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Evaluation of the androgenic activity of some medicinal plants in the case of andropause (Côte d'Ivoire)

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Abstract

Description of the subject. Andropause is a relatively important health and social problem that consequences are harmful to humans. In the therapy of this condition, the administration of testosterone is recommended.

Objective. This study aims to evaluate the androgenic power of some medicinal plants from Côte d'Ivoire.

Methods. Biological investigations on sperm parameters and testosterone levels were carried out by oral administration of different aqueous extracts of *Cissus aralioides*, *Palisota hirsuta*, *Zanthoxylum gilletii* and *Euphorbia hypericifolia* to mature male rat. This administration was done over 35 successive days, at a dose of 100 mg/Kg body weight.

Results. Administration of the different extracts induced an increase in serum testosterone levels in the treated animals. The aqueous extracts of the whole plant of *E. hypericifolia* and the stem bark of *Z. gilletii* respectively induced the greatest increase in serum testosterone (15.61±0.34 ng/mL and 11.87±0.35 ng/mL) versus 0.83±0.18 ng/mL for the control group. The sperm densities of the *E. hypericifolia* (~78.10^6 spz/mL ± 2.93) and *Z. gilletii* (~62.94 × 10^6 spz/mL ± 2.44) groups were higher than those of the control group (~63.95 × 10^6 spz/mL ± 2.93) with ($p < 0.001$). The percentage of motile sperm was higher in the *E. hypericifolia* group (~79%±0.00%) than in the control group (~17±0.01%), with $p < 0.001$.

Conclusions. These different results show that these plant species improve spermatogenesis.

Keywords: Medicinal plants; Sperm parameters; Testosterone; Côte d'Ivoire

1. Introduction

Andropause is the set of physiological symptoms associated with a decline in testosterone secretion in men. These symptoms have been observed mainly in men whose testosterone levels were linked to age [1,2,3]. According to Matsumoto [4], testosterone levels decline with ageing at a rate of 1% per year, and this decline is more pronounced in

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free testosterone levels due to alterations in globulin, the sex hormone binding protein. This is generally due to androgen deficiency in ageing men. This deficiency is often total or partial. It can also be linked to late-onset symptomatic hypogonadism. The symptoms are as diverse as they are varied, leading to a drop in testosterone, which is at the root of loss of libido, erectile dysfunction, sexual weakness, fatigue, insomnia, mood disorders and low motivation [5]. Stress, professional fatigue, loss of interest in one's partner, the emergence of psychological conflicts, the side-effects of medication, the onset of depression and hormonal upheaval are often the source of the changes and symptoms observed in ageing men.

However, this decline in testosterone levels varies between individuals and is affected by chronic illnesses such as obesity, metabolic diseases (diabetes, hypertension, etc.), severe emotional stress and medication [6]. But this decline can be slowed by managing health and lifestyle factors [6,7]. Testosterone is the hormone responsible for the secondary sexual characteristics that appear at puberty, so it has a powerful effect on stimulating libido, sexual desire and arousal. It also has an anabolic action, improving metabolic processes in muscles, bones and bone marrow (erythropoiesis), immune system function, cognition and mood (Bain, 2001). Fertility and sometimes virility decline with age, accompanied by signs of sex hormone deficiency.

It should be noted that data on the prevalence of andropause is lacking, and the elderly population (aged 65 and over) represents the fastest-growing age group in the population [4]. As a result, 52% of men over 60 and 55% of men over 80 have serum total testosterone levels below normal [8,9]. This decline in testosterone and other anabolic hormones in men from their mid-thirties onwards may influence age-related deteriorations in body function (e.g. frailty, obesity, osteopenia, cognitive decline and erectile failure). Testosterone insufficiency in older men has been shown to be associated with an increased risk of death over the past 20 years, independent of several risk factors and pre-existing health problems [10,11].

Faced with this situation, modern medicine offers treatments using testosterone enanthate, the main advantage of which is that it is inexpensive compared with other treatments. However, its disadvantages are that frequent intramuscular injections are necessary and can cause pain [12,13].

This provides an additional incentive to seek new, even more effective phytomedicines for the treatment of patients, and thus to explore traditional medicine. Traditional medicine is an alternative to modern medicines because it is widely used by the local population and should be exploited.

The aim of this study is to assess the androgenic potential and phytochemical composition of four plant species in order to help improve the health of people undergoing andropause.

2. Materials and methods

2.1. Biological material

The plant species studied were selected from the literature on plants with androgenic potential. This literature enabled us to choose *Cissus aralioides, Euphorbia hypericifolia, Palisota hirsuta and Zanthoxylum gilletii* because they have never been the subject of a study on androgenic hormones in Côte d'Ivoire [14,15,16,17]. Plant organs were harvested and dried for a fortnight under air conditioning (18 ± 2°C). These plant organs were then pulverised to obtain powders. The plant name was verifed using http://www.worldforaonline.org.

The animal material consisted of rats, *Rattus norvegicus* (Muridae) of the Wistar strain, weighing between 160-180 g and 120 days old. The rats came from the animal house of the Laboratory of Botany and Valorization of Plants Diversity of the Nangui ABROGOUA University. The animals were kept at room temperature under a photoperiod of 12 hours of light and 12 hours of darkness. The animals had free access to water and food consisting of pellets supplied by Ivograin®-Abidjan. Te animals were acclimatized for two weeks. The study was carried out following the NIH guidelines for animal care. The rats were treated according to good laboratory practices of OECD [18]. 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 [19].

2.2. Preparation of plants extracts for phytochemical tests

For the decoction, 10 g of plant powder were mixed with 100 mL of distilled water, then the whole was brought to the boil on a hot plate. After boiling, the decoction was filtered under vacuum using a funnel and Whatman No. 4 filter paper.

To prepare the macerates, 10 g of plant powder was mixed with 100 mL of distilled water, then the whole mixture was left to macerate under mechanical agitation for 24 hours. The macerate was filtered under vacuum on Whatman No.4 filter paper.

The ethanolic and methanolic extracts were prepared by maceration. To 10 g of plant powder, 100 mL of 96% ethanol or 100 mL of methanol was added for each powder, then the whole was left to macerate under mechanical agitation for 24 hours. The macerate was filtered under vacuum using a funnel and Whatman No.4 filter paper. The filtrates obtained are concentrated in a rotary evaporator (Rotavapor).

The extracts obtained are then dried in an oven at 45°C (Memmert® UM-400 oven) for 72 hours.

2.3. Phytochemical screening of extracts prepared

Phytochemical screening is a set of tests for detecting the major groups of chemical compounds present in plants. Detection is based on colorimetric reactions and the precipitation of different reagents in tubes in order to highlight the major groups of chemical compounds present in plants (Table 1). The tests are carried out according to Harbone [20] et Fofana [21].

Table 1 Summary of the main tests used for phytochemical screening

Phytochemical compounds	**Extraction solvents**	**Reagents**	**Positive reaction**
Polyphenols	Distilled water / ethanol	2% $FeCl_3$	Blue-blackish color
Flavonoids	Distilled water / ethanol	10% NaOH	Orange color
Sterols and polyterpenes	Distilled water / ethanol	Acetic anhydride/ concentrated H_2SO_4	Appearance of violet colour /turns blue and then green
Anthocyanins	Distilled water / ethanol	Concentrated HCl	Red color
Catechic tannins	Distilled water / ethanol	Formol 30% / concentrated HCl	Light brown flakes
Gallic tannins	Distilled water / ethanol	Sodium acetate / 2% $FeCl_3$	Blue-black color
Alkaloids	Distilled water / ethanol	Iodobismutate / Iodo-iodide	Precipitate/reddish-brown color
Protein	Distilled water/ethanol	20% NaOH/ 2% $CuSO_4$	Violet colour, or a reddish tinge
Saponosides	Distilled water	None	Persistent moss (1 cm)
Gums and mucillages	Distilled water/ethanol	Absolute alcohol	Flaky precipitate

2.4. Study of androgenic activity

2.4.1. Preparation of extracts for administration

To prepare the macerates, 100 g of plant powder was mixed with 1000 mL of distilled water, then the whole was left to macerate under mechanical agitation for 24 hours. The macerate was filtered under vacuum on Whatman No. 4 filter paper. The extracts obtained were then dried in an oven with a current of air at 45°C (Memmert® UM-400 oven) for 72 hours.

The plant extract solutions administered to the animals were prepared by solubilising dry extracts in distilled water.

2.4.2. Preparation of the reference androgen solution

An intramuscular injectable solution of testosterone enanthate (Androtardyl®, Bayer) was used. The dose required, according to the manufacturer's instructions, is 3.6 mg/Kg in man or 0.54 mg for a 140-150 g rat. For administration, two successive dilutions were made to obtain a 2.5 mg/mL solution. We therefore administered 0.2 mL of this solution to each animal intramuscularly on a single occasion [22].

2.5. Animal care and experimentation

2.5.1. Administration of plant extracts

The macerated aqueous plant extracts were administered orally via an oesophageal tube.They were administered once a day for 35 successive days at a dose of 100 mg/Kg body weight [23]. Microscopic and macroscopic parameters characteristic of spermatozoa (motility, vitality, concentration, morphology and pH of spermatozoa) were assessed. For this study, 30 animals weighing between 160 and 180 g were divided into six batches of five rats male. The animals received the different substances over 35 days, at a dose of 100 mg/Kg body weight (bw), between 13-14 hours PM.

- Lot 1: 1 mL distilled water/100 g ;
- Lot 2: 0.86 mg/Kg bw testosterone enanthate (reference molecule) intramuscularly;
- Lot 3: 100 mg/Kg bw of aqueous macerated extract, *Z. gilletii* (stem bark);
- Lot 4: 100 mg/Kg bw of aqueous macerated extract, *P. hirsuta* (stem);
- Lot 5: 100 mg/Kg bw of aqueous macerated extract, *E. hypericifolia* (whole plant);
- Lot 6: 100 mg/kg bw of aqueous macerated extract, *C. aralioides* (stem).

2.5.2. Sperm collection and analysis technique

Spermatozoa were collected according to the method of Ngoula et al. [24]. A. Immediately after sacrifice, the tail of the left epididymis of each mouse was removed by opening the scrotum, then dilacated in 10 mL of 0.9% NaCl previously incubated in a water bath at 36°C. This allows the spermatozoa to diffuse into the solution. A fine drop of the solution was then placed between the slide and the coverslip for analysis of sperm parameters.

2.6. Evaluation of aqueous extracts of the plant species studied on certain sperm characteristics

The effects of macerated extracts of the plants studied on certain microscopic sperm characteristics were studied in order to assess the effects of the extracts on sperm function (motility, vitality, concentration, morphology and PH of the spermatozoa). To do this, after the animals had been sacrificed with ethyl ether and the organs removed, the tail of the left epididymis of each rat was excised, weighed and dilacated in a Petri dish containing 10 ml of a 0.9% NaCl solution, then incubated in a water bath at 36°C [25,24].

2.6.1. Sperm motility

Motility is important because it shows the vitality of the spermatozoa. In low-quality ejaculations, sperm move slowly and jerkily. Good quality semen should contain at least 60 to 70% motile sperm with a motility grade of 4 or 5 [26]. 20 µL of this solution was placed between a slide and a coverslip (previously maintained at 36°C). Assessment was carried out using a light microscope (Olympus CX31RBSF, Philippine) at x40 magnification. Motile and immobile spermatozoa were determined using the following formula:

$$\%\ spermatozoa = \frac{Number\ of\ motile\ spermatozoa}{Total\ number\ of\ spermatozoa} \times 100$$

2.6.2. Sperm concentration

Sufficient sperm production in the ejaculate is an important criterion for assessing sperm quality; insufficient production could lead to infertility in the subject [27,28]. Sperm counts were performed using the Malassez cell. A drop of epididymal macerate was removed and placed on the Malassez cell and covered with a coverslip. The number of spermatozoa per mm^3 was estimated using a light microscope (Olympus CX31RBSF, Philippines) (x 400) [29].

2.6.3. pH and sperm vitality

The pH was not measured from pure spermatozoa, but directly from the suspension of spermatozoa in 2 mL of physiological water, and the pH was read using urine strips. Vitality was assessed using a methodology: slide preparations used to count live and dead spermatozoa; staining of spermatozoa, then light microscopy reading

(objective 40) of 200 spermatozoa. On the smears, live spermatozoa stained red with 2% eosin and dead spermatozoa were not stained [30].

2.6.4. Sperm morphology

Sperm morphology is an important parameter in the exploration of spermatozoa in infertile men [31]. Sperm morphology is analysed using a sperm smear. Briefly, a drop of the above solution is placed on a slide and spread using a coverslip. The smear is stained with an eosin solution. The smear was examined for normal spermatozoa and abnormal spermatozoa (abnormalities of the head and tail) under a light microscope at x 400 magnification. The percentages of abnormal and normal forms were determined using the formula followed proposed by Ngoula et al. [24].

$$\%\ of\ normal\ spermatozoa = \frac{Number\ of\ normal\ spermatozoa}{Total\ number\ of\ spermatozoa\ counted} \times 100$$

$$\%\ of\ abnormal\ spermatozoa = \frac{Number\ of\ abnormal\ spermatozoa}{Total\ number\ of\ spermatozoa\ counted} \times 100$$

2.7. Sacrifice of animals and collection of blood from rats treated with plant extracts

After 35 days of treatment, the rats were sacrificed on day 38 under anaesthetic between 08-11 AM in order to minimise hormonal variations due to the nycthemeral rhythm. The animal was then sacrificed and the blood collected in special glass tubes and centrifuged at 3000g/5 minutes. The supernatant was transferred to microtubes and stored at -20°C for testosterone assays.

2.8. Statistical analysis

The results obtained are given as the mean followed by the standard error of the mean (M ± SEM). Statistical analysis was performed using Graph Pad Prism 8.01 software (San Diego, California, USA). For each parameter (sperm motility, vitality, concentration, morphology and PH), the Shapiro and Wilk test was used to check the normality of the distribution. We used Bartlett's test to check the homogeneity of the variance. Student's t-test and ANOVA1 (One-way Analysis of Variance) followed by Dunett's comparison test were used to identify differences between treated and control batches. Differences are considered significant for $p < 0.05$.

3. Results

3.1. Phytochemical screening of the plants studied

Preliminary phytochemical investigations on *C. aralioides, E. hypericifolia, P. hirsuta* and *Z. gilletii* revealed the presence of large groups of chemical compounds. The essential secondary metabolites with androgenic properties, namely polyphenols, flavonoids, sterols and polyterpenes, saponins and alkaloids, are predominantly present (Table 2).

Table 2 Chemical compounds characterized in the plant species studied

	Aqueous extract								**Ethanolic extract**				**Methanolic extract**			
	Macerated				**Decocted**											
Plant species	***Pal. hirs.***	***Zan. gill.***	***Euph. hyp.***	***Cis. ara.***	***Pal. hirs.***	***Zan. gill.***	***Euph. hyp.***	***Cis. ara.***	***Pal. hirs.***	***Zan. gill.***	***Euph. hyp.***	***Cis. ara.***	***Pal. hirs.***	***Zan. gill.***	***Euph. hyp.***	***Cis. ara.***
Organs	Stem	Stem Bark	whole Plant	Stem	Stem	Stem Bark	whole Plant	Stem	Stem	Stem Bark	whole Plant	Stem	Stem	Stem Bark	whole Plant	Stem
Phytochemical compounds																
Alkaloids	-	-	**+**	+	-	-	+	-	-	-	+	+	-	-	+	-
Polyphenols	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
Sterols and polyterpenes	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	+

Saponins	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-
Catechic tannins	+	+	+	-	+	+	+	-	-	-	+	-	-	-	+	-
Gallic tannins	+	+	+	-	+	+	+	-	-	-	+	+	-	-	+	+
Anthocyanins	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	+
Proteins	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gums and mucilages	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+

Zan. gill. : *Zanthoxylum gilletii* ; Pal. hirs. : *Palisota hirsuta* ; Euph. hyp. : *Euphorbia hypericifolia* ; Cis. ara. : *Cissus aralioides*

3.2. Effect of aqueous extracts of the plant species studied on the microscopic and macroscopic characteristics of sperm

The effects of aqueous extracts of the plant species studied on sperm characteristics are summarised in Table 3. The results show that oral administration of aqueous extracts of the plant species studied (100 mg/Kg bw) to rats resulted in significant increases ($p < 0.05$; $p < 0.01$ and $p < 0.001$) in sperm concentration and motility compared with rats from the control batch treated with distilled water. However, sperm vitality and normal sperm counts were only significant ($p < 0.01$) with E. hypericifolia and Z. gilletii extracts at 100 mg/Kg bw, respectively, compared with negative controls. Furthermore, sperm pH showed no variation ($p > 0.05$) regardless of the doses administered. These results show a significant increase ($p < 0.01$) in sperm concentration and vitality levels in testosterone enanthate-treated rats compared to animals in the control lot given distilled water.

Table 3 Effects of aqueous extracts of the plant species studied on sperm parameters

	Distilled water control 1 mL/100g	**Testosterone enanthate 3.6 mg/Kg**	**Treatment with macerated extract (100 mg/Kg pc)**			
			Zanthoxylum gilletii	***Palisota hirsuta***	***Euphorbia hypericifolia***	***Cissus aralioides***
pH	6,0±0.28	6,5±0,40	6,1±0,30	6,2±0,40	6,0±0,2	6,4±0,3
Sperm motility rate (%)	17 ± 0,01	28±0,02	76±5,49***	72± 0,01***	79±0,00***	34±2,44 **
Number of spermatozoa / epididymal tail (x 10^6 spz/mL)	12±0,5	20±0,40**	62.94±2.44***	20±0,81**	78±2,93***	14±4,80 *
Sperm vitality (%)	60±0,04	77±4,08***	88±4,07***	80±4,09***	90±4,09***	63±4,4
Normal sperm (%)	60±0,04	65±0,06ns	80±0,00**	77±0,00**	88±0,00***	65±0,05
Abnormal sperm (%)	40±0,04	35±0,10ns	20±0,00**	23±0,00**	12±0,00***	35±0,05

Values are means ± MSE, with n = 5. * : $p < 0.05$, ** : $p < 0.01$ and *** : $p < 0.001$ significant difference compared to controls (distilled water), ns : no significant

3.3. Influence of aqueous plant extracts on testosterone production in animals

After 35 days of treatment, we obtained 15.61±0.34 ng/mL in animals treated with the aqueous extract of *E. hypericifolia* and 11.87±0.35 ng/mL in animals treated with the aqueous extract of *Z. gilletii*. These results show an increase in testosterone levels in animals treated with extracts of these two plants compared with the controls.

Compared with the distilled water control (0.83±0.18 ng/mL) and the testosterone enanthate control (9.35±0.51 ng/mL), testosterone levels were significantly higher in animals treated with the aqueous extract of *E. hypericifolia* ($p < 0.001$), *Z. gilletii* ($p < 0.01$) and *C. aralioides* ($p < 0.05$). In animals treated with aqueous extracts of *P. hirsuta*, the difference was not significant (Table 4). The aqueous extract of *E. hypericifolia* contained 18.80 times more than in the serum of control animals (distilled water). Aqueous extracts of *Z. gilletii* contained 14.30 times more than in the serum

of control animals (distilled water). Aqueous extracts of *C. aralioides* contained 5.59 times more than in the serum of control animals (distilled water). Aqueous extracts of *P. hirsuta* had 2.23 ng/mL (2.68 times more than in the serum of control animals (distilled water).

Table 4 Comparative effects of plant extracts and control on testosterone production

			Treatment with macerated extract (100 mg /Kg pc)			
	Control distilled water 1 mL/100g	Testosterone enanthate 3.6 mg/Kg	*Zanthoxylum gilletii*	*Palisota hirsuta*	*Euphorbia hypericifolia*	*Cissus aralioides*
Mean ±ESM (ng/mL)	0.83±0.18	9.35±0.51**	11.87±0.35***	2.23±0,05	15.61±0.34****	4.64±0.49*

**** P<0.0001: highly significant difference/distilled water-treated control; *** P<0.0001: highly significant difference/distilled water treated control; ** P<0.001: significant difference/distilled water-treated control; * P<0.05: small significant difference/distilled water treated control

4. Discussion

In this study, we aim to evaluated the androgenic properties of aqueous extracts of various organs of *Euphorbia hypericifolia* (Euphorbiaceae), *Zanthoxylum gilletii (Rutaceae), Palisota hirsuta* (Commelinaceae) and *Cissus aralioides* (Vitaceae), four plant species used to treat andropause-related disorders, was carried out on mature male rats. These animals received aqueous extracts of stems, stem barks and whole plants of these species at a dose of 100 mg/Kg of body weight [24].

The androgenic effects of a substance are noticeable at the level of certain organs known as androgen-dependent. In humans, the testicles, seminal vesicle, epididymis, and adrenal glands are some of the organs influenced by androgens [18].

The evaluation of the effects of plant extracts on sperm parameters showed a significant increase in the percentage of motile spermatozoa in *Palisota hirsuta* and with *Euphorbia hypericifolia* (100 mg/Kg bw). Indeed, sperm motility is recognized as an index of fertility in males [32]. Thus, aqueous extracts could have positive effects on male fertility. This result confirms the effects of these plants on androgen-dependent organs. Furthermore, spermatogenesis is under the regulatory influence of pituitary gonadotropins and testosterone. The improvement in sperm quality and quantity is dependent on the quality of spermatogenesis and its transit to the caudal epididymis [33].

The results of sperm parameter analyses support this idea. Indeed, the comparison showed that the Z. gilletii (~62.94.106 spz/mL ± 2.44) and *E. hypericifolia* (~78.106 spz/mL ± 2.93) extracts increased sperm density with a highly significant difference ($p < 0.001$) compared to the control (~12.106 spz/mL ± 0.5). These data show that the aqueous macerates of *Z. gilletii* and *E. hypericifolia* contain compounds that stimulate spermatogenesis with a more intense effect of the E. hypericifolia extract.

A highly significant increase ($p < 0.001$) in motility was observed for *E. hypericifolia* extracts (~79% ± 0.00%) compared to the control (~17% ± 0.01%). *Z. gilletii extract* (~76% ± 5.49%) also increased sperm motility compared to the control, with a highly significant difference. The aqueous macerated extracts of *Z. gilletii and E. hypericifolia* also increased the percentage of normal sperm compared to the control group with a highly significant difference ($p < 0.001$). For motility and morphology, the values obtained with *E. hypericifolia* extract were nevertheless higher than *Z. gilletii* extract with a significant difference ($p < 0.01$). These results showed that the extracts contain active compounds improving spermatogenesis and sperm motility, with a more intense effect of the *E. hypericifolia* extract.

To our knowledge, very few data describe the effects of macerated aqueous extracts of *Cissus aralioides*, *Euphorbia hypericifolia, Palisota hirsuta and Zanthoxylum gilletii* on sperm parameters in albino wistar rats. Although data on rats are poorly documented, our observations of the increase in these parameters are nevertheless consistent with those of other authors [34,35] using the concentration of 100 mg/Kg body weight of macerated aqueous extracts of *Cissus aralioides*, *Euphorbia hypericifolia, Palisota hirsuta* and *Zanthoxylum gilletii* in rats. Indeed, it is well known that flavonoids present in the plant species studied increase testosterone levels and that this hormone stimulates spermatogenesis [36,37,38,39].

The testosterone assay carried out on the serum of animals treated with aqueous plant extracts showed an increase in the testosterone level in these animals. This increase is very significant ($P < 0.0001$) in animals treated with aqueous extracts of *E. hypericifolia* organs, significant ($P < 0.001$) in animals treated with aqueous extracts of *Z. gilletii* stem root and weakly significant in animals treated with aqueous extracts of *C. aralioides* organs compared to the control (saline water). The increase in the testosterone level in the serum of animals treated with aqueous extracts of plant organs could be explained by the ability to synthesize the hormone endogenously from certain elements from the administered extracts. For example, the presence of flavonoids in the plants studied could explain this androgenic activity because these compounds have the ability to stimulate testosterone production. According to Van-Meeuwen et al. [40], flavonoids present in aqueous extracts of *Fadogia agrestis* (Rubiaceae) are responsible for the ability of this plant to increase blood testosterone levels.

Other phytocompounds such as alkaloids present in *E. hypericifolia* would be the basis of its potential to stimulate testosterone production in animals. Furthermore, alkaloids are aromatase inhibitors that transform testosterone into estrogen. Thus, these alkaloids contribute to inhibiting estrogen and subsequently lead to an increase in testosterone levels [41].

In addition, it is possible that the saponins present in the aqueous extract of *Z. gilletii* are the basis of the increase in testosterone levels in animals. According to Jacot, [42] it would act by promoting the growth of testosterone levels.

The study of phytochemical composition of differents extracts performed on *C. aralioides, E. hypericifolia, P. hirsuta and Z. gilletii* showed the presence of sterols and polyterpenes, polyphenols, coumarins, anthocyanins, saponins, catechol tannins, gallic tannins, quinone derivatives, flavonoids and alkaloids. Among these phytocompounds, others have already been revealed as having androgenic properties. It is therefore likely that these different plants studied owe their androgenic properties to the presence of these detected secondary metabolites. Indeed, chrysin, a flavonoid from *Passiflora coerulea* (Passifloraceae), has the capacity to increase free testosterone, to decrease excess estrogens while generating an anti-stress effect [40]. Also, plants studied for their action on sexual desire such as *Tribulus terrestis* and ginseng, have shown an activity improving the levels of free testosterone. This effect is due to saponins such as protodioscin (steroidal saponin) present in *Tribulus* and ginsenosides (triterpenic saponin) in ginseng [43].

In addition, different studies have shown that ferutinin (terpenoids) is the phytohormone responsible for the aphrodisiac effect of *Ferula hermonis*. In addition, sexual activity has been demonstrated by in vivo studies on male and female rats. This activity of ferutinin depends on its dose and duration of administration. For example, chronic administration of the molecule (0.25 mg/Kg, daily for 10 days) increases the level of testosterone [44]. In addition, it is reported that anabasine, nicotine and cotinine (alkaloids), aromatase inhibitors, inhibit aromatase and estrogen biosynthesis in its terminal phase, leading to an increase in testosterone [41].

5. Conclusions

The aim of this study was to know the androgenic properties of *Euphorbia hypericifolia* (Euphorbiaceae), *Zanthoxylum gilletii* (Rutaceae), *Palisota hirsuta* (Commelinaceae) and *Cissus aralioides* (Vitaceae), four plant species used to treat andropause-related disorders in Côte d'Ivoire. The administration of the different extracts induced an increase in serum testosterone levels in the treated animals. Compared to the negative control, the aqueous extracts of the whole plant of *E. hypericifolia* and stem bark of *Z. gilletii* respectively led to the greatest increase in serum testosterone (15.61±0.34 ng/mL and 11.87±0.35 ng/mL versus 0.83±0.18 ng/mL for the control). Also, the phytochemical screening carried out showed the presence of chemical compounds with androgenic properties such as alkaloids, flavonoids, saponins and polyterpenes. These different results validate the traditional use of *E. hypericifolia, Z. gilletii, P. hirsuta* and *C. aralioides* in the treatment of certain disorders related to andropause. These data open many perspectives for the treatment of andropause. In particular, the aqueous extract of *E. hypericifolia* could be specifically recommended for the treatment of oligospermia, asthenospermia and teratospermia. Further research in this direction would therefore be relevant. It would also be relevant to elucidate the fertilizing power of the spermatozoa produced during treatments with the extracts *E. hypericifolia, Z. gilletii, P. hirsuta and C. aralioides.*

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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