf extracts at a dose of 100 mg/kg b.w have been increasing body weight during the experiment. While in rats that were received the root extracts have been a weight gain at the dose 250 mg/kg b.w. From this analysis it had appeared that organ extracts of *Euadenia trifoliolata* induce weight growth in rats. This weight induction was not depended on dose. It differed from one organ to another. This weight induction was 2.5 higher with root extracts at 250 mg/kg b.w than with leaf extracts at 100 mg/kg b.w. The leaves were therefore better indicated for weight gain in rats. It should be noted that the organs of the wild and cultivated plants have the same tendencies on the body weight of the rats.

3.3. Effect of Wild and Cultutived Leaf Extracts on Serum Testosterone



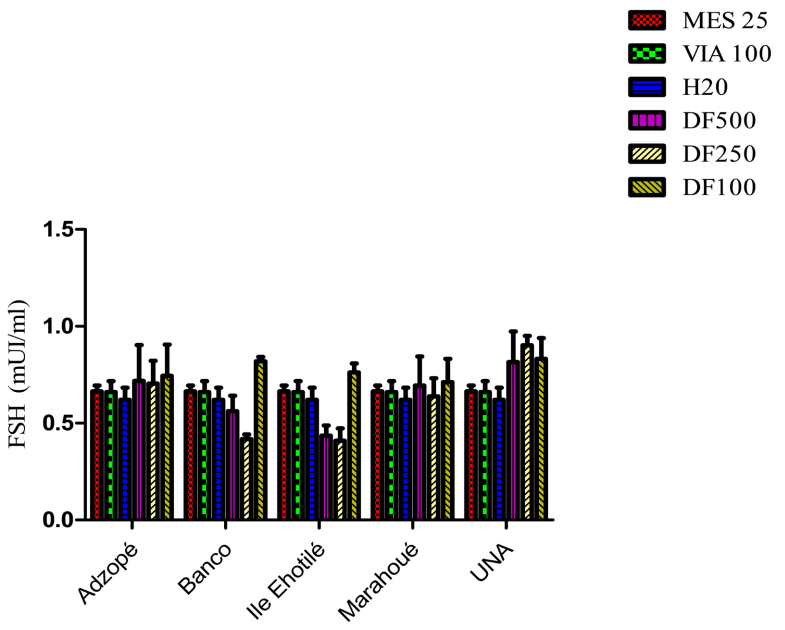
Mes: mesteronone; Via: viagra (sildenafil); DF: dose of leaves.

Figure 2. Serum rate of testosterone according to the areas of harvest of the leaves.

Figure 2 presents the impact of aqueous extracts of *Euadenia trifoliolata leaves* on testosterone in rats. The administration of aqueous leaf extracts were a highly significant effect ($f = 15.67$, $p < 0.0001$). The rats were crammed by cultivated leaf extracts were higher testosterone levels than the wild ones. Intra analysis of wild extracts were showed that rats were treated with Marahoué extracts were increased testosterone levels more than Adzopé, Banco and Éhotilé Island. Adzopé extracts were the lowest testosterone level. From this analysis, it were appeared that wild and cultivated *E. trifoliolata* were induced the production of testosterone. Cultivated plants further were stimulated the production of testosterone.

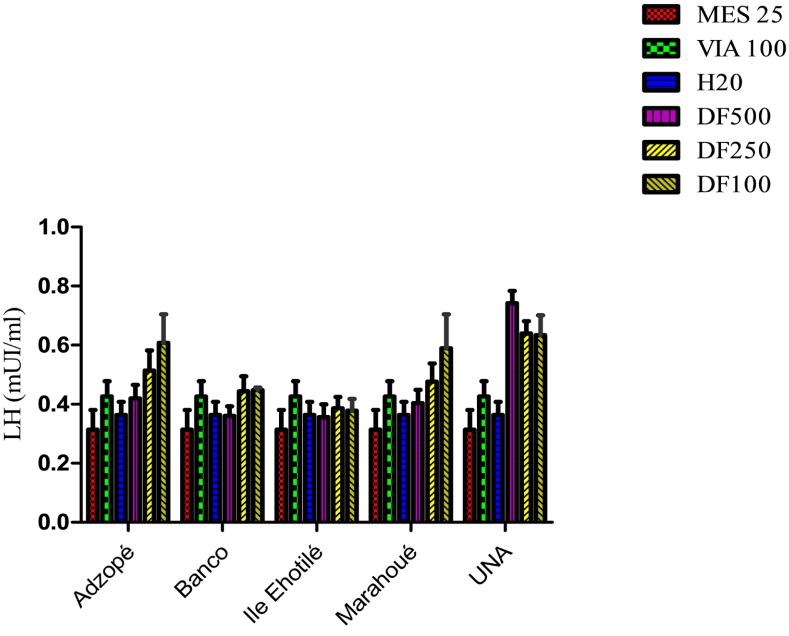
This induction activity were depended on the areas where the leaves were harvested. The best dose of induction of testosterone were 250 mg/kg b.w.

3.4. Effect of Wild and Cultured Leaf Extracts on Serum Levels of FSH and LH



Mes: mesteronone; Via: viagra (sildenafil); DF: dose of leaves.

Figure 3. Serum rate of FSH according to the areas of harvest of the leaves.



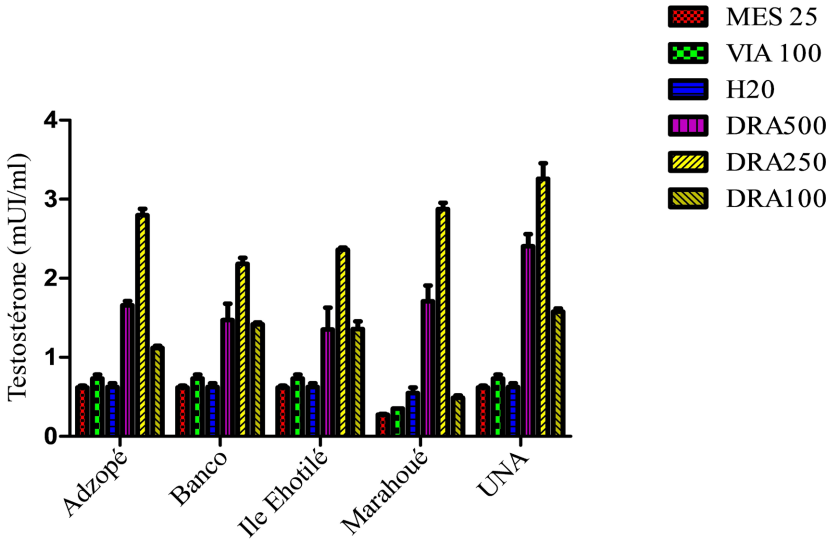
Mes: mesteronone; Via: viagra (sildenafil); DF: dose of leaves.

Figure 4. Serum rate of LH according to the areas of harvest of the leaves.

The observations made in Figure 3 showed that aqueous extracts of *E. trifoliolata* leaves were induced FSH production in rats ($f = 8.45$, $p < 0.0001$). In addition, there were differences between the effect of cultivated and wild plants and between the wild plants themselves. Induction of FSH production was not varied. But it depended on the harvest areas. This analysis showed that the extracts of the cultivated plants increased the level of serum FSH in the rats more.

The analysis of Figure 4 revealed that aqueous extracts of *E. trifoliolata* leaves were induced LH production in rats ($f = 5.42$, $p < 0.0001$). In addition, there were differences between the effect of cultivated and wild plants and between the wild plants themselves. Induction of LH production was also varied with dose, but it was a function of the harvest area. From this analysis, it appeared that the extracts of the cultivated plants were induced to a high-level production of serum LH of the rats.

3.5. Effect of Wild and Cultured Root Extracts on Serum Testosterone

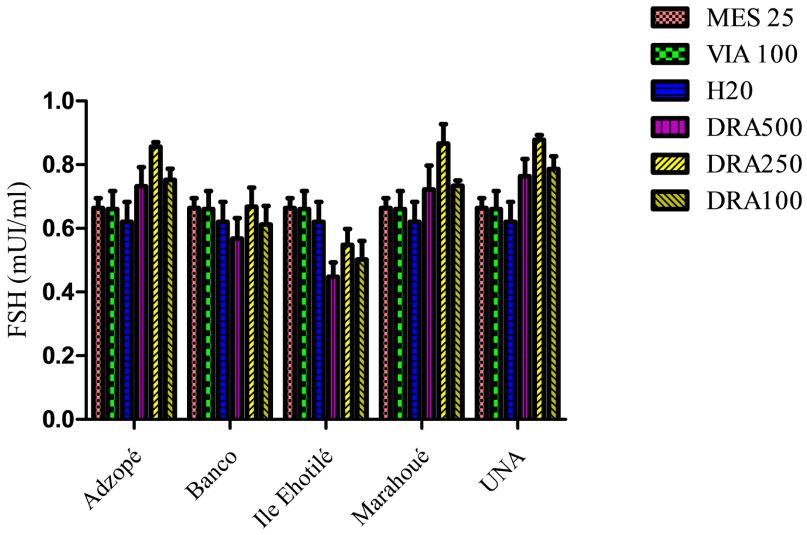


Mes: mesteronone; Via: viagra (sildenafil); DRA: dose of roots.

Figure 5. Serum rate of testosterone according to the zones of harvest of the roots.

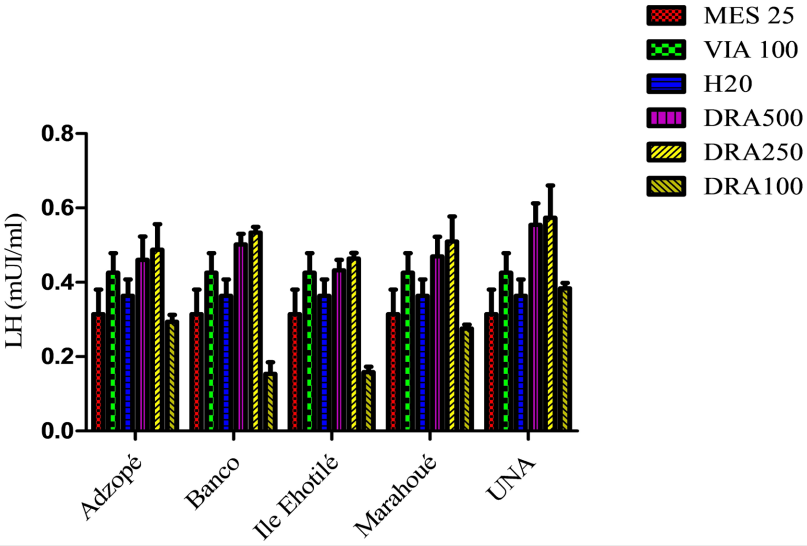
Figure 5 showed the impact of aqueous extracts of *E. trifoliolata* roots on testosterone induction in rats. The administration of aqueous root extracts has a highly significant effect ($f = 6.46$, $p < 0.0001$). The rats received the cultivated root extracts were higher testosterone levels than the wild ones. The intra analysis of the wild extracts showed that the rats those were treated with the extracts of the Marahoué and Adzopé have been a testosterone level higher than those treated with extracts of Banco and Ehotilé Island. From this analysis, it appeared that wild and cultivated *E. trifoliolata*'s roots were induced the production of testosterone. This induction activity was depended on the harvested areas of leaves. It was also not dependent on dose. And the best dose of testosterone was 250 mg/kg b.w.

3.6. Effect of Wild and Cultured Root Extracts on Serum Levels of FSH and LH



Mes: mesteronone; Via: viagra (sildenafil); DRA: dose of roots.

Figure 6. Serum rate of the FSH according to the zones of harvests of the roots.



Mes: mesteronone; Via: viagra (sildenafil); DRA: dose of roots.

Figure 7. Serum rate of the LH according to the zones of harvests of the roots.

Figure 6 showed that aqueous extracts of *E. trifoliolata* roots were induced FSH production in rats ($f = 99.60$; $p < 0.0001$). The rats received from the plant extracts of Adzopé, Marahoué and UNA have the same effects. The comparison of the wild extracts between them showed a weaker production in rats were received the extracts from Ehotilé Islands. Induction of FSH production were not depended doses, but it varies witch the harvest area. However, there are some peaks at a dose of 250 mg/kg b.w.

Analysis in Figure 7 revealed that aqueous extracts of *E. trifoliolata* roots were induced LH production in rats ($f = 29.43$, $p < 0.0001$). In addition, there were differences between the effect of cultivated and wild plants and between the wild plants themselves. Induction of LH production did not function on doses. But it depended on the area of harvest. From this analysis, it appeared that the extracts of the cultivated plants were induced a massive production of serum LH of the rats.

4. Discussion

Investigations of the androgenic properties of wild and cultivated *Euadenia trifoliolata* (Schum. & Thonn.) Oliv. (Capparaceae) were made through estimation of body weight, testosterone, FSH and serum LH after administration of leaf order root extracts at different doses (100 mg/kg b.w, 250 mg/kg b.w and 500 mg/kg b.w) in rats. For this fact, it was discussed to study the impact of these extracts on an average yield, body weight, serum levels of testosterone, FSH and LH.

4.1. Average Yield

Aqueous extracts of the regenerated plants showed the highest yields. These observations could be related to the compatibility between the polarity of the regenerated plants and that of the solvent used. Indeed, the tissues of these cultivated plants contain a multitude of secondary and primary metabolites that have an affinity for water. This work differs from that of [27] who demonstrated in their study that despite the high polarity of water, the yield is low. In addition, other studies have indicated that the quantity and quality in terms of phytochemical composition is a function of the nature of the extraction solvent [28].

4.2. Evaluation of the Effects of *Euadenia trifoliolata* Leaf and Root Extracts on Body Weight

Plant organ extracts promote weight gain in rats. This is observed as weight gain, which could be explained by the presence of nutrients, proteins, hormones, or compounds such as corticotropin-releasing hormone (CRH), vitamins, and testosterone. Indeed, these compounds appear to have stimulated weight gain. These results could also be explained by the stimulation of intestinal flora and antidepressant (serotonin) activity [25] [29] [30]. According to these authors, the activity of the intestinal flora and these compounds involve in food intake and responsible for weight gain. This weight gain did not depend on the dose; then it were differed from one organ to another. Furthermore, weight gain was 2.5 times greater with leaf extracts at 100 mg/kg than with root extracts at 250 mg/kg body weight. Therefore, the leaves would be more suitable for weight gain in rats. Similar studies conducted on plants have shown the same trends [31]-[33]. According to these authors, plants such as moringa (*Moringa oleifera*), shatavari (*Asparagus racemosus*) and fenugreek (*Trigonella foenum-graecum*) contribute to weight gain.

4.3. Effect of Wild and Cultured Leaf Extracts on Serum Testosterone

The results of the effect of wild and cultured *E. trifoliolata* leaves extracts on serum testosterone were shown that they induce the production of testosterone. It was higher in the rats that were received the cultivated plants extracts. This activity depended on the zone of harvest, and the best induction dose were 250 mg/kg b.w. Testosterone is produced by the tests but in case of pathology by the adrenal glands. Since the rats under study were normal, this increase may be due to stimulation of testicular leydig cells. Indeed, these cells synthesize testosterone from cholesterol through pegnolone [4]. Also, leaf extracts would have prevented cleavage of the cholesterol side chain by P450 protein [34]. The high activity of the cultivated leaves could be explained by the cultivation practices and the age of these leaves. Thus, the ambient environment of the culture medium and the young age of these leaves would have favored the accumulation of the compounds involved in the biosynthesis of cholesterol.

The variability in serum production of testosterone was function of harvesting sites. It could be explained by the effect of the environment on plants (leaves). Indeed, the harvest zones Adzopé, Banco, Marahoué and UNA are continental environments while the Ehotilé sites were islands. In addition, the environment of the different areas would have militated in favor of other compounds that were not involved in the biosynthesis of cholesterol, hence the low level of testosterone in the rats that received the extracts of Adzopé. The small quantities of testosterone were due to Adzopé extracts may be related to the physiological reaction of the rats. Also, genetic factors at both plant and animal levels could be responsible for this low production of testosterone [35]. However, the stimulation dose of testosterone production in rats remains the same (250 mg/kg b.w.). This is explained by the fact that the production of testosterone is a physiological phenomenon. When the threshold of 250 mg/kg b.w. were reached, testosterone was produced.

4.4. Effect of Wild and Cultured Leaf Extracts on Serum FSH and LH Levels

The results of the effect of wild and cultivated *E.trifoliolata's* leaves on the production of FSH and LH were shown that they have stimulated their production. This stimulation was more observed in the rats receiving the leaf extracts from cultivated plants. These leaf extracts act on anterior pituitary gonadotropic cells in order to secrete LH and FSH gonadotropins via GnRH-R receptors [36]. In addition, these extracts would have increased the pulses, rhythms and half-life of FSH and LH. This would explain the high serum levels of these hormones.

4.5. Influence of Wild and Cultured Root Extracts on Serum Testosterone

The results of the effect of wild and cultivated *E. trifoliolata* root extracts on serum

testosterone showed that it was induced the production of testosterone. It was higher in the rats receiving the cultivated root extracts. This activity depended on the place of harvest, and the best induction dose were 250 mg/kg b.w. Stimulation of testosterone production by root extracts may be due to the presence of flavonoids in these roots. The same observations were made in rats treated with aqueous extracts of *Anacyclus pyrethrum* [14] [37]. According to these authors the flavonoids have an androgenic effect. In addition, flavonoids modulate the action of neurotransmitters at the target cells, increasing the serum level of testosterone. These compounds induce an increase in the serum levels of androgens (testosterone) [38] [39]. This massive production would be attributed to the chemical composition of the root extracts. Thus, phytochemical analysis of root extracts revealed the presence of polyphenols, tannins, alkaloids and sterols and polyterpenes in addition to flavonoids. These phytochemicals have androgenic potentialities. Stimulation of testosterone production may also be due to the presence of the alkyl amide [40]-[42]. According to these authors the alkyl amide stimulates the synthesis of testosterone.

4.6. Serum Variations in FSH and LH Induced by Extracts of Wild and Cultivated Roots

The results of the effect of wild and cultivated roots on the production of FSH and LH generally were showed that they were induced in their production. *Euadenia trifoliolata* root extracts were improved LH production in rats [32] [33]. These authors reported a significant improvement in serum luteinizing hormone (LH) levels during work on *Muccuna pruriens*. While the results on follicle-stimulating hormone (FSH) level do not corroborate. However, there was a difference in the serum levels of FSH and LH in cultivated and wild roots, but also in wild roots themselves. This difference could be explained by the environmental and genetic factors related to the plants whose roots were derived and to rats that received the root extracts. Indeed, the action of these factors induces an iso-synchrasia reaction both in the plant and in the rats subjected to the experimentation. Hence the variability of serum levels of FSH and LH. The increase in serum levels of FSH and LH may be due to the harmonious balance of enzymatic activity of the metabolic pathways and energy metabolism involved in the production of these hormones.

5. Conclusion

This study reveals that extracts of *Euadenia trifoliolata* (Schum. & Thonn.) Oliv. (Capparaceae) exhibit androgenic properties, promoting weight gain and enhancing sexual performance. Firstly, the regenerated plants had a high extract yield. The superior action of the aqueous extract obtained from the leaves, particularly pronounced at the optimal dose of 250 mg/kg, demonstrates a highly effective dual anabolic and pro-erectile activity. These significant scientific results fully justify the use of this plant in the traditional treatment of male reproductive disorders. Therefore, future research will focus, firstly, on bio-guided fractionation of this

specific extract to isolate the active ingredients and assess their long-term toxicological safety, and secondly, on a comprehensive comparative study with plants cultivated *in vitro* to standardize an Improved Traditional Medicine (ITM) while promoting the eco-responsible and sustainable use of the plant resource.

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Author Contributions

Monh Alice Fah designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Witabouna Mamidou Kone and Irié Arsène Zoro Bi managed the analyses of the study. Ehilé Hervé Ehilé managed the literature searches, and he participated in the laboratory experiment. All authors read and approved of the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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