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## Antiplasmodial and Antioxidant Activities of Saye: A Traditional Herbal Remedy for Malaria

<sup>1,6</sup>O. Da, <sup>1,3</sup>M. Traoré/Coulibaly, <sup>1</sup>R.S. Yerbanga, <sup>1</sup>B. Koama, <sup>2</sup>N. Ouedraogo, <sup>1</sup>S. Tamboura, <sup>4</sup>Z.P. Dakuyo, <sup>5</sup>M.P. Sekhoacha, <sup>7</sup>J.B. Nikiéma, <sup>4</sup>G.A. Ouedraogo, <sup>5</sup>M.G. Matsabisa and <sup>1,3</sup>J.B. Ouedraogo

<sup>1</sup>Regional Research Institute for Health Sciences (ICES-DRO/CNRST), Burkina Faso

<sup>2</sup>Research Institute for Health Sciences (ICES), 03 BP 7192 Ouagadougou, Burkina Faso

<sup>3</sup>Centre Muraz, BP390, Bobo-Dioulasso, Burkina Faso

<sup>4</sup>Pharmacy Comoe, Phytofla, BP 293 Banfora, Burkina Faso

<sup>5</sup>Indigenous Knowledge System Unit of the Medical Research Council Cape Town, South Africa

<sup>6</sup>Polytechnique University of Bobo-Dioulasso, Science Research Laboratory of Health and Animal Biotechnology (LARESBA), BP 1091 Bobo-Dioulasso, Burkina Faso

<sup>7</sup>General Direction of Pharmacy, Medicines and Laboratories, (DGPML), 03BP 7009 Ouagadougou 03, Burkina Faso

*Corresponding Author: Ollo Da, Regional Research Institute for Health Sciences (ICES-DRO/CNRST), Burkina Faso  
Tel: +226710742977*

### ABSTRACT

Saye is an antimalarial recipe containing *Cochlospermum planchonii* Hook. F. (Cochlospermaceae), *Phyllanthus amarus* Schumach and Thonn (Euphorbiaceae) and *Cassia alata* L. (Fabaceae). This study assessed the antiplasmodial and antioxidant activity of the aqueous extracts of the individual plants and their combinations. Extracts were assessed on *Plasmodium berghei* infected mice according to the 4 day suppressive test, for their total phenol contents according to Folin-Ciocalteu method and their antioxidant potential by FRAP assay and by the inhibition of ion induced lipid peroxidation in rat liver homogenate. Macerated extracts from *Cochlospermum planchonii*, *Phyllanthus amarus* and *Cassia alata* gave 50.6, 46.3 and 44.9% inhibition of the parasites at 100 mg kg<sup>-1</sup> body weight (b.wt.), respectively. At 250 mg kg<sup>-1</sup> b.wt. the decocted extract of *Cochlospermum planchonii* gave 54% inhibition. Decocted extracts of the combinations *Cassia alata*+*Phyllanthus amarus* (2:1) and *Phyllanthus amarus*+*Cochlospermum planchonii* (2:1) reduced mice parasitemia by about 20-30% at 100 mg kg<sup>-1</sup> b.wt. At the same dose, a 4.9-15.9% inhibition was observed with the decocted extract of *Cassia alata*+*Cochlospermum planchonii* (1:1) and the whole "Saye" but a higher effect of 43.7 to 50.3% was observed at 250 mg kg<sup>-1</sup> b.wt. *Phyllanthus amarus* extract exhibited the highest total phenol content (294 µg TAEs/mg). The highest content in flavonoids 43 µg QE/mg and the highest inhibition of lipid peroxidation (22.56%) were found for *Cassia alata*+*Phyllanthus amarus* (2:1) and the best reducing power (41.38 µmol TE/mg) for Saye. Saye appears as promising antioxidant and could be used as preventive agent in oxidative stress diseases such as malaria.

**Key words:** Saye, antiplasmodial activity, *P. berghei*, antioxidant activity, polyphenol content

## INTRODUCTION

“Saye” is a multiherbal formulation composed of 3 plants widely used for the treatment of uncomplicated malaria in Burkina Faso. The three plants are *Cassia alata* (leaves), *Cochlospermum planchonii* (roots) and *Phyllanthus amarus* (whole plant) respectively combined in the ratio 2:2:1.

In a previous study, the decocted extract of Saye administered at 250 mg kg<sup>-1</sup> b.wt. per day during 4 days, reduced *Plasmodium berghei* parasitaemia in infected mice by 74% (Traore *et al.*, 2008). More recently, the prophylactic activity of Saye was demonstrated; 52.0% parasitaemia reduction was obtained at 100 mg kg<sup>-1</sup> b.wt. with the decocted extract (Yerbanga *et al.*, 2012).

In preliminary phytochemical screening, Saye has revealed the presence of terpenoids, flavonoids, anthraquinones, tannins and traces of alkaloids. Alkaloids and quinones are known to have antiparasitic activity and polyphenolic recognized as antioxidant compounds and recommended today as therapeutic agents against diseases involving free radical damage.

Oxidative stress exists in malaria infection and occurs during its complications. During the infection, the parasites digest hemoglobin and produces heme which triggers the production of Reactive Oxygen Species (ROS) and can lead to the development of anemia and apoptosis (Reis *et al.*, 2010). Antioxidants are reducing agents, which limit the oxidative damage to biological structures and found to prevent the development of cerebral complications of malaria in animals (Reis *et al.*, 2010).

*Cassia alata* (leaves), *Cochlospermum planchonii* (roots) and *Phyllanthus amarus* as well as Saye were found to possess diverse medicinal properties (Aliyu *et al.*, 1995; Ajala *et al.*, 2011; Veerachari and Bopaiah, 2012). Previous phytochemical studies have shown the presence of flavonoids and tannins in *Phyllanthus amarus* (Foo, 1993, 1995; Obianime and Uche, 2009), in *Cochlospermum planchonii* (Nafiu *et al.*, 2011; Paul *et al.*, 2011) and only flavonoids in *Cassia alata* (Moriyama *et al.*, 2003). Then, Saye constituent plants contain flavonoids and tannins known to have free radical scavenging activity and oxidant reducing capacity, property more and more demonstrated today to be beneficial in the management of malaria.

The current study was carried out to investigate the antiplasmodial and antioxidant activity of extracts from each of the three plants, their content in phenolics, comparatively to whole Saye formulation. The study was also an occasion to verify the claim that multiherbal therapies have synergistic and potentiative pharmacological effects compared to monoherbal therapy.

## MATERIALS AND METHODS

**Plant identification and collection:** The leaf powder of *Cassia alata*, the root powder of *Cochlospermum planchonii* and the whole plant powder of *Phyllanthus amarus* were all supplied by Phytofla laboratory. Each plant material and the whole Saye were used according to the recipe provided by Phytofla laboratory.

**Extraction:** A maceration (24 h) and a decoction (10 mm) with distilled water (100 mL) of each plant powder (50 g) were prepared. Extracts from the combined plant material *Cochlospermum planchonii*+*Cassia alata* (1:1); *Cochlospermum planchonii*+*Phyllanthus amarus* (2:1) and *Cassia alata*+*Phyllanthus amarus* (2:1) were also prepared. After filtration the extracts were frozen at -20°C and freeze-dried using a Christ® Alpha 1-2 (Bioblock scientific) freeze dryer.

**Experimental animals:** Female NMRI mice (8 weeks old and weighing 25.7±3 g) purchased from the International Centre for Research and Development on Livestock in Sub-humid areas (ICRDLS), Bobo-Dioulasso, Burkina Faso were used. Animals were maintained in the same

environmental conditions (temperature 24±3°C and 12 h photoperiod); fed with standard food provided by “Service Régional d'élevage de Bobo Dioulasso, Burkina Faso”.

***In vivo* antiplasmodial activity testing:** The experiment was performed in mice based on the 4-day suppressive test described by Peters and Robinson (1992). Three groups of six mice were used. At day 0, mice were inoculated intraperitoneally with 10<sup>7</sup> red blood cells parasitized with *Plasmodium berghei* ANKA strain. 2 h post infection, 200 µL of plant extract at 100 and 250 mg kg<sup>-1</sup> b.wt. were administered to the animals by oral route, once a day, from day 0 to day 3. The control group received only distilled water. On day 4 post infection, thin blood smears were performed with the blood taken from the tail of the mice, fixed in methanol, stained with Giemsa 10% and investigated microscopically. The parasitaemia (mean±95% confidence interval) for each group of mice was recorded and the percentage suppression of parasitaemia calculated.

#### ***In vitro* antioxidant tests**

**Ferric Reducing Antioxidant Power (FRAP) assay:** The ability of the extracts to reduce iron (III) was assessed by the method of Oyaizu (1986). A 0.5 mL aliquot of extract was dissolved in water (0.1 mg mL<sup>-1</sup>) and mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of a 1% aqueous potassium hexacyanoferrate [K<sub>3</sub>Fe(CN)<sub>6</sub>] solution. After 30 min incubation at 50°C, 1.25 mL of trichloroacetic acid (10%) was added and the mixture centrifuged at 2000 rpm for 10 min. Three aliquots of 0.625 mL were prepared from the upper layer; each 0.625 mL aliquot was mixed with 0.625 mL of water and 0.125 mL aqueous FeCl<sub>3</sub> (0.1%) and the absorbance was recorded at 700 nm using a spectrophotometer. Distilled water without extract prepared in the same conditions was used as the control. The results on the reducing compounds were expressed in mmol Trolox Equivalent (TE) per gram.

**Lipid peroxidation assay:** The degree of lipid peroxidation was assessed by estimating the thiobarbituric acid (TBA) reactive compounds using the modified method previously described by Ardestani and Yazdanparast (2007).

Male rats (250 g mean weight) were sacrificed by cervical dislocation after anaesthesia and dissected whole liver was cut in small pieces and mashed to prepare a homogenate at 10% (m/v) in phosphate buffer saline, 50 mM, pH 7.4 then centrifuged at 3000 g for 10 min. The supernatant was used for the *in vitro* peroxidation assay. To 0.1 mL of different concentrations of extracts was added 0.5 mL of homogenate, 0.9 mL of phosphate buffer saline (50 mM, pH 7.4), 0.25 mL of ferrous sulphate (FeSO<sub>4</sub>) (0.01 mM), 0.25 mL of ascorbic acid (0.1 mM). The reactive mixture was incubated for 30 min at 37°C and the reaction was stopped by adding 1 mL of 20% trichloroacetic acid (TCA) and 1 mL of 0.67% TBA. The mixture was heated at 100°C for 15 min and centrifuged at 3000×g for 10 min. The absorbance was measured at 532 nm in a spectrophotometer and the percentage inhibition of lipid peroxidation was calculated as follows:

$$\text{Inhibition (\%)} = \frac{A_0 + A_1}{A_0} \times 100$$

Where:

A<sub>0</sub> = Absorbance of the control reaction

A<sub>1</sub> = Absorbance of the sample

(+) = Catechine was used as positive control

**Total phenol content:** The total phenolic content of the extracts was estimated according to the Folin-Ciocalteu method (Singleton *et al.*, 1999). Add 1 mL of undiluted Folin-Ciocalteu reagent and 3 mL of 20% (w/v) carbonate ( $\text{Na}_2\text{CO}_3$ ) to 1 mL of extract. The mixture was allowed to stand for 40 min at ambient temperature for colour development and the absorbance was then measured at 760 nm in a spectrophotometer and compared to a tannic acid calibration curve. The control contained all the reaction reagents except the extract. Total phenolic content was determined as tannic acid equivalents (mg tannic acid/g extract).

**Tannins content:** Tannin content was assessed as previously described by Tibiri *et al.* (2007). Polyvinyl polypyrrolidone (PVPP) selectively precipitates tannins by formation of a complex. 100 mg of PVPP are enough to precipitate 2 mg of total phenolics. To 1 mL of 0.50 mg mL<sup>-1</sup> of extract is added PVPP at a quantity necessary to precipitate the total phenolics; the mixture was vortexed, kept at 4°C for 15 min and then centrifuged at 3000 g for 10 min. The supernatant contains other phenolics and not tannins, which have been precipitated by PVPP. Total phenolic content of the supernatant was determined as described (Tibiri *et al.*, 2007). Tannin content was determined as the difference between total phenolics (containing tannins) and the total phenolics (in the absence of tannins).

**Flavonoid content:** The total flavonoid content in the extracts was estimated according to the method of Abdel-Hameed (2009). Hundred milliliter of 2% of  $\text{AlCl}_3$  in methanol solution and one drop of acetic acid were added to 100  $\mu\text{L}$  of extract diluted in methanol (10 mg mL<sup>-1</sup>); the total volume was adjusted with methanol to 5 mL. After 40 min at room temperature, the absorbance was measured at 415 nm in a spectrophotometer (Agilent 8453). The control consisted of 100  $\mu\text{L}$  of extract in 5 mL of methanol with one drop of acetic acid. The absorbance of quercetin (0.1 mg mL<sup>-1</sup>) measured in the same conditions served as a reference. Total flavonoid content in the extract was calculated as quercetin equivalent (EQ) (mg mg<sup>-1</sup>) using the following equation:

$$T_{\text{Flav}} = \frac{A \times m_0}{A_0 \times m}$$

Where:

- $T_{\text{flav}}$  = Flavonoïds content of the extract estimated as EQ in mg mg<sup>-1</sup>
- A = Absorbance of the extract
- $A_0$  = Absorbance of Quercetin
- m = Extract mass in mg
- $m_0$  = Quercetin mass in mg

**Flavonol content:** Flavonol content was estimated according to Abdel-Hameed (2009). The measurement is based on the formation of an aluminium complex with maximum absorption at 440 nm.

One milliliter of methanol extract (10 mg mL<sup>-1</sup>) was mixed with 1 mL of aluminium trichloride (20 mg mL<sup>-1</sup>) and 3 mL of sodium acetate (50 mg mL<sup>-1</sup>). The absorption was measured after 150 min. Quercetin (0.025 mg mL<sup>-1</sup> in methanol) was used as reference compound and absorbance

was measured under the same conditions. The tests were performed in triplicate. The content of the extract in flavonoids is expressed as Quercetin Equivalent (QE).

**Statistical analysis:** Data is the average of triplicates analyses. Statistically significance was achieved when the p-value was less than 0.05 using xlstat 2012.

## RESULTS

**Antiplasmodial activity results:** The *in vivo* antiplasmodial activity of an extract is classified as moderate, good or very good when the extract displays a percentage growth inhibition equal to or greater than 50% at 500, 250 and 100 mg kg<sup>-1</sup> b.wt./day, respectively of extract (Munoz *et al.*, 2000). Based on this classification, the decocted and macerated aqueous extracts of *Cochlospermum planchonii* exhibited a good to very good antiplasmodial activity *in vivo* against *Plasmodium berghei* parasites (Fig. 1a).

The antiplasmodial activity of the macerated aqueous extract of *Cassia alata* was also significantly higher (44.9%) than the activity displayed by the decocted extract (19.2%) (p = 0.540) (Fig. 1b).

The single plant extract of *Phyllanthus amarus* with 40% inhibition at 100 mg kg<sup>-1</sup> b.wt. can be considered as a moderate to good antiplasmodial extract. There was no significant difference between the antiplasmodial activity of the macerated and the decocted extracts of *Phyllanthus amarus* (p = 0.563) (Fig. 1c).

The decocted extract of "Saye" or the combination *Cassia alata*+*Cochlospermum planchonii* (1:1) revealed moderate to good antiplasmodial activity at 250 mg kg<sup>-1</sup> b.wt. (Fig. 1b and c). Combined extracts of *Cassia alata*+*Phyllanthus amarus* (2:1), *Phyllanthus amarus*+*Cochlospermum planchonii* (2:1) had low *in vivo* antiplasmodial activity compared to the activity of each single plant extract (Fig. 1b and c).

The results did not demonstrate any synergism between the chemical constituents of the combined plant extracts.

## Reducing power, total of phenolics, flavonoids and flavonols

**Total of phenolics, flavonoids and flavonols:** The amount of total phenolics measured by Folin-Ciocalteu method varied widely in plant extracts and ranged from 86 to 294 µg TAE mg<sup>-1</sup> (Table 1). The highest content was found in *Phyllanthus amarus* aqueous extract and the lowest was found in *Cochlospermum planchonii* aqueous extract (86 µg TAE mg<sup>-1</sup>).

Table 1: Total yield and total phenolic and flavonoids content of the plant extracts

Extracts	Yield (%)	Total phenolics (µg TAEs/mg)	Flavonoids (µg QE/mg)	Flavonol (µg QE/mg)	Tannins (µg TAEs/ mg)
CA	13.46	145.36±1.75	33.55±0.90	18.67±0.27	11.39±0.17
CP	8.59	86.80±1.32	14.41±0.39	9.49±0.14	2.54±0.04
PA	11.47	294.94±0.90	19.16±0.52	10.96±0.16	38.79±0.59
CA+CP	11.02	101.59±1.54	29.62±0.80	10.30±0.15	12.42±0.19
CA+PA	9.60	233.78±0.71	43.78±1.18	9.81±0.14	31.70±0.48
CP+PA	7.16	-	-	-	-
TSAYE	10.85	191.07±0.58	27.71±0.75	3.87±0.06	35.57±0.54

CA: *Cassia alata*, CP: *Cochlospermum planchonii*, PA: *Phyllanthus amarus*, CA+CP: Combination *Cassia alata*+*Cochlospermum planchonii* (1/1), CA+PA: Combination *Cassia alata*+*Phyllanthus amarus* (2/1), CP+PA: Combination *Cochlospermum planchonii*+*Phyllanthus amarus* (2/1)

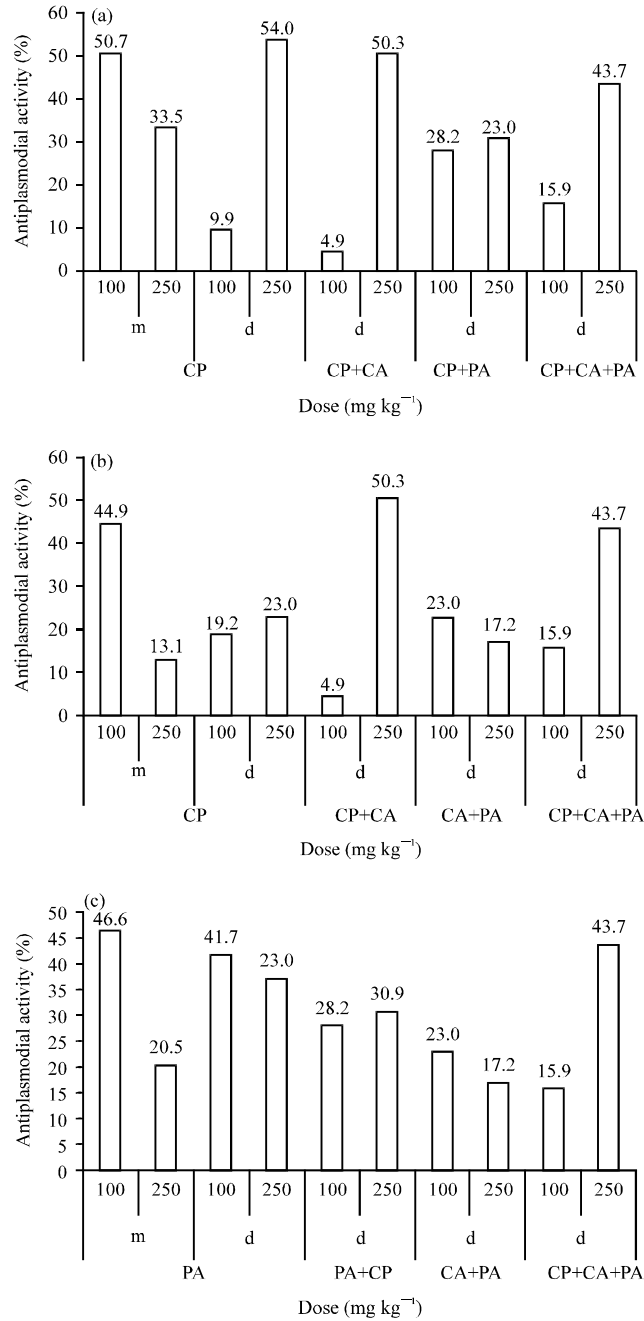


Fig. 1(a-c): Antiplasmodial activity of the extract of (a) *Cochlospermum planchonii* compared to the activities of the extract of *Cochlospermum planchonii*+*Cassia alata* and the extract of *Cochlospermum planchonii*+*Phyllanthus amarus* and the extract of Saye, (b) *Cassia alata* compared to the activities of the extract of *Cassia alata*+*Cochlospermum planchonii* and the extract of *Cassia alata*+*Phyllanthus amarus* and the extract of Saye and (c) *Phyllanthus amarus* compared to the activities of the extract of *Phyllanthus amarus*+*Cochlospermum planchonii* and the extract of *Phyllanthus amarus*+*Cassia alata* and the extract of Saye

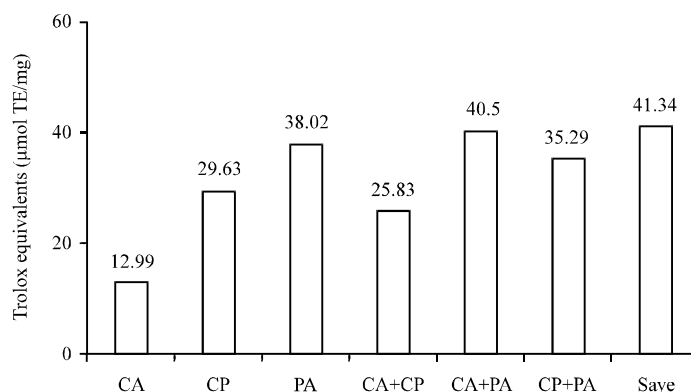


Fig. 2: Antioxidant activity of Saye based plant extracts on iron reduction, CA: *Cassia alata*; CP: *Cochlospermum planchonii*, PA: *Phyllanthus amarus*, CA+CP: Combination *Cassia alata* +*Cochlospermum planchonii* (1/1), CA +PA: Combination *Cassia alata* +*Phyllanthus amarus* (2/1), CP+PA: Combination *Cochlospermum planchonii*+*Phyllanthus amarus* (2/1)

The aqueous extract of the combination *Cassia alata*+*Phyllanthus amarus* (233 µg TAE/mg), Saye extract (191 µg TAE/mg) and aqueous extract of *Cassia alata* (145 µg TAE/mg) had also very high level of phenolics.

The amount of flavonoids in the tested extracts was ranged from 14 to 43 µg QE mg<sup>-1</sup> (Table 1). The highest level was found in the aqueous extract of the combination *Cassia alata*+*Phyllanthus amarus*, followed by *Cassia alata* extract. The lowest was also found in *Cochlospermum planchonii*. Saye extract displayed an appreciable level of flavonoids.

The concentration of flavonols was two times higher in *Cassia alata* (18.67±0.3 µg QE/mg) extract than in *Cochlospermum planchonii* (9.49±0.14 µg QE/mg) and *Phyllanthus amarus* (10.96±0.16 µg QE/mg). The content in Saye was very weak (3.87±0.1 µg/QE mg) (Table 1).

The highest content of tannins was displayed by *Phyllanthus amarus* extract (38.79±0.59 µg TAEs/mg) followed by Saye extract (35.56±0.54 µg TAEs/mg) and *Cassia alata*+*Phyllanthus amarus* extract (Table 1).

**Reducing power assay:** The reducing activity expressed in Trolox equivalent (µmol TE/mg) was ranked from 12.99 µmol TE/mg to 41.38 µmol ET/mg extract. The reducing power was found in the order Saye>CA+PA>PA>CP+PA>CP> CA+CP>CA (Fig. 2). The best activities were found for Saye, CA and PA extracts.

**Inhibition of lipid peroxidation:** The combined *Cassia alata*+*Phyllanthus amarus* extract displayed the highest inhibition (22.56%), of lipid peroxidation in liver homogenate followed by *Cochlospermum planchonii* (20.76%), *Cassia alata* (17.06%) and *Phyllanthus amarus* (16.96%) extracts. Low inhibition (5.06%) was observed with the whole Saye extract (Fig. 3).

## DISCUSSION

Complex organic molecules are synthesized and accumulated in plants as secondary metabolites which biological activity has been reported several times. These molecules are mainly composed of polyphenols, terpenoids and alkaloids which in plant extracts act synergistically or antagonistically and might explain the biological activity of medicinal plants. Several compounds exist in plant



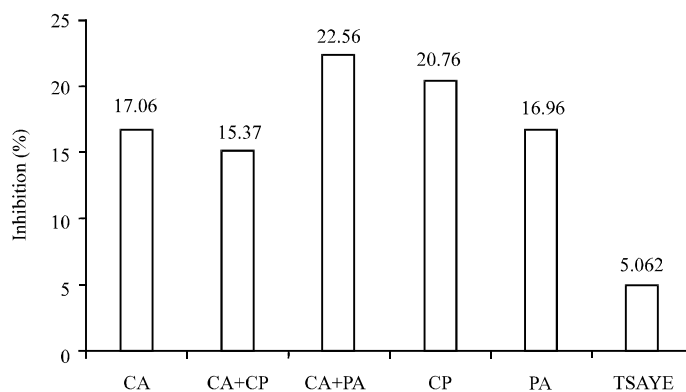


Fig. 3: Antioxidant activity of Saye based plant extracts on lipid peroxidation, CA: *Cassia alata*, CP: *Cochlospermum planchonii*, PA: *Phyllanthus amarus*, CA+CP: Combination *Cassia alata*+*Cochlospermum planchonii* (1/1), CA+PA: Combination *Cassia alata*+*Phyllanthus amarus* (2/1), CP+PA: Combination *Cochlospermum planchonii*+*Phyllanthus amarus* (2/1)

extract and when dealing with a polyherbal formulation comprising 2 or more plants, the number of compounds in the extract might probably be multiplied and thus improve the biological activity.

**Antiplasmodial activity of the plant extracts:** In a previous study, a decocted extract of the roots of *Cochlospermum planchonii* gave 45.5% antiplasmodial activity in mice (Yerbanga *et al.*, 2012), the macerated extract of *Cochlospermum planchonii* displayed higher activity than the decocted extract of the same plant material. *Cochlospermum planchonii* roots are traditionally used as boiled aqueous extracts. Temperature effect may explain the difference in activity observed between the boiled and the macerated extracts. High temperature was found to alter chemical compounds in plants (Bassene, 2012). In a clinical study, after 5 days of treatment using *Cochlospermum planchonii* root extract in patients suffering from uncomplicated *Plasmodium falciparum* malaria there was a 52% parasitological cure (Benoit-Vical *et al.*, 2003).

*Cassia alata* leaves were reported to possess several pharmacological properties (Dalziel, 1937; Villasenor *et al.*, 2002; Moriyama *et al.*, 2003; Hennebelle *et al.*, 2009; Chatterjee *et al.*, 2010; Veerachari and Bopaiah, 2012). Crude ethanolic extract from *Cassia alata* L. leaves previously tested for *in vitro* antiplasmodial activity against *Plasmodium falciparum*, exhibited 83% parasites inhibition at 25  $\mu\text{g mL}^{-1}$  and 53% inhibition at 12.5  $\mu\text{g mL}^{-1}$  (Zirihi *et al.*, 2005).

These results confirmed the previous moderate to good antiplasmodial activity obtained with the macerated aqueous extract of the leaves of *Phyllanthus amarus* on *Plasmodium berghei* (Dapper *et al.*, 2007).

Macerated aqueous extracts of *Phyllanthus amarus* were previously tested for antiplasmodial activity, instead of the decocted aqueous extracts, used in this study. Indeed, in previous studies the macerated aqueous extract of the leaves of *Phyllanthus amarus* gave 51.58% inhibition at 165.5  $\text{mg kg}^{-1}$  b.wt. against *Plasmodium berghei* (Dapper *et al.*, 2007) and 56.07% inhibition at 200  $\text{mg kg}^{-1}$  b.wt. on *Plasmodium yoelii* with the macerated whole plant extract (Ajala *et al.*, 2011).

The good to moderate antiplasmodial activity of Saye was already obtained by Traore *et al.* (2008) and Yerbanga *et al.* (2012).

**Antioxidant activity of the plant extracts:** Plant derived polyphenolic compounds such as flavonoids, anthocyanidins, flavanones and flavones commonly consumed in the diet are found to possess biochemical and antioxidant effects. Antioxidants protect cells against the damaging effects of Reactive Oxygen Species (ROS), such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Poljsak *et al.*, 2013) and in long term to neurodegenerative diseases such as Parkinson and Alzheimer's, cancer, aging, atherosclerosis, inflammations.

The reducing capability of an extract is a significant indicator of its potential antioxidant activity that is associated with the presence in the extract of polyphenolic compounds. The study investigated both the antioxidant activity of the extracts and their content in phenolics.

The FRAP assay is an easy method to evaluate the antioxidant activity of a plant extract. The root extract of *Phyllanthus amarus* was previously found to be a potent antioxidant (Maity *et al.*, 2013). Indeed, during the present investigation *Cassia alata* based extracts have shown the highest amount in flavonoids. In a previous study, the content in total flavonoids of the leaf extracts of *C. alata* was estimated at  $275.16 \pm 1.62 \mu\text{mol mL}^{-1}$  Quercetin Equivalents (QE) (Akinmoladun *et al.*, 2010). *Phyllanthus amarus* extract in our study had an higher total phenolic content compared to those presented by Poompachee and Chudapongse (2011) who have previously reported the total phenolic content of two species of *Phyllanthus*. The greater antioxidant activity by FRAP (Saye>CA+CP>PA) and inhibition of lipid peroxidation (CA+PA>PA) assays was obtained with *P. amarus* based extracts. It is the first time that the antioxidant activity ( $29.62 \pm 3.19 \mu\text{mol TE/mg}$ ) of *Cochlospermum planchonii* was demonstrated. The analgesic and anti-inflammatory activities of the plant were previously demonstrated by Paul *et al.* (2011). The plant is used to treat jaundice and hepatic disorders (Aliyu *et al.*, 1995) and this antioxidant activity may explain the activity of the plant.

**Antiplasmodial and antioxidant activities of the extracts:** Oxidative stress is involved in the pathophysiology of malaria and plants and compounds with antioxidant activity are believed to be an alternative source for malaria disease therapy. During the study, Saye and its constituent plants gave a significant antioxidant activity. The occurrence of flavonoids, flavonols and tannins in the studied plants is likely to be responsible of the antioxidant activity. These results suggest that Saye and its constituent plants are able to inhibit the parasites and also to protect against the oxidative damage induced by malaria parasite.

## CONCLUSION

The findings of the study corroborate the therapeutic indications of Saye. The results demonstrate that the decoction of Saye (the whole combination of the three plants) and the combination *Cassia alata*+*Cochlospermum planchonii* (1:1) are the best extracts to inhibit *Plasmodium berghei* parasites in mice. Macerated aqueous extracts of single *Cochlospermum planchoii* and single *Phyllantus amarus* exhibited a good to moderate antiplasmodial activity whereas decocted extracts of *Cochlospermum planchonii*, *Cassia alata* and *Phyllantus amarus* exhibited individually a moderate to weak antiplasmodial activity. Single and combined extracts from the three plants revealed more and less a good content in flavonoids, flavonols, tannins and total phenols that would justify the appreciable antioxidant activity of the extracts. For both, the antiplasmodial and antioxidant activity, this study was unable to demonstrate the pharmacological

superiority of combined extracts compared to single plant extracts. Regarding the total phenol content and the antioxidant activities of the extracts, Saye and its component plants are efficient antioxidants. SAYE may contribute to limit the expansion of oxidative stress during malaria and other infections.

The antioxidant and antiplasmodial activities demonstrated in the study confirm the multiple biological activities recognized to medicinal plants.

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