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In vitro screening of the potential of six Cameroonian medicinal plants on male reproductive biomarkers

Paulin Teko Keumedjio, Sara N. Edjenguèlè Béboy*, Paul Fewou Moundipa

Abstract

Background: Male infertility and erectile dysfunction are the main conditions associated with male reproductive function disorders. Testosterone is the main hormone that regulates male reproductive function, while nitric oxide is a potent vasodilator involved in the erectile process.

Methods: This study was conducted to evaluate *in vitro* the potential of the aqueous extract of six Cameroonian medicinal plants traditionally used for male reproductive function: *Palisota ambigua*, *Terminalia superba*, *Rauvolfia macrophylla*, *Pycnanthus angolensis*, *Pausynistalia yohimbe,* and *Shumanniophyton magnificum*.

Results: Their effect on testosterone and nitric oxide production was evaluated using rat interstitial and macrophage cells, respectively. Then, the antioxidant properties, flavonoids, and total phenolic content were assessed. The phytochemical screening was also performed.

Conclusion: Each plant extract significantly increased ($p<0.05$) testosterone production. At 100 µg/mL, the extract of *Schumanniophyton magnificum* showed the highest level (3.730 ng/mL). Each plant extract significantly increased ($p<0.05$) the production of nitric oxide. The aqueous extract of *Rauvolfia macrophylla* showed the highest level of nitric oxide with an EC_{50} = 8.554 µg/mL. For antioxidant activities, *Terminalia superba* showed the highest antiradical activity (SC_{50} = 4.796 µg/mL), the highest reducing power (OD = 2.235 at 700 nm), and the highest total antioxidant capacity (276.035 mgEAA/g of plant extract) and while *Pausynistalia yohimbe,* showed the highest inhibition on lipid peroxidation (15.170 µg/mL). *Terminalia superba* also showed the highest flavonoid (13.419 mgEQ/g) and total phenolic (300.813 mgEGA/g) content. The phytochemical screening of the plant extracts showed many active secondary metabolites such as alkaloids, phenols, flavonoids, saponins, and glycosides. All the extracts showed androgenic effects and antioxidant activities without significant cytotoxicity, thus supporting the use of these medicinal plants by traditional healers for managing male infertility.

Keywords: Antioxidant properties; aqueous extracts; medicinal plants; nitric oxide; testosterone.

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Background

During life, the reproductive system is exposed to many conditions that can disrupt its proper functioning. This can include certain dietary habits, the use of some drugs, and environmental conditions such as exposure to gonadotoxins [1]. These different conditions can disrupt male reproductive function by three main mechanisms: Firstly, they generate a state of oxidative stress in germ cells and all other cells involved in fertility and erectile process; secondly, they disrupt the biosynthesis, availability, and action of androgens, the most important of which is testosterone. Finally, they can hinder reproductive tracts. Infertility is generally defined as the inability of a couple to achieve a pregnancy after a year or more of regular unprotected sexual intercourse [2]. This problem affects about 15% of couples of childbearing age [3] and the male factor contributes to 50% of the cases [4]. Erectile dysfunction (ED) is the recurrent inability to obtain and/or maintain an erection sufficient for satisfactory sexual activity [5]. ED is a common sexual dysfunction that can lead to male infertility [6]. Nitric oxide (NO) is the main mediator released during the erectile process [7]. The overall prevalence of ED ranges from 3 to 76.5% and increases with age and the existence of risk factors [8, 9]. The drugs used to treat these two diseases are not always effective, some of them have harmful side effects, such as Sildenafil which causes prolonged erections. However, medicinal plants have been found to be a natural source of compounds with various therapeutic properties which most of the time are triggered by the phytochemicals that they contain. Most of those phytochemicals are antioxidants such as phenols, flavonoids, or flavonols. Antioxidants play a significant role as they can prevent DNA fragmentation, improve semen quality in smokers and stimulate spermatozoa production [10].

Thus, the '*Baka*' Pygmies who live in the forest of the Southern region of Cameroon do not generally use modern medicine, but successfully use of medicinal preparations to solve all their health problems. These Pygmies use extracts of *P. ambigua*, *T. superba,* and *R. macrophylla* to treat male infertility; while extracts of *P. angolensis*, *P. yohimbe,* and *S. magnificum* are used to improve erectile dysfunction. However, no scientific evidence had been made to support the use of these plants for the treatment of male infertility in traditional medicine. Then, the aim of this work was to assess the *in vitro* effects of the aqueous extracts of these plants on male reproductive function biomarkers. In addition, the antioxidant properties as well as the major groups of secondary metabolites of the plant extracts were determined.

Methods

Experimental animals

The animals used for this work were provided by the animal house of the Laboratory of Pharmacology and Toxicology of the University of Yaoundé I. Male and female rats *Wistar* albino aged 3 months were used to prepare testicular homogenates and to isolate macrophage and testicular interstitial cells.

Harvest of plants and preparation of aqueous extracts

Fresh leaves of *P. ambigua*, stem bark of *T. superba*, *R. macrophylla*, and *P. yohimbe*, stem wood of *P. angolensis,* and roots of *S. magnificum* were harvested in March 2019 in the forest of '*Baka*' Pygmies. This forest is located in Assok village, Subdivision of Djoum, Division of Dja-et-Lobo, and Southern region of Cameroon. All plants were identified at the Cameroon National Herbarium under voucher numbers: 29555; 55546; 43413; 31619; 2359 and 52761, respectively. The harvested parts were cleaned, dried, and ground into powder which was used to prepare a 10 % (m/v) aqueous extract for each plant by decoction.

Effect of extracts on testosterone production by primary interstitial cells

After the rats were killed, testes were removed aseptically, minced, and incubated thrice in 20 mL of DMEM/Ham F12 culture medium containing 2 mg/mL collagenase type I; 0.2 mg/mL STI and 20 µg/mL DNase. Then, testicular interstitial cells were isolated according to the method described by Moundipa et al. [11]. Thus, 500 µL of DMEM/Ham F12 culture medium containing 500,000 cells were incubated with 50 µL of plant extract (0.1; 1; 10; 100 and 1000 µg/mL) for 4 hours at 32°C and in the presence of 5% CO_2. Human chorionic gonadotropin (hCG) at 25 UI and 50 UI was used as a positive control. After incubation, all tubes were centrifuged at 180×g, 18 °C for 10 min. The supernatant was frozen for testosterone determination by competitive ELISA testosterone assay using the Calbiotech Testosterone ELISA kit (Cat. No: TE373S) according to the manufacturer's protocol. The cell pellet was used for the MTT assay [12] to determine the viability of cells after exposure to different concentrations of plant extracts. The percentage of cell viability was calculated by the following formula.

$$\% V = \frac{OD_s}{OD_c} \times 100$$

% V: Percentage of cell viability
OD_s: Optical density of sample
OD_c: Optical density of control

Effect of extracts on nitric oxide production by primary macrophages

After an intraperitoneal injection of 2 mL of 0.1 M saline buffer (pH 7.4) containing 2%, rat primary macrophages were elicited and isolated as described by Bansal [13]. Thus 150 µL of DMEM culture medium containing 10,000 macrophages were incubated in a microtiter plate, in the presence or absence of 50 µL of plant extract (0.1; 1; 10; and 100 µg/mL) [14]. The mixture was incubated at 37 °C for 4 h. After incubation, the supernatant was recovered for the nitric oxide assay using Griess reagent according to the method described by Grisham et al [15]. The cell pellet was used for the MTT assay to determine the viability of the cells after exposure to different concentrations of plant extracts.

Determination of antioxidant properties

DPPH (2, 2-Diphenyl-1-picryl hydrazine) free radical scavenging test

This test was used to determine the anti-radical power and was carried out according to the method described by Zengin et al [16]. Thus, 3100 µL of a methanolic solution of DPPH (40 µg /ml) was added to 50 µL of plant extract (0.1; 1; 10 and 100 µg/mL) or 50 µL of water (control tubes) or 50 µL of ascorbic acid. After incubation at room temperature in the dark for 30 minutes, the optical density

was measured at 517 nm. The scavenging percentages were calculated using the formula below the scavenging concentration fifty (SC_{50}) were determined, then the effective concentration fifty (EC_{50}) and the antiradical powers (AP) were respectively calculated by the formula below.

$$\% SC = \frac{OD_c - OD_s}{OD_c} \times 100$$

$$EC_{50} = \frac{SC_{50}}{C} \times 100$$

$$AP = \frac{1}{EC_{50}} \times 100$$

% SC: Scavenging percentage
OD_s: Optical density of sample
OD_c: Optical density of control
C: Concentration of methanolic solution of DPPH

Ferric Reducing Antioxidant Power (FRAP) test

This test was used to determine the reducing power of plant extracts. It was carried out according to the method described by Pulido et al [17]. Five hundred microliters of phosphate buffer (0.2 M; pH 6.75), 200 µL of plant extract (0.1; 1; 10 and 100 µg/mL) or distilled water (blank), and 500 µL of a 1% aqueous solution of potassium ferricyanide were mixed. Then, the mixture was incubated at 50 °C for 20 minutes. After incubation, 500 µL of a 10 % trichloroacetic acid (TCA) solution was added to each tube and the whole set of tubes was centrifuged at 45×g and 4 °C for 10 minutes. After centrifugation, 1 mL of supernatant was added to 1 mL of distilled water and 200 µL of 0.1 % aqueous ferric chloride solution. The tubes were homogenized, and their optical densities were measured at 700 nm.

Determination of total antioxidant capacity

The total antioxidant capacity of the different plant extracts was determined by the phosphomolybdenum method described by Prieto et al [18]. In test tubes containing 300 µL of plant extract, 1000 µL of each of the following reagents were introduced: 0.6 M HCl; 28 mM $NaPO_4$; 4 mM $H_{32}MO_7N_6O_{28}$ (Ammonium molybdate). In the control tubes, 300 µL of distilled water was added in place of the extract. All tubes were homogenized and incubated at 90 °C for 90 minutes. Then, the optical densities were measured at 695 nm.

Inhibition of lipid peroxidation

The ability of the extracts to inhibit lipid peroxidation was also assessed, and for this purpose, a testicular homogenate was prepared by the modified method of Akomolafe et al [19]. Thus, adult male rats, weighing between 200 and 250 g, were sacrificed by cervical dislocation, and testes were carefully removed and used to prepare a 10 % (w/v) homogenate in a 1.15 % KCL solution. The homogenate was then centrifuged at 1260×g, 4 °C for 15 min, and the supernatant was used for the evaluation of lipid peroxidation according to the method described by Su et al [20]. Thus, 25 µL of plant extract or distilled water (control tubes) or 25 µL of 15 % KCl (blank tubes), 500 µL of 10 % testicular homogenate, 25 µL of 31.5 mM $FeCl_2$ and 25 µL of 31.5 mM H_2O_2 were successively introduced in the test tubes which were incubated at 37 °C for 1h. After incubation, 500 µL of 15 % TCA and 500 µL of 0.67 % TBA were added to all tubes, and 500 µL of homogenate was added to the blank tubes. The tubes were shaken and then boiled in a water bath for 15 minutes. After cooling, all tubes were centrifuged for 15 min at 1620×g, 4 °C. The optical density was read at 532 nm. The percentages of lipid peroxidation inhibition were calculated using the formula below and the percentages of inhibition fifty were determined.

$$\% I = \frac{OD_c - OD_s}{OD_c} \times 100$$

% I: Percentage of inhibition
OD_s: Optical density of sample
OD_c: Optical density of control

Phytochemical Screening and quantification of total polyphenols and flavonoids

Phytochemical screening

Phytochemical screening was performed on secondary metabolites that may be responsible for the activities of the plant extracts. The plant extracts were prepared at 1 mg/mL and the following phytochemical tests were performed. Test for phenolic compounds, flavonoids, tannins, and terpenoids were done according to the method described by Prabhavathi et al. [21]. Test for coumarins, anthocyans, and saponins were done according to the method described by Savithramma et al. [22]. Test for alcaloïds (test of Mayer) and glycosides; Test for steroids (test of Libermann-Burchard**)** were carried out according to the methods described by Trease and Evans [23] and by Finar [24], respectively.

Determination of total phenolic content

Total phenolic content was assessed according to the method of phosphomolybden previously described by Nantia et al. [25], based on the reduction of the Folin-Ciocalteau reagent**.** Two thousand microliters of distilled water, 100 µL of plant extract (at 100 µg/mL), and 200 µL of 2N Folin-Ciocalteau reagent were mixed. The mixture was incubated at room temperature for 3 minutes. After this incubation, 1000 µL of 20 % Sodium Carbonate was added to each tube and the mixture was incubated at room temperature in the dark for 1 hour. Then, the optical density of each tube was measured at 765 nm using a spectrophotometer. The standard curve was done with gallic acid at different concentrations (0; 0.60; 1.2; 1.8; 2.4; 3 µg/mL). The results were expressed as milligram Equivalent of Gallic Acid per gram of plant extract (mgEGA/g).

Determination of flavonoids content

Total flavonoid content was assessed according to the method previously described by Nantia et al. [25]. Five hundred microliters of plant extract (at 100 µg/mL) and 500 µL of 2 % aluminum trichloride were mixed and incubated at room temperature for 1 hour. Then, the optical density was measured at 430 nm using a spectrophotometer. The standard curve was done with Quercetin at different concentrations. The results were expressed as milligram Equivalent of Quercetin per gram of plant extract (mgEQ/g).

Ethical considerations

The study received the agreement of the Cameroon Institutional National Ethics Committee and was conducted according to the principles and procedures of the European Union on Animal Care (reference FWA-IRD 0001954).

Statistical analysis

All experiments were carried out in triplicate, results were expressed as mean ± standard deviation. Graph Pad Prism software (version 8.0.2) was used for all analyses. Means were compared by ordinary one-way ANOVA followed by the multiple comparison tests of Tukey and Dunnett. The threshold of significance was set at $p< 0.05$.

Results

Effect of plant extracts on testosterone production by primary interstitial cells

Cell viability of primary interstitial cells treated with plant extracts

Cells were incubated with different concentrations of plant extract afterward their viability was assayed, and the results are present in Figure 1. Cell viability is higher than 90 % for each plant extract, implying no adverse effect on cell survival. This result shows that even up to 1000 µg/mL, these plant extracts did not exhibit cytotoxic effects against primary interstitial cells.

Production of testosterone by stimulated primary interstitial cells

The quantity of testosterone (ng/mL) produced by primary interstitial cells after stimulation with different concentrations of plant extracts is shown in Figure 2. Each plant extract significantly ($p< 0.05$) increased in a dose-dependent manner the production of testosterone. *T. superba* and *S. magnificum* showed activities (at 100 µg/mL) close to that of the standard (hCG) at 50 UI/mL. Among all the plant extracts, *S. magnificum* showed the highest activity.

Effect of plant extracts on nitric oxide production by primary macrophages

Cell viability of primary macrophages treated with plant extracts

The viability of primary macrophages treated with different concentrations of plant extracts is shown in Figure 3. With percentages of viability higher than 90 %, the plant extract did not exhibit any harmful effect on macrophage viability.

Production of nitric oxide by stimulated primary macrophages

Each plant significantly ($p<0.05$) increased in a dose-dependent manner the production of nitric oxide (Figure 4). *R. macrophylla* showed the highest activity (EC_{50} = 8.55 ± 0.24 µg/mL). The EC_{50} values are presented in Table 1.

Antioxidant assays

Antiradical activity

The antiradical activity of plant extracts was evaluated through the $DPPH^{\bullet}$ scavenging assay, the results are shown in Figure 5. This figure shows that the extracts of *R. macrophylla*, *T. superba* and *P. yohimbe* presented a percentage of scavenging greater than 50 % within the range of concentration used. Their scavenging concentrations fifty, effective concentrations fifty, and antiradical power are presented in Table 2. *T. superba* presented antiradical activity which is comparable to that of ascorbic acid used as a standard antioxidant.

Ferric Reducing Antioxidant Power (FRAP)

The reducing power of plant extracts was evaluated through the ferric reducing antioxidant power and the results are presented in Figure 6. At 100 µg/mL, only the extract of *T. superba* showed a reducing power (2.235 ± 0.015) similar to the reducing power of the standard (2.253 ± 0.006). The extract of *P. yohimbe* also showed the ability to reduce oxidants at the highest concentration (100 µg/mL).

Total antioxidant capacity (TAC)

The results of total antioxidant capacity expressed in terms of milligram equivalent of ascorbic acid per gram of plant extract (mgEAA/g) are presented in Figure 7. *T. superba* presented the highest total antioxidant capacity (276.035 ± 1.153 mgEAA/g of plant extract) followed by *P. yohimbe* (175.067 ± 2.081 mgEAA/g of plant extract).

Inhibition of lipid peroxidation

The capacity of plant extracts to inhibit lipid peroxidation was evaluated on rat testicular homogenate (Figure 8). *P. yohimbe*, *T. superba*, *R. macrophylla* and *P. angolensis* showed inhibition percentages greater than 50 % at the range of concentrations used. Their effective concentrations fifty showed the highest activities (Table 3).

Phytochemical screening and total phenolic and flavonoids content

Phytochemical screening

The phytochemical screening of the plant extracts showed many active secondary metabolites such as alkaloids, phenols, flavonoids, saponins, and glycosides (Table 4). *T. superba* especially contained high amounts of components associated with antioxidant activity such as phenols.

Total phenolic and flavonoids contents

Results obtained from this study revealed that all the plant extracts presented considerable levels of phenolic and flavonoid contents. *T. superba* also showed the highest flavonoids and total phenolic contents.

Discussion

The main aim of the present study was to evaluate the *in vitro* effect of aqueous extracts of six medicinal plants on the production of two markers using appropriate cells, primary rat interstitial cells for testosterone and macrophages for nitric oxide. The latter is a powerful vasodilator and a major physiological stimulus for the relaxation of the penile vasculature and trabecular smooth muscle which are necessary for penile erection [1]. Testosterone is the main androgen produced by the Leydig cells. which controls both male fertility and erectile function [2, 26]. Compared to the negative control, results showed that all these plant extracts significantly increased the production of both testosterone and nitric oxide, supporting their use by the *Baka* Pygmies of Southern Cameroon

to improve male reproductive dysfunction. Results showed that *P. yohimbe* is a potent aphrodisiac plant [27]. The obtained results of this study sustained that this activity should be due to the α_2-adrenoreceptors antagonistic activity [7]. Since there is a positive correlation between *in vitro* production of nitric oxide by macrophages and *in vivo* penile erection in rats [14], *R. macrophylla* could be a good inducer for penile erection. *S. magnificum* extract exhibited the greatest ability to induce testosterone production and strongly increased nitric oxide production. Some previous studies showed that the aqueous extract of the stem bark of *S. magnificum* can improve sexual maturation and fertility in immature female rats [28], suggesting that this plant extract could act on steroidogenesis and nitric oxide synthesis. *T. superba* slightly increased testosterone production and exhibited the greatest antioxidant activity.

To explain the biological effects of the plant extracts, phytochemical screening was performed to know the groups of secondary metabolites which may be responsible for the improvement of testosterone and nitric oxide production and also for antioxidant activities. The results revealed the presence of numerous secondary metabolites, thereby justifying the activities of these medicinal plants. The extract of *R. macrophylla* presented the highest content in saponins as well as the highest capacity to induce nitric oxide production. Indeed it has been shown that saponins isolated from *Panax ginseng*, were able to induce nitric oxide production through the activation of endothelial nitric oxide synthase [29]. However, despite that the extract of *S. magnificum* exhibited the greatest ability to induce testosterone production, this extract did not reveal the presence of particular class of secondary metabolites in significant amounts suggesting that the secondary metabolites responsible for this activity were not detected by the phytochemical screening assays.

Oxidative stress is a process associated with the pathophysiology of male infertility which can lead to cell membrane damage and DNA fragmentation [30]. The antioxidant properties of these plant extracts were then evaluated using four antioxidant assays. According to the results, the extract of *T. superba* presented the best antioxidant activity, followed by the extracts of *R. macrophylla*, *P. yohimbe,* and *P. angolensis*. These results could be justified by their total polyphenol and flavonoid contents. *In vivo* antioxidant activity of *T. superba* has been previously proven [31]. The results of antioxidant activity showed that, in addition to their ability to stimulate male reproductive function, these plant extracts also can protect cells and tissues from oxidative stress.

The six plant extracts used in this study exhibited different activities. Some presented the ability to improve male fertility while others showed the capacity to improve penile erection or to protect the reproductive organs against damage due to reactive oxygen species. Sometimes traditional healers use a mixture of these plants to get additive effects but unfortunately for this study, the different extracts were not mixed. It should be beneficial to study the combination of these plants to maximize their effects. Moreover, *in vivo* prospective studies should be done with extracts that showed significant improvement in the male reproductive function to confirm the present study.

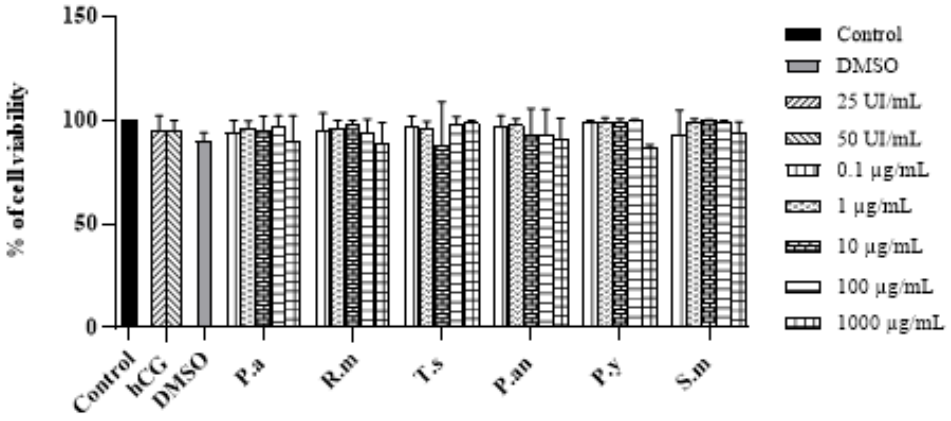

Figure 1. Percentage of viability of primary interstitial cells after exposition to plant extracts.
hCG: Human Chionic Gonatropine, DMSO: Dimethylsufoxide, Pa: *Palisota ambigua,* Rm: *Rauvolfia macrophylla,* Ts: *Terminalia superba,* Pan: *Pycnanthus angolensis*, Py: *Pausynistalia yohimbe,* Sm: *Shumaniophyton magnificum*

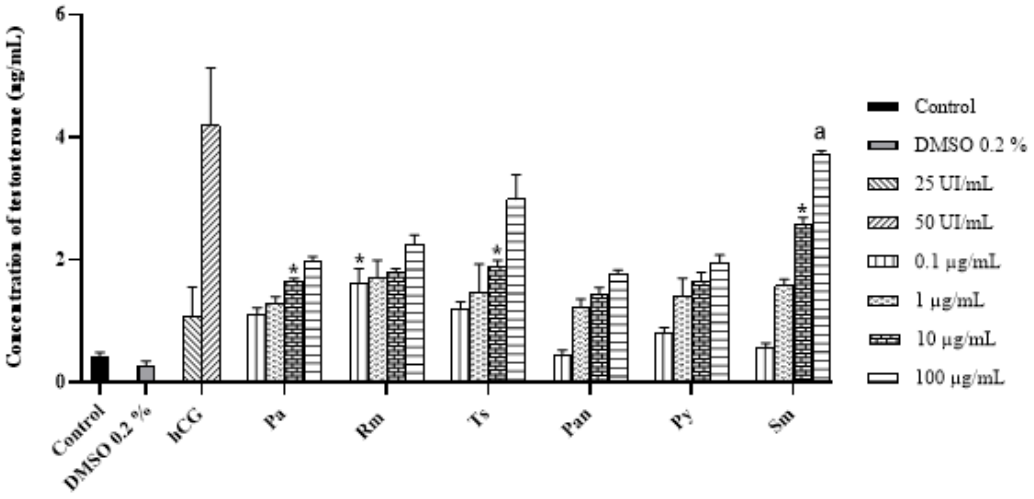

Figure 2. Concentration of testosterone produced by stimulated interstitial cells.
*: significant difference compared to hCG 25 UI/mL (P<0.05), a: no significant difference compared to hCG 50 UI/mL
hCG: Human Chionic Gonatropine, DMSO: Dimethylsufoxide, Pa: *Palisota ambigua,* Rm: *Rauvolfia macrophylla,* Ts: *Terminalia superba,* Pan: *Pycnanthus angolensis*, Py: *Pausynistalia yohimbe,* Sm: *Shumaniophyton magnificum*

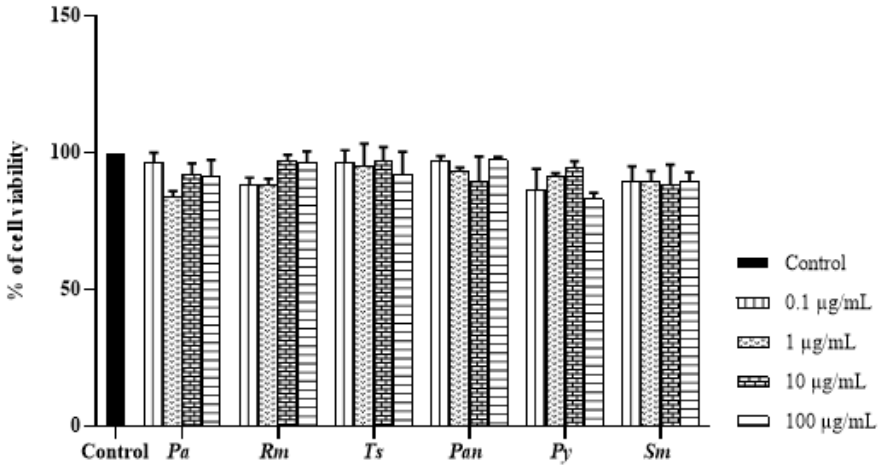

Figure 3. Viability of macrophages treated with different concentrations of plant extracts.
Pa: *Palisota ambigua,* Rm: *Rauvolfia macrophylla,* Ts: *Terminalia superba,* Pan: *Pycnanthus angolensis*, Py: *Pausynistalia yohimbe,* Sm: *Shumaniophyton magnificum*

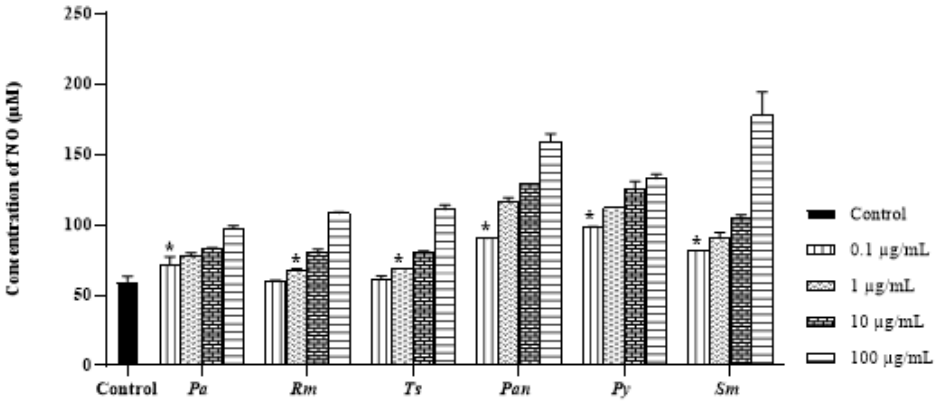

Figure 4. Concentrations of nitric oxide produced by stimulated macrophages.
Pa: *Palisota ambigua,* Rm: *Rauvolfia macrophylla,* Ts: *Terminalia superba,* Pan: *Pycnanthus angolensis*, Py: *Pausynistalia yohimbe,* Sm: *Shumaniophyton magnificum.* *: significant difference (p<0.05) compared to control

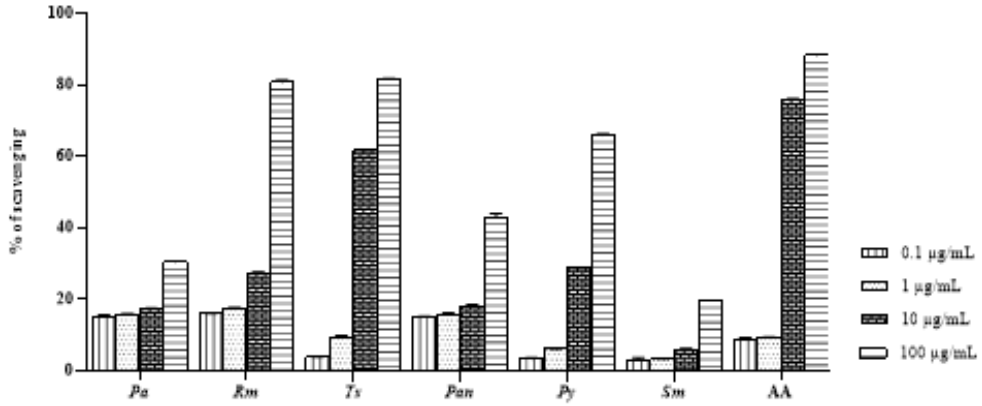

Figure 5. DPPH radical scavenging percentages of plant extracts.
Pa: *Palisota ambigua,* Rm: *Rauvolfia macrophylla,* Ts: *Terminalia superba,* Pan: *Pycnanthus angolensis*, Py: *Pausynistalia yohimbe,* Sm: *Shumaniophyton magnificum.* AA: Ascorbic acid

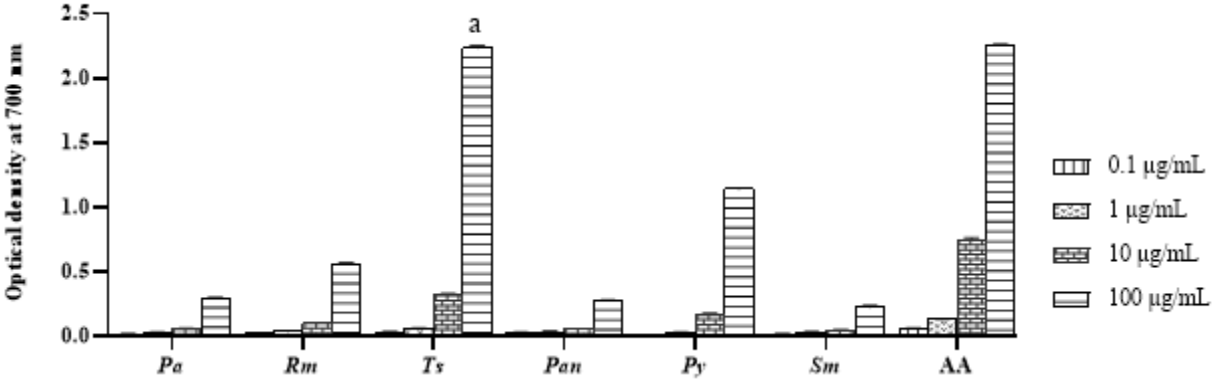

Figure 6. Ferric reducing antioxidant power of plant extracts.
Pa: *Palisota ambigua,* Rm: *Rauvolfia macrophylla,* Ts: *Terminalia superba,* Pan: *Pycnanthus angolensis*, Py: *Pausynistalia yohimbe,* Sm: *Shumaniophyton magnificum.* AA: Ascorbic acid. a: no significant difference ($p<0.05$) compared to control ascorbic acid.

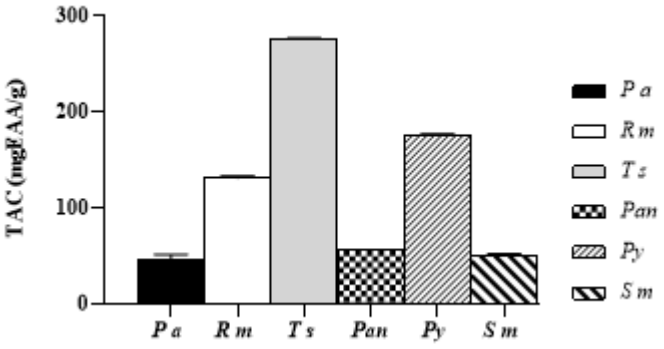

Figure 7. Total antioxidant capacity of plant extracts.
Pa: *Palisota ambigua,* Rm: *Rauvolfia macrophylla,* Ts: *Terminalia superba,* Pan: *Pycnanthus angolensis*, Py: *Pausynistalia yohimbe,* Sm: *Shumaniophyton magnificum.*

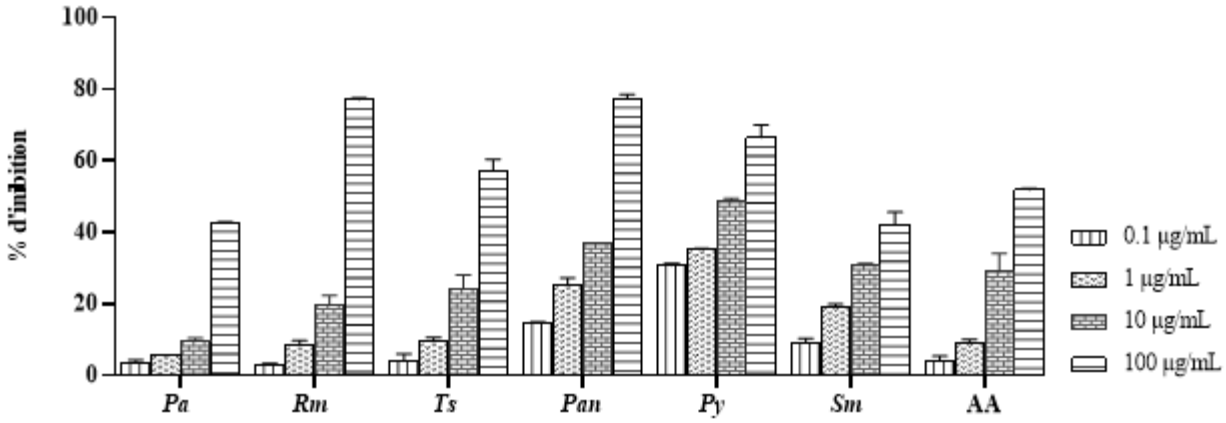

Figure 8. Lipid peroxidation inhibition percentages of plant extracts.
Pa: *Palisota ambigua,* Rm: *Rauvolfia macrophylla,* Ts: *Terminalia superba,* Pan: *Pycnanthus angolensis*, Py: *Pausynistalia yohimbe,* Sm: *Shumaniophyton magnificum.* AA: Ascorbic acid.

Table 1. Effective concentration 50 (EC_{50}) for nitric oxide production.

Plant extracts	Pa	Rm	Ts	Pan	Py	Sm
EC_{50} (µg/mL)	ND	8.55± 0.24[a]	ND	37.94 ± 0.60[b]	28.17± 0.87[c]	21.83± 1.65[d]

ND: not determined. a, b, c and d are significantly ($p<0.05$) different from each other. Pa: *Palisota ambigua, Rm: Rauvolfia macrophylla, Ts: Terminalia superba, Pan: Pycnanthus angolensis, Py: Pausynistalia yohimbe, Sm: Shumanniophyton magnificum*

Table 2. Antiradical parameters of plants extracts.

Plant extracts	SC_{50} (µg/mL)	EC_{50} (µg of plant extracts /mol DPPH) × 10^9	AP (mol of DPPH/µg of plant extract) × 10^{-9}
Rm	109.33 ± 4.13	1.095 ± 0.041	0.91 ± 0.03
Ts	4.79 ± 0.04[a]	0.048 ± 0.0004[a]	20.81 ± 0.18[a]
Py	18.86 ± 0.41	0.188 ±0.004	5.29 ± 0.11
Pa	ND	ND	ND
Pan	ND	ND	ND
Sm	ND	ND	ND
AA	4.03 ± 0.03	0.040± 0.0003	24.74 ± 0.21

ND: not determined, a: No significant difference ($p>0.05$) compared to ascorbic acid (standard). Pa: *Palisota ambigua, Rm: Rauvolfia macrophylla, Ts: Terminalia superba, Pan: Pycnanthus angolensis, Py: Pausynistalia yohimbe, Sm: Shumanniophyton magnificum,* AA: Ascorbic acid

Table 3. Effective concentration 50 for lipid peroxidation inhibition assay.

Plant extracts	EC_{50} (µg/mL)
Rm	51.46 ± 2.34
Ts	18.33 ± 1.32[a]
Py	15.17 ± 0.38[a]
Pan	29.32 ± 0.57
Pa	ND
Sm	ND
AA	15.43 ± 0.40

a: No significant difference ($p>0.05$) compared to ascorbic acid. Pa: *Palisota ambigua, Rm: Rauvolfia macrophylla, Ts: Terminalia superba, Pan: Pycnanthus angolensis, Py: Pausynistalia yohimbe, Sm: Shumanniophyton magnificum,* AA: Ascorbic acid

Table 4. Major groups of secondary metabolites of plant extracts.

Phytochemical classes	Pa	Rm	Ts	Pan	Py	Sm
Alkaloids	+	++++	+	+	+++	+
Phenols	+	+	++++	+	++	+
Flavonoids	++	+++	++++	++	+	+
Tannins	-	-	++	-	-	-
Coumarins	-	-	++++	-	-	-
Anthocyans	-	-	-	-	++	-
Terpenoids	-	-	-	+	-	-
Steroids	-	-	-	+	-	-
Saponins	-	+++	+	+	+	-
Glycosides	-	++	++++	++	-	+

The sign (+) indicates the presence of the compound and the sign (-) indicate the absence of the compound.
Pa: *Palisota ambigua*, Rm: *Rauvolfia macrophylla*, Ts: *Terminalia superba*, Py: *Pycnanthus angolensis*, Py: *Pausynistalia yohimbe*, Sm: *Shumanniophyton magnificum*

Table 5. Flavonoids and total phenolic contents of plant extracts.

Plant extract	Total phenolic content (mgEGA/g of plant extract)	Flavonoids content (mgEQ/g of plant extract)
Pa	25.55 ± 4.02	3.63 ± 0.22
Rm	110.33 ± 8.04	6.19 ± 0.07
Ts	300.81 ± 10.05	13.41 ± 0.15
Pan	20.90 ± 6.03	5.67 ± 0.30
Py	101.04 ± 6.03	9.48 ± 0.00
Sm	6.96 ± 1.05	6.75 ± 0.13

Pa: *Palisota ambigua, Rm: Rauvolfia macrophylla, Ts: Terminalia superba, Pan: Pycnanthus angolensis, Py: Pausynistalia yohimbe, Sm: Shumanniophyton magnificum,* AA: Ascorbic acid

Conclusion

The results of this study showed that only the aqueous extracts of *Terminalia superba*, *Rauvolfia macrophylla*, *Pycnanthus angolensis*, and *Schumanniophyton magnificum* could improve the functioning of the male reproductive system by increasing the production of testosterone and nitric oxide and preventing oxidative stress. According to the best results obtained by the aqueous extract of *Schumanniophyton magnificum*, *Terminalia superba,* and *Rauvolfia macrophylla*, these plants could be considered as a potential source of androgens, of antioxidants and vasorelaxants, respectively.

Abbreviations

AA: Ascorbic acid
ANOVA: Analysis of variances
AP: Antiradical power
DMEM: Dulbecco's Modified Eagles Medium
DMSO: Dimethylsulfoxid
DPPH: 2, 2-diphenyl-1-picrylhydrazyle
EC: Efficient concentration
ED: Erectile dysfunction
ELISA: Enzyme-Linked Immunosorbent Assay
hCG: human chorionic gonadotropin
mgEAA: milligram equivalent of ascorbic acid
mgEGA: milligram equivalent of gallic acid
mgEQ: milligram equivalent of quercetin
MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyl tetrazolium
NO: Nitric oxide
ND: Not determined
OD: Optical density
Pa: *Palisota ambigua*
Pan: *Pycnanthus angolensis*
Py: *Pausynistalia yohimbe*
Rm: *Rauvolfia macrophylla*
SC_{50}: Scavenging percentage fifty
Sm: *Shumaniophyton magnificum*
Ts: *Terminalia superba*
w/v: weight/volume

Authors' Contribution

PTK carried out the experiments and analyzed the data. PTK and SNEB designed the study and wrote the manuscript. PFM supervised the study. All the authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest

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