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Antiplasmodial, antioxidant, antipyretic and cyto-toxic activities of hydroethanolic extract of *Phyllanthus niruri* (Linn), *Sida acuta* (Burm) and their combination

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ABSTRACT

The resistance of *Plasmodium*, to medications is hindering malaria eradication efforts. As a reason, the search for treatments has become crucial. Therefore, this study aimed to explore the effectiveness of a malaria treatment recipe containing extracts from *Phyllanthus niruri* Linn and *Sida acuta* Burm. The study evaluated antioxidant activity by phosphomolybdate reduction and the FRAP method; antimalarial activity was assessed on *Plasmodium falciparum* field isolates *in vitro;* antipyretic activity using yeast induced hyperthermia and measured cytotoxicity with the MTS (3-(4,5-dimethylthiazol-2-yl) -5- (3- carboxymethoxyphenyl) -2- (4-sulfophenyl)-2H-tetrazolium) assay. The *P. niruri* extract showed the best antioxidant activity with concentrations of 0.09 ± 0.00 mg AAE/g extract using the phosphomolybdate reduction method and 267.17 ± 25.66 µmol Eq Fe^{2+}/mg extract using the FRAP (Ferric Reducing Antioxidant Power) method respectively. The different extracts and their combination demonstrated effects on *P. falciparum* fields with IC_{50} (Concentration of the substance that inhibited 50% of parasites) values ranging between 0.19 to 2.68 µg/ml (IC_{50} < 5 µg/mL). Additionally antipyretic activity results indicated that the combined extracts had effects compared to paracetamol a common reference drug. The results, from the cytotoxicity tests indicated that both extracts had a CC_{50} (The cytotoxic concentration for 50%) above 100 µg/mL**.** It was found that all plant extracts are non-toxic and when combined they exhibited intriguing biological effects.

Keywords: *Malaria*, Plants, Extract combination, biological activities.

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INTRODUCTION

The *Plasmodium* parasite is responsible, for malaria which stands out as a disease transmitted by vectors (Jeje et al., 2023). The World Health Organization reported 249 million cases of malaria and 608,000 deaths worldwide in 2022, with a high prevalence in the WHO African Region (WHO, 2023). In Togo, the National Malaria Control Program (NMCP) , reported 1,737,469 cases, and 929 deaths in 2020, with a much higher incidence among children under five years of age (PNLP, 2020). With the growing resistance of *Plasmodium* to drugs, there is an increasing demand for treatments. In response to this challenge, antiplasmodial plants and their active components emerge as promising sources, for developing medications (Lemma et al., 2017).

In this sense, a review carried out by Poli et al. (2023), on the antiplasmodial activity of medicinal plants used in West Africa showed very good activities *in vitro* for 37 plants out of 78 studied. It is therefore necessary to carry out scientific evaluations to verify the efficacy of these plants and to identify the compounds responsible for their antimalarial properties (Karou, 2006). Nowadays, the use of medicinal plants in the fight against malaria must be based on scientific results for safety and quality (Karou, 2006). Based on their traditional uses, our attention was turned to *Sida acuta* and *Phyllanthus niruri*, two plants used in traditional medicine in Togo and belonging to the *Malvaceae* and *Phyllanthaceae* family respectively. Several studies have also confirmed the antipyretic activity (Sharma et al., 2012; Jansen et al., 2015) antioxidant activity (Rusmana et al., 2017 ; Radha and Saragna 2020) of these two plants

A review of literature showed that there are few documentations, on *in vitro* toxicity, antipyretic properties and antiplasmodial effects of these plants in Togo. This study was undertaken to assess the effects, antiplasmodial properties, antipyretic effects and *in vitro* toxicity of a blend of hydroethanolic extracts (HE), from *Sida acuta* and *Phyllanthus niruri*.

MATERIALS AND METHODS

Study framework

The study was conducted at the Laboratory of Microbiology and Food Quality Control (LAMICODA), in the laboratory complex of the Higher School of Biological and Food Techniques (ESTBA) and in the laboratory complex of the Regional Centre of Excellence on Avian Sciences (CERSA) at the University of Lome (Togo) and at the Laboratory of Tropical Infectious Diseases Research Centre (TIDRC) at the Regional Institute of Public Health / University of Abomey Calavi (Benin).

Plant material

Fresh leafy twigs of *S. acuta* Burm. F. and *P. niruri* L. were collected in september 2021 from the agricultural area of Agoe-Nyivé ((6°14'11.0"N 1°10'27.3"E) 6,236377, 1.174243) and Anfoin ((6°19'46.5"N 1°36'30.4"E) 6.329583, 1.608442), in the maritime region of Togo. Identification and authentication of the plants were carried out at the Botany and Plant Ecology Laboratory of the University of Lome with their respective codes, TOGO 15650 and TOGO 15651. After air-drying for one week at room temperature in laboratory, the twigs were all pulverized to obtain a powder.

Extraction

The hydroethanolic extract was prepared by stirring 250 g of the powder for 48 h in 3000 ml of an ethanol-water mixture of 70:30. The mixture was then filtered with Whatman N°1 filter papers. Alcohol was evaporated under reduced pressure using a rotary evaporator (Heidolph, Hei-VAP Precision, Germany), and then the extract was frozen and freeze-dried. The blend of extracts was made by mixing the two extracts in proportions; A (50% of *S. acuta* and 50% of *P. niruri*), B (25% of *S. acuta* and 75% of *P. niruri*), C (75% of *S. acuta* and 25% of *P. niruri*). These extract blends were tested for their antimalaria properties and their capacity in reducing fever.

Solvents and reagents

Various chemicals and reagents such as ammonium molybdate, 0.6 M sulfuric acid, 0.1% sodium phosphate, ascorbic acid, distilled water, 2,4,6- tripyridyl-s-triazine (TPTZ), iron III chloride, iron II sulfate ferric chloride, chloroform, 95% ethanol, distilled water, iron sulfate, and methanol were procured from Fisher (USA). Parasitized and non-parasitized erythrocytes, mixed gas (1% O_2, 3% CO_2 and 96% N_2), RPMI 1640 culture medium (with 2 mM L-glutamine, 25 mM HEPES buffer, 0.85 g sodium bicarbonate) supplemented with 50 mg/L hypoxanthine (Gibco), 0.25% albumax II (Gibco) without gentamycin, Giemsa, paracetamol (Tongmei), brewer's yeast (Fisher), 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyméthoxyphényl)-2-(4- sulfophényl)-2H- tétrazolium) (MTS), DMEM medium, Antibiotics (Penicillin/streptomycin); Fetal calf serum (FBS) (SIGMA, USA).

***Plasmodium* strains**

Plasmodium strains used in the study were field strains of *Plasmodium falciparum* obtained from the Tropical Infectious Diseases Research Centre, at the Regional Institute of Public Health, University of Abomey Calavi, Benin.

Animals

The animals for the present study were procured from the Laboratory of Microbiology and Food Quality Control (LAMICODA), of ESTBA. The animal experiment was approved by the Committee for Animal Experimentation Ethics of ESTBA-UL. A minimum number of animals were used to obtain reliable results.

Antiradical activity of extracts by the phosphomolybdate reduction method

Phosphomolybdate reduction was carried out according to the method described by Prieto et al. (1999) taken over by Miwonouko et al. (2023). A phosphomolybdate reagent (100 ml) was prepared as follows: sulfuric acid 0.6 M (90 ml), sodium phosphate 0.1% (5 ml), ammonium molybdate 1% (5 ml). Each extract (1 ml) was combined with 9 ml of the reagent and heated to 95°C in a water bath for 90 minutes, before optical densities were measured at 695 nm. The antioxidant standard used was ascorbic acid and results were reported in milligrams of ascorbic acid equivalent per gram of extract (mg AAE/g). The experiments were conducted three times for accuracy.

Determination of antioxidant activity according to the Ferric Reducing Antioxidant Power (FRAP) method

The antioxidant activity of the extracts was determined according to the method described by Agbodan et al. (2015). Briefly, the FRAP reagent was prepared by mixing 3 reagents: Acid buffer pH 3.5 (50 ml), 2,4,6 tripyridyls triazine (TPTZ) solution (5 ml) and iron III chloride solution (5 ml). For standard curve plotting, 3 ml of FRAP reagent was added to 100 µl of iron II sulfate solution, ranging from concentrations of 0 to 2000 µmol/l. After vigorous vortexing; the optical density was measured at 593 nm on a spectrophotometer (METASH UV-5200PC UV/VIS Spectrophotometer (Shanghai, China). For the test of extract, 100 µl of each extract (1mg/ml) was added to 3 ml of FRAP reagent in the same proportions as for standard curve plotting. Optical density was read after 5 minutes at 593 nm. The antioxidant capacity of the extracts was measured using the calibration curve and expressed in µmol Eq $FeS0_4$/mg dry extract. Assays were performed three times.

Antiplasmodial activity

Hydroethanol extracts, from *S. acuta* and *P. niruri* were tested individually and in combinations (A B, C) on *Plasmodium falciparum* field isolates in a laboratory setting following a method outlined by Frederich et al. (2000) with some adjustments. The extracts were tested at 5 concentrations 0.39, 1.56, 6.25, 25, and 100 µg/ml in the wells of a 96 well culture plate. Each extract concentration (100 µl) was mixed with 100 µl of parasite suspension (1% parasitaemia and 4% haematocrit). Artesunate (Artesun® injectable, Fosun Pharma) was taken as standard, and for the positive and negative control, infected and

uninfected erythrocytes were added. After incubation at 37◦C up to 72 h under an oxygen-reduced atmosphere of 1% O_2, 3% CO_2, and 96% N_2 (Air Liquid, France Industry), parasitaemia was assessed. After the incubation period, thin-film smears were prepared from each well. The smears were fixed with absolute methanol and stained with 10% Giemsa for 20 min. The smears were observed under a light microscope using a 100x oil immersion objective lens, and antiplasmodial activity of each plant extract has been assessed by determining parasite density. Parasite growth inhibition percentages (PI) were calculated according to the formula of Fidock et *al.,* (2004) (Fidock et al., 2004) **:**

$$PI = 1 - \left(\frac{PT}{Pt}\right) \times 100$$

Where PT= Mean parasitaemia of the triplicate of each dilution and Pt = Mean parasitaemia of the triplicate of control wells.

Finally, the concentration of the substance that inhibited 50% of parasites (IC_{50}) was estimated from dose-response curves using logarithmic non-linear regression analysis in Graph Pad Prism version 8.02 (San Diego, California USA).

Antipyretic activity

The antipyretic activity of extracts was evaluated using hyperthermia induced by subcutaneous injection of brewer's yeast *(Saccharomyces cerevisiae*) in the animal model (Parimalakrishnan et al., 2007). After temperature measurement, the rats received a 20% aqueous yeast suspension (1 ml per 100 g body weight) subcutaneously in the dorsolateral region. Seventeen (17) hours after administration of the aqueous yeast suspension, a new temperature reading was taken and four (04) groups of three rats each were formed, with rats showing a temperature increase of between 0.5°C and 1°C. Between the two temperature measurements, the animals were deprived of food. The different groups received either distilled water, the extract or the reference substance (paracetamol, 100 mg/kg bw). One hour after administration of the extracts per os, temperatures were taken every hour for six (06) hours. The antipyretic effect was estimated by calculating the percentage reduction in temperature (%RT) according to the following formula:

$$\%\boldsymbol{RT} = \mathbf{1} - \left(\frac{\mathbf{\Delta To}}{\mathbf{\Delta Tn}}\right) \times \mathbf{100}$$

Where ;

ΔTo = T0h - T 16h = variation in mean temperature before antipyretic treatment.

ΔTn = Tnh - T16h = variation in mean temperature at the ^{nth} hour after antipyretic treatment.

T 0h = initial batch mean temperature

T16h = Average temperature of the batch after induction of hyperthermia, i.e. 16 hours after pyrogen administration.

Tnh= Batch mean temperature at the nth hour after antipyretic treatment.

Cytotoxicity test

Cytotoxicity was evaluated with the MTS ((3-(4,5-dimethylthiazol-2-yl) -5- (3-carboxyméthoxyphényl) -2- (4- sulfophényl)-2H- tétrazolium)) assay (CellTiter 96 AQueous One Solution Cell Proliferation Assay, from Promega, Madison, Wisconsin, USA) using NCM 356 normal colon epithelial cells (Lau et al., 2004). These cells were acquired from the cell culture unit of Centro de Instrumentación Científica of the University of Granada, Spain. NCM 356 cells were cultured in DMEM Advanced medium freshly prepared, made up of, 10% FBS L glutamine 2 mmol/l, penicillin 100 units/ml, streptomycin 1 mg/ml at 37°C in a 5% CO_2 atmosphere. For the experimentation 3.10^4 NCM 356 cells were seeded in each well of a 96 well plate and were allowed to attach for a day. After they were exposed to concentrations of extracts (2, 5,10, 50 and 100 µg/ml) or untreated with extracts (controls) for 48 hours at 37°C. After 48 hours, the old culture medium was swapped with 90 µl, per well of medium mixed with 10 µl, per well of MTS solution. Afterwards the mixture was incubated for 3 to 4 hours at a temperature of 37°C and at 5% CO_2. An absorbance levels were read at a wavelength of 490 nm using a colorimetric plate reader. The process was conducted three times (n=3). The cytotoxic concentration for 50% (CC_{50}) of cells was

estimated from dose-response curves using logarithmic non-linear regression analysis in Graph Pad Prism version 8.02 (San Diego, California USA).

Selectivity Index (SI)

This was used as a parameter of clinical significance of the samples tested, by comparing cytotoxicity and selective inhibitory effect of *P. falciparum* using the following formula:

$$SI = \left(\frac{CC_{50} \text{ of cytotoxicity}}{IC_{50} \text{ of antiplasmodial activity}}\right)$$

Data analysis

The results are presented as mean ± standard deviation. The generated graphs were developed using Graph Pad Prism version 8.02. The IC_{50} which is the concentration that inhibits the parasite growth by 50% of the antiplasmodial activities were determined using dose-response curve analysis by non-linear regression with the aid of GraphPad Prism version 8.02, San Diego, California, USA.

RESULTS

Antioxidant activity

Antioxidant activity of *S. acuta* and *P. niruri* was assessed using the phosphomolybdate reduction and FRAP methods. Hydroethanolic extract of *P. niruri* recorded the highest activity with a concentration of 0.09 ± 0.00 mg AAE/g extract with the phosphomolybdate reduction method and 267.17 ± 25.66 µmol Eq $^{Fe2+/mg}$ extract with the FRAP method. The results are reported in Table 1.

Antiplasmodial activity

Extract inhibition percentages ranged from 37.29 to 93.15%, depending on extract concentration. The dose-response curves obtained show that inhibition percentages are dose-dependent. *S. acuta* recorded the highest percentage inhibition at 100 µg/ml. On the other hand, *P. niruri* extract and combinations B and C recorded the highest inhibition percentages at concentrations of 25; 6.25; 1.56 and 0.39 µg/ml (Figure 1). The calculated inhibition percentages were used to estimate the concentrations of extracts inhibiting 50% of parasite IC_{50}. Different extracts and combinations showed very excellent anti-plasmodial activity with IC_{50} values ranging from 0.19 to 2.68 µg/ml (IC_{50} < 5 µg/ml). Combination C which constituted 75% *S. acuta* extract and 25% *P. niruri* extract gave the highest activity of the antiplasmodial. His IC_{50} was 0.19 µg/ml. *P. niruri* extract recorded the lowest activity with an IC_{50} of 2.68 µg/ml (Figure 2).

Antipyretic activity

Physiological water and paracetamol represent the negative and the positive controls respectively. All groups recorded a temperature increase of more than 0.5°C, 16 hours after pyrogen induction. A decrease in temperature was observed from the first hour after administration of the various treatments until the 6th hour. Apart from the group receiving physiological water, a considerable decrease in temperature was observed in all groups (Table 2).

The C extract combination recorded higher antipyretic activity than the reference drug paracetamol. At a dose 400 and 800 mg/kg.bw the combined extract had the percentage reduction in the high temperature recording 85.25 ± 0.77% and 83.25 ± 0.39% respectively, while that with paracetamol recorded 78 ± 0.53% at the 6th hour after the administration of the various treatment (Table 3).

Cytotoxicity

Cytotoxic activity did not show significant cellular damage. The percentage lethality of the cell at the highest concentration with *S. acuta*, *P. niruri*, and C was 12.5%, 7.5% and 10% respectively (Table 4). The cytotoxic concentration for 50% of cells (CC_{50}) is respectively 70.36 mg/ml for *S. acuta*, 146.42 mg/ml for *P. niruri* and 89.90 mg/ml for the combination of C extracts (Figure 3). The CC_{50s} of extracts and combination C were > 100 µg/ml, so the extracts and combination C were not cytotoxic.

Selectivity index

The ratio between the CC_{50}s and IC_{50}s obtained was used to calculate the selectivity index (SI) of the extracts on the *P. falciparum* strain tested *in vitro*. The Selectivity index indicates if the extracts have a good therapeutic potential or not. All extracts recorded high selectivity indices. Combination C recorded the highest selectivity index with an SI of 473158 (Table 5). These results indicate a good therapeutic potential for extracts.

Table 1*:* Antiradical activity results

Extracts	Antiradical activity	
	Phosphomolybdate reduction (mg AAE/g)	FRAP (µmol Eq Fe^{2+}/mg)
HE de *S. acuta*	0.08 ± 0.00	237.17 ± 7.64
HE de P. niruri	0.09 ± 0.00	267.17 ± 25.66

HE: hydroethanolic extract

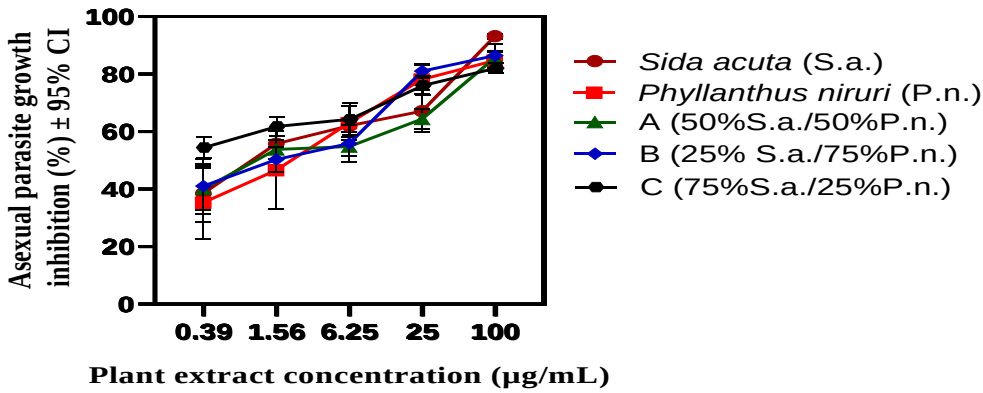

Figure 1: Inhibitory effects of plant extracts on asexual *P. falciparum.*

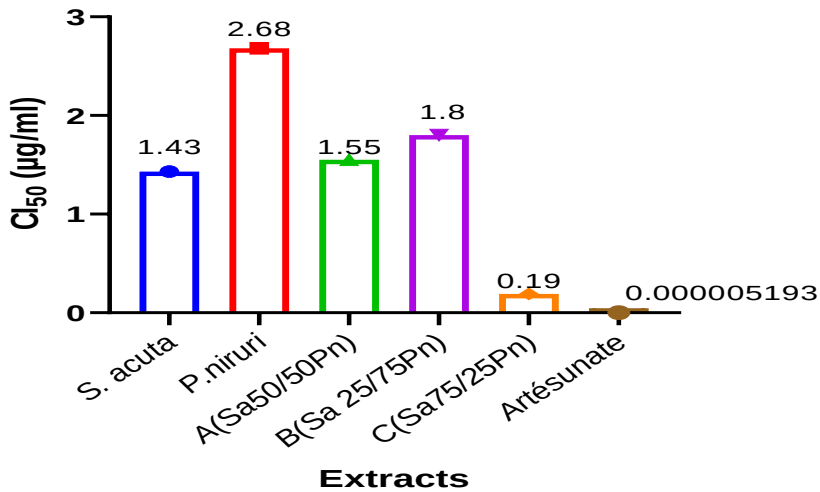

Figure 2: IC_{50} values (µg/ml) for extracts and their combinations.

Table 2: Temperature Changes Against Time.

Extracts	physiological water	C (400 mg/kg)	C(800mg/kg)	Paracetamol (100 mg/kg)
Time (hours)				
T0h	36.7± 0.36	36.5± 0.63	36.7± 0.76	37.13 ± 0.75
T16h	37.45 ± 0.31	37.43 ± 0.47	37.6 ± 0.74	38.05± 0.71
T1h	37.38± 0.26	37.25 ± 0.52	37.4 ± 0.74	37.78± 0.66
T2h	37.23 ± 0.30	37.18 ± 0.45	37.33 ± 0.77	37.68± 0.81
T3h	37.21± 0.36	37.03 ± 0.57	37.15 ± 0.73	37.70± 0.62
T4h	37.10 ± 0.40	36.85 ± 0.61	37.15 ± 0.64	37.48± 0.60
T5h	37.03 ± 0.42	36.73 ± 0.59	36.98± 0.68	37.35 ± 0.65
T6h	37.03 ± 0.42	36.65 ± 0.58	36.85± 0.77	37.38± 0.66

T0h: temperature before induction of brewer's yeast; T16h: temperature after 16 hours after induction of the hyperthermia; T1h trough T6h: temperature after administration of different treatments.

Table 3: Effect of C extract combination (Sa75/25Pn) on brewer's yeast-induced temperature evolution.

Extracts	Physiological water	C (400 mg/kg)	C (800 mg/kg)	Paracetamol (100 mg/kg)
T1h	11.75 ± 3.5	21.00± 0.27	25.00 ± 0.89	38.50± 1.81
T2h	24.00 ± 0.43	35.00± 0.83	27.50± 0.42	48.50± 0.74
T3h	41.00± 0.42	46.75 ± 0.84	57.00± 0.83	50.00± 0.78
T4h	46.00 ± 1.42	62.50± 0.40	57.75± 0.29	62.75± 0.36
T5h	55.50 ± 1.62	74.75 ± 0.42	72.25± 0.57	73.75± 0.17
T6h	55.50 ± 0.58	85.25± 0.77	83.25± 0.39	78.00± 0.53

C: extract combination (Sa75/25Pn*)*

Table 4: Percentages lethality of the different extracts and their combination C (Sa75/25Pn).

Extracts	The percentage lethality of the cell (%)
S. acuta	12,5
P. niruri	7,5
C (Sa 75/25 Pn)	10

C: extract combination (Sa75/25Pn)

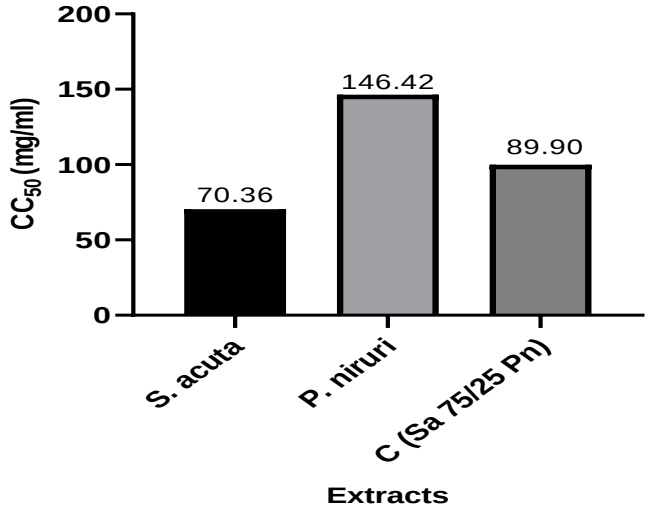

Figure 3: The cytotoxic concentration for 50% of cells (CC_{50}) of extracts and their combination.

Table 5: Selectivity index of different extracts.

Extracts	CC_{50} (mg /ml)	CI_{50} (µg/ml)	Selectivity index (SI)
S. acuta	70.36	1.48	47541
P. niruri	146.42	2.43	60255
C(Sa75/25Pn)	89.90	0.19	473158

C (Sa 75/25 Pn): Combination of 75% *S. acuta* extract and 25% *P. niruri* extract; CC_{50}: The cytotoxic concentration for 50% of cells; IC_{50}: (Concentration of the substance that inhibited 50% of parasites).

DISCUSSION

The aim of the present work was to contribute to the valorization of an antimalarial recipe made up of extracts of *Phyllanthus niruri* and *Sida acuta* through its ability to treat malaria infection. Hence the bioactivities of hydroethanolic extracts of *Phyllanthus niruri* and *Sida acuta* were evaluated.

The antioxidant activity of hydroethanolic extracts of *S. acuta* and *P. niruri* was assessed by two methods: the molybdate reduction method and the FRAP method. The molybdate VI to molybdate V reduction method was carried out under heat and acidic conditions. The green phosphomolybdate V complex ($Mo_3(PO_4)_5$) formed following the reduction of molybdate VI (Mo^{+6}) to molybdate V (Mo^{+5}) by the reducing compounds in the extracts, showed an absorption maximum at 695 nm (Ouadja et al., 2018). The antioxidant activity of the reducing compounds thus measured is quantified in milligram equivalents of ascorbic acid per gram of extract. The *S. acuta* results obtained corroborate the work of Subramanya et al. (2015) and Muneeswari et al. (2016) who obtained similar antioxidant activity using the ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) decolorization method. Karou (2006) reported a good correlation between the ABTS decolorization method and that of Phosphomolybdate (r = 0.92). The results of the antioxidant activity of *P. niruri* EH are

similar to the work of Rusmana et al. (2017) and Radha and Saragna (2020).

The FRAP method corresponds to the reduction of a ferric tripyridyltriazine complex $[(Fe(III)TPTZ_{)2}]^{3+}$ (orange-yellow) to a ferrous tripyridyltriazine complex $[(Fe(II)TPTZ_{)2}]^{2+}$ (dark blue) by an electron-donating antioxidant, at a pH of 3.6 to maintain iron solubility (Agbodan et al., 2015). *P. niruri* extract recorded the highest value at 267.17 ± 25.66 µmol Eq Fe^{2+}/mg extract. In Indonesia, Rusmana et al. (2017) found similar results. The *S. acuta* results obtained corroborate the work of Arciniegas et al. (2016) who obtained similar antiradical activity (220.54 ± 6.47 µmol Eq $Fe^{2+/}$mg extract). It will not be a mistake to say that entire antioxidant activities observed would be a function of phenolic compounds in these extracts, since most of the biological properties of polyphenols are determined by their ability to inactivate free radicals. In this respect, a very good relationship was observed between the phenolic compound content and activities of the phosphomolybdate reduction method for anti-free radical power ($r^2 = 0.99$), and between the total phenol content and antioxidant activities measured by FRAP method ($r^2 = 0.98$). Antioxidants block the effect of free radicals. They are chemical compounds that can trap free radicals and/or slow down their production and the creation of lipid peroxides in human bodies (Alara et al., 2018) . Also the detection of phytochemical compounds with antioxidant activity would be valuable in battling against numerous diseases such as: cancer; diabetes; cardiovascular disease (Alara et al., 2018).

S. acuta and *P. niruri* are plant species employed in indigenous treatment of malaria. The combined and individual extracts are shown to have a very strong *in vitro* antiplasmodial activity with IC_{50} values of 0.19 – 2.68 µg/ml. The extracts were considered to have a very strong antiplasmodial activity according to WHO recommendation and few earlier scientific studies on the antiplasmodial of plant extracts and pure compounds if their IC_{50} is ≤ 5 µg/ml (Bero et al., 2009). The IC_{50} values obtained for *S. acuta* (1,43 µg/ml) and *P. niruri* (2,68 µg/ml) are respectively similar to that obtained by Banzouzi et al. (2004) who found an IC_{50} of 3.9 µg/ml with ethanolic extracts on a chloroquino-resistant strain (FcM29) in Ivory Coast and that of Mustofa et al. (2007) who found an IC_{50} of 2.3 µg/ml for a chloroquine-resistant strain (FCR-3) with methanolic extracts of *P. niruri*. Soh et al. (2009) found an IC_{50} values ranging from 14 to 19 µg/ml for aqueous extracts of *P. niruri*. These results are inconsistent with the present. Edaphic and climatic conditions, the different solvent and *Plasmodium* strain used could explain these contradictory results.

As the *S. acuta* extract showed greater anti-plasmodial activity than that of *P. niruri*; the combinations of these two extracts, A, B and C, recorded IC_{50} values of 1.55, 1.80 and 0.19 µg/ml respectively. Combination C, made up of 75% *S. acuta* extract and 25% *P. niruri* extract, recorded the highest antiplasmodial activity. The activity of this combination is almost 8 times greater than that of the individual extracts. This level of synergy is very rare. The results obtained show an increase in the activity of *S. acuta* in the presence of *P. niruri*. Given that the cryptolepin (Karou, 2006) , is the compound responsible for the anti-plasmodial activity of *S. acuta*. *P. niruri* compounds would therefore have increased quantitatively the activity of cryptolepin, which was identified as the alkaloid involved in the anti-plasmodial activity of *S. acuta*. Combination therapies are an innovation in addressing the malaria challenge. Previous works have confirmed synergy between extracts of plants traditionally combined (Azas et al., 2002). Synergy was demonstrated *in vitro* between extracts of *Mitragyna inermis*, *Nauclea latifolia*, *Guiera senegalensis* and *Feretia apodanthera*, which are traditional Malian first line antimalarial treatments (Azas et al., 2002). Although the synergistic relationships between the various plant extracts have been proven, the

mechanisms of this interaction have yet to be elucidated (Rasoanaivo et al., 2015) .

The results of the antipyretic activity showed an increase in temperature within the first few hours of extract administration. Combination C showed higher antipyretic activity than the reference drug paracetamol. Body temperature was virtually restored 24 hours after pyrogen administration. Inhibition of the synthesis of prostaglandins and other inflammatory mediators could explain this reduction (Kilimozhi et al. 2009). This activity is probably due to the synergistic action of the molecules in the two extracts. In fact, *S. acuta* and *P. niruri* are known for their antipyretic activities (Sharma et al., 2012; Jansen et al., 2015).

Given the cellular scope of the extract's actions, it is important to assess their safety at cellular level. Malebo et al. (2009), classified cytotoxicity with CC_{50} < 1 µg/ml as highly cytotoxicity, a CC_{50} between 1- 10 µg/ml as moderately cytotoxic, CC_{50} between 10–30 µg/ml as low cytotoxic and CC_{50} > 30 µg/ml non-cytotoxic. Therefore, extracts of *S. acuta* and *P. niruri*, and their combinations are non-cytotoxic. Figure 3, show that the cytotoxicity of extracts is dose-dependent. Controls compared with tests showed that there was no statistically significant difference at $P > 0.05$ between the negative control group and the tests groups. This means that the extracts, used in these proportions, had no significant impact on cell viability.

The highest dose of *P. niruri* (100 µg/ml) caused only 7.5 ± 0.01% cell lethality, showing a non-significant difference ($P > 0.05$) between it and the negative control.

Similarly, Tang et al. (2010) reported no significant cytotoxicity of *P.niruri* extracts; aqueous and methanolic on normal human skin and prostate cells. The present findings are also consistent with the work of Lee et al. (2011) who reported the non-significant cytotoxicity of *Phyllanthus* species including *P. niruri* extracts; aqueous and methanolic on normal cells using MTS assay. However these results seem contradicting, with *in vitro* toxicity results according to Asare et al. (2012). performed according to the LDH (Lactate dehydrogenase) method on peripheral blood mononuclear cells (PBMCs) (26 µg/ml). This insimilarity may result from various methodological and pedological differences. Moreover, according the phytochemical composition, cytotoxic activity of the same plants, can diverge sharply depending on the place of growth. Combination C, which is also non-toxic, would-be evidence of its use by local populations. Furthermore, biological efficacy is generally considered not to be due to *in vitro* cytotoxicity when the selectivity index (SI) is equal to or greater than 1 (Vonthron-Sénécheau et al., 2003). All the extracts tested showed high selectivity indices, indicating good therapeutic potential. Knowledge of plant toxicity information, in particular therapeutic and lethal doses, is an essential criterion for their use.

Conclusion

The present work enabled a scientific evaluation of the hydroethanolic extracts of *S. acuta* and *P. niruri*. Antiradical activity showed that *P. niruri* extract have better antiradical activity. Both the extracts and their combinations showed highly interesting anti-plasmodial activity. Cytotoxic tests revealed that both plants and their combination are non-toxic. Plants possess numerous phytochemical compounds which are responsible for their biological properties. The results of the present work confirm the use of these plants in traditional medicine. However, further studies are needed to better understand molecular pathway leading to hight antiplasmodial activity displayed by combinations of tested plant extracts.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Conceptualization: SP and DSK; Data collection and processing: SP, AM, LG, DSV, AGT, LSD; Analysis, interpretation and writing: SP, EA, AM, EHG, AGT; resources: EA, GK, DSK; visualization: AA, LG, YH, MESM. All authors have read and agreed to the published version of the manuscript.

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