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Research Article

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Comparative antiplasmodial, cytotoxic and antioxidant activities of aqueous and hydroethanolic extracts of Nauclea pobeguinii (Pobéguin ex Pellegr.) Petit and Artemisia annua Linne

Viviane Raïssa Sipowo Tala[1], Stéphanie Youdom Meudje[1,2], Patrick Yamen Mbopi1, Narcisse Herve Bayaga[2], Venant Tchokonte-Nana[1], Joseph Ngoupayo[2*]

[1]Higher Institute of Health Sciences, Université des Montagnes, PB 208, Bangangté, Cameroon
[2]Laboratorry of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Medicine and Biomedical Sciences, University of Yaounde I, BP 1364, Yaounde, Cameroon
*Corresponding author: ngoupayo@gmail.com

Abstract The genus Plasmodium is a causative protozoan of Malaria. The treatment of malaria has been since the 1970s based on the use of Artemisinin derivatives extracted from Artemisia annua. Despite the use of Artemisinin Combination Therapy to limit the emergence of the Plasmodium resistant strains, therapeutic failures still exist because of these strains. Thus, in a search for new antimalarial molecules, two medicinal plants commonly used in traditional medicine to fight malaria in Cameroon are here explored. The aim of this work was to evaluate comparatively the antiplasmodial, the cytotoxic, and the antioxidant activities of aqueous and hydroethanolic extracts of N. pobeguinii and A. annua, our reference plant. Maceration and decoction methods were used. Different classes of secondary metabolites of the crude extracts were qualitatively highlighted by phytochemical screening. The toxicity evaluation of the extracts was carried out in culture and the antiplasmodial activity of the extracts were studied and revealed by spectrophotometry. Results show that the extraction yields varied from 3.5 to 15.5%. The phytochemical screening revealed the presence of phenols, saponins, alkaloids, flavonoids in the extracts. However, the reference plant A. annua showed a better antiplasmodial IC50 activity (3.82 and 4.89 µg/mL) compared to N. pobeguinii IC50 (10.35 and 25.85 µg/mL). There was no indication of toxicity of these extracts to healthy erythrocytes and, to RAW and VERO cells. The hydroethanolic extract of A. annua leaves and the aqueous extract of N. pobeguinii barks presented very good antioxidant activities. These results justify the use of N. pobeguinii in traditional medicine; although it showed less activity compared to A. annua for the treatment of malaria. This observation warrants further investigations of N. pobeguinii barks for its use as an alternative remedy for the control of malaria.

Keywords Plasmodium, malaria, antioxidant, antiplasmodium, Nauclea pobeguinii, Artemisia annua,

1. Introduction

Malaria is a disease of parasitic origin widespread in the world. It is transmitted by a vector, the female Anopheles mosquito which inoculates the pathogenic agent, a parasite of the genus *Plasmodium*.[1] In addition to the production of free radicals, *Plasmodium* infection is accompanied by a general depression of total plasma antioxidants that can contribute to morbidity and mortality due to malaria.[2]Endemic in more than 100 countries whose GNP (Gross

National Product) are among the lowest, 228 million cases and 411,000 deaths were recorded in 2018 compared to 229 million cases and 409,000 deaths in 2019.[3,4] The global annual incidence of malaria is between 300 to 500 million clinical cases with at least 1 million deaths; among which more than 90% occur in Africa and mainly in children under 5.[3] In Cameroon, malaria is the leading cause of hospitalization,[5,6] and is among 15 countries most affected worldwide.[3] The emergence of the *Plasmodium* resistant strains coupled with the inaccessibility to conventional antimalarial drugs have forced people to turn to the readily available plants widely used in the African traditional medicine which are believed to fight malaria and malaria-like symptoms more efficiently.[7-9]

Artemisia annua, an annual herbaceous native plant from China,[10,11] is widely used in Asia and Africa for many years as a dietary spice [12] and as herbal tea in traditional medicine for the treatment of malaria and fever.[13,14] This plant belongs to the Asteraceae family. Similarly, *Nauclea pobeguini*, a family of Rubiaceae is used in african traditional medicine to treat fever, including malarial fever;[15,16] because of its iridoid-indole alkaloids contents that have important antimalarial activities,[17] with significant antioxidant properties.[18] Both *Artemisia anuua* and *Nauclea Pobeguinii* are found worldwide, especially in tropical and warm regions. Although, the antiplamodial activities these medicinal plants have been demonstrated,[19,15] their comparative efficacy remains of big research interests. Thus, in order to comfort the use of these plants as an antimalarial phytotherapy, and establish them as an alternative remedy for the control of malaria, we seek in this study to evaluate and compare the antiplasmodial, antioxidant and cytotoxic properties of *N. pobeguinii* with *A. annua*.

2. Materials and Methods

2.1 Materials

2.1.1 Plant material

The plant materials used in this work are the leaves and stems of Artemesia annua then the bark and the leaves of Nauclea pobeguinii. The plants were harvested at the Symbock swamp and in a garden in Messassi area in the city of Yaoundé, and were identified at the Cameroon National Herbarium under reference numbers 67448 HNC and 31575 HNC, respectively. The resulting leaves, barks and stems were dried separately at room temperature in the dark, before reducing to powder (800µ to 1600µm) and kept for further investigations.

2.1.2 Microorganisms and cells

The Plasmodium strains used to test for the antiplasmodial activity of different extracts were the NF54-E Chloroquino-sensitive plasmodium strain and the Dd2 multi-resistant plasmodium strain. Meanwhile, the cells used to assess the cytotoxicity of the different extracts were the Vero cell line (ATCC CRL 1586) obtained from the normal kidney of the African green monkey, and the Raw cell line (ATCC #TIB-71) of murine macrophages.

2.2 Methods

2.2.1 Preparation of extracts

About 60g of the resulting powders of leaves, barks and stems obtained from the plants (*Artemesia annua* and *Nauclea pobeguinii*) were each macerated in 1 liter of distilled water, while, another equal weight of leaves, barks and stems were macerated in 1 liter of hydro-ethanolic mixture (70/30: v/v) for 72 hours at room temperature. They were exposed to a constant stirring (800 rpm) on a PRO HPS-7 Lab Plus Series magnetic stirrer with solvent changes every 24 hours.

In addition, 250g of leaf and bark of *Nauclea pobeguinii* powders were boiled in 1.5 L of distilled water each, for 20 minutes. The hydro-ethanolic and aqueous solutions were filtered on Whatman N°1 paper and then concentrated to dryness on a BUCHI R-201 rotary evaporator under reduced pressure at 40°C. The raw extracts obtained were weighed and the extraction yield was calculated and expressed in percentage (%) using the formula below:

$$Yield\ (\%) = \frac{\text{mass of crude extracts (g)}}{\text{mass of powders (g)}} x\ 100$$

2.2.2 Phytochemical screening

The crude extracts obtained were subjected to phytochemistry screening methodology to search for alkaloids, polyphenols,[20] flavonoids, coumarins,[21] tannins,[22] phlobotannins,[23] anthocyanins, saponosides[24] and Oxalates,[25] through a series of coloring and precipitation reactions.

2.2.3 In vitro evaluation of antiplasmodial activity

2.2.3.1 Preparation of stock solutions of extracts, chloroquine and artemisinin

The stock solution of the different extracts (100mg/mL) was prepared by dissolving 100mg of total extracts in 1mL of dimethylsulfoxide (DMSO), Chloroquine (Sigma-Aldrich) and Artemisinin (98%, Sigma-Aldrich) were prepared at a concentration of 1 mM. The preparation of the intermediate concentrations was carried out by adding 10 µL of the various extracts (100mg/mL) and 20 µL of Chloroquine and Artemisinin (1 mM) in 190 µL and 180 µL of incomplete RPMI1640 medium respectively in a 96-well microplate followed by a geometric dilution of order 5.
Intermediate concentrations ranged from 8 to 5000 µg/mL for different extracts and from 0.016 to 10 µM for Artemisinin and Chloroquine.

2.2.3.2 Cultivation of the malaria parasite

The culture was carried out on two strains of Plasmodium falciparum (chloroquine-sensitive NF54-E and multi-resistant Dd2) according to the method described by Trager and Jensen in 1976.26, 27 The strains were cultured in Rhesus positive group O human red blood cells at 4% hematocrit in RPMI 1640 medium (500 mL) supplemented with 25 mM HEPES, 0.50% Albumax I, 45 µg/mL of hypoxanthine and 20 µg/mL gentamicin (0.5mL) and incubate at 37ºC in a humidified incubator consisting of 92% N2, 5% CO2 and 3% O2. The medium was replaced daily with complete RPMI medium to facilitate the growth of the parasites in culture. Subsequently, fine blood smears were made and stained with Giemsa then observed under the microscope at the 100 objective with immersion oil in order to follow all the stages of the cell cycle and evaluate the parasitaemia

2.2.3.3 Antiplasmodial activity test

The antiplasmodial activity test was evaluated using the SYBR Green I fluorescence method described by Smilkstein et *al* in 2004.[27,28] 90 µL of the parasite suspension synchronized at the stage in rings at 2% parasitaemia and 1% hematocrit was incubated with 10 µL of the different concentrations of each extract, pre-diluted Artemisinin and Chloroquine. The plates were then incubated at 37°C in a humidified incubator consisting of 92% N_2, 5% CO_2 and 3% O_2 for 72 hours. The final concentrations in the test plates ranged from 0.8 to 500 µg/mL (DMSO < 1%) for each extract and from 0.0016 to 1µM (DMSO 0.1%) for Artemisinin and Chloroquine in the final volume of 100 µL. The experiments were carried out in duplicate.
After 72 hours of incubation, 100 µL of SYBR Green I buffer [6 µL of 10,000 × SYBR Green I (Invitrogen) + 600 µL of red blood cell lysis buffer {Tris (25 mM; pH 7.5)} + 360 µL of EDTA (7.5 mM) + 19.2 µL of parasite lysis solution {saponin and 28.8 µL of Triton X-100 (0.08%; v/v)}] were gently added to each well, followed by incubation at 37 °C for 1 hour in the dark. After 1 hour of incubation, the fluorescence was measured using the Infinite M200 (Tecan) Microplate reader at an excitation and emission wavelength of 485 and 538 nm, respectively. The activity will be considered high if $IC_{50} < 5$ µg/mL, active if $5 \leq IC_{50} < 50$ µg/mL, moderate if $50 \leq IC_{50} < 100$ µg/mL and inactive if $IC_{50} > 100$ µg /mL.
The resistance index was calculated through the ratio of the IC_{50} of the extract on the multi-resistant strain (PfDd2) and the IC_{50} of the same extract on the sensitive strain (PfNF54-E). A resistance index below 1 indicates that the inhibitor (plant extract) acts preferentially on the resistance strain.

$$IR = IC_{50} \text{ extracted on PfDd2} / IC_{50} \text{ of the same extract on PfNF54-E}$$

2.2.4 Cytotoxicity assay

The evaluation of the cytotoxicity of the total extracts was evaluated according to the method described by Bowling et *al* in 2012.[29] Vero cell line (ATCC CRL 1586) from normal African green monkey kidney and Raw (ATCC

#TIB-71) from murine macrophages were maintained in modified Dulbecco Eagle complete medium supplemented with 10% fetal bovine serum, bicarbonate of sodium 0.2% (w/v) with a penicillin-streptomycin combination 1% (v/v). A cell density of 10,000 cells per well in a 100 µL suspension was seeded on 96-well plates and incubated for 24 hours at 5% CO_2 and 37°C to reach 90% confluence. 10 µL of the total extracts and of the control were added after 24 hours at a starting concentration of 500 µg/ml. Positive control (Podophyllotoxin) was added at a concentration of 20µM. subsequently, 10 µL of Resazurin solution (0.15 mg/mL in sterile PBS) was added to all the wells and incubated for an additional 4 hours in the same state. Fluorescence was subsequently read via a Magelan Infinite M200 (Tecan) multi-well plate fluorescence reader at excitation and emission wavelengths of 530 and 590 nm respectively.

The Selectivity Index (SI) was used as a parameter of clinical significance of the test samples by comparing the general toxins and the selective inhibitory effect of *P. falciparum* using the following equation[30]:

$$Selectivity\ Index\ (SI) = \frac{\text{IC50 of the vero or Raw cell lines}}{\text{IC50 of the Plasmodium cell lines}}$$

In addition, the cytotoxicity activity of the extracts was evaluated according to the cytotoxicity criteria for crude extracts as established by the American National Cancer Institute (NCI) stipulating that the IC_{50} of the extract < 30µg/mL.[31]

2.2.5 Evaluation of the antioxidant activity of the different extracts

2.2.5.1 DPPH radical scavenging

The determination of the antioxidant power of the extracts was carried out using the DPPH radical according to the method previously described by Dieng et *al* in 2015.[32] A 0.02% ethanolic solution of DPPH was prepared. Then to 25 µL of each solution of the extracts, was added 75 µL of the DPPH solution. All the extracts as well as the positive control (L-ascorbic acid) were tested at different concentrations (500; 250; 125; 62.5; 31.25; 15.62; 15.62; 7.81; 3.90 µg/mL). Absorbance was measured at 517 nm after incubation in the dark for 30 min. The tests were carried out in triplicate.

2.2.5.2 ATBS radical scavenging

The evaluation of the antioxidant power of the extracts was then carried out using the ATBS radical according to the method previously described by Khan et *al* in 2012.[33] To 25 µL of each solution of extracts at different concentrations, was added 75 µL of an ATBS solution (0.175mM). All the extracts as well as the positive control (L-ascorbic acid) were tested at different concentrations (500; 250; 125; 62.5; 31.25; 15.62; 15.62; 7.81; 3.90 µg/mL). Absorbance was measured at 734 nm after incubation in the dark for 30 min. The tests were carried out in triplicate.

2.2.5.3 FRAP (Ferric Reducing Antioxidant Power Assay)

The evaluation of the antioxidant power of the extracts was also carried out using the Fe3+ reduction technique according to the method previously described by Gohari et al in 2011.34 To 25 µL of each solution of extracts at different concentrations, was added 25 µL of an iron (III) chloride solution at 1.2mg/ml. After 15 minutes of incubation in the dark, 50 µL of the 0.2% orthophenanthroline solution was added and the optical density of the content of the wells was read at 505nm with a plate reader (TECAN M200) after a second incubation of 15 minutes. All extracts as well as positive controls (L-ascorbic acid; gallic acid) were tested at different concentrations (500; 250; 125; 62.5; 31.25; 15.62; 15.62; 7.81; 3.90 µg/mL). The tests were carried out in triplicate.

3. Results

3.1 Yields from various types of extractions

The yields from various extractions are presented in Table 1 below. The results show that in general high percentage yields were recorded for the hydroethanolic extract of the leaves of *N. pobeguinii* (15.5%) and *A. annua* (13.5%)

while the decoction of the barks and the aqueous extracts of the leaves of *N. pobeguinii* recorded the lowest yields (3.6%; 3.5% respectively).

Table 1: Percentage yields of different extractions

Plants	Parts	Powder (g)	Type of extraction	Solvent used	extract (g)	Yields (%)
N. pobeguinii	Barks	60	Maceration	HE	6.74	**11.23**
				Water	2.4	**4**
			Decoction	Water	2.2	**3.6**
	Leaves	60	Maceration	HE	9.3	**15.5**
				Water	2.1	**3.5**
			Decoction	Water	4.3	**7.16**
A. annua	Stems	60	Maceration	HE	5.67	**9.45**
				Water	5.4	**9**
	Leaves	60	Maceration	HE	8.1	**13.5**
				Water	4.61	**7.69**

HE: Hydro-ethanolic

3.2 Phytochemical analysis

Table 2 shows the results of the phytochemical analysis of secondary metabolites tested on all the plant extracts. Six out of nine secondary metabolites sought were present on all the extracts.

Table 2: Phytochemical analysis

Secondary metabolites	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Alkaloids	+	+	+	+	+	+	+	+	+	+
Polyphenols	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+	+	+	+	+
Anthocyans	-	-	-	-	-	-	-	-	-	-
Saponosids	+	+	+	+	+	+	+	+	+	+
Terpenoids	-	+	+	+	+	+	+	-	-	-
Steroids	-	+	+	+	+	+	+	+	+	+

S1: leaves of N. pobeguinii aqueous extract; S2: leaves of N. pobeguinii hydroethanolic extract; S3: leaves of A. annua hydroethanolic extract; S4: leaves of A. annua aqueous extract; S5: stem of A. annua hydroethanolic extract; S6: stem of A. annua aqueous extract; S7: bark of N. pobeguinii hydroethanolic extract; S8: bark of N. pobeguinii aqueous extract; S9: aqueous decoction of the leaves of N. pobeguinii; S10: aqueous decoction of bark of N. pobeguinii. ; +: present; - : absent.

3.3 IC_{50} values of the antiplasmodial activity and resistance index

The IC_{50} values of the antiplasmodial activity of different extracts and resistance index were obtained and represented in Table 3; indicating high activity ($IC_{50} < 5$ µg/mL), active activity ($5 \leq IC_{50} < 50$ µg/mL), moderate activity ($50 \leq IC_{50} < 100$ µg/mL), and inactive ($IC_{50} > 100$ µg /mL).[35] Among all aqueous and organic extracts of the different parts of the plants which were tested against both Dd2 (resistant to Chloroquine) and NF54-E (sensitive to Chloroquine) of P. falciparum, 10% showed high activity ($IC_{50} < 5$ µg/mL), 30 % showed active activity ($5 \leq IC_{50} < 50$ µg/mL), 20 % showed moderate activity ($50 \leq IC_{50} < 100$ µg/ mL), while 40% showed inactive activity ($IC_{50} > 100$ µg/mL).

However, the hydroethanolic extract of the leaves of *A. annua* showed high antiplasmodial activity (IC50 < 5 µg/mL), while both the hydroethanolic extracts and aqueous decoction of *N. pobeguinii* bark were active against the

two *P. falciparum* strains (PfDd2 and PfNF54-E). Aqueous extracts of the leaves of *A. annua* and hydroethanolic *A. annua* stems showed moderate antiplasmodial activity ($50 \leq IC_{50} < 100$ µg/mL) on the Dd2 strain and were active on the NF54-E strain. Hydroethanolic extracts of *N. pobeguinii* leaves and aqueous extracts of *N. pobeguinii* bark showed moderate antiplasmodial activity ($50 \leq IC_{50} < 100$ µg/mL) on NF54-E strain, but were inactive ($IC_{50} > 100$ µg/mL) on Dd2 strain. Unfortunately, aqueous extracts and aqueous decoction of *N. pobeguinii* leaves were inactive ($IC_{50} > 100$ µg/mL) against both strains.

Table 3: IC_{50} values of the antiplasmodial activity of different extracts and resistance index

	IC_{50} (µg/mL)		**Resistance index**
Extracts (codes)	**PfDd2**	**PfNF54-E**	
S1	> 100	> 100	Nd
S2	> 100	65 ± 1.76	Nd
S3	3.82 ± 0,14	4.89 ± 0,30	0.78
S4	99.32 ± 0,09	41.36 ± 1,18	2.40
S5	62.69 ± 0,08	35.40 ± 1,29	1.77
S6	> 100	> 100	Nd
S7	25.85 ± 1,50	10.35 ± 1,30	2.49
S8	> 100	70 ± 0,07	Nd
S9	> 100	> 100	Nd
S10	40.75 ± 0,07	20 ± 1.02	2.03
Positif control			
Artémisinine (nM)	30.64 ± 0,21	98.88 ± 0,14	0.30
Chloroquine (nM)	245.70 ± 0,20	51.04 ± 0,19	4.81

S1: leaves of N. pobeguinii aqueous extract; S2: leaves of N. pobeguinii hydroethanolic extract; S3: leaves of A. annua hydroethanolic extract; S4: leaves of A. annua aqueous extract; S5: stem of A. annua hydroethanolic extract; S6: stem of A. annua aqueous extract; S7: bark of N. pobeguinii hydroethanolic extract; S8: bark of N. pobeguinii aqueous extract; S9: aqueous decoction of the leaves of N. pobeguinii; S10: aqueous decoction of bark of N. pobeguinii.; Nd: Not determined.

3.4 Cytotoxicity and selectivity index of the different extracts

The different extracts tested in this study did not show any cytotoxic effects on RAW and VERO cells up to a concentration of 100 µg/mL. Clearly, all the extracts have a CC50 > 30 µg/mL and a selectivity index (IS) > 1 indicating that the extracts have a selectivity towards the parasite and not the host (Table 4).

Table 4: Results of cytotoxicity and selectivity index of the different extracts.

Extracts codes	**IC_{50} (µg/mL)**		**CC_{50} (µg/mL)**	**Selectivity index (CC_{50} /IC_{50})**	
	PfDd2	PfNF54-E	Raw 264,7 et Vero	PfDd2	PfNF54-E
S1	>100	>100	>100	> 1	> 1
S2	>100	65	>100	> 1	> 1.53
S3	3.82	4.89	>100	> 26.17	> 20.44
S4	99.32	41.36	>100	> 1	> 2.41
S5	62.69	35.40	>100	> 1.59	> 2.82
S6	> 100	> 100	>100	> 1	> 1
S7	25.85	10.35	>100	> 3.86	> 9.66
S8	> 100	70	>100	> 1	> 1.42
S9	> 100	> 100	> 100	> 1	> 1
S10	40.75	20	> 100	> 2.45	> 5

S1: leaves of N. pobeguinii aqueous extract; S2: leaves of N. pobeguinii hydroethanolic extract; S3: leaves of A. annua hydroethanolic extract; S4: leaves of A. annua aqueous extract; S5: stem of A. annua hydroethanolic extract; S6: stem of A. annua aqueous extract; S7: bark of N. pobeguinii hydroethanolic extract; S8: bark of N. pobeguinii aqueous extract; S9: aqueous decoction of the leaves of N. pobeguinii; S10: aqueous decoction of bark of N. pobeguinii.

3.5 SC_{50} values for different extracts and standards

The antiradical activity profiles obtained were tested using different methods (DPPH, FRAP, ATBS); and the SC50 values are represented in Figure 1 below. The SC50 values revealed that all extracts possess significantly lower antioxidant activities than ascorbic acid and gallic acid. The hydroethanolic extracts of the leaves of A. annua and aqueous barks of N. pobeguinii exhibited better antioxidant activities out of all three methods performed .

In general, depending on the type of extraction and the used part of each plant, the aqueous extract from the barks of N. pobeguinii exhibited better antioxidant activity compared to other parts of the same plant; similarly, the hydroethanolic extract of the leaves of A. annua exhibited better antioxidant activity compared to other parts of the same plant.

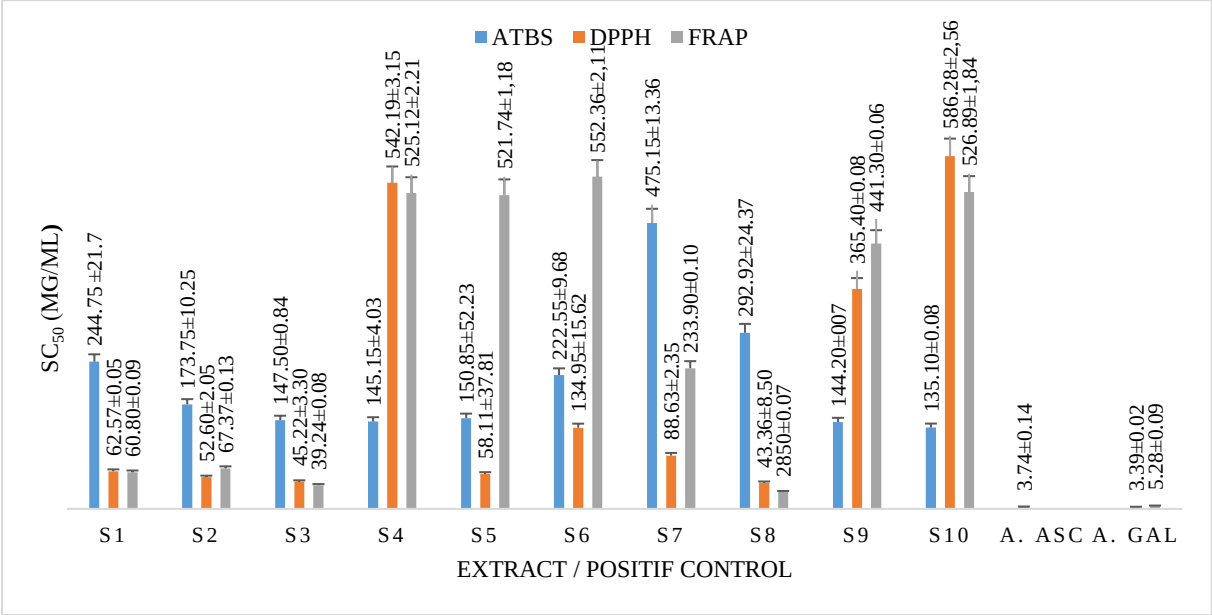


Figure 1: SC50 values for different extracts and standards

S1: leaves of *N. pobeguinii* aqueous extract; S2: leaves of *N. pobeguinii* hydroethanolic extract; S3: leaves of *A.annua* hydroethanolic extract; S4: leaves of *A. annua* aqueous extract; S5: stem of *A. annua* hydroethanolic extract; S6: stem of *A. annua* aqueous extract; S7: bark of *N. pobeguinii* hydroethanolic extract; S8: bark of *N. pobeguinii* aqueous extract; S9: aqueous decoction of the leaves of *N. pobeguinii*; S10: aqueous decoction of bark of *N. pobeguinii*. ; A. ASC: Ascorbic Acid; A. GAL: Gallic Acid.

4. Discussion

The present work aimed to evaluate and compare the antiplasmodial, antioxidant and cytotoxic activities of different extracts of *A. annua* and *N. pobeguinii*. The results obtained showed that the best extraction yields were obtained by hydro ethanolic maceration of the leaf powders of *A. annua* (13.5%), and *N. pobeguinii* (15.5%). This result could be justified on the one hand by the nature of the solvent used, and the other hand, by the concentration of metabolites in the parts of the plant material. Indeed the leaves are the seat of photosynthesis and therefore of the

synthesis and accumulation of molecules.[36] Meanwhile, the hydroethanolic mixture would make it possible to extract compounds of high and low polarity.[37]

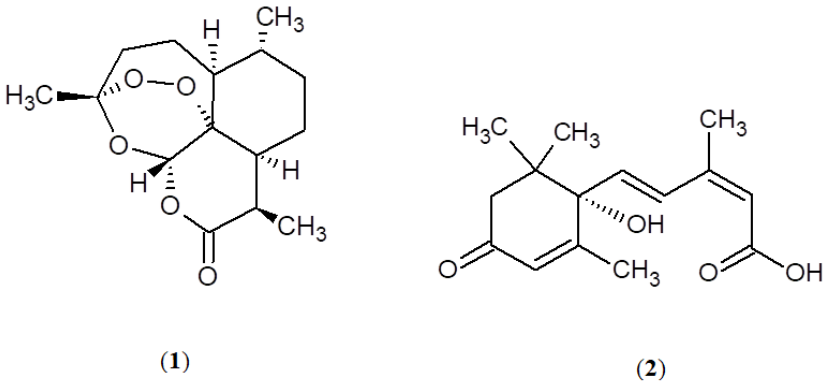
Phytochemical tests on all ten extracts used in this study showed that there are six out of nine secondary metabolites common to the extracts (polyphenols, flavonoids, alkaloids, tannins, coumarins, saponosids). Four of the extracts did not show the presence of terpenoids, while steroids were not present in one extract. These results are similar to the work of Tsafack *et al* in 2020[38] on the aqueous and methanolic extract of the barks of *N. pobeguinii*.[38] Another work on *N. latifolia* (species of the same genus with *N. pobeguinii*) showed similar secondary metabolites present in the same parts of the plant.[39] However, previous studies[40,41,42] have isolated compounds belonging to different classes of secondary metabolites found in our study. Indeed, Artemisinin (figure 2) and Abscisic acid (figure 2) are two compounds belonging to terpinoids isolated from Artemisia annua.[40,41] Similarly, Seukep et *al*[42] isolated four compounds from *N. pobeguinii*; which are *p*-coumaric acid belonging to coumarins, resveratrol and resveratrol *β-D*-glucopyranoside belonging to polyphenols, and strictosamide belonging to alkaloids (figure 3).

The antiplasmodial activity of the plant extracts showed higher activities of the hydroethanolic extract of the leaves of A. annua compared to that of hydroethanolic extracts and aqueous decoction of *N. pobeguinii* barks. The leaves of *A. annua* contain Artemisinin[43] which are extracted by the hydroethanolic mixture and which have shown their antiplasmodial properties,[44] justifying the activity of the hydroethanolic extract of leaves *A. annua*. Meanwhile, the barks of *N. pobeguinii* contain alkaloids[45,46] which have antiplasmodial properties.[47] Indeed, compounds of alkaloid nature (19-O-methylaugustoline, 5(S)-5-carboxystrictosidine) isolated from the bark of *N. pobeguinii* have demonstrated high anti-plasmodial properties,[15,46] supporting the high activity of both hydroethanolic and aqueous extracts of *N. pobeguinii*. Furthermore, some authors have reported that saponins could also be responsible for the anti-plasmodial properties of the bark of the genus *Nauclea*.[45]

It is generally considered that biological efficacy is not due to *in vitro* cytotoxicity when the selectivity index (SI) is equal or superior to one.[48] In this study, the cytotoxicity activity of the extracts was evaluated according to the cytotoxicity criteria for crude extracts as established by the American National Cancer Institute (NCI).[31] The measurement of the parameters demonstrated no adverse effects on the tested cells, indicating that the various extracts did not exhibit any toxicity. This result is in line with that of a previous study[49] carried out on *in vivo* toxicity of barks of the trunk of *N. pobeguinii* in the Democratic Republic of Congo, which demonstrated no adverse effects or abnormalities of vital organs, following administration for 30 days.

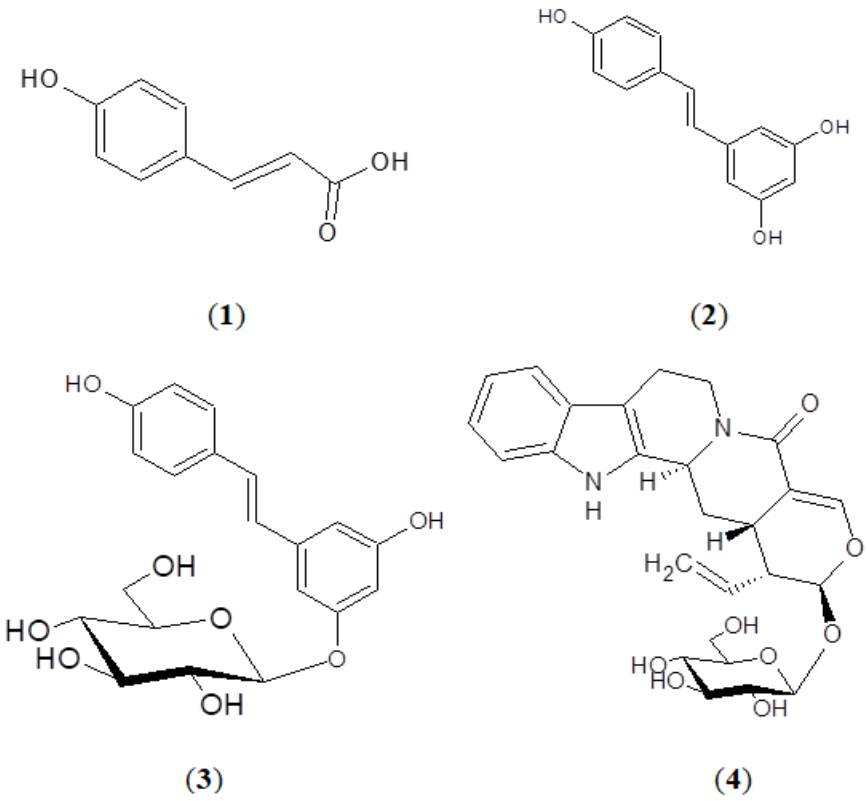
The common use of hydroalcoholic and aqueous solvents makes it possible to extract polyphenols – a polar compound from plants,[50] which are also found as a plant compound possessing an antioxidant activity.[32] However, in this study, the antioxidant activity carried out on all the ten extracts showed that the hydroethanolic extracts of the leaves of *A. annua* and aqueous barks of *N. pobeguinii* exhibited better antioxidant activities compared to the rest of the extracts using all three methods for testing antioxidant activities. As a result, the barks of *N. pobeguinii* and the leaves of *A. annua* may contain a high level of polyphenols of high polarity in view of the water (solvent) used and would be responsible for the antioxidant activity observed. A previous work[38] on the aqueous and methanolic extract of *N. pobeguinii* bark supports our observations, where it demonstrates that the aqueous extract of *N. pobeguinii* had higher antioxidant activity compared to the methanolic extract with the same concentrations when using all three methods for testing antioxidant activities.

Following observations made in our study, the aqueous extract of the bark of *N. pobeguinii* showed a weak antiplasmodial activity, while the hydro ethanolic extract of the barks presented a high antiplasmodial activity, which could justify the hypothesis that *N. pobeguinii* alkaloids mediate antiplasmodial activities.[46-47, 49] Hence, the type of solvent and the method used are important in the extraction of alkaloids. It is therefore clear that polyphenols may not be responsible for antiplasmodial activity observed in our study.



(**1**) (**2**)

Figure 2: Chemical structures of compounds isolated from Artemesia annua. (1): artemisinin; (2): abscisic acid.



(**1**) (**2**)

(**3**) (**4**)

Figure 3: Chemical structures of compounds isolated from Nauclea pobeguinii. (1): p-coumaric acid; (2): resveratrol; (3): resveratrol β-D-glucopyranoside; (4): strictosamide.

5. Conclusion

This work has shown low cytotoxicity of N. pobeguinii and A. annu, and has demonstrated their antiplasmodial and antioxidant activities. The hydroethanolic mixture of barks and leaves of these plants showed significant activities as an extraction solvent. Therefore N. pobeguinii and A. annua could be considered potential candidates for the development of new drugs.

Declaration of competing interest

The authors declare no competing interests.

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