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Comparative study of leaves and root bark from *Securidaca longepedunculata* Fresen (Polygalaceae): Phytochemistry and antiplasmodial activity

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Abstract
The ant plasma dial activity of roots bark from *Securidaca ongepedunculata* was previously demonstrated in accordance with its ethno pharmacological use as antimalarial drug. Unfortunately, the uncontrolled harvesting of roots and massive destruction of *S. ongepedunculata* by uprooting is incompatible with the sustainable management of that antimalarial plant, particularly within Sahelian areas in the context of climate change. This paper intends to compare the phyto chemistry and antiplasmodial activity of roots bark and leaves from *S. longepedunculata* harvested during cold and warm period to recommend or not leaves instead of roots for malaria treatment. Phytochemical comparison was performed though spectrophotometric quantification of total polyphenol, flavonoid, alkaloid and DPPH radical scavenging test. Antiplasmodial bioassays were conducted *in vitro* by evaluating the viability of *Plasmodium falciparum* strains and the inhibition of ß-himation formation. The results showed that the methanol and chloroform leaves extracts exhibit a good inhibitory activity in strain K1 development with an IC_{50} as 2.2 and 2.6µg / mL respectively. The same type extracts of root barks show a moderate activity with an IC_{50} as 5.7µg/mL and 6.2µg/mL. Interestingly, the alkaloids were presented a best inhibition effect of strain K1 development with 1.05µg/mL as an IC_{50}. The similar observations were obtained concerning the strain 3D7 inhibition. The leaves extract and alkaloid inhibition activities on ß-hematin formation are respectively 56.3% and 69.17%. The biological activities were supported by the different metabolites like flavonoid, phenolic and alkaloids. The finding was that the leaves could substitute to the root uses in malaria treatment.

Keywords: Securidaca longepedunculata, *antiplasmodial activity; ß-hematin, polyphenol, flavonoid; alkaloid*

1. Introduction
The raw material source of many of populations for diseases treatment was still currently the plants. So, the knowledge of traditional medicine was a source of discovery of new active principle against a big number of diseases [1, 2]. Among of 2067 species of well-known plants in Burkina Faso, 50% were used traditionally by the populations [3]. The most commonly health problems that were concerned with this medicinal plants are in general: infections/infestations, digestive system disorders and genitourinary disorder [3]. Ethno botanical studies in the west regions of Burkina Faso (Cascade, Haut-Bassin, Mouhoun), revealed that many antimalarial plant species were endangered singularly *Securidaca longepedunculata*. Its roots were many used in medicinal preparations by population [4, 5]. Again this plant is used in others countries in the treatment of anthrax, stomach pain and abdominal problems schistosomiasis fever; chronic stomach pain; headache; vaginal itching, malaria, pneumonia, skin infections, skin cancer, toothache, tuberculosis and typhoid fever [6, 9]. In previous phytochemical investigation, the presence of quercetin, rutin, apigenin, gallic, chlorogenic, coffee, cinnamic and p-coumaric acids were reported in the root barks extracts [10]. Some secondary metabolite groups like alkaloids, flavonoids, saponins and tannin were detected in root bark extracts [11]. The present study aimed to compare the phytochemical profile and the antimalarial effect of *S. longepedunculata* roots barks and leaves organic extracts. The data could be a best strategy to develop the leaves uses in malaria medicinal treatment and *S. longepedunculata* protection management.

2. Materials and Methods

2.1. Chemicals

All solvents used in this study were analytical grade. RPMI-1640 (liquid without L-Glutamine), NaOH, Hypoxanthine, Albumax II, Hepes buffer, L-glutamine, Gentamicin, 3-acetylpiridine adenine di-nucleotide (APAD), Nitro Blue Tetrazolium (NBT), Phenazine ethosulfate (PES), Lithium L-Lactate, Trizma base, Hemin chloride, Triton X100, Chloroquin, Atropine, Bromocresol green (BCG) and Folin-Ciocalteu reagent (FCR), 2,2-diphenyl-1-picrylhydrazyl (DPPH) were provided from Sigma-Aldrich.

2.2. Plant material

Roots bark and leaves from *Securidaca longepedunculata* Fresen (Polygalaceae) were harvested in Badara locality (Region of "Haut Bassin") in December 2013 (Cold season) and April 2014 (Warm season). Voucher specimen (CI: 16713) was deposited in the herbarium of "Université de Ouagadougou", Burkina Faso. Collected plant materials were dried for one week in the laboratory (25°C) and ground into fine powder.

2.3. Parasitic strains

K1 and 3D7 *Plasmodium falciparum* strains were provided by the Parasitology Laboratory from the London School of Hygiene and tropical Medicine, England. Parasitic strains maintained in continuous *in vitro* culture according to the methodology described by Trager *et al*. [12] Culture medium was made of RPMI 1640 medium supplemented with 1% Albumax, 0.01% Hypoxanthine, 2% Hepes buffer, 2% L-Glutamine and 0.5% Gentamicin. Sterile flasks, containing culture medium, washed O^+ erythrocytes (4% final hematocrit) and parasites (1 to 2% starting parasitemia), were incubated for 24 hours at 37 °C in a CO_2 incubator (2% O_2, 5% CO_2, 93% N_2 and 95% humidity, HERA cell 150). Supplemented media was renewed daily and subculture made when the parasitemia reached 6%.

2.4. Extracts preparation

Powdered leaves and root barks from *S. longepedunculata* were defatted with petroleum ether in a Soxhlet apparatus and residual powder macerated (16 h, continuous agitation) with methanol or chloroform (500 mL) to get methanol and chloroform extracts. Methanol and chloroform extracts were used for phytochemicals determination, antiradical assay, β-hematin formation and antiplasmodial bioassays. The remains were then dried and alkalinized (NH4OH 30%) before maceration with dichloromethane (16 h, continuous agitation). Dichloromethane extract was concentered and extracted using sulfuric acid solution (pH 3). Aqueous layer was then alkalinized (NH4OH 30%) until pH 8 and extracted with dichloromethane. The dichloromethane layer was evaporated in a vacuum to yield the total alkaloid extract. Total alkaloid extract was used for the β-hematin formation and antiplasmodial bioassays.

2.5. Secondary metabolites content determination

Total polyphenol was determined according to the colorimetric method of Folin–Ciocalteu [13]. Plant extract (25 µL, 100 µg/mL in methanol) was mixed with Folin–Ciocalteu Reagent (125 µL, 0.2N) and 5 min later, with sodium bicarbonate (100 µL, 75 g/L). After incubation (1 h, room temperature), absorbance was measured at 760 nm against a methanol blank. Gallic acid (0 – 100 mg/L) was used to generate a standard calibration curve ($y = 0.0063x + 0.1053$ $r^2 = 0.99$) and total polyphenol content expressed as mg Gallic acid equivalent to 1 g of plant extract (mg GAE/g).

Total flavonoid was estimated according to the Dowd method as adapted by Arvouet-Grand *et al*. [14]. Plant extract (75 µL, 100 µg /mL) was mixed with Aluminium trichloride (75 µL, 2%). Absorbance was subsequently read at 415 nm after incubation (10 min, room temperature) against a methanol blank. Quercetin was used to plot a standard calibration curve ($y = 16.819x + 0.0898$, $r^2 = 0.99$) and total flavonoid content expressed as mg of Quercetin equivalent to 1g of extract (mg QE/g).

Total alkaloid was determined according to previous described procedure [15]. Briefly, 1 mL of plant methanol extract (40mg/mL in HCl 2N) was filtered washed with chloroform (10 mL) and the pH readjusted to neutral with Na OH (0.1N). 5mL of Bromo cresol green (69.8mg in 3mL of 2N Na OH and water to 69.8mg/L), 5mL of phosphate buffer (2M) were added. The mixture was shaken vigorously, and complex extracted with 1, 2, 3 and 4 mL of chloroform by vigorously shaking. Chloroform layer was collected and completed to 10 ml. Absorbance was read at 470 nm against the blank prepared as above but without extract or atropine. Atropine was used to plot a standard calibration curve ($y = 0.0014x-0.0018$, $r^2= 0.98$) and total alkaloid content expressed as µg of Atropine equivalent to 1 g of plant extract (µg AE/g).

2.6. Scavenging of DPPH radical test

Extracts capacity to inhibit DPPH radical was evaluated [16]. Briefly, in a 96 micro-well plate, 200 mL of DPPH (20 mg/L) and 100 mL of sample from successively dilution of initial concentration (1mg/mL) were incubated in dark for 15 min, following a reading at 517 nm (Bio Tek Instruments, USA). The data expressed in concentration scavenging 50% of free radicals (IC50) using the curve of anti-radical activity as a function of the extract concentrations.

2.7. Antiplasmodial activity determination

2.7.1. Inhibition of *P. falciparum* proliferation

Antiplasmodial activity was determined according to Makler *et al*. method [16]. Test sample was mixed within culture medium (O^+ blood: 2% hematocrit, parasitemia: 1.5%). After incubation (72 hours, 37°; 5% CO_2, 2% O_2, 93% N_2 and 95% of humidity) parasite growth was measured with Malstat reagent (Distilled water: 160 ml, Triton X-100: 200 µl, Lithium L-Lactate: 2 g, Trizma base: 660 mg APAD: 66 mg, pH 9 with 1 M HCl) and NBT/PES solution (Distilled water: 100 mL, NBT: 160mg, PES: 8 mg). The absorbance was recorded at 650nm against a blank (culture medium without test sample). Data were expressed as inhibition percentage of parasite growth and calculated by the equation: Inhibition (%) = $[1-(Abs_{test} - Abs_{blank}) / (Abs_{control})] \times 100$. Concentration inhibiting 50% of the parasite growth (IC_{50}) was determined through software Table Curve 2D version 5.0. Chloroquine was used as positive control.

2.7.2. ß-hematin formation inhibition test

The inhibition of β-hematin formation was assessed according to literature [17, 18]. Freshly prepared hemin chloride solution (100 µL, 0.5mg/ mL in DMSO) was mixed with sodium acetate buffer (200 µl, 0.5M in water, pH4.4) and test sample at 100µg/mL in mixture. Test mixture was incubated (37°C for 18 to 24h) and centrifuged (4000 rpm for 10 min) to discard supernatant. Total pellet was suspended in DMSO

(400 µL) and centrifuged (4000 rpm for 10 min) to dissolve remaining hemin chloride. β-hematin pellet within tubes is solubilized with NaOH (400 µL, 0.1M). The negative control contains no inhibitor but water. The absorbance of β-hematin solution is read at 405nm. Data were expressed as the percentage of inhibition of β-hematin formation and calculated by the equation: Inhibition (%) = [(Abs $_{control}$ - Abs $_{test}$) / (Abs $_{control}$)] × 100. The concentration inhibiting 50% of the β-hematin formation (IC_{50}) is determined through software Table Curve 2D version 5.0.

2.8. Statistics
Results was expressed as mean value of three (n=3) independent experiments ± standard deviation. Table curve 2D v.2 was used to determine IC50. Statistical analysis of data was performed with the XLSTAT pro 7.5. ANOVA $p<0.05$.

3. Results and discussion
3.1. Phytochemical data
As shown in the Figure 1a, chloroform extracts from root bark (warm and cold periods) exhibited higher amount of total polyphenol than chloroform extracts from leaves (warm and cold periods) on the contrary of methanol extracts. Moreover, chloroform extracted more poly phenolic compounds in plant materials collected in cold period than leaves and root bark harvested in warm season, in the opposite of methanol used as extraction solvent. Interestingly, the same scheme is observed with the total flavonoid content of plant materials (Figure 1b). Taking in account chloroform extracts from leaves, flavonoid content was slightly higher in cold period (25.51 ± 1.08 mg QE/g) than in warm period (21.44 ± 0.78 mg QE/g) while methanol extracted more flavonoids in leaves from warm period (40.96 ± 0.19 mg QE/g) than leaves collected in cold period (28.74 ± 0.39 mg QE/g). Meanwhile, the spectrophotometric method used did not allow flavonoids quantitation in root bark methanol and chloroform extracts. Previously, it was demonstrated that *S. longepedunculata* root bark methanol aqueous extracts contained 9.86 mg gallic acid equivalents/ g dw and 5.85 mg catechin equivalents/ g dw of total phenolic and total flavonoid compounds respectively [10]. Within dry season (November to May), plant materials (leaves and root bark) harvested in the cold period (December) contain more non-polar total polyphenols than those collected during the warm period (April). This finding constitutes some preliminary data on the phytochemical pattern of leaves and root bark from *S. longepedunculata* regarding climatic conditions (Temperature, rainfall). Previous studies highlighted that root from *S. longepedunculata* harvested in the dry season contained more alkaloids than those collected in the rainy season [19]. Considering the total alkaloid content from leaves collected during cold and warm periods (Figure 1c) no significant difference was noticed (246.43 ± 20.62 and 252.38 ± 17.86 µg AE/g).

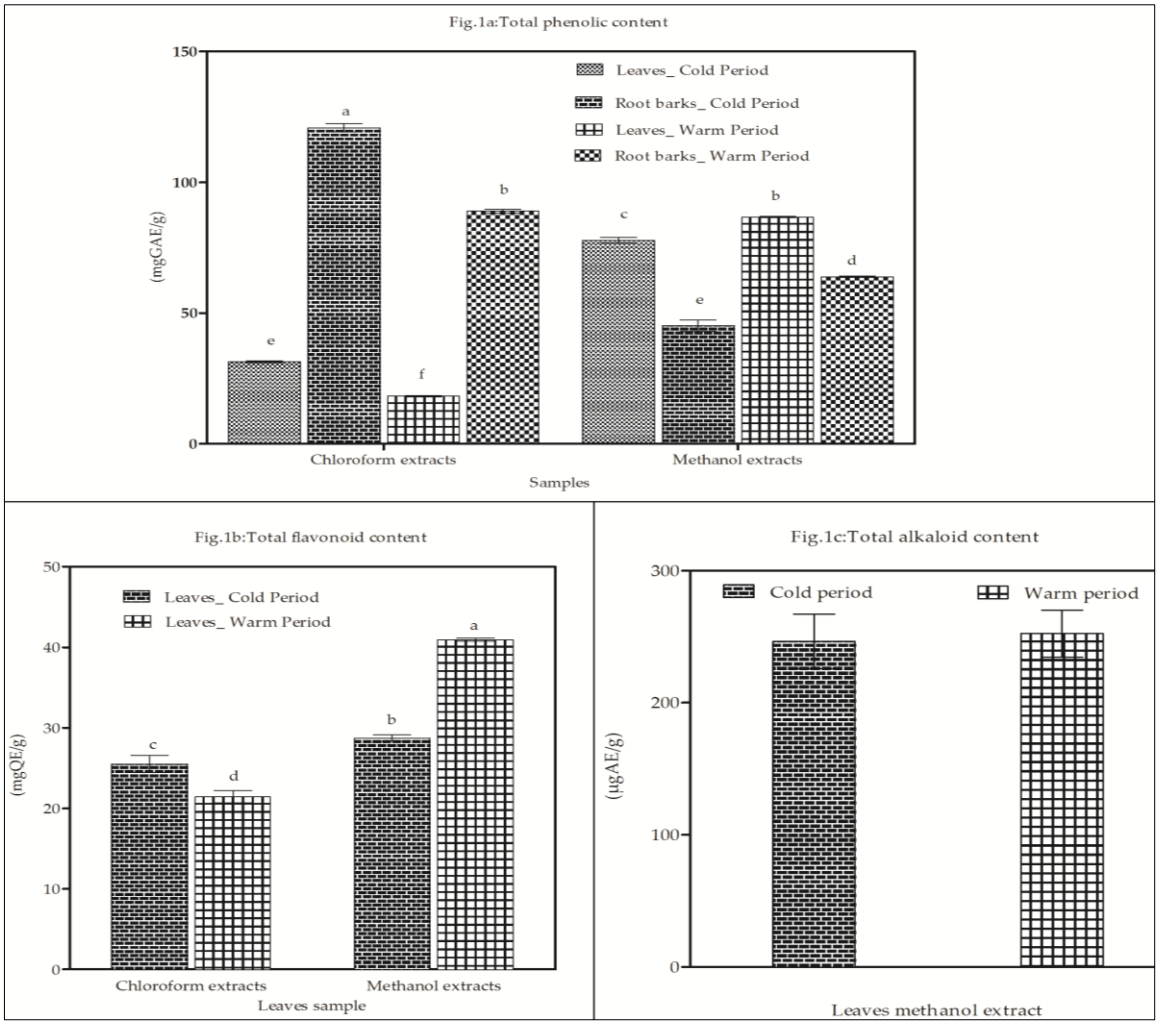

Fig 1: Total polyphenol, flavonoid and alkaloid content from *S. longepedunculata*. Data followed by the same letter (a-f) are not significantly different at $p<0.05$

3.2. Inhibition percentage of DPPH radical

The results of DPPH radical scavenging activity are summarized in figure 2. Using the chloroform, the extract from the roots bark of cold period (42.22 µg/mL) are more active than extract from root bark of warm period (81.11µg/mL), in the opposite of methanol used as extraction solvent. On the other hand, the chloroform extract of the leaves (warm and cold period) showed no activity, yet, extract by methanol, leaves from warm period are more active (IC_{50}=74.44 µg/mL) than those of cold period leave (IC_{50}=93.33 µg/mL). The antioxidant activity and phenolic content showed a good correlation with R^2= 0.98 as coefficient correlations. Muanda *et al.* were found that root (aqueous methanol) extract from *S. longepedunculata* show 5.5µg/mL as IC_{50} [10].

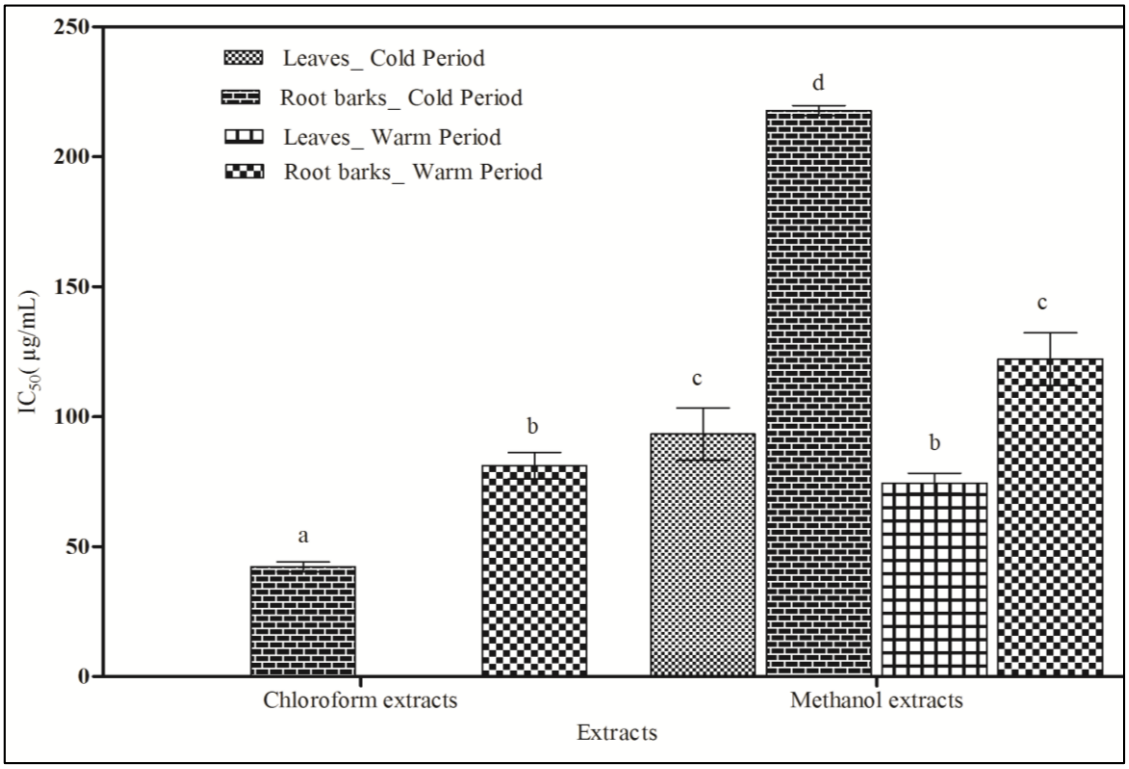

Fig 2: Antiradical DPPH activity of *S. longepedunculata*. IC50: Concentration of plant extract inhibiting 50% of DPPH radical. Data followed by same letter (a-d) are not significantly different at $p<0.05$

3.3 Antiplasmodial activity

3.3.1 Inhibition of P. falciparum grown

The antiplasmodial activity of *S. longepedunculata* extracts on chloroquine-resistant and chloroquine-susceptible strains are shown below (Figure 3). The leaves extracts (all period, any solvent) were twice active than root barks extract on chloroquine-resistant strain K1 (Figure 3a). The leaves extracts showed high activity (IC_{50} < 5 µg /mL) while the roots bark extract showed moderate activity according to Deharo *et al.* classification [20]. The leaves alkaloid extract has showed the high activity (IC_{50} = 1.05±0.13 µg /mL). All extracts from leave and root bark (warm and cold period) showed high activity on the susceptible strain 3D7 (figure 3b). The polar compounds of leaves and root bark (warm and cold period) showed an IC_{50} less than 2 µg / mL, contrary of non-polar compound which only, those from the leaves extract (warm and cold period) showed an IC_{50} less than 2 µg/mL. These two strains differ in terms of sensitivity by the presence of a chloroquine resistance gene (*pfcrt*) in the strain K1 [21]. This gene, encoding a transporter located on the membrane of the digestive vacuole causes an efflux of chloroquine [22]. The difference in susceptibility of strain K1 and 3D7 to the extracts would due to the action of this gene. While, the resistant strain K1 was more susceptible on alkaloid extract than susceptible strain 3D7. Some authors have showed that mutated gene that confer resistance to the parasite could make it susceptible to other drug [21].The antiplasmodial activity of the alkaloids would be due to nitrogenous heterocycle [23].

Recently, some studies showed that Methanol and chloroform roots extract of *Securidaca longepedunculata* were able to inhibit strain W2 proliferation [24]. Others authors were showed that roots petroleum ether extract were exhibited 7.34 µg/mL as an IC_{50} on chloroquine resistant K1 strains [25]. Bah *et al.*, reported that dichloromethane leaf extract of *S. longepedunculata* showed an IC_{50} = 6.9µg / mL on the chloroquine-sensitive 3D7 strain [26]. Indeed, studies have shown that in addition to having antiplasmodial activity, flavonoids can enhance the effectiveness of other molecules by increasing their uptake or by decreasing their efflux out of the cell [22].

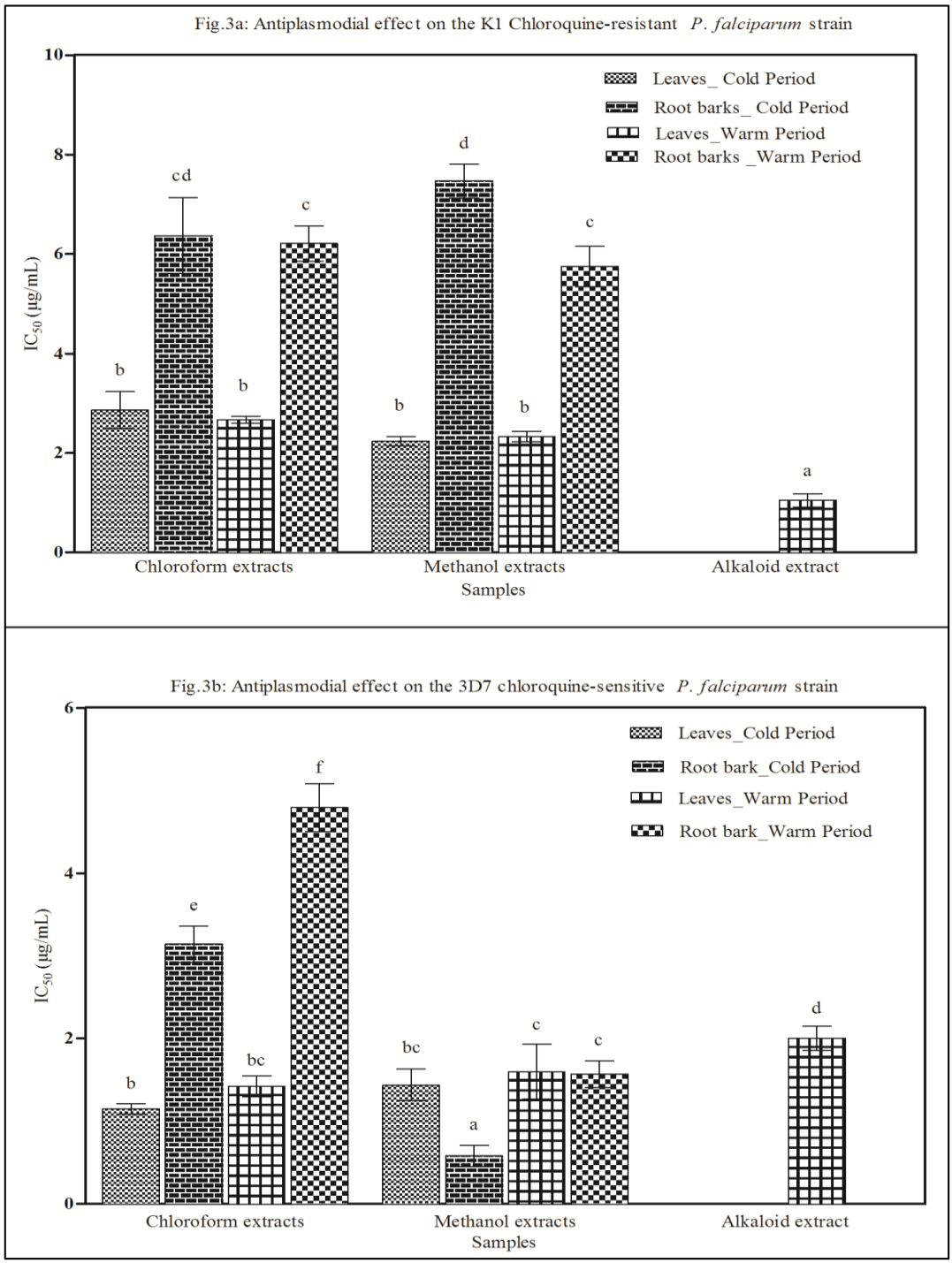

Fig 3: Antiplasmodial activity on chloroquine- resistant and sensitive strains. IC_{50}: The concentration inhibiting 50% of the parasite growth. Data followed by same letter (a-f) are not significantly different at $p<0.05$

3.3.2 Inhibition percentage of ß-hematin formation

Inhibition of Hemozoin formation is a validated target for most of the well-known existing antimalarial drugs and was a suitable target to develop some new antimalarial [27, 28]. Methanol and chloroform extracts of the leaves inhibited β-hematin formation at 53.4 and 56.63% respectively while using root bark, the activity was only 38.39% with methanol extract and 27.65% with the chloroform extract (Figure 4). Among the extracts, the leaf alkaloids showed the greatest inhibitory activity (69.19%) although that of the chloroquine used as a reference was 91.50% (Figure. 4). The chloroquine resistant strain K1 is most susceptible to leaves and alkaloid extract and it is these extracts that have the best inhibitory activity on β-hematin formation. The combination of these two extracts activities would be in accordance with the relationship that exists between the effect of hemozoin inhibition and the antiplasmodial IC_{50} like as suggested by some authors [29].

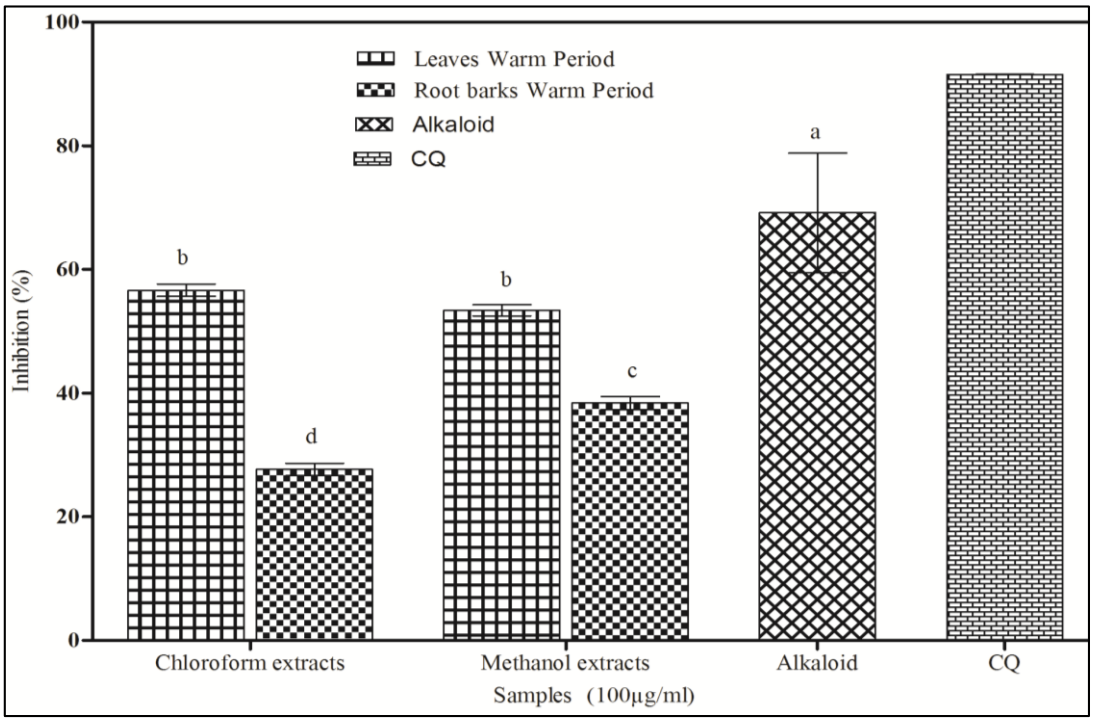

Fig 4: Inhibition of β-hematin formation. The data were expressed as the percentage of inhibition. Data followed by same letter (a-d) are not significantly different at $p<0.05$. CQ: chloroquine

4. Conclusions

This study confirmed that *Securidaca longepedunculata* extracts contained many bioactive compounds that can be effective to fight malaria. Leaves extracts, and alkaloids showed high activity on the chloroquine-resistant strain K1 while root extracts were only moderately active on this strain. Leaf alkaloids and leaf extracts inhibited about 70% and 56% β-hematin formation respectively while root bark inhibited less than 40%. Importantly, this finding was an interesting high light of traditional medicine uses. The next word will be the isolation of active compounds in Methanol and Chloroform fractions of leaves to promote the leaves used in malaria treatment and the best management exploitation of *Securidaca longepedunculata*.

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