

In vitro antioxidant activity and *in vivo* aphrodisiac activity in male *Cavia porcellus* of *Albizia adianthifolia* (Schum.) W. Wight and *Pericopsis angolensis* (Baker) Meeuwen

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World Journal of Biology Pharmacy and Health Sciences, 2021, 05(03), 044–052

Publication history: Received on 04 February 2021; revised on 06 March 2021; accepted on 09 March 2021

Article DOI: <https://doi.org/10.30574/wjbphs.2021.5.3.0024>

Abstract

This study was carried out to evaluate the aphrodisiac and antioxidant activities of aqueous and methanolic extracts of two plants used as aphrodisiac in traditional medicine in Kampemba municipality, in Lubumbashi DR Congo. Aqueous and methanolic root bark extracts of both *Albizia adianthifolia* and *Pericopsis angolensis* were administered orally to male *Cavia porcellus* before evaluating their physical behavior in the presence of females and their testosterone, cholesterol and triglyceride contents. The antioxidant activity was determined by the DPPH method. At 200 and 250 mg kg⁻¹ body weight (bw), the extracts exhibited a decrease in rise time and intromission time and an increase in ejaculation time, ascending frequency, intromission frequency, ejaculation frequency and testosterone compared to the control group that received sildenafil (10 mg kg⁻¹ bw) used as a reference. A positive relationship between testosterone level and antioxidant activity has also been observed. The aphrodisiac and antioxidant activity observed in the two species justify their uses in traditional medicine as aphrodisiacs. Further work is needed in order to proceed to the isolation and the identification of the active compounds.

Keywords: Erectile dysfunction; Congolese traditional medicine; Kampemba; DPPH-test; Fabaceae.

1. Introduction

Erectile dysfunction (ED) refers to the constant inability of a man to achieve or maintain an erection for satisfactory sexual activity [1]. The prevalence and severity of ED increases with age [2] and not only affects the life expectancy, but also the well-being and quality of life of exposed people [3]. In the past, plants called aphrodisiacs, in memory of Aphrodite, the Greek god of love, such as *Pausinystalia johimbe* (K. Schum.) Pierre, Rubiaceae [4] or *Zingiber officinalis* L, Zingiberaceae [5], have been used to improve sexual pleasure. Several plants are still used today as aphrodisiacs in several cultures.

In Lubumbashi, *Albizia adianthifolia* (Schum.) W. Wight (Fabaceae), [Synonyms: *Albizia fastigiata* (E.Mey.) Oliv. *Albizia intermedia* De Wild. & T. Durand, *Albizia ealaensis* De Wild., *Albizia gummifera* auct. non (JFGmel.) CASm.] and, *Pericopsis angolensis* (Baker) Meeuwen (Fabaceae), [synonym: *Afrormosia angolensis* (Baker) Harms.], are two plants widely used in traditional medicine in the treatment of sexual dysfunction in the Kampemba municipality in DR Congo. *Albizia adianthifolia* is also used as a purgative and antidiabetic as well as in eye, gastrointestinal, hemorrhoidal, cephalic,

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neurodegenerative diseases [6, 7]. A part from ED, *Pericopsis angolensis* is also used in traditional medicine in respiratory and eye conditions and in gastrointestinal disorders [8].

This study aimed to evaluate the aphrodisiac and antioxidant activities of these two plants, thus contributing to the scientific validation of this aphrodisiac use which they enjoy in the city of Lubumbashi.

2. Material and methods

2.1. Plant material

The root bark of *Albizia adianthifolia* and *Pericopsis angolensis* were collected at Kashamata (south part of Lubumbashi town) in November 2017 respectively at GPS data: alt. 1226 m, south latitude 11 ° 41'57 " , longitude 27 ° 42'32 " East and alt. 1201m, South latitude 11 ° 34'20.1 " , East longitude 27 ° 23'32.6 " , then identified at the Kipopo herbarium under the respective numbers KIP0011123 and KIP0011124.

2.2. Experimental animals

A local strain of *Cavia porcellus* L. on average weight 380.38 ± 10.9 g of both sexes was obtained from the Faculty of Agronomic Sciences of the University of Lubumbashi. These animals were fed with a standard diet (MIDEMA / RD Congo) and acclimatized for 10 days under the experimental environmental conditions (28 °C, alternating light dark every 12 h, meal and water at will).

2.3. Preparation of extracts and determination of experimental doses

The methanolic extract (ME) was prepared by maceration of 350 g of crushed dried plant material in 1.5 L of methanol (67-56-1 Sigma Aldrich, Belgium) for 48 h. The aqueous extracts (EA) were prepared according to the protocol used in traditional medicine by decoction for 30 minutes of 600 g of plant material in 4 L of water for *A. adianthifolia* and by decoction of 750 g of plant material in 2.5 L of water for 45 minutes for *P. angolensis*. The macerated or decocted solutions were filtrated under filter paper ($\varnothing = 0.25 \mu\text{m}$, No. 1172.000002.05 Whatman, USA), and were evaporated under a rotary evaporator (Büchi R-210, Switzerland). The doses administered in Guinea pigs (Table 1) were deduced from the doses administered in traditional medicine in humans by applying the formula:

$$D_H = \frac{K_{mA}}{K_{mH}} \times D_A \text{ [9].}$$

With D_H = Dose in humans (in $\text{mg} \cdot \text{kg}^{-1}$); D_A = Dose in animals (in $\text{mg} \cdot \text{kg}^{-1}$); K_{mH} = Body surface constant in humans of $70 \text{ kg} = 37 \text{ mg} \cdot (\text{m}^2)^{-1}$, K_{mA} = body surface consistency in *Cavia porcellus* of $0.4 \text{ g} = 8 \text{ mg} \cdot (\text{m}^2)^{-1}$.

Table 1 Determination of the experimental dose in humans versus in *Cavia porcellus*.

Plants	Extract used in traditional medicine	EB (g)	D_H (mg / kg)	D_A (mg / kg)	Adjusted dose (mg/kg)
<i>A. Adianthifolia</i>	Decoction of 2 handfuls of root bark powder in 2L of water for 30 minutes. Drink 2 cups 1 hour before the act.	3.01 ± 0.2	42.9 ± 0.2	198.4 ± 0.2	200
<i>P. Angolensis</i>	Decoction of 2 and a half handfuls of root bark powder for 45 minutes in 2.5 L of water, drink a cup 1 hour before the act.	3.71 ± 0.1	52.9 ± 0.1	244.7 ± 0.1	250
<i>Sildenafil</i>	NA	NA	1.43	6.6	10

1 handful = 28 g for *A. adianthifolia* and 35 g for *P. angolensis*, 1 cup = 450 mL $D_H = EB \cdot 100/7$, the results are expressed as the mean \pm standard deviation of 3 repetitions. D_A = dose administered in animals, D_H : dose administered in humans; EB: crude extract; NA: not applicable.

2.4. Aphrodisiac activity assessment

The aphrodisiac activity evaluation test was inspired by a previous protocol [10]. Briefly, fifty male *Cavia porcellus* were randomized into 10 groups (GA) of 5 animals each. The animals of group A, which served as control, received per 2 mL of 0.9% saline solution and those of group B, used as positive control, received 10 mg.kg⁻¹ of weight of sildenafil (MFCD09026931; Sigma-Aldrich, UK). Animals of groups C, D, (aqueous extract) E, F (methanolic extracts), for *A. adianthifolia*, and G, H (aqueous extract), I, J (methanolic extract) for *P. angiogenesis*, were treated with 200 and 250 mg.kg⁻¹ of extract. All the administrations were carried out in a single dose at 8:00 am by gavage probe and 45 minutes after administration, the female *Cavia porcellus* were introduced into metal cages (0.5 m x 0.5 m x 0.5 m) where male *Cavia porcellus* were monitored for 2 h using a camera (SONY FDR-AX53 HANDYCAM 4K). The sexual behavior of male *Cavia porcellus* based on physical parameters including rise time (RT), intromission time (IT), ejaculation time (ET), post-ejaculatory interval (PEI), number of displays (ND), the number of intromissions (NI) and the number of ejaculations (NE) as well as the measurements of biochemical parameters such as total cholesterol, triglyceride and testosterone concentration were used to evaluate the aphrodisiac activity. The assays were carried out as described in the Total cholesterol assay kit (STA-38, CellBiolabs.inc.), Triglyceride Colorimetric Assay Kit (No. 10010303, Cayman) and Testosterone ELISA Kit (KA0309, Abnova), at the university clinics of Lubumbashi.

2.5. Antioxidant activity evaluation

Antioxidant activity was evaluated using the DPPH method developed previously [11]. Briefly, 50 µL of extract or ascorbic acid, positive control, prepared at different dilutions in methanol from a 100 µg / mL solution were interacted with 1950 µL of DPPH (0.002% in a 96-well plate (Nunc WVR, Germany). After mixing and incubating in the darkness for 30 minutes, the solution was read in a spectrophotometer (Thermo Fisher Scientific Inc., Waltham, USA) at 492 nm. The tests were performed in triplicate and the 0.002% DPPH solution was used as a negative control. The percentage of antioxidant activity was calculated by the formula: $I (\%) = (Ab - Ae) \times 100 \times Ab^{-1}$ with Ab = absorbance measured in the presence of the negative control; Ae = absorbance measured in the presence of the extract, $I (\%)$ = Percentage of inhibition. This percentage of activity allowed to generate IC₅₀.

2.6. Statistical analysis of data

Statistical analysis of the data was made possible using GraphPad Prism version 6 software (GraphPad Software, La Jolla, USA). The comparison between two means was carried out by Student's t test and that of several variables by one-way ANOVA, the significance level was set at 95%.

3. Results

3.1. Aphrodisiac activity

3.1.1. Physical parameters of sexual behavior

The different physical parameters vary significantly compared to the negative control group (with a $p < 0.01$). Declines are recorded for RT, IT and PEI unlike ET, ND, NI and NE which increased. At equal doses, *Albizia adianthifolia* (*A. adianthifolia*) exhibits more expressive performance indicators than *Pericopsis angolensis* (*P. angolensis*). The EM of *A. adianthifolia* seem more efficient than the EA unlike *P. angolensis* where the performances between the two extracts (EA and EM) seem to be of the same amplitude. *A. adianthifolia* at 200 mg.kg⁻¹ and *P. angolensis* at 250 mg.kg⁻¹ exhibit better physical parameters than Sildenafil, used as a positive control (Table 2). The rise time (RT) of the extracts varies between 9.3 ± 0.13 (EMAA 250 mg. kg⁻¹) and 27.1 ± 1.2 min (EAPA200 mg.kg⁻¹), that of intromission (IT) varies between 0.38 ± 0.13 (EMAA250 mg. kg⁻¹) and $5, 1 \pm 0.13$ (EMPA 200 mg. kg⁻¹) min and that of ejaculation (ET) varies between 1.5 ± 0.11 (EAPA200 mg. kg⁻¹) and 3.6 ± 0.13 (EAAA200 mg. kg⁻¹). The post-ejaculatory interval (PEI) varies between 0.30 ± 0.10 (EM AA 200 mg. kg⁻¹) and 11.5 ± 2.13 (EMPA 200 mg. kg⁻¹). Except for PA 250 mg. kg⁻¹, RT is lower in the methanolic extract than the aqueous extract and overall a very significant drop in TM is observed in comparison with the negative control group. With the exception of *P. angolensis* 200 mg. kg⁻¹, the extracts show a lower RT compared to the positive control group ($p < 0.01$) (Table 2).

Table 2 Variation of physical parameters during the evaluation of aphrodisiac activity.

Groups	RT (min)	IT (min)	ET (min)	PEI (min)
NaCl 0.9 %	57.7 ± 1.5	6.47 ± 0.0	0.45 ± 0.11	20.4 ± 0.13
Sildenafil10mg.kg ⁻¹	15.3 ± 2.3 ^d	2.41 ± 0.6 ^d	2.6 ± 0.13 ^b	0.57 ± 0.13 ^d
EAAA 200 mg kg ⁻¹	12.3 ± 1.7 ^d	2.3 ± 0.3 ^d	3.6 ± 0.13 ^d	0.45 ± 0.01 ^d
EMAA 200 mg kg ⁻¹	10.1 ± 0.11 ^d	0.42 ± 0.11 ^d	1.8 ± 0.14 ^a	0.30 ± 0.10 ^d
EAAA 250 mg kg ⁻¹	13.1 ± 0.13 ^d	2.7 ± 0.11 ^d	3.4 ± 0.13 ^d	0.51 ± 0.13 ^d
EMAA 250 mg kg ⁻¹	9.3 ± 0.13 ^d	0.38 ± 0.13 ^d	1.5 ± 0.13 ^a	0.49 ± 0.13 ^d
EAPA 200 mg kg ⁻¹	27.1 ± 1.2 ^d	3.1 ± 0.20 ^d	1.5 ± 0.11 ^a	10.4 ± 1.10 ^d
EMPA 200 mg kg ⁻¹	20.2 ± 0.13 ^d	5.1 ± 0.13 ^c	4.1 ± 0.13 ^d	11.5 ± 2.13 ^d
EAPA 250 mg kg ⁻¹	12.1 ± 1.2 ^d	2.1 ± 0.2 ^d	3.5 ± 0.11 ^d	0.40 ± 0.13 ^d
EMPA 250 mg kg ⁻¹	12.3 ± 0.12 ^d	1.9 ± 0.37 ^d	3.8 ± 1.02 ^b	0.46 ± 0.15 ^d

Results expressed as the mean ± SD of 5 replicas, RT: rise time, IT: intromission time, ET: ejaculation time, PEI: post-ejaculatory interval. ANOVA test treatment with Tukey's multiple comparisons test: ^a p <0.01, ^b p <0.001 and ^c p <0.0001.

The frequencies of ascension (ND) and intromission (NI) vary between 0.03 ± 0.01 (EM PA 200 mg.kg⁻¹) and 0.07 ± 0.01 (EM AA 250 mg. kg⁻¹) as well as ejaculation frequency (NE) (Table 3).

Table 3 Variation of physical parameters during the evaluation of aphrodisiac activity.

Groups	ND / min	NI / min	NE / min
NaCl 0.9%	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01
Sildenafil10mg kg ⁻¹	0.06 ± 0.01 ^a	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a
EAAA 200 mg kg ⁻¹	0.07 ± 0.02 ^c	0.07 ± 0.02 ^b	0.06 ± 0.01 ^b
EMAA 200 mg kg ⁻¹	0.06 ± 0.01 ^b	0.06 ± 0.01 ^a	0.06 ± 0.01 ^b
EAAA 250 mg kg ⁻¹	0.06 ± 0.02 ^b	0.06 ± 0.01 ^a	0.06 ± 0.01 ^b
EMAA 250 mg kg ⁻¹	0.07 ± 0.01 ^c	0.07 ± 0.01 ^b	0.07 ± 0.01 ^c
EAPA 200 mg kg ⁻¹	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01
EMPA 200 mg kg ⁻¹	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
EAPA 250 mg kg ⁻¹	0.06 ± 0.01 ^b	0.06 ± 0.02 ^a	0.04 ± 0.01
EMPA 250 mg kg ⁻¹	0.07 ± 0.01 ^c	0.06 ± 0.01 ^a	0.06 ± 0.01 ^b

Results expressed as the mean ± SD of 5 replicas, FM: ascending frequency, IF: intromission frequency, FE: ejaculation frequency. Frequencies are related to time. ANOVA test treatment with Tukey's multiple comparisons test: ^a p <0.01, ^b p <0.001 and ^c p <0.0001.

3.1.2. Biochemical parameters of libido

Overall, we observe a decrease in triglyceride levels (Figure 1a), compared to the negative control (p <0.00001) as well as an increase in cholesterol (Figure 1b) and testosterone (Figure 1c) with p <0.001, except for AP at 200 mg. kg⁻¹. The aphrodisiac index (Figure 1d), calculated for the testosterone levels and by contribution to sildenafil, considered as a positive control, is <1 for AP at 200 mg. kg⁻¹ and > 1 in the rest of the extracts (Figure 1). The triglyceride levels vary between 83.1 ± 1.1 and 98.3 ± 2.8 mg. dL⁻¹, those of cholesterol between 31.2 ± 11.6 mg. dL⁻¹ and those of testosterone between 3.32 ± 0.34 and 5.11 ± 0.19 ng. mL⁻¹ (Figure 1).

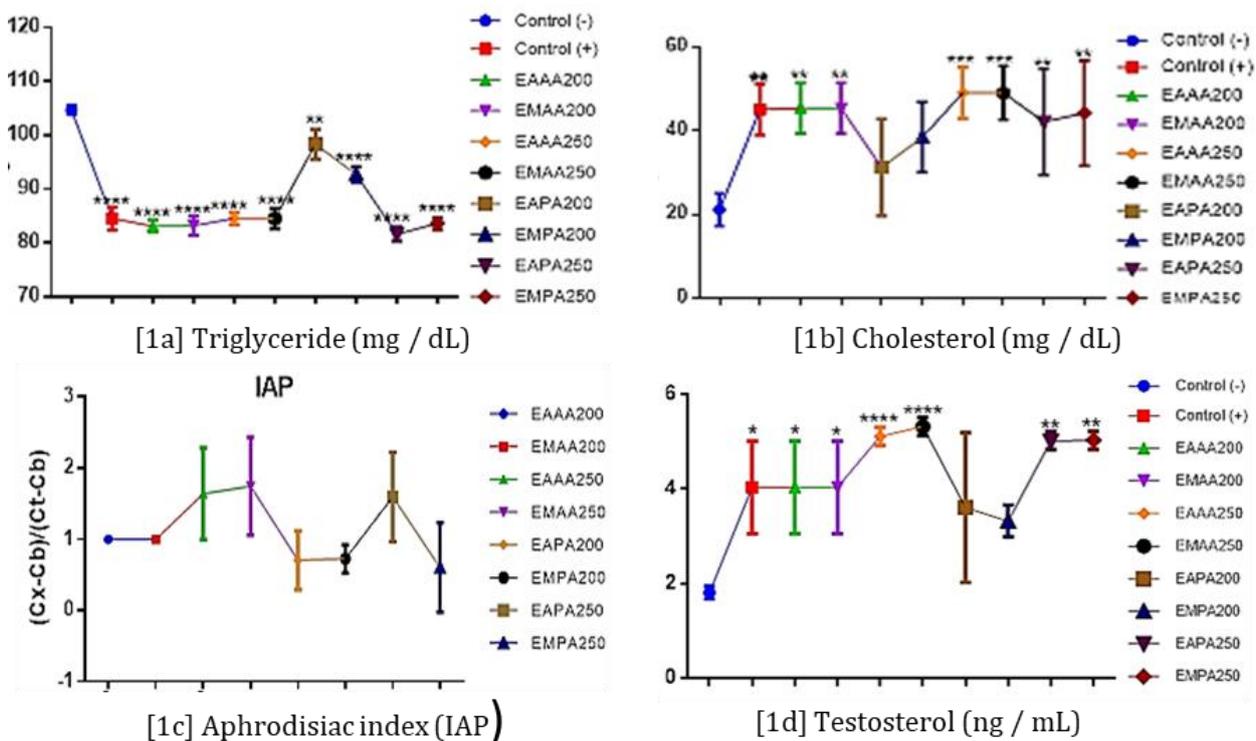


Figure 1 Biochemical parameters measured during the evaluation of the aphrodisiac activity in *Albizia adianthifolia* and *Pericopsis angolensis*.

EA: aqueous extract, EM: methanolic extract, AA: *Albizia adianthifolia*, PA: *Pericopsis angolensis*, Control (-), NaCl 0.9%, Control (+): Sildenafil 10 mg / kg; results of mean \pm SD n = 5, statistical ANOVA treatment with Tukey's multiple comparisons test, *p < 0.01, ** p < 0.001, *** p < 0.0001, **** p < 0.00001.

3.2. Antioxidant activity and correlation with testosterone level

3.2.1. Antioxidant activity

The antioxidant activity of the extracts, evaluated as a function of the anti-free radical activity on DPPH and expressed as $1 / IC_{50}$ ($\mu\text{g} \cdot \text{mL}^{-1}$) varies between 0.079 ± 0.001 and 0.204 ± 0.001 ($\mu\text{g} \cdot \text{mL}^{-1}$) (Figure 2a), but when it is expressed in the form of ascorbic acid equivalent per 100 g of extract, it varies between 15.36 ± 0.06 and 41.27 ± 0.12 mg E vit C / 100 g of extract (Figure 2b). In both forms of expression, AA extracts are the more antioxidant than sildenafil (p < 0.001) (Figure 2).

3.2.2. Correlation between antioxidant activity and testosterone level

A positive linear correlation with high amplitude ($R = 0.9996$) is observed between the antioxidant activity and the testosterone level unlike the totals of triglycerides for which a negative and low amplitude correlation ($R = 0.251$) is observed with antioxidant activity (Figure 2c and 2d).

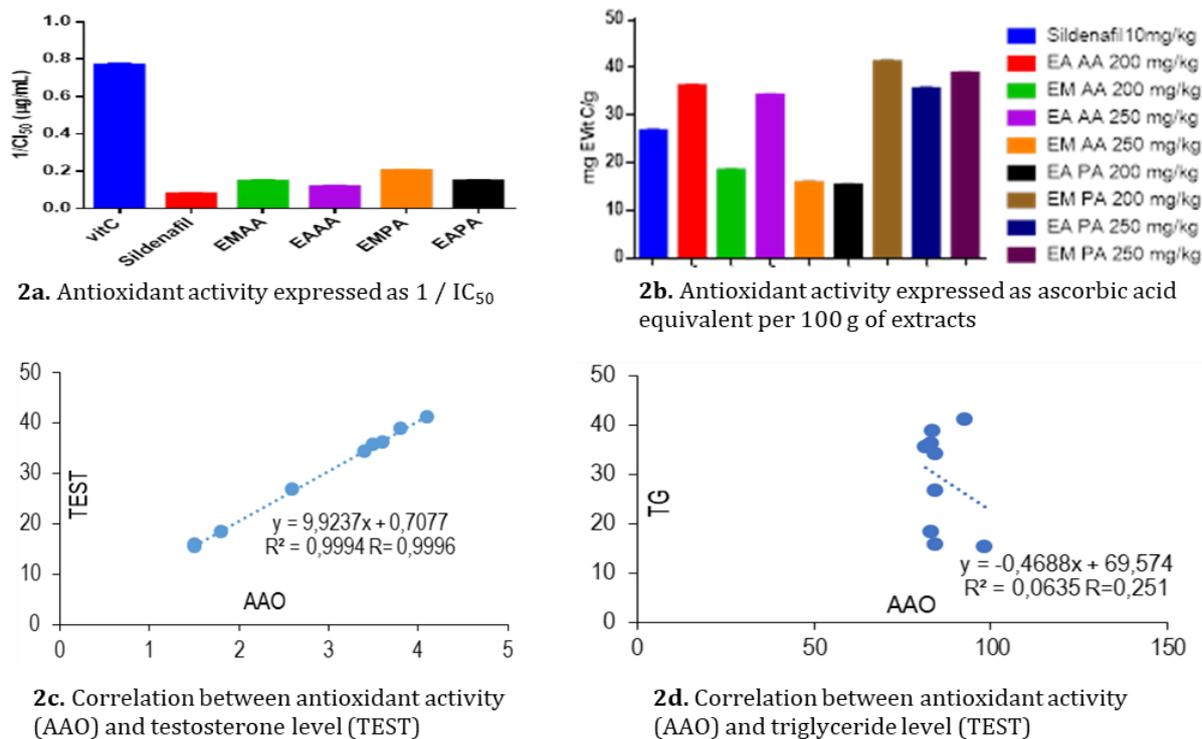


Figure 2 Antioxidant activity and correlation antioxidant activity versus aphrodisiac activity

4. Discussion

During this study, we evaluated the aphrodisiac properties of aqueous and methanolic extracts of 2 plants used in traditional medicine in the commune of Kampemba in Lubumbashi-DRC, *Albizia adianthifolia* (Schum.) W. Wight (Fabaceae) and *Pericopsis angolensis* (Baker) Meeuwen (Fabaceae) [12], by examining sexual behavior and hormonal variation. The study also evaluated the antioxidant activity of the same extracts before establishing a correlation between the antioxidant activity and the aphrodisiac index.

The various observations recorded during this study reveal that the administration of doses of 200 mg.kg⁻¹ bw of extract of *A. adianthifolia* modifies the physical parameters, indicators of libido on *Cavia porcellus* and that these effects appear to be dose dependent. "Thus", we observed a significant variation between oral administration of the doses of 200 and 250 mg.kg⁻¹ bw ($p < 0.01$). The performances induced by the oral administration of the extracts of *A. adianthifolia* consist of a decrease in time of RT, IT, PEI and an increase of ND, NI and NE frequencies, as during the oral administration of Sildenafil at 10 mg. kg⁻¹ bw. Furthermore, the oral administration of *P. angolensis* shows a significant difference between the doses of 200 and 250 mg. kg⁻¹ bw, however, these extracts behave in the same way as sildenafil. *A. adianthifolia* at 200 mg. kg⁻¹ bw and, *P. angolensis* at 250 mg. kg⁻¹ bw exhibit very similar aphrodisiac activity. On the other hand, the methanolic extracts exhibit a lower activity ($p < 0.01$) than their aqueous extracts for *A. adianthifolia* unlike *P. angolensis* for which, no significant difference is observed. These data make it possible to understand not only why traditional medicine uses *A. adianthifolia* at doses equivalent to 200 mg. kg⁻¹ bw and, *P. angolensis* at doses of 250 mg. kg⁻¹ bw but also the need to prefer the aqueous extract of the species *A. adianthifolia* to the detriment of alcoholic extract [12]. On the other hand, for *P. angolensis*, these results suggest that the alcoholic extract is also used in the same way as the aqueous extract insofar as they could produce similar results from the point of view of physical activity the methanolic extracts exhibit a lower activity ($p < 0.01$) than their aqueous extracts for *A. adianthifolia* unlike *P. angolensis* for which, no significant difference is observed. The methanolic extracts exhibit a lower activity ($p < 0.01$) than their aqueous extracts for *A. adianthifolia* unlike *P. angolensis* for which, no significant difference is observed.

The literature reports that TG levels vary inversely with cholesterol and testosterone levels and serum testosterone levels increase during sexual intercourse [13–16] as a result of an olfactory stimuli leading to the activation of the hypothalamic-pituitary axis [17]. These variations were observed in the same order during this study. In addition, it has been reported that testosterone acts on the periphery by facilitating nitrergic neurotransmission, accentuating the release of nitric oxide in the cavernous bodies thus contributing to penile erection [18,19]. For this purpose, it constitutes a biomarker of aphrodisiac activity as observed during this study in the groups treated with sildenafil and

the test groups (Figure 1). It is in this context that we calculated the aphrodisiac index as the ratio between the testosterone levels of the trial group and sildenafil (Figure 1c); moreover, the increase in serum cholesterol levels may be justified by the increase in serum testosterone levels since cholesterol constitutes a synthetic precursor [20] and that it increases *in vitro* in proportion to the levels of testosterone [21,22]. Some studies have even found a positive correlation between increased serum cholesterol levels and increased erectile function [23,24]. It could within certain limits constitute another biomarker of aphrodisiac activity in the absence of the possibility of resorting to conventional biomarkers.

A positive correlation was observed between antioxidant activity and testosterone level (Figure 2c). Previous work [25,26] reported that antioxidant plants increase libido and at the same time it has been argued that flavonoids and other polyphenols have an aphrodisiac property due to their androgenic properties [27] and estrogenic [28] as well as their inhibitory action of phosphodiesterases [29]. These observations can justify the positive correlation observed during this study between aphrodisiac activity and the level of testosterone which would be due to the presence of the natural polyphenols present in the various extracts examined.

Among the compounds identified or isolated in *A. adianthifolia* [6], some are also found in other species known to be aphrodisiacs. This is notably the case with Aurantiamide acetate isolated from *Hybanthus enneaspermus* (L.) F. Muell, Violaceae [30], Octadecanoic acid identified from *Sida cordata* (Burm.f.) Borss.Waalk., Malvaceae [31] and Prosapogenin isolated from *Tribulus terrestris* L., Zygophyllaceae [32]. However, no study has so far evaluated the contribution of these compounds in the expression of the aphrodisiac activity of the aforementioned plants. It would be desirable for a further study to do so. For *Pericopsis angolensis*, no sophisticated phytochemical studies are reported. However, preliminary phytochemical screens performed on the species revealed flavonoids, saponins and tannins [8]. Of all these phytochemical groups, only the tannins, through protodioscin and protogracillin, isolated from *Tribulus terrestris* L., Zygophyllaceae are responsible of the aphrodisiac activity [33]. It would be desirable to evaluate the activity of the major groups of secondary metabolites and to determine among them the phytochemical groups with aphrodisiac property.

5. Conclusion

A. adianthifolia and *P. angolensis* at 200 mg. kg⁻¹ bw improve the physical parameters of libido as well as the testosterone level and to this end exhibit an aphrodisiac effect similar to their antioxidant activity. These results constitute a scientific basis likely to justify the use of these two plant species as aphrodisiacs in traditional medicine of Lubumbashi. Further studies should focus on the isolation of these antioxidant and aphrodisiac compounds preferably for *P. angolensis* whose phytochemistry is not reported.

Compliance with ethical standards

Acknowledgments

The authors thank the traditional healers who agreed to share their knowledge as well as Ir Dédé Mbungu of Kipopo herbarium for his collaboration in the scientific identification of plants.

Disclosure of conflict of interest

All the authors declare that they have no conflict of interest.

Statement of ethical approval

The project proposal and procedures were reviewed and approved by the Department of Pharmacology in the faculty of Pharmaceutical Sciences from the University of Lubumbashi, DR Congo (UNILU/FSP/DPCOL/PT/02/2017).

Authors' contributions

Bashige chiribagula V designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Alombong Alonie G, managed the execution of the protocol and the collection of data. Many Mboni H, Bakari Amuri S and Okusa Ndjolo P managed analyzes of the study and the literature searches. All authors read and approved the final manuscript.

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