Original Research Article

Antioxidant properties and effects on nitric oxide release of alcoholic extracts of some selected Cameroonian medicinal plants

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ABSTRACT

Aims: In the Southern region of Cameroon, some medicinal plants are used for the treatment of the male reproductive system disorders; including *Palisotaambigua*, *Rauvolfia macrophylla*, *Terminalia superba*, *Pycnanthusangolensis*, *Pausynistalia yohimbe* and *Schumanniophytonmagnificum*. In order to support the medicinal use of these plants, their phytochemical substances and antioxidant properties as well as their effects on nitric oxide release were investigated.

Study design: Experimental design.

Place and Duration of Study: Laboratory ofPharmacologyandToxicology,DepartmentofBiochemistry,UniversityofYaoundé1(Cameroon),betweenMarch2019andFebruary 2021.

Methodology: Rat peritoneal macrophages were isolated and used to assess in vitro effects of plant extracts on nitric oxide release. Then, antioxidant properties of alcoholic extracts of the plants were screened through DPPH test, total antioxidant capacity test and ferric reducing power test and lipid peroxidation inhibition test. Phytochemical substances, total phenol and flavonoid contents were also investigated.

Results: Results showed that only the extracts of *T. superba* and *P. yohimbe* were found to be strong DPPH free radical scavengers with low EC_{50} (8.40 [8.31, 8.53]; 17.73 [17.67, 17.74] µg of plant extract/mol DPPH). The plants extracts exhibited better inhibitions of the lipid peroxidation in concentration-dependent manner; especially *R. macrophylla* with an IC_{50} of 2.38 (2.13, 4.92) µg/mL.No significance difference was observed neither among the total phenolic nor flavonoids contents of the plant extracts.

Conclusion: Extract of *T. superba* exhibited strong antioxidant properties. Though, the extracts of *R. macrophylla* and *P. angolensis* and *S. magnificum* were also able to protect against lipid peroxidation and free radicals. The phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, tannins, coumarins, glycosids in most of the plants extracts. These properties sustain their use for treatment of male infertility and erectile dysfunction.

Keywords: *alcoholic extract, Cameroonian medicinal plants, antioxidant activities, nitric oxide*.

Abbreviations

AA: Ascorbic acid
AP: Antiradical power
DMEM: Dulbecco's Modified Eagles Medium
DMSO: Dimethylsulfoxid
DPPH: 2,2-diphenyl-1-picrylhydrazyle
EC_{50}: Efficient concentration fifty
mg EAA: milligram equivalent of ascorbic acid
mg EGA: milligram equivalent of gallic acid

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mg EQ: milligram equivalent of quercetin
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyl tetrazolium
NO: Nitric oxide
OD: Optical density
Pa: *Palisotaambigua*
Pan: *Pycnanthusangolensis*
Py: *Pausynistaliayohimbé*
Rm: *Rauvolfia macrophylla*
SC_{50}: Scavenging percentage fifty
Sm: *Schumaniophytonmagnificum*
Ts: *Terminalia superba*
TBA: Thiobarbituric acid
TCA: Trichloroacetic acid
w/v: weight/volume

1. INTRODUCTION

Oxidative stress is a common pathology which results from the imbalance between ROS and antioxidants in the body. This imbalance induces cellular damage, sperm DNA damage, denaturation of cellular protein and lipid peroxidation in the sperm plasma membrane leading to male infertility [1,2]. Moreover, some studies have demonstrated the relationship between total decreased antioxidant capacity and male infertility [3,4]. Nitric oxide plays a significant role in male reproductive functions, mainly in the regulation of steroidogenesis in Leydig cells and the control of testicular blood flow. Hence, it can be a marker of erectile function. Male infertility can be managed by conventional medicine through hormonal therapy, surgical treatment or assisted reproduction techniques. Meanwhile, these therapies are expensive and the success rates are very low [5]. Plants and derivatives of plant play a key role in world health and have long been known to possess biological activities due to their phytochemical substances which are mostly antioxidants such as phenols, flavonoids or flavonols. Antioxidants are substances which scavenge, quench and suppress the formation of reactive oxygen species (ROS) which can lead to oxidative and sperm damages [6,7]. Antioxidants play an important role in protecting the gonad's physiology by maintaining a balance between ROS generation and scavenging activities. These substances have been shown to prevent DNA fragmentation, improve semen quality in smokers, reduce cryodamage to spermatozoa and stimulate spermatozoa production [8]. The World Health Organization (WHO) estimates that almost 75 % of the world's population use medicinal plants for their healthcare [9,10] because herbal remedies may have fewer side effects, enhance the effects of conventional agents or be an alternative treatment [9]. About 80% of Africans rely on traditional practitioners and medicinal plants for their daily healthcare needs [11,12].

Numerous plants are used to improve male fertility by treating different aspects of male infertility such as sexual asthenia, libido, sperm abnormalities like azoospermia and oligospermia, as well as ejaculatory and erectile disorders [13]. Extracts from the leaves of *Palisotaambigua* (Pa), as well as from the bark of the trunk of *Rauvolfia macrophylla* (Rm) and *Terminalia superba* (Ts) are used by the 'Baka' Pygmies of Southern region of Cameroon for the treatment of male and female infertility. Traditional healers also use extracts from the wood of *Pycnanthusangolensis*(Pan), the bark of the trunk of *Pausynistalia yohimbe* (Py) and the roots of *Schumanniophytonmagnificum*(Sm) to treat erectile dysfunction

The aim of the present work was designed to study the antioxidant properties of the alcoholic extracts of those medicinal plants and investigate their effect on nitric oxide release.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1 Experimental animals

Male *Wistar* albino rats aged of 3 months were provided by the animal house of the Laboratory of Pharmacology and Toxicology of the University of Yaoundé I. Rats were used to isolate peritoneal macrophages and to prepare testicular homogenate.

2.2 Preparation of plant extracts

Fresh leaves of *P. ambigua*, roots of *S. magnificum*, stem bark of *T. superba*, *R. macrophylla*, and *P. yohimbe* and the stem wood of *P. angolensis* were harvested in March 2019 in the Subdivision of Djoum, Division of Dja-et-Lobo in the Southern region of Cameroon. The medicinal plants were respectively identified at the Cameroon National Herbarium under voucher numbers: 29555; 52761; 55546; 43413; 31619 and 2359. Then, the collected parts were washed, dried and ground into powder to prepare a 10 % (M/V) alcoholic extracts for each plant by maceration during 48 hours under gentle stirring.

2.3 Effect of extracts on nitric oxide production by primary macrophages

Rat primary macrophages were elicited by intraperitoneal injection of 2 mL of saline buffer 0.1 M, pH 7.4 containing 2 % starch. Four days later, animals were killed and peritoneal macrophages were isolated as described by the modified method of Bansal [14]. Cells number was determined with a hemocytometer and viability was assessed using Trypan blue exclusion test. According to the method described by Thakur *et al.*[15], macrophages were seeded in 96 well plates at 50000 cells /mL in DMEM culture medium (150 µL) in presence or absence of 50 µL of each concentration (0.1; 1; 10; and 100 µg/mL) of plant extract at 37 °C for 4 h. The effects of the extracts on cell viability of macrophages were determined by the reduction of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT). After incubation, cell media was assayed for the nitric oxide assay using Griess reagent according to the method described by Grisham *et al.*[16].

2.4 Antioxidant assays

2.4.1 Scavenging effects on DPPH free radical

The DPPH scavenging effect was carried out to determine the anti-radical power according to the method described by Zengin *et al.*[17]. Thus, 50 µL of plant extract at various concentrations (0.1; 1; 10 and 100 µg/mL) was mixed to 3100 µL of a methanolic solution of DPPH (40 µg /ml). The mixture was shaken and allow to stand at room temperature in the dark for 30 minutes. Absorbances were read at 517 nm against a blank. Ascorbic acid was used as a reference compound. The scavenging percentages as the effective concentrations fifty (EC_{50}) and the antiradical powers (AP) were respectively calculated by the formulas below.

$$\% SC = \frac{A_c - A_s}{A_c} \times 100$$

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

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

Where % SC is the scavenging percentage; A_s the absorbance of the sample; A_c the absorbance of the control and C the concentration of the methanolic solution of DPPH.

2.4.2 Reducing ability assay

The reducing power of the plant extracts was determined according to the method described by Pulido *et al.*[18]. Two hundred microliters of each various concentrations of plant extracts were mixed with500 µL of phosphate buffer (0.2 M; pH 6.75) and 500 µL of a 1 % aqueous solution of potassium ferricyanide. After 20 minutes of incubation at 50 °C, 500 µL of a 10 % aqueous trichloroacetic acid (TCA) solution was added to each tube. Then, the mixture was centrifuged at 45×g and 4 °C for 10 minutes. A volume of 1 mL of supernatant was added to 1 mL of distilled water and 200 µL of 0.1 % aqueous ferric chloride solution. Then, the absorbances were read at 700 nm against the blank (distilled water).

2.4.3 Determination of Total antioxidant capacity (TAC) assay

The total antioxidant capacity of the different plant extracts was determined by the phosphomolybdenum method described by Prieto *et al.*[19]. Three hundred microliters of each plant extract were mixed with 1000 µL of each of the following reagents were: 0.6 M HCl; 28 mM $NaHPO_4$; 4 mM $H_{32}MO_7N_6O_{28}$ (Ammonium Molybdate). The mixture was incubated at 90 °C for 90 minutes, and the absorbances were measured at 695 nm against the blank.

2.4.4 Lipid peroxidation inhibition of rat testes

In order to assess the ability of the plant extracts to inhibit lipid peroxidation, a 10 % (w/v) testicular homogenate was prepared in a 1.15 % KCL solution [20]. Then lipid peroxidation was carried out using the method of Su *et al.*[21]. Thus, 25 µL of each plant extract or distilled water (control tubes) or 25 µL of 1.15 % KCl (blank tubes) were added to 500 µL of 10 % testicular homogenate, 25 µL of 31.5 mM $FeCl_2$ and 25 µL of 31.5 mM H_2O_2. After an incubation at 37 °C for 1h, 500 µL of 15 % TCA and 500 µL of 0.67 % TBA were added. The mixture was then allowed to boil in a water bath for 15 minutes and then after cooling, centrifuged for 15 min at 1620×g, 4 °C. Absorbances were read at 532 nm. The percentages of lipid peroxidation inhibition were calculated using the formula below. The percentages of inhibition fifty were determined.

$$\%\, I = \frac{A_c - A_s}{A_c} \times 100$$

Where %I is the percentage of inhibition, A_c the absorbance of control and A_s the absorbance of sample.

2.5 Phytochemical screening

In order to determine the bioactive components of these plant extracts, a phytochemical screening was performed. The plant extracts were prepared at 1 mg/mL and the following phytochemical assays were performed: test for phenolic compounds, flavonoids, tannins, and terpenoids [22]; test for coumarins and anthocyans[23]; test for alcaloïds and glycosides [24]and test for steroids [25].

2.6 Phytochemical contents

2.6.1 Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteau reagent [26]. One hundred microliters of each plant extract (at 100 µg/mL) were added to 2000 µL of distilled water and 200 µL of 2N Folin-Ciocalteau reagent. The mixture was incubated at room

temperature for 3 minutes. Then, 1000 µL of 20 % Sodium Carbonate were added for another incubation at room temperature in the dark for 1 hour and absorbances were measured at 765 nm using a spectrophotometer against the blank (distilled water). Gallic acid was used as standard for the calibration curve. The results were expressed as milligram Equivalent of Gallic Acid per gram of plant extract (mgEGA/g).

2.6.2 Determination of flavonoids content

Total flavonoids content was assessed according to the method previously described by Ordonez *et al.* [27]. The reaction mixture was consisting of 500 µL of each plant extract (at 100 µg/mL) and 500 µL of 2 %aluminium trichloride. After 1 hour incubation at room temperature, the absorbances were measured at 430 nm against the blank. Total flavonoid content was expressed as milligram Quercetin Equivalent per gram of plant extract (mgEQ/g) using the equation obtained from the calibration curve.

2.7 Data analysis

Data were analyzed using R software (version 4.2.3, Lyon) [28] and expressed as the median of three replicate (interquartile range). Analysis of data between the groups were done using the Kruskal-Wallis test followed by a post-hoc Dunn's test. Correlation analyses were carried out using R software.Results were considered statistically significant at *P* values < 0.05.

3. RESULTS

3.1 Effects of extracts on cell viability of macrophages

The effects of plant extracts were assessed on peritoneal macrophages viability using MTT method. No significances were observed comparing to the control group but significant decreases were observed with the highest concentration of the extract of *P.yohimbe* and *T. superba* while compared to the macrophages cultured with the lowest concentration of *P. angolensis* extract. Results showed that cell viability of macrophages was not affected by the different concentrations of the alcoholic extracts (Fig.1.).

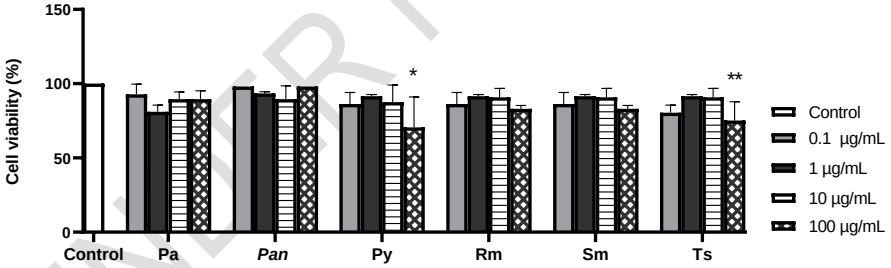

Fig. 1.Cell viability of macrophages cultured with alcoholic extract of the medicinal plants

Pa= *Palisotaambigua*; Rm= *Rauvolfia macrophylla*; Ts= *Terminalia superba*; Pan= *Pycnanthusangolensis*; Py= *Pausynistalia yohimbe;* Sm=*Schumanniophytonmagnificum*. **P*<0.01 versus Pa at 0.1 µg/mL; **p<0.05 versus Pa at 0.1 µg/mL

3.2 Stimulatory effects of extracts on nitric oxide production

Fig. 2. shows the effects of plants extracts on nitric oxide production by peritoneal macrophages. Incubation of macrophages with plants extracts stimulate the NO production in a significant concentration-dependent manner compared to the control. The alcoholic extract of *S. magnificum* presented the highest stimulation of NO production with a lower EC_{50} while the highest EC_{50} was exhibited by the extract of *P. ambigua* (table 1). The

alcoholic extracts of *R. macrophylla* and *T. superba* were found to be less active than the extract of *S. magnificum* since their EC_{50} were found to be higher.

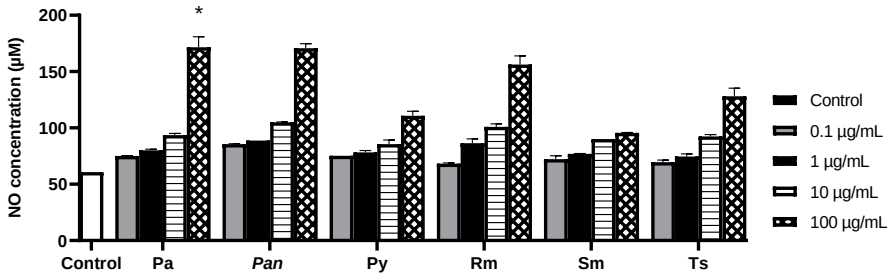

Fig. 2.NO production of macrophages cultured with alcoholic extract of the medicinal plants

Pa= *Palisotaambigua*; Rm= *Rauvolfia macrophylla*; Ts= *Terminalia superba*; Pan= *Pycnanthusangolensis* ;Py= *Pausynistalia yohimbe;* Sm=*Schumanniophytonmagnificum*. **P*<0.05 versus Pa at 0.1 µg/mL.

Table 1. Effective Concentration 50 (EC_{50}) for NO production

Plant extract	*P. ambigua*	*R. macrophylla*	*T. superba*	*P. angolensis*	*P. yohimbe*	*S. magnificum*
EC_{50} (µg/mL)	111.5 (87.28, 132.50)	23.93 (23.34, 30.64)	24.08 (18.58, 27.00)	62.66 (59.64, 64.60)	48.63 (36.44, 59.25)	4.32 (3.24, 4.32)

Values are presented in term of median (25e - 75e percentile). *P. ambigua= Palisotaambigua*; *R. macrophylla= Rauvolfia macrophylla*; *T. superba= Terminalia superba*; *P. angolensis= Pycnanthusangolensis*; *P. yohimbe = Pausynistalia yohimbe; S.magnificum =Schumanniophytonmagnificum*.

3.3 Antioxidant assays

3.3.1 Scavenging effects of extracts on DPPH free radical

The capacity of the plant extracts to scavenge free radical was assessed using the DPPH scavenging assay. Results showed that the extracts of *T. superba*, *P. yohimbe* were found to be strong free radical scavengers displaying low EC_{50} values, while the other plants extracts exhibited high values of EC_{50} (table 2). The ascorbic acid displayed a value of 4.04 (4.03, 4.09) µg/mol. Only the plant extract of *T. superba* showed an antioxidant power close to the oneof the standard. A significant difference was observed between the EC_{50} ($p<0.05$) of *Palisotaambigua*and the reference. The antioxidant power of the reference was 24.78 (24.43, 24.80) mol of DPPH/ µg of ascorbic acid. The antioxidant power of *Pycnanthusangolensis* was significantly different ($p<0.05$) from the one of the reference. Among the six selected plants extracts, *T. superba* displayed the highest antioxidant power (11.90 [11.73, 12.03] mol of DPPH/ µg of plant extract).

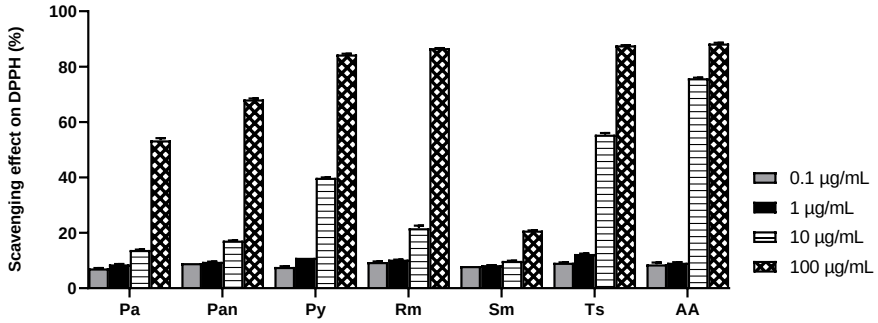

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Fig. 3.The DPPH scavenging activity of the alcoholic plants extracts

Pa= *Palisotaambigua*; Rm= *Rauvolfia macrophylla*; Ts= *Terminalia superba*; Pan= *Pycnanthusangolensis* ;Py= *Pausynistalia yohimbe;* Sm=*Schumanniophytonmagnificum*; AA= Ascorbic acid.

Table 2. Antiradical parameters of plant extracts

Plant extract	Pa	Rm	Ts	Pan	Py	Sm	AA
EC_{50} (µg of plant extract/mol DPPH	225.60 (207.85, 234.25) *	124.70 (123.25, 152.45)	8.40 (8.31, 8.53)	210.40 (210.00, 215.05)	17.73 (17.67, 17.74)	215.00 (199.10, 225.40)	4.04 (4.03, 4.09)
AP (mol of DPPH/ µg of plant extract	0.44 (0.43, 0.48)	0.80 (0.68, 0.81)	11.90 (11.73, 12.03)	0.48 (0.47, 0.48) *	5.64 (5.64, 5.66)	0.47 (0.44, 0.51)	24.78 (24.43, 24.80)

Values are presented in term of median (25e – 75e percentile). Pa= *Palisotaambigua*; Rm= *Rauvolfia macrophylla*; Ts= *Terminalia superba*; Pan= *Pycnanthusangolensis*; Py= *Pausynistalia yohimbe;* Sm=*Schumanniophytonmagnificum*; AA= Ascorbic acid. **P*<0.05 versus ascorbic acid.

3.3.2 Ferric reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power of the extracts using the FRAP method is illustrated in figure 4. Among the plant extract, *T. superba* showed the highest reducing power while *S. magnificum* showed the lowest power. The reducing power of *T. superba* is close to the one exhibited by the reference ascorbic acid (*P*> 0.05).

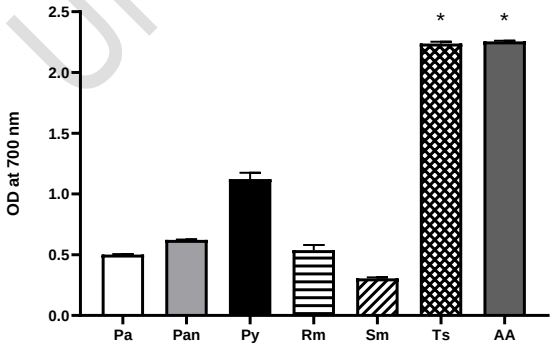

Fig. 4.Ferric reducing antioxidant power of plants extracts

Pa= *Palisotaambigua*; Rm= *Rauvolfia macrophylla*; Ts= *Terminalia superba*; Pan= Pycnanthusangolensis ;Py= Pausynistalia yohimbe*;* Sm=*Schumanniophytonmagnificum*. **P*< 0.05 versus *Schumanniophytonmagnificum*.

3.3.3 Total antioxidant capacity

Fig. 5. depicted the total antioxidant capacity of the plants extracts. Among the six plants tested, *T. superba* showed the highest total antioxidant capacity followed by *P. yohimbe*, *R.macrophylla*, *P. angolensis*, and *P.ambigua*. The alcoholic extract of *S. magnificum* showed the lowest capacity.

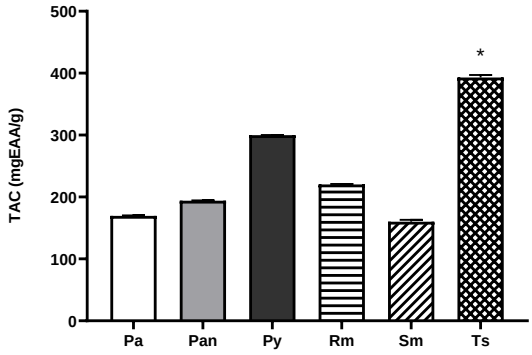

Fig. 5. Total antioxidant capacity of plants extracts

Pa= *Palisotaambigua*; Rm= *Rauvolfia macrophylla*; Ts= *Terminalia superba*; Pan= *Pycnanthusangolensis*; Py= *Pausynistalia yohimbe;* Sm=*Schumanniophytonmagnificum*. **P*< 0.05 versus *Palisotaambigua*.

3.3.4 Lipid peroxidation inhibition

Figure 6 shows the effects of plant extracts on the *in vitro* inhibition of the lipid peroxidation in the rat testicular homogenate. At the same concentrations, the plants extracts exhibited better inhibitions of the lipid peroxidation in concentration-dependent manner; especially *R. macrophylla* with an IC_{50} of 2.38 (2.13, 4.92) µg/mL which was lower than thatof the ascorbic acid. *P. yohimbe* and *S. magnificum* presented IC_{50} close to that of the reference. The IC_{50} of *T. superba* and *P. angolensis* were significantly higher than the one of *R. macrophylla* (*P>* 0.05).

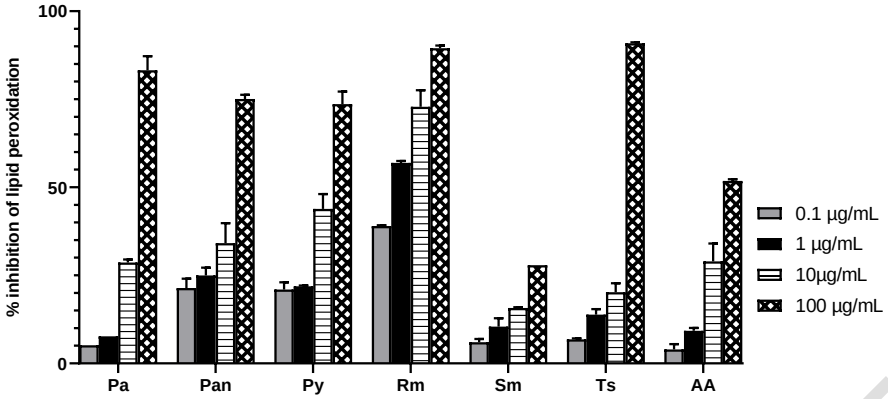

Fig. 6.Lipid peroxidation inhibition in rat testicular homogenate
Pa= *Palisotaambigua*; Rm= *Rauvolfia macrophylla*; Ts= *Terminalia superba*; Pan= Pycnanthusangolensis ;Py= Pausynistalia yohimbe*;* Sm=*Schumanniophytonmagnificum*. **P*< 0.05 versus *Palisotaambigua*.

Table 3. Inhibitory Concentration 50 (IC_{50}) for lipid peroxidation inhibition assay

Plant extract	*P. ambigua*	*R. macrophylla*	*T. superba*	*P. angolensis*	*P. yohimbe*	*S. magnificum*	AA
IC_{50} (µg/mL)	36.16 (32.17, 37.60)	2.38 (2.13, 4.92)	240.30 (191.45, 481.65) *	96.12 (60.32, 182.61) *	17.22 (15.29, 18.02)	16.81 (16.50, 17.78)	13.40 (10.07, 14.54)

Values are presented in term of median (25e -75e percentile). *P. ambigua= Palisotaambigua*; *R. macrophylla= Rauvolfia macrophylla*; *T. superba= Terminalia superba*; *P. angolensis= Pycnanthusangolensis*; *P. yohimbe = Pausynistalia yohimbe; S.magnificum =Schumanniophytonmagnificum*. **P*< 0.05 versus *Rauvolfia macrophylla.*

3.4 Major groups of secondary metabolites of the alcoholic plants extracts

The qualitative phytochemical analysis of the plants extracts revealed the following bioactive substances in most of the plants extracts: alkaloids, flavonoids, phenols, tannins, coumarins, glycosids while saponins were absent in all plants extracts. Terpenoids and anthocyans were only found in the extract of *P. yohimbe* (table 4). High contents of alkaloids and steroids were found in *R. macrophylla*, *P. yohimbe* and *P. ambigua*, respectively.

Table 4. Bioactive secondary metabolites identified in the alcoholic extracts of the plants

Medicinal plants	Alkaloids	Flavonoids	Phenols	Tannins	Coumarins	Anthocyans	Terpenoids	Saponins	Glycosides	Steroids
P. ambigua	++	++	-	+	++	-	-	-	-	+++ ++
R. macrophylla	+++ +++	+++	-	-	++	-	-	-	+++	-
T. superba	+++	+++	++	+++ +	+++	-	-	-	++	-
P. angolensis	+++	+++	-	+	++	-	-	-	+++	-
P. yohimbe	+++ ++	-	+	+++	-	++++	+	-	-	-
S. magnificum	++	++	-	-	-	-	-	-	+	-

++++++ = Abundant presence, ++++ = fairly abundant presence, ++/+ = slight presence, - = absence. *P. ambigua*= *Palisotaambigua*; *R. macrophylla*= *Rauvolfia macrophylla*; *T. superba*= *Terminalia superba*; *P. angolensis*= *Pycnanthusangolensis*; *P. yohimbe* = *Pausynistalia yohimbe; S.magnificum* =*Schumanniophytonmagnificum*

3.4.1 Determination of total phenolic and flavonoids contents

The total phenolic and flavonoid contents of the plant extracts are shown in table 5. The highest total phenolic content was obtained with the extract of *T. superba* (253.21 [251.46, 254.95] mg EGA/g of plant extract)while the highest flavonoid content was observed with the extract of *P. ambigua* (11.60mg EGA/g of plant extract). No significance difference was observed neither among the total phenolic nor flavonoids contents ($P< 0.05$).

Table 5. Total phenolic and flavonoid contents of plant extracts

Plant extract	Pa	Rm	Ts	Pan	Py	Sm
Total phenolic content (mg EGA/g of plant extract	58.08 (56.34, 58.08)	232.30 (225.33, 239.27)	253.21 (251.46, 254.95)	207.91 (200.94, 207.91)	145.19 (143.45, 145.19)	26.72 (24.98, 28.47)
Flavonoid content (mg EQ/g of plant extract	11.60 (11.41, 11.67)	6.28 (5.95, 6.28)	5.89 (5.82, 5.89)	6.93 (6.93, 6.93)	2.51 (2.51, 2.51)	1.99 (1.99, 1.99)

Values are presented in term of median (25e – 75e percentile). Pa= *Palisotaambigua*; Rm= *Rauvolfia macrophylla*; Ts= *Terminalia superba*; Pan= *Pycnanthusangolensis*; Py= *Pausynistalia yohimbe;* Sm=*Schumanniophytonmagnificum*.

3.4.2 Relationship between total antioxidant activity and the contents of phenolic compounds and flavonoids

The relationship was investigated by the method of Kendall which indicated a negative coefficient for TAC and flavonoids (-0.15) while the coefficient between total antioxidant activity(TAC) and phenolic compounds was 0.70 (P<0.05).

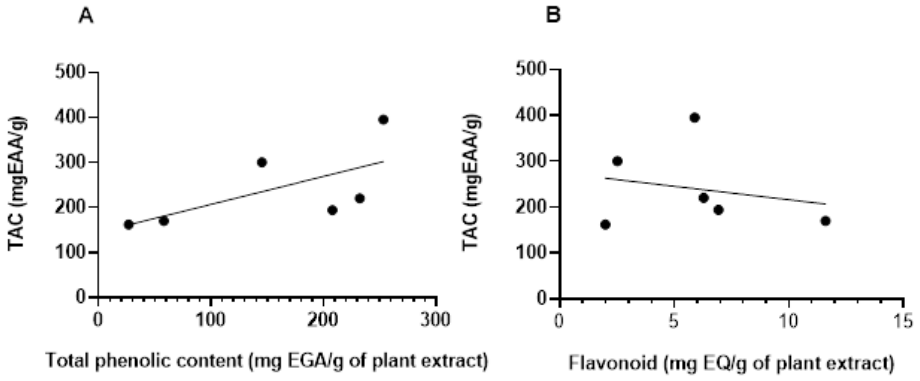

Fig. 7.Correlation between total phenolic content (A), flavonoids (B) and TAC

3.4.3 Relationship between DPPH and the contents of phenolic compounds and flavonoids

A positive correlation was observed between the DPPH scavenging activity and the flavonoids content with a coefficient of 0.26. Though, a negative correlation was observed between DPPH scavenging activity and the content of phenolic compounds.

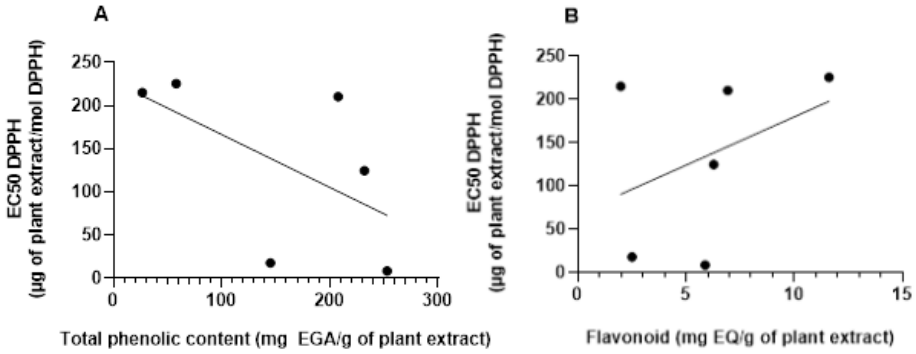

Fig. 8.Correlation between total phenolic content (A), flavonoids (B) andDPPH scavenging activity

4. DISCUSSION

The present study was undertaken to investigate the antioxidant properties of the alcoholic extracts of six Cameroonian selected plants and their *in vitro* effects on nitric oxide release. Macrophages were incubated in presence of the alcoholic extracts in order to evaluate their capacity to stimulate nitric oxide production. Comparing to the control group, no significances were observed regarding the cell viability of the macrophages stimulated in presence of the plants extracts. Nitric oxide is a signaling molecule that is involved in inflammation and immunological processes [29] but it has also a key role in the erection mechanism [30]. It is the main mediator of penile erection because of its involvement in the

relaxation of the penile smooth muscle, thus in the penileerection. Results showed that compared to the control, all extracts stimulated the release of NO in a concentration-dependent manner. The alcoholic extract of *S. magnificum* presented the best stimulation of NO production with the lowest EC_{50} followed by the extracts of *R. macrophylla*, *T. superba* and *P. yohimbe*. These results suggest that these extracts could stimulate the NO production by activating the NO/cGMP signaling pathway which is involved in the penile erection. Thus, these plant extracts could contain natural substances which can act as potent stimulators of the penile erection. *S. magnificum* is a medicinal plant used by the *Baka* Pygmies of the Southern region of Cameroon to treat erectile dysfunctions. Therefore, the alcoholic extract of that plant could enhance the erectile dysfunction by activating the NO/cGMP signaling pathway.

Testicular oxidative stress due to production of ROS may result in Leydig cells and germ cells lipid peroxidation, lipoprotein damage, protein aggregation and DNA fragmentation, as well as inhibition of steroidogenic enzymes [31]. This phenomenon may reduce the production of testosterone, a key regulator of erectile dysfunction in stimulating nitric oxide [32,33].The antioxidant properties of the extracts were investigated by assessing their scavenging effects of DPPH free radicals, inhibiting effects of lipid peroxidation and their total antioxidant capacity.

The DPPH assay allows to measure the ability of plant extracts to scavenge free radicals. Among the six extracts, the ones of *T. superba* and *P. yohimbe* exhibited significant scavenging effects against the DPPH free radicals. Results suggest that those extracts could be potent radical scavenger displaying very low EC_{50} values (8.40 [8.31, 8.53]; 17.73 [17.67, 17.74] µg of plant extract/mol DPPH). Compared to the extract of quinoa leaves [34], better results were obtained with the extracts of *T. superba*, R. *macrophylla* and *P. yohimbe* at the same concentration. *T. superba* showed the highest total antioxidant capacity followed by the extract of *P. yohimbe*, *R. macrophylla* and *P. angolensis*. *T. superba* also exhibited the highest content of phenolic compounds while *P. ambigua* displayed the highest content of flavonoids (11.60 [11.41, 11.67] mg EQ/g of plant extract). The lowest phenolic compounds and flavonoid contents were observed in the alcoholic extracts of *S. magnificum* and *R. macrophylla*, respectively. In this study, the alcoholic extract of *R. macrophylla* exhibited better inhibitory properties of lipid peroxidation than the control, ascorbic acid. Lipid peroxidation is a process of oxidative degradation of lipids especially polyunsaturated fatty acids which can lead to various diseases. The ability of plants extracts to inhibit the lipid peroxidation was investigated using a 10 % (w/v) testicular homogenate. The alcoholic extract of *R. macrophylla* had the best peroxidation inhibition with an IC_{50} of 2.38 (2.13, 4.92) µg/mL, lower than the IC_{50} of the positive control. Other extracts with low IC_{50} values among the evaluated extracts were those of *P. yohimbe* and *S. magnificum*. Results indicate that those extracts could be considered as potent inhibitors of lipid peroxidation inhibition.

The correlation of Kendall has been used to study the relationship between the antioxidant capacity and the contents of phenolic compounds and flavonoids. The correlation coefficient between TAC and phenolic compounds was 0.70 (P<0.05) indicating a strong correlation between the two parameters. These results illustrate a good matching of the high content of phenolic compounds in the alcoholic extract of *T. superba* with its high total antioxidant power. Thus, results could suggest that the high total antioxidant power of the extracts might be attributed to the presence of phenolic compounds. The correlation coefficient between TAC and flavonoids was negative suggesting that the flavonoids present in the plant extracts may not be responsible of the total antioxidant power f the extracts. The DPPH scavenging activity had a weak correlation (R=0.26) with the flavonoid contents of the plants extracts suggesting that their ability to scavenge the free radicals could be attributed to the flavonoids [35]. A negative correlation was observed between the DPPH scavenging activity and the phenolic contents.

The phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, tannins, coumarins, glycosids in most of the plants extracts while saponins were absent in all

plants extracts. Terpenoids and anthocyans were only found in the extract of *P. yohimbe*. It has been reported that the stimulation of NO release by plant extracts could be due to the presence of natural substances such as alkaloids and phenolic compounds [36] which are present in most of the plant extracts and are capable to stimulate the activity of the nitric oxide synthase (NOS) in the penile tissue [37,38].
Results suggest that the antioxidant properties of the studied plants extracts could be attributed to flavonoids and phenolic compounds which are well known to display antioxidant activities [35]. Antioxidants are very useful in preventing the formation of free radical causing diseases through oxidation process.
No previous studies on the antioxidant properties of the alcoholic extract of those medicinal plants has been reported but results confirmed that *T. superba* is a good source of antioxidants because even its aqueous extract revealed the same properties [39].

5. CONCLUSION

Results showed that among the six alcoholic extracts tested, the extract of *T. superba* contained the highest amount of phenolic compounds and exhibited highest total antioxidant capacity and the greatest DPPH scavenging activity while *R. macrophylla* showed the best inhibition of lipid peroxidation. The present study suggests those extracts as potential sources of natural antioxidants compounds.

ETHICAL APPROVAL

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

REFERENCES

1. Tremellen K. Oxidative stress and male infertility- a clinical perspective. Hum Reprod Update. 2008; 14(3):243-58.

2. Makker K, Agarwal A, Sharma R. Oxidative stress & male infertility. Indian J Med Res. 2009; 129(4):357-67.

3. Pasqualotto FF, Sharma RK, Nelson DR, Thomas AJ, Agarwal A. Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. Fertil Steril. 2000; 73(3):459-64. Available from: http://www.fertstert.org/article/S0015028299005671/fulltext

4. Agarwal A, Makker K, Sharma R. Clinical relevance of oxidative stress in male factor infertility: an update. Am J Reprod Immunol. 2008; 59(1):2-11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18154591

5. Liu PY, Handelsman D. The present and future state of hormonal treatment for male infertility. Hum Reprod Update. 2003; 9(1):9-23. Available from: http://humupd.oupjournals.org/cgi/doi/10.1093/humupd/dmg002

6. Sheweita SA, Tilmisany AM, Al-sawaf H. Mechanisms of Male Infertility : Role of Antioxidants. Curr Drug Metab. 2005; 6:495-501.

7. Gutteridge JMC, Halliwell B. Antioxidants: Molecules, medicines, and myths. Biochem Biophys Res Commun. 2010; 393(4):561-564. Available from: http://dx.doi.org/10.1016/j.bbrc.2010.02.071.

8. Badade Z, Samant P. Role of Oxidative Stress in Male Infertility and Antioxidant Supplementation. J Biomed Sci Res. 2011;3(2):385–91.

9. Fabricant D, Farnsworth N. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect. 2001; 109:69-75. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/pmc1240543/

10. Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol Aspects Med. 2006; 27:1-93. Available from: http://www.sciencedirect.com/science/article/pii/S0098299705000348.

11. Johnson Q, Syce J, Nell H, Rudeen K, Folk WR. A randomized, double-blind, placebo-controlled trial of Lessertia frutescens in healthy adults. PLoS Clin Trials. 2007; 2(4):e16. Available from: http://journals.plos.org/plosclinicaltrials/article?id=10.1371/journal.pctr.0020016.

12. McKay DL, Blumberg JB. Review of the bioactivity of south african herbal teas: Rooibos (*Aspalathus linearis*) and Honeybush (*Cyclopia intermedia*). Phyther Res. 2007; 21:1-16.

13. Singh R, Singh S, Jeyabalan G, Ali A, Semwal A. Medicinal plants used to treat sexual dysfunction : A Review. Int J Recent Adv Pharm Res. 2013; 3(1):29-35.

14. Bansal SK. Carbohydrate metabolism in the rat peritoneal macrophages. Journal of Biosciences. 1987;12(4): 415-420. Available from: https://doi.org/10.1007/BF02898591.

15. Thakur M, Thompson D, Connellan P, Deseo MA, Morris C, Dixit VK. Improvement of penile erection, sperm count and seminal fructose levels *in vivo* and nitric oxide release *in vitro* by ayurvedic herbs: Proerectile function of some Ayurvedic herbs. Andrologia. 2010; 43(4): 273-277. Available from: https://doi.org/10.1111/j.1439-0272.2010.01068.x.

16. Grisham MB, Johnson GG, Lancaster JR (1996). Quantitation of nitrate and nitrite in extracellular fluids in Methods in Enzymology, Elsevier. 1996;268*:* 237-246. Available from: https://doi.org/10.1016/s0076-6879 (96)68026-4.

17. Zengin G, Cakmak YS, Guler GO, Aktumsek A. *In vitro* antioxidant capacities and fatty acid compositions of three *Centaurea* species collected from Central Anatolia region of Turkey. Food and Chemical Toxicology. 2010;48(10): 2638-2641. Available from: https://doi.org/10.1016/j.fct.2010.06.033.

18. Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry. 2000;48(8): 3396-3402. Available from: https://doi.org/10.1021/jf9913458.

19. Prieto P, Pineda M, Aguilar M. Spectrophotometric Quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of Vitamin E. Analytical Biochemistry.1999;269(2): 337-341. Available from: https://doi.org/10.1006/abio.1999.4019.

20. Akomolafe S, Oboh G, Olasehinde T, Oyeleye S, Ogunsuyi O. Modulatory effects of Aqueous extract from Tetracarpidium conophorum leaves on key enzymes linked to erectile dysfunction and oxidative stress-induced lipid peroxidation in penile and testicular tissues. J Appl Pharm Sci. 2017; 7: 51–56. doi:10.7324/JAPS.2017.70107

21. Su X-Y, Wang Z-Y, Liu J-R. *In vitro* and *in vivo* antioxidant activity of *Pinus koraiensis* seed extract containing phenolic compounds. Food Chemistry. 2009;117(4), 681-686. Available from: https://doi.org/10.1016/j.foodchem. 2009.04.076.

22. Prabhavathi RM, Prasad MP, Jayaramu M. Studies on Qualitative and Quantitative Phytochemical Analysis of *Cissus quadrangularis*. Advances in Applied Science Research. 2016;*7*(4): 11-17. ISSN: 0976-8610.

23. Savithramma N, Linga RM, Suhrulatha D. Screening of Medicinal Plants for Secondary Metabolites. MiddleEast J. Sci. Res. 2011; 8(3): 579-584.

24Trease GE Evans WC. Trease and Evan's Textbook of Pharmacognosy. 13th Edition: Cambridge University Press; London; 1989.

25Finar IL. Organic chemistry Stereochemistry and the chemistry of natural products. 5thEdition: Longmans Green and Co: London; 1988.

26. Gao X, Ohlander M, Jeppsson N, Björk L, Trajkovski V. Changes in Antioxidant Effects and Their Relationship to Phytonutrients in Fruits of Sea Buckthorn (*Hippophae rhamnoides* L.) during Maturation. J Agri Food Chem. 2000; 48(5):1485–1490.

27. Ordonez A, Gomez J, Vattuone M, Lsla M. Antioxidant activities of *Sechium edule* (*Jacq*.) Swartz extracts. Food Chemistry.2006;97(3), 452-458. Available from: https://doi.org/10.1016/j.foodchem.2005.05.024.

28. R version 4.2.2 (2022-10-31 ucrt) -- "Innocent and Trusting". Copyright (C) 2022 The R Foundation for Statistical Computing. Platform: x86_64-w64-mingw32/x64 (64-bit)

29. Adebayo S, Ondua M, Shai L, Lebelo S. Inhibition of nitric oxide production and free radical scavenging activities of four South African medicinal plants. J Inflamm Res. 2019;12: 195–203.

30. Bivalacqua TJ, Champion HC, Hellstrom WJG, Kadowitz PJ. Pharmacotherapy for erectile dysfunction. *Trends in Pharmacological Sciences.* 2000;21(12): 484-489. https://doi.org/10.1016/S0165-6147 (00)01587-X.

31. Darbandi S, Darbandi, M. Lifestyle modifications on further reproductive problems. Cresco J. Reprod. Sci. 2016; 1(1): 1-2.

32. Zirkin B.R.; Chen, H. Regulation of Leydig cell steroidogenic function during aging. Biol. Reprod. 2000, 63, 977–981.

33 Turner TT, Bang HJ, Lysiak JJ. Experimental testicular torsion: Reperfusion blood flow and subsequent testicular venous plasma testosterone concentrations. Urology. 2005; 65: 390-394.

34. Chen H, Lan X, Wu Y, Ou Y, Chen TC, Wu W. Original article The antioxidant activity and nitric oxide production of extracts obtained from the leaves of Chenopodium quinoa Willd. 2017;7: 24–28. doi:10.1051/bmdcn/2017070424.

35. Tepe B. Food Chemistry Antimicrobial and antioxidant activities of the essential oil and various extracts of Salvia tomentosa Miller (Lamiaceae). 2005;90: 333–340. doi:10.1016/j.foodchem.2003.09.013.

36. Kenmogne H, Koloko B, Hambe C, Domkam J, Njila NM, Bend E, Oundoum OP, Lembè MD, Dimo T. Effects of aqueous root extract of *Carpolobia alba G . Don* on sexual behaviour in adult male rats. Andrologia. 2016: 48:908-914.

37. Gratzke C, Angulo J, Chitaley K, Dai YT, Kim NN, Paick JS, Simonsen U, Ulf Ückert S, Wespes E, Andersson KE, Lue TF, Stief, CG. Anatomy, physiology, and pathophysiology of erectile dysfunction. J Sex Med. 2010; 7:445-475.

38. Yakubu MT, Jimoh RO. *Carpolobia lutea* roots restore sexual arousal and performance in paroxetine-induced sexually impaired male rats. Rev Int Androl. 2014;12(3): 90-99.

39. Keumedjio PT, Béboy SNE, Moundipa PF.*In vitro* screening of the potential of six Cameroonian medicinal plants on male reproductive biomarkers. Investigational Medicinal Chemistry and Pharmacology. 2023; 6(1): 1-10.