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Androgenic and Antioxidant Effects of Ethanolic Extract of *Strychnos camptoneura* (longaniaceae) Trunk Bark in Male Wistar Rats

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Abstract The main purpose of the present study was to evaluate the androgenic and antioxydant effects of ethanolic extract of *Strychnos camptoneura* trunk bark in Wistar rats. For this purpose, 20 rats were randomly arranged in 4 lots of 5 animals each and received respectively for lots 1 and 2 distilled water and testosterone enanthate and lots 3 and 4 treated with ethanolic extract of *Strychnos camptoneura* (100 and 250 mg /kg). It appears from this study that the administration of ethanolic extract of *Strychnos camptoneura* orally at the doses studied causes significant increases in body weight gain ($p < 0.05$; $p < 0.01$ and $p < 0.001$) in rats in contrast to the animals of the negative control lot that received distilled water. Administration of *Strychnos camptoneura* extract at the doses studied did not induce any significant change ($P > 0.05$) in the relative weights of testes, vas deferens, seminal vesicles, and penis compared to negative control rats. Furthermore administration of ethanolic extract of *Strychnos camptoneura* at the doses studied induced a significant increase ($p < 0.01$ and $p < 0.001$) in serum and testicular testosterone levels in rats compared to those treated with distilled water. Chemical analyses performed in this study revealed the presence of total polyphenols (TPP) and flavonoids in the ethanolic extract of *Strychnos camptoneura*. These results suggest that the ethanolic extract of *Strychnos camptoneura* would have androgenic and antioxidant properties and would justify its use in traditional Congolese medicine for the treatment of male infertility.

Keywords: *Strychnos camptoneura, androgenic, hypogonadisme and antioxidant*

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1. Introduction

Hypogonadism is traditionally defined as the inability of the testicle to produce testosterone at sufficient levels during the normal phases of testicular activation [1,2]. This dysfunction may be the consequence of alterations in the hypothalamic-pituitary and/or testicular complex on the one hand and also the influence of endocrine disruptors (xenobiotics, heavy metals and pesticides) on the other hand, which are at the origin of male infertility [3,4]. Faced with this situation, two main scientific approaches can be envisaged: firstly, the use of synthetic drugs and secondly, the use of medicinal plants. Many therapeutic advances have been made in recent years, notably with the use of pharmaceutical specialities. Unfortunately, these drugs are too expensive, hence the need to resort to the use of medicinal plants that are more accessible to the populations of sub-Saharan Africa [5].

Several plant species with androgenic potential have already been the subject of research work such as: Study the effect of *Eruca sativa* leaves extract on male fertility [6]; evaluation of the fertility activity of the aqueous leaves extract of *Zanthoxylum macrophylla* [7]; androgenic effects of the aqueous extract of *Pausinystalia yohimbe* [8]; androgenic potential of the aqueous extract of *pathodea Campanulata* [9] and evaluation of the androgenic potential of the hydroethanol extract of *Strychnos camptoneura* [10]. Among the plants presumed to be androgenic is *Strychnos camptoneura*. It is a plant touted in traditional Congolese medicine for the treatment of male infertility. However, to the best of our knowledge, no study on the androgenic and antioxidant effects of the

trunk bark of the ethanolic extract of *Strychnos camptoneura* has been conducted. Therefore, this study was initiated to evaluate the biological potential of this plant.

2. Materiel and Methods

2.1. Animal Material

Male Wistar rats weighing between 130 and 230 were used. These rats were provided by the animal house of the National Institute for Health Sciences Research (NIHR). They were fed as a standard with free access to tap water.

2.2. Plant Material

Strychnos camptoneura trunk barks were used. They were collected in Mvoula, a village located 740 km from Brazzaville in the sub-prefecture of Itoumbi, Cuvette Ouest, northern Congo. The sample was authenticated at I.R.S.E. N (a research institute, formerly ORSTOM in Brazzaville), registered under number 271. These barks were dried at room temperature in the Biochemistry and Pharmacology laboratory of the Health Sciences Faculty. They were then pulverised using a wooden mortar and a sieve.

2.3. Preparation of Ethanolic Extract of *Strychnos camptoneura*.

The ethanolic extract of *Strychnos camptoneura* trunk bark was prepared by maceration. Thus 100g of powdered *Strychnos camptoneura* trunk bark were mixed in 1000 ml of ethanol for 72 hours. The macerate obtained was filtered with cotton wool and then with filter paper and concentrated using a rotary evaporator. The dry extract obtained, brown in colour, was weighed and then stored in a bottle for pharmacological tests.

2.4. Preparation of the Reference Androgen Solution

Testosterone enanthate (Androtardyl) intramuscular injection was used. The dose required, according to the manufacturer's instructions, is 3.6 mg/kg in man or 0.54 mg for a 150 g rat. To facilitate administration, two successive dilutions were made to give a 2.5 mg/ml solution. We therefore administered 0.2 ml of this solution to each animal by the intramuscular route in a single dose [8,10].

2.5. Study of the Androgenic Effects of the Ethanolic Extract of *strychnos camptoneura* in Male Rats

To evaluate the androgenic effects of this extract, 20 rats were randomly divided into 4 lots of 5 animals each and received for 26 days:

- distilled water at a dose of 0.5 ml/100g/ (negative control lot);
- testosterone enanthate at 3.6 mg/kg/ (reference molecule lot);
- *Strychnos camptoneura* extract at 100 and 250 mg/kg/po respectively.

2.5.1. Effect of Ethanolic Extract of *strychnos camptoneura* on Weight Development

The weight of each animal was measured every two days for twenty-six (26) days using a QUIGG brand scale (capacity 5000 g and accuracy 1).

2.5.2. Effect of Ethanolic Extract of *strychnos camptoneura* on the Relative Weight of Androgen-dependent Sex Organs

This study was conducted to evaluate the effects of the presumptive trophic extract on the weight of androgen-dependent organs. According to the results of the study [11], the weight, size and secretory functions of the testes, epididymides, seminal vesicles, vas deferens and penis are regulated by androgens. Thus, after 26 days of *strychnos camptoneura* administration at doses of 100 and 250 mg/kg, the rats per lot were sacrificed by overdose with cooper's ether. The testicles, epididymides, vas deferens, seminal vesicles and penis were removed, cleared of fatty material and weighed using a branded precision balance (Mettler-Tdedo) of capacity (160g) and accuracy (10-3 g) [8].

2.5.3. Effect of Ethanolic Extract of *strychnos camptoneura* on Biochemical Parameters

This study was conducted to evaluate the effects of the presumed steroidogenic extract on biochemical reproductive parameters in order to determine the hormonal profile of the ethanolic extract of *strychnos camptoneura*. Androgens, of which the main hormone is testosterone, are synthesised from cholesterol. These androgens have an anabolic action, causing an increase in protein synthesis and thus in muscle mass, and contribute to an increase in the volume of the testicles and epididymides [11]. In rats, any increase in serum and testicular testosterone or treatment with androgens is accompanied by an increase in the secretory activity and weight of these organs [12].

2.5.3.1. Obtention and Conditioning of the Serum

24 hours after the last treatment with ethanolic extract of *strychnos camptoneura* (100 and 250 mg/po), the animals were anaesthetised with Cooper's ether using haematocrit tubes, the blood of each animal was taken orally and then collected in dry tubes without anticoagulant. The supernatant collected after 4 hours was distributed into the marked tubes and stored at -20°C for serum testosterone determination.

2.5.3.2. Obtention and Conditioning of Homogenates

The right testicle collected after sacrifice of the animals by cooper ether overdose was then ground in a mortar containing a 0.9% NaCl solution to obtain 10% homogenates. The crushed material was centrifuged (Centromix centrifuge) at 3000 rpm for 30 minutes at room temperature. The supernatant was collected and stored at -20°C for testicular testosterone determination [8].

Figure 1. Bark and powder of *Strychnos camptoneura*

2.5.3.3. Determination of Serum Testosterone and Testicular Testosterone

Testicular testosterone and serum testosterone were determined using the Accu DiagTM ELISA kit (Diagnostic Automation Inc).

- **Principle**

The ELISA testosterone assay is based on the competition type technique. In the presence of the specific antibody, the labelled and unlabelled hormone compete to bind to the antibody. The binding of one or the other of the two antigens (hormones) will depend on its proportion in the medium.

2.6. Anti-free Radical Activities of the Hydroethanol Extract of *Strychnos Camptoneura*

This test was performed according to the DPPH radical reduction method as described [13]. This method was also used [14]. It consists in reducing the DPPH by the antioxidant substances contained in the hydroethanol extract of *Strychnos camptoneura*. This method consists of mixing the solution of 1.1-diphenyl-2-picrylhydrazyl (DPPH) at 10 mg/250mL in ethanol and 100 µL of extract at concentrations of 40; 20; 10; 5; 2.5 mg/ml with the hydroethanol extract. The activity was then measured at 517 nm after 30 minutes of incubation in the dark using a UV-visible spectrophotometer. The calculation of the inhibition percentages was done according to this relationship:

$$\% I = \frac{[D.O_{blank} - D.O_{EI}]}{D.O_{blank}} x100$$

D.O blanc= Optical density of the negative control; D.OEI= Optical density of the extract/inhibitor.

The concentration that inhibits 50% of DPPH (IC50) is determined graphically.

2.6.1. Determination of Total Polyphenols

The determination of TPP was carried out using a spectrophotometer. We determined the optical densities of our extracts and then compared the result with that obtained by a gallic acid standard of known concentration. The assay was carried out as follows: to 0.1 ml of plant extract introduced into a test tube, were added 0.9 ml of distilled water; 0.9 ml of Folin-Ciocalteu reagent (1N); then immediately 0.2 ml of a Na2CO3 solution (20%). The resulting mixture was incubated at room temperature for 40 minutes in the dark. The absorbance was then measured with a spectrophotometer at 725 nm against a methanol solution used as a blank. It should be noted that a calibration line was previously carried out before the analysis with gallic acid under the same conditions as the samples to be analysed. The results obtained were expressed in mg gallic acid equivalent per 100 grams of dry matter (mgEGa/100 gMs). [15].

2.6.2. Determination of Total Flavonoids

Total flavonoids were also determined using a spectrophotometer, as follows: 250 µl of the extract and 1 ml of distilled water were successively introduced into a test tube. At the initial time (0 minutes), 75 µl of a NaNO2 solution (5%) was added, followed by 75 µl of AlCl3 (10%) 5 minutes later. After 6 minutes, 500 µl of NaOH (1N) and 2.5 ml of distilled water were successively added to the mixture. The absorbance of the obtained mixture was directly measured with a UV-visible spectrophotometer at 510 nm and the results were expressed as mg rutin equivalent per 100 grams of dry matter (mgERu/100g Ms). A calibration curve was developed with standard Rutin solutions prepared at different concentrations. [16].

2.7. Statistical Analysis of the Collected Data

Statistical analysis of the collected data was performed using analysis of variance (ANOVA), Student's t-test and Mann-Whhitney test to compare the "test" groups. The results are expressed as mean ± standard error with $p < 0.05$ as the significance level.

3. Results

3.1. Effect of Ethanolic Extract of *Strychnos camptoneura* Trunk Bark on Weight Growth

Figure 2 shows the effect of ethanolic extract of *Strychnos camptoneura* on weight growth. From this figure, it can be seen that the administration of ethanolic extract of *Strychnos camptoneura* orally (100mg/kg and 250mg/kg) in rats caused significant increases (*p < 0.05, **p< 0.01 and ***p<0.001) from D8, D14 and D26 of (104.36±0.96%, 116.56±1.66% and 132.33±4.73%; 103.72±1.74%, 116.79±2.72% and 128.10±3.16%) compared to the negative controls treated with distilled water that are (92.06±9.97%; 106.79±9.41% and 122.09±11.01%). This increase was also comparable in rats treated with the reference molecule (3.6 mg/kg) (106.57±3.95%; 121.72±1.08% and 142.49±4.43%).

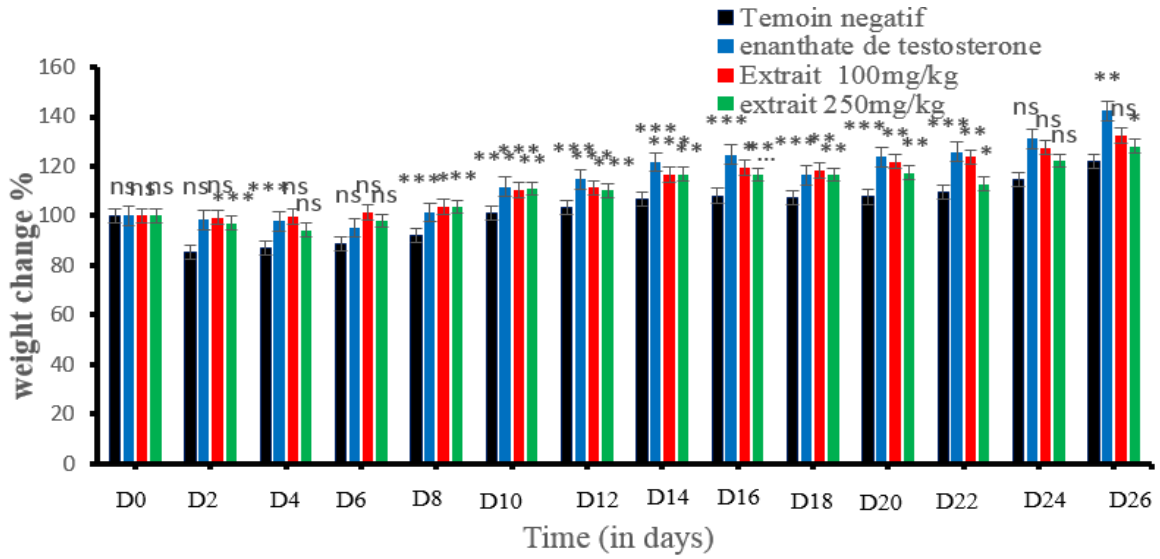

Figure 2. Effect of ethanolic extract of *Strychnos Camptoneura* on weight development (With n: 5. *p < 0.05; **p < 0.01; *** p < 0.001 significant difference from controls (distilled water), ns: P > 0.05 non-significant difference from controls (distilled water). D0 = Day 1, T-: Negative control, ET+: Testosterone enanthate and EESC: Ethanolic extract of *Strychnos Camptoneura*)

3.2. Effect of Ethanolic Extract of *Strychnos camptoneura* on the Relative Weight of Androgen-dependent Organs

Table 1 shows the effect of ethanolic extract of *Strychnos camptoneura* on the relative weight of androgen-dependent sex organs. From this table, it can be seen that, administration of ethanolic extract of *Strychnos Camptoneura* orally (100 and 250 mg /kg/, po) caused no significant change in the relative weights of testicles, vas deferens, seminal vesicles and penis (P>0.05) compared to negative and positive control rats. Furthermore, the relative weight of the epididymides was significantly decreased (p < 0.05) in rats treated with 250 mg /kg *Strychnos Camptoneura* compared to animals from the lots that received distilled water and the reference molecule.

3.3. Effects of Ethanolic Extract of *Strychnos camptoneura* on Biochemical Parameters of Reproduction

The effect of ethanolic extract of *Strychnos camptoneura* on biochemical parameters is summarised in Table 2. Oral administration of ethanolic extract of *Strychnos camptoneura* at the doses studied (100 and 250 mg /kg) caused a significant increase (p < 0.01 and p < 0.001) in serum and testicular testosterone levels compared to rats treated with distilled water. This increase is comparable to the reference molecule.

Table 1. Effect of ethanolic extract of *Strychnos Camptoneura* on relative androgen-dependent organ weights

androgen-dependent parameters (g)	Treatments			
	Distilled water (0.5ml/100g)	testosterone enanthate (3.6mg/ kg)	*S.Camptoneura* (100mg/kg)	*S . camptoneura* (250mg/kg)
Testicles	1.29±0.13	1.36±0.05	1.30±0.02	1.37±0.09ns
Epididymes	0.23±0.02	0.26±0.01	0.25±0.01ns	0.16±0.02*
vas deferens	0.08±0.02	0.09±0.01ns	0.11±0.01ns	0.08±0.00ns
seminal vesicles	0.52±0.18	0.52±0.07ns	0.76±0.01ns	0.75±0.12ns
penis	0.26±0.03	0.24±0.01ns	0.30±0.02ns	0.30 ±0.00ns

Values are means ± MSE, with n = 5. *: p < 0.05, significant difference from controls (distilled water), ns : P > 0.05 non-significant difference from controls (distilled water) and S.: Strychnos.

Table 2. Effects of ethanolic extract of *Strychnos Camptoneura* on biochemical parameters of reproduction

biochemical parameters (ng/mL)	Treatments			
	Distilled water (0.5ml/100g)	Testosterone enanthate (3.6mg/ kg)	*S. camptoneura* (100mg/kg)	*S. camptoneura* (250mg/kg)
Testicular testosterone	47.64±2.02	73.24±2.75***	71.76±1.88***	68.71±1.21***
Serum testosterone	0.41±0,11	1.22±0.02***	1.12±0.02**	1.37±0.08***

Values are means ± MSE, with n = 5. **: p < 0.01 and ***: p < 0.001 significant difference from controls (distilled water), ns: P > 0.05 non-significant difference from controls (distilled water) and S.: Strychnos.

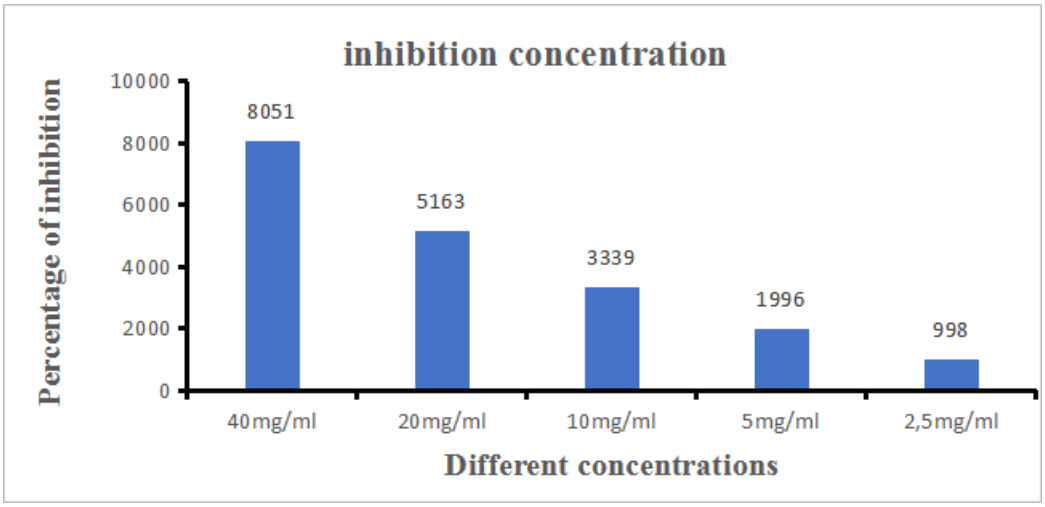

Figure 3. The percentage of inhibition on anti-radical activity

3.4. Anti-radical Activity of the Ethanolic Extract of *Strychnos camptoneura*

The figure shows the evolution of the percentage of radical reduction according to the different concentrations. It should be noted that the strong reduction of free radicals at 50% was observed in the concentration interval between 20-10 mg/ml with a respective IC50 of 14.97 mg/mL.

3.5. Chromatogram of the Anti-free Radical Activity of the Ethanolic Extract of *Strychnos camptoneura*

The demonstration of the anti-free radical activity by the revelation of the plate with DPPH, showed the light green compounds, present in the ethanolic extract, fluoresce yellow under visible light on a violet background (figure), which shows, these compounds are free radical scavengers (DPPH).

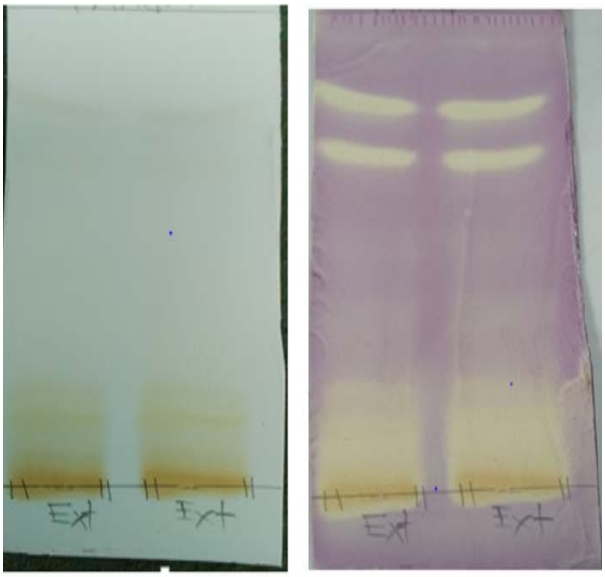

Figure A : TLC profile to the visible

Figure B : TLC profile to the visible after revelation with the DPHH

Figure 4. Chromatogram of the anti-radical activity (Eluent: Ethyl acetate/formic acid/water (8/1/1), Ext: Extract)

3.6. Chemical Analysis of Ethanolic Extract of *Strychnos camptoneura* Trunk Bark

The results of the quantitative analysis by UV-visible spectrophotometer of the ethanolic extract of *Strychnos camptoneura* showed that total polyphenols are quantitatively higher than total flavonoids. In view of these results, we can say that the polyphenol contents are quantitatively richer than total flavonoids

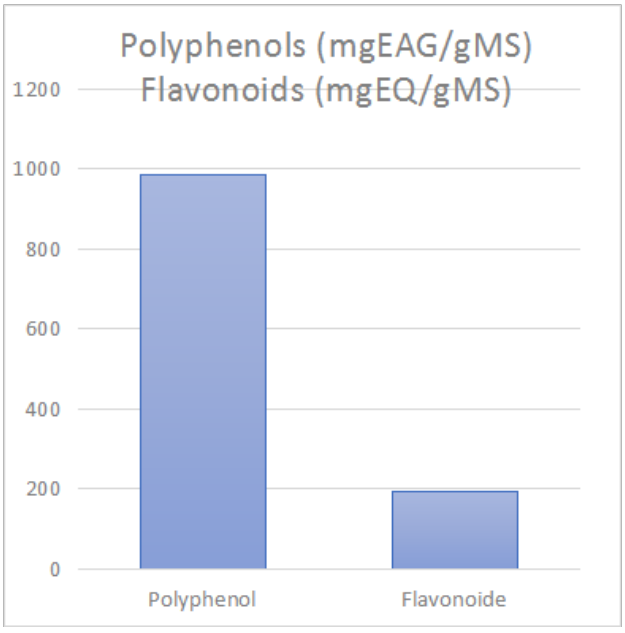

Figure 5. Total polyphenol and flavonoid content

4. Discussion

The objective of the present study was to evaluate the androgenic and antioxidant effects of ethanolic extract of *Strychnos camptoneura* trunk bark in male Wistar rats. The results obtained showed that the administration of the ethanolic extract of *Strychnos camptoneura* orally at the doses studied caused significant increases in weight gain in the rats in contrast to the negative control animals that received distilled water. These results suggest that the ethanolic extract of *Strychnos camptoneura* stimulates weight

growth resulting in good food and water consumption in rats. *Strychnos camptoneura* could therefore have anabolic potential. These results corroborate those obtained [10], in rats treated with the hydroethanol extract of *Strychnos camptoneura* and are similar to those obtained [8], in rats treated with the aqueous extract of *Pausinystalia yohimbe*. Administration of *Strychnos camptoneura* extract at the doses studied did not induce any significant variation in the relative weights of the testicles, vas deferens, seminal vesicles and penis compared to negative control rats. Our results are similar to those obtained [17], because no significant variation in the relative weight of the testicles and reproductive appendages was noted in rats treated with methanol extract of *Basella alba*.

These results showed a significant decrease in the relative weight of the epididymides in rats treated with ethanolic extract of *Strychnos camptoneura* at 250 mg/kg. These results are in disagreement with those of [8,10] who observed a significant increase in the relative weights of the epididymides in rats treated respectively with the aqueous extract of *Pausinystalia yohimbe* and the hydro-ethanolic extract of *Strychnos camptoneura*. This difference could be attributed on the one hand to the type of solvent used, and on the other hand to the chemical composition and concentration of the various secondary metabolites present in the extract. It is known that the androgenic potential of a plant depends on the different organs of the plant, its chemical composition, its concentration, the doses used and the extraction techniques used [8,10,18]. Administration of ethanolic extract of *Strychnos camptoneura* at the indicated doses induced a significant increase in serum and testicular testosterone levels in rats compared to those treated with distilled water. These results corroborate those of [10] and are in line with [10,19], who noted significant increases in serum and testicular testosterone levels in rats treated with hydroethanol extract of *Strychnos camptoneura*, aqueous extract of *Pausinystalia Yohimbe*. The significant increase in serum and testicular testosterone levels observed during this experiment showed that this extract possesses steroidogenic activity and probably acts on the hypothalamic-pituitary-testicular complex by stimulating the synthesis of testosterone via the specific receptors of Leydig cells. The increase in serum and testicular testosterone levels is attributed to the presence in the extract of phenolic compounds and flavonoids with androgenic and antioxidant properties [8]. The free radical scavenging activity of the ethanolic extract of *Strychnos camptoneura* revealed a respective inhibitory concentration (IC50) of 14.97 mg/mL. This high antiradical activity could be explained by the high content of polyphenolic compounds in general and flavonoid compounds in particular, which have antioxidant potential in medicinal plants. These results contradict those observed [19] having observed a high inhibitory concentration (IC50) lower than that found with the hydro-ethanolic extract of *Strychnos camptoneura* trunk bark, i.e. 23.64 mg/ml. It is scientifically proven that the lower the value of the inhibitory concentration is, the stronger the antiradical activity becomes. This difference could be justified either by a high content of polyphenolic compounds on the one hand and could be attributed to the extraction solvents on the other hand. The chemical analyses carried out during this study revealed the presence of total polyphenols and flavonoids in this extract. These results corroborate those of [19] with the hydroethanolic extract of *Strychnos camptoneura*. Similar results were also obtained by [20] from phytochemical analysis of aqueous extracts of *Spathodea campanulata* and methanoic extracts of *Peltophorum africanum* respectively. Polyphenols and flavonoids are the main secondary metabolites of the plant. These results corroborate those of [19] with the hydroethanol extract of *Strychnos camptoneura*. Similar results were also obtained by [21] following phytochemical analysis of aqueous extracts of *Spathodea campanulata* and methanoic extracts of *Peltophorum africanum* respectively. Polyphenols and flavonoids are the most abundant secondary metabolites in medicinal plants and confer androgenic and antioxidant potential to the plant [20,21]. The improvement in the reproductive parameters observed in this study would be attributed to the chemical composition of this extract, particularly the presence of total polyphenols and flavonoids, justifying the androgenic and antioxidant potential of the ethanolic extract of *Strychnos camptoneura* in male rats.

5. Conclusion

These results suggest that the ethanolic extract of *Strychnos camptoneura* would have androgenic and antioxidant properties and would justify its use in traditional Congolese medicine for the treatment of male infertility.

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