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Antiplasmodial activity of the leaves and stem bark *Ocotelea procera* and *Alstonia boonei*

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ABSTRACT

There is an urgent need for the development of effective anti-malarial drugs due to emergence of resistant strains of *P. falciparum*. Medicinal plants represent a plethora of compounds to achieve this feat. The stem and leaf extracts of *Alstonia boonei* and *Carapa procera* are used in various traditional medicines for the treatment of malaria. In the present study, the petroleum ether and hydro-alcoholic extracts of these two plants were tested in vitro on chloroquine sensitive (3D7) strains of *Plasmodium falciparum* for their anti-malarial activity. Growth inhibition was determined in vitro by counting Giemsa-stained parasites by light microscopy. The petroleum ether extract of the leaves and stem bark of *A. boonei* were both inactive ($IC_{50} > 100 \mu\text{g/ml}$). Their soxhlet ethanol extracts were also inactive with $IC_{50} > 100 \mu\text{g/ml}$. However, the leaf extract, obtained by cold maceration, showed weak activity ($IC_{50} = 71.24 \mu\text{g/ml}$) whereas that of the stem was $88.15 \mu\text{g/ml}$. The petroleum ether extract of the stem bark of *C. procera* inhibited the growth of the chloroquine sensitive (3D7) *Plasmodium falciparum* parasite with IC_{50} value of $19.52 \mu\text{g/ml}$ and the soxhlet extracted ethanol extract giving $IC_{50} = 11.41 \mu\text{g/ml}$. Column chromatography of the bioactive soxhlet ethanol extract afforded fractions with significant antimalarial activities ($IC_{50} < 10 \mu\text{g/ml}$). The present study has revealed that the leaves and stem bark of *A. boonei* show weak antiplasmodial activity. Extracting the constituents of *A. boonei* by cold maceration retains considerable antiplasmodial activity. The stem bark of *C. procera* however, showed significant antiplasmodial activity. Chromatographic fractionation afforded more potent antiplasmodial fractions.

Keywords: Antiplasmodial, *Carapa procera*, *Alstonia boonei*, column chromatography, TLC, parasitaemia

INTRODUCTION

Malaria contributes substantially to the poor health situation in Africa. It is on record that, Sub-Saharan Africa accounts for 90% of the world's 300 – 500 million cases and 1.5 – 2.7 million deaths annually. About 90% of all these deaths in Africa occur in young children. Between 20 and 40 percent of outpatient visits and between 10 and 15 percent of hospital admissions in Africa are attributed to malaria [1]. It is a massive problem, which plagues all segments of the society.

On a continent-wide basis, malaria has severe socio-economic effect through increased poverty, impaired learning and decreasing attendance of school and work, as well as direct costs that include a combination of personal and public expenditures on both prevention and treatment of the disease [2]. The cost of treating malaria, in Ghana, amounted to US \$772.4 million in 2009. This amount, that equaled to Ghana's entire health budget for 2008, represents 10% of the country's entire Gross Domestic Product for 2006 [2].

Self prescribed medication is a widespread phenomenon in Ghana. Majority of the malaria victims only seek medical examination and treatment from health facilities when the initial attempts have failed resulting in late presentation [3-4]. Very often malaria treatments in Ghana occur at home with only a few of such home-based treatments being correct and complete. Accessibility to orthodox medical treatment in Ghana is low with per capita outpatient visit of 0.46 in 2000 [5].

The search for new anti-malaria drugs remains indispensable in the face of resistant strains of *P. falciparum*. One of the most promising targets in the search for new anti-malaria drugs is the large repository of medicinal plants used in the treatment of malaria in traditional societies. The first anti-malaria drug provided by ancestral treatment was quinine, derived from the bark of the Peruvian *Cinchona* tree [6]. Despite the cost and adverse effects, a standard treatment for severe malaria in Africa and in Europe is still the intravenous administration of quinine [7]. Still now, resistance against quinine in Africa is absent.

The world's poorest are the worst affected by the disease, and many treat themselves with traditional herbal medicines. These traditional herbal remedies are often more available and affordable, and sometimes are perceived as more effective than conventional anti-malaria drugs. Some Ghanaians living in rural areas depend on traditional herbal medicine for treatment of many infectious diseases including malaria. The reputed efficacies of these plants have been experienced and passed on from one generation to the other. Apparently, lack of scientific proof of efficacies claimed by traditional medical practitioners in Ghana called for this study. The present research was aimed at investigating the anti-plasmodial activity (*in vitro*) of two Ghanaian medicinal plants, *Carrapaprocera* DC (Meliaceae) and *Alstonia boonei* De Willd (Apocynaceae), used in ethnomedicine for the treatment of malaria and also explore the effect of different methods of extraction on the antiplasmodial activity.

MATERIALS AND METHODS

Plant collection and identification

The stem bark of *Carapa procera* DC (Meliaceae) and the leaves and stem bark of *Alstonia boonei* De Willd (Apocynaceae) were harvested from a farm in Effiduase, a town in the Sekyere-East district of the Ashanti Region. The plants were identified by Dr. George Henry Sam of the herbarium section of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical sciences, Kwame Nkrumah University of Science and Technology.

Plant material processing and extraction

The leaves and stem barks were initially screened for foreign matter and air dried for seven days. The materials, thus dried, were coarsely milled and packed into a brown paper bag and kept in the laboratory until required for use.

Petroleum ether and ethanol (70%) were the main solvents used for the extraction of the plant materials. They were supplied from B.D.H. Ltd (England) and were of the British pharmacopoeia (BP) grade. 65.5g of coarsely powdered stem bark of *C. procera* and 72.5g of *A. boonei* leaves and stem bark were soxhlet extracted successively with petroleum ether and 70% ethanol for over 48hours until the material was completely exhausted. Each extract was concentrated under reduced pressure to a small volume by means of rotavapor (R-114, Buchi, Switzerland) at a temperature of 50°C. Similar weights of powdered plant materials were extracted again by cold maceration. The extracts were kept in a desiccator for subsequent bioassays and phytochemical analysis.

ANTI-MALARIA ASSAY

In vitro cultivation of malaria parasite

Frozen laboratory strains of *P. falciparum* (chloroquine sensitive 3D7 strain) were cultured according to the method described by Houtet *al.*, [8] with slight modifications. Parasite vials were taken from a liquid nitrogen tank, thawed quickly in a water bath set at 37 °C. The vials were spun 10 minutes at 2000rpm and the supernatant discarded. An equal volume of thawing mixture (3.5% NaCl in distilled water) was added to each of the pellet, thoroughly mixed and spun at 2000rpm for 10 minutes. The pellets were gently disengaged and 1ml of complete parasite medium (5ml of L-glutamine, 2.5ml of 10mg/ml and 50ml Albumax in 500ml of RPMI 1640) was added and span again at 2000rpm for 10 minutes. This washing step was repeated and the supernatant discarded. The parasites were suspended in 25ml culture flask (BD Falcon) containing 200µl freshly prepared pack RBC (sickling negative; O rhesus positive) and 5ml of complete parasite medium to have a haematocrit of 4%. The culture was then gassed with a special gas mixture (2% Oxygen, 5.5% Carbon dioxide and 92.5% Nitrogen) for 30 seconds for 25ml culture flask or 90 seconds for 75ml culture flask. The flasks were quickly closed and placed in an incubator (RS Biotech) set at 37°C. The spent culture medium was changed daily using fresh complete parasite medium. Spent complete parasite medium was aspirated out from the culture flask using pipette and each time a thin smear was prepared on microscope slides under sterile conditions in the laminar flow safety cabinet (Hitachi Clean Bench, Japan). The

slides were fixed in absolute methanol, stained with 10% giemsa in phosphate buffer for 10 minutes and after adding a drop of immersion oil (Fluka, BDH England) examined using 100X magnification light microscope to check the level of parasitaemia, growth stages and viability of the parasites. The culture flasks were then sent back into the incubator after adding appropriate amount of complete parasite medium and gassing (Houtet *al.*, 2006). The parasitaemia was determined as below:

$$\% \text{ Parasitaemia} = \frac{\text{number of infected RBCs}}{\text{total RBC count (IRBCs + URBCs)}} \times 100$$

Where: IRBCs = RBCs infected with *P. falciparum*

URBC = RBCs not infected with *P. falciparum*

At a parasitaemia of about 5%, subcultures (of the 3D7) were made by adding known amount of fresh sickling negative O rhesus positive (O⁺) RBCs to the culture to reduce the parasitaemia and always maintaining 200ul or 1ml of the pack red cells in 25ml or 75ml culture flask respectively. The parasites were maintained in continuous culture to obtain a stable parasitaemia before they were used for the efficacy assay.

Incubation of Parasites with Plant Extracts

Thin smears were prepared and the level of parasitaemia determined. The parasites were then diluted with non-infected O⁺ RBC to obtain 1% parasitaemia. Complete parasite medium was then added to the culture in the culture flask to have a haematocrit of 4.44%. Nine hundred microlitres (900ul) aliquots of the culture at 1% parasitaemia were separately dispensed into each of twenty-four micro titre plates at haematocrit. One hundred microlitres (100ul) of the prepared plant extract at the various dilutions (100-0.09mg/ml) were then added to the labelled wells except the first 2 wells which served as positive controls and the last 4 in order to observe the effect of the drug on normal RBC while the remaining 2 wells served as drug negative controls while the remaining two wells served as non-drug negative controls. Standard antimalarial drug artesunate was tested at dilutions between 0.000048-12.5µg/ml. Assays were performed in duplicates and under aseptic conditions. In all the cases, the addition of 100 ul/medium to the culture in the microtitre plate brought the haematocrit to 4%. The micro titre plates were covered and placed in a clean modular incubator chamber (California, USA) tightly closed and gassed for 6 minutes. The chamber containing the plates were then placed in the incubator previously set at 37°C for 48 hrs. After 48 hours, cultures were removed from incubator and 2 thin smears prepared from each of the wells on double frosted microscope slides (Fisher, USA). Slides were air-dried, fixed with methanol and stained with 10% giemsa in phosphate buffer (Fluka, BDH England). The slides were then examined using the light microscope at 100X magnification after a drop of immersion oil was added.

The same procedure was used for the fractions H2B, H2C, H2D and H2E except that they were tested at dilutions between 0.03 and 31.25 µg/ml.

Column chromatography

Silica gel 60 (70-230 mesh ATSM) was used as the stationary phase for the column. The packing of the column was done by the dry method while gradient elution was employed in developing the column.

Detection for analytical thin layer chromatography

The zones on TLC plates corresponding to separated compounds were detected under U.V light 254nm and 365nm and also by spraying with anisaldehyde 0.5% w/v in HOAc-H₂SO₄-MEOH (10:5:85) followed by heating at 105°C for 5-10 minutes.

Column chromatographic fractionation of extract

90g of silica gel 60 (70-230 mesh ASTM) was packed dry into a glass column (90cm × 5cm). 15g of the soxhlet extracted stem bark extract was dissolved in about 50 ml of ethanol (90%) and mixed with 30g of silica gel 60 (70-230 mesh ASTM), allowed to dry to attain the same consistency as the silica in the column, and spread gently on top of the packed column. The column was eluted with a gradient of Pet-ether, chloroform and methanol. Aliquots of 50ml each were collected based on the polarity of the eluent. By means of thin layer chromatography (TLC), the profiles of the aliquots were determined employing anisaldehyde reagent as detecting agent. Similar fractions were bulked together based on the TLC profiles resulting in four bulked fractions (H2A, H2B, H2C and H2D). The fractions were subjected to thin layer chromatographic studies to ascertain some compounds which may be present. The identification of major chemical groups was carried out by TLC on silica gel 60 F254 Merck (layer thickness 0.25 mm) as follows; for polyphenols (flavonoids), *n*-butanol/acetic acid/water 4:1:5 (top layer) was used as solvent system, 5% vanillin (BDH laboratory) in concentrated HCl as detecting reagent [9]. The developed chromatogram

was exposed to fumes of ammonia and observed under UV at 365 nm. Flavanols give a blue-green fluorescence whereas catechins and proanthocyanidins form a pinkish red colour after spraying with vanillin in Conc. HCl[9]. Alkaloids were detected with Dragendorff's reagent and chloroform/ acetone/diethylamine: 5:4:1 was used as mobile phase. Anthraquinones detected with NaOH 10% using ethylacetate/methanol/water: 8:1:1 as mobile phase. Coumarins were detected under UV (366 nm) by their blue fluorescence which becomes intense after spraying with KOH 10%. Terpenes or steroids were identified with Liebermann–Burchard test [10].

STATISTICAL ANALYSIS OF DATA

Growth inhibition due to each extract defined as the difference between the % parasitaemia of each treatment group and the corresponding positive control was calculated as follows [11].

$$\% \text{ Growth inhibition} = \frac{(\% \text{ Parasitaemia CIRBC} - \% \text{ parasitaemia DIRBC}) \times 100\%}{\% \text{ parasitaemia CIRBC}}$$

Where CIRBC = % parasitaemia of infected RBC without extracts i.e. control

DIRBC = % parasitaemia of infected RBCs incubated with extract or standard drug

Total parasitaemia over the 48 hour period was calculated in arbitrary unit as the area under the curve (AUC). Differences in AUCs were analyzed by one way analysis of variance (ANOVA) followed by Student-Newman-Keuls' *post hoc t* test.

Doses and concentrations responsible for 50% of the maximal effect (IC₅₀) for each drug/extract were determined using an iterative computer least squares method [12-13]. Using regression equations of best fit of plotted growth inhibition versus concentration curves, the IC₅₀ of each plant extract against each of the parasite strains were obtained. Graph Pad Prism for Windows version 5.0 (Graph Pad Software, San Diego, CA, USA) was used for all statistical analyses. *P* < 0.05 was considered statistically significant [14].

RESULTS

Antimalarial activity was determined *in vitro* by counting GIEMSA-stained parasites by light microscopy [15]. The plant materials were extracted by cold maceration and also by soxhlet extraction using petroleum ether and 70% ethanol. This was to investigate the effect of heat on the secondary metabolites which may be responsible for the antiplasmodial activity (if any) as well as the appropriate solvent for the extraction of the bioactive constituents towards further development of appropriate dosage form. The yields of the various extracts are shown Table 1.

Table 1: Percentage yield of extracts of *A. boonei* and *C. procera*

Solvents	% yield of extracts (%/w)		
	<i>A Boonei</i> stem bark	<i>A boonei</i> leaves	<i>C procera</i> stem bark
Pet-ether	PS1= 2.51	PSL = 4.1	PS2=3.6
Hot ethanol (70%)	H1 =7.2	H3 =11.1	H2=8.81
Cold ethanol (70%)	C1 = 6.8	C3= 8.7	C2=6.51

Antiplasmodial activity of Alstonia boonei extracts

Petroleum ether extracts of the leaves and stem bark (PSL and PSS) as well as soxhlet extracted (ABLs and ABSs respectively) and cold macerated (ABLc and ABSc respectively) 70% ethanol extracts of the leaves and stem were tested for their antiplasmodial activity at concentrations of 0.09-100 mg/ml. The extracts showed a dose dependent chemo suppressive with varying degrees of growth inhibition against the *Plasmodium falciparum* parasite. The pet-ether extracts of the leaves and stem as well as the soxhlet extracted and the cold macerated ethanol stem bark extract of *A. boonei* were practically inactive (IC₅₀>100 µg/ml) (Table 2). However, the cold macerated ethanol extracts of the leaves (ABLc) and stem bark (ABSc) showed some considerable level of activity (Table 2). The cold macerated ethanol extract of the leaves of *A. boonei* showed the highest antimalarial potential (IC₅₀=71.24 µg/ml) (Table 2).

Table 2: *In vitro* antiplasmodial activity of extracts of *A. boonei*

Extracts	IC ₅₀ /μgml ⁻¹
PSL	>100
ABLs	>100
ABLc	71.24
PSS	>100
ABSs	>100
ABSc	88.15

Petroleum ether extracts of the leaves and stem bark (PSL and PSS);soxhlet extracted ethanol leaf and stem extracts (ABLs and ABSs respectively); cold macerated ethanol leaf and stem extracts (ABLc and ABSc respectively). IC₅₀<10 μg/ml (good activity); IC₅₀>100 μg/ml (inactive); IC₅₀ between 10–50 μg/ml (moderate activity); and IC₅₀ of 50–100 μg/ml(low activity) (Bascoet al., 1994).

Antiplasmodial activity of *Carapa procera* extracts

Petroleum ether (CPP) and ethanol (soxhlet extracted CPE₁ and cold macerated CPE₂) stem bark extracts of *Carapa procera* were tested at concentrations of 0.09-100 mg/ml. The dose dependent chemo-suppressive effect of the extracts at the various doses employed is shown in Figure 1. There was total clearance of the parasites at concentrations greater than 25mg/ml for the petroleum ether extract and the hot ethanolic extract. The total parasitemia is represented by the area under the curve (AUC) shown in Figure 1b. The extracts showed considerable degree of growth inhibition against the *Plasmodium falciparum* parasite. The soxhlet extracted ethanol extract (CPE₁) showed a significant anti-malarial activity (p<0.001) followed by the pet-ether extract and the cold macerated ethanol extracts respectively (Table 3).

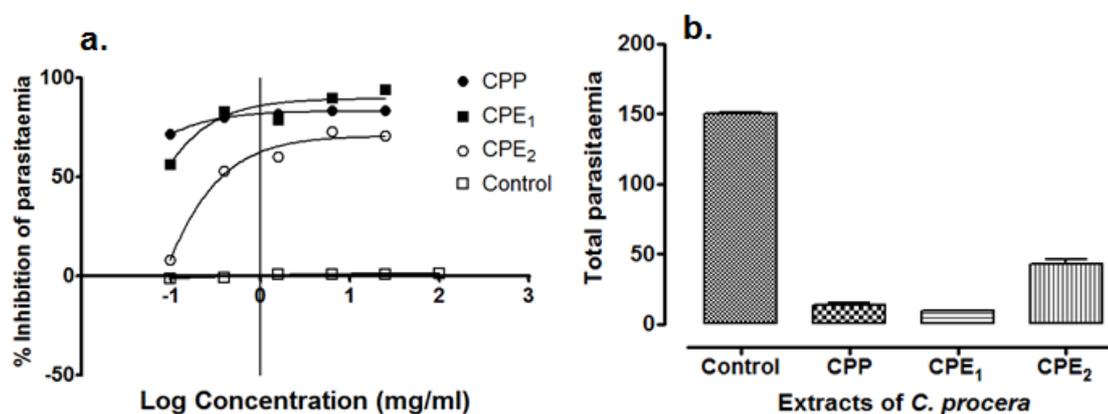


Figure 1: Effect of CPP, CPE₁ and CPE₂ (0.09-100 mg/ml) on the total parasitaemia for 48 hours. Values are means ± S.E.M (n=3) *** p < 0.001, ** p < 0.01. *P<0.05 compared to control group (One-way ANOVA followed by Newman-Keul's post hoc test. CPP (*C. procera* pet-ether extract), CPE₁ (soxhlet extracted *C. procera* ethanol extract), CPE₂ (cold macerated *C. procera* ethanol extract)

Table 3: *In vitro* antiplasmodial activity of *C. procera* extracts

Extract	CPP	CPE ₁	CPE ₂
IC ₅₀ (μg/ml)	19.52	11.41	33.35

CPP (*C. procera* pet-ether extract), CPE₁ (soxhlet extracted *C. procera* ethanol extract), CPE₂ (cold macerated *C. procera* ethanol extract)

Due to the relatively high antiplasmodial activity shown by CPE₁, it was column chromatographed on silica gel to afford four bulked fractions H2A, H2B, H2C and H2D.

In vitro antiplasmodial assay of the fractions revealed combined fraction H2A to have the highest activity (IC₅₀= 0.1916μg/ml). However it was 108 fold less potent than the standard antimalarial drug artesunate (IC₅₀= 0.00177μg/ml)(Table 4).

Table 4: *In Vitro* antiplasmodial activity of fractions of *C. procera* stem extract

Fractions	IC ₅₀ (μg/ml)
H2A	0.1916
H2B	23.14
H2C	0.3084
H2D	8.999
ATS	0.00419

ATS; Artesunate

Phytochemical constituents of the fractions were analysed by thin layer chromatography (TLC). The presence or otherwise of the secondary metabolites were established by spraying the TLC plates with specific derivatising reagents. The results are presented in table 5.

Table 5: Phytochemical constituents of fractions of ethanol extract of *C. procera*

<i>Fraction</i>	<i>H2A</i>	<i>H2B</i>	<i>H2C</i>	<i>H2D</i>
Constituents	Sterols Terpenoids	Flavonoids Catechin Proanthocyanidins	Flavonoids Catechin Proanthocyanidins	Flavonoids Catechin Proanthocyanidins

DISCUSSION

Alstonia boonei (stem and leaves) is widely used in traditional medicine in some African countries to treat malaria. In the present investigation, the petroleum ether extract of the

leaves (PSL) and stem bark (PS1) were both inactive ($IC_{50} > 100 \mu\text{g/ml}$). Also the soxhlet extracted ethanolic extracts of both plant parts were inactive ($IC_{50} > 100 \mu\text{g/ml}$). However, the cold macerated extracts of both the leaves and stem bark showed weak activity (Table 2). This result indicates that extracts of the leaves and stem bark of *A. boonei* have very weak antiparasitic activity and therefore does not justify their use as antimalarial agents. However, in many cases, plants reportedly used in a number of antimalarial products are found to have antipyretic properties and had no real antiparasitic properties [16]. Antipyretic agents relieve fevers associated with malaria. Thus a patient may be asymptomatic (for a short period) but present with high parasitaemia levels. Herbal preparations containing extracts of *A. boonei*, for the treatment of malaria, are found in combination with other plants such as *Picralima nitida* and *Azadirachta indica* [17]. The root, stem bark and fruits of *P. nitida* were found to exhibit potent antiparasitic activities with respective IC_{50} values of 0.188, 0.545 and 1.581. This activity was attributed to the active alkaloid akuammine [18]. Therefore antimalarial preparations containing extracts of *A. boonei*, may owe their activity to the other plant extracts used.

Carapa procera (meliaceae) stem bark is used in many traditional societies in Ghana and other African countries for the treatment of malaria. Members of the meliaceae have been used for generations in Africa to treat malaria [18]. In this present study extracts of the stem bark of *C. procera* exhibited significant antimalarial activity. The petroleum ether extract of the stem bark (CPP) inhibited the growth of the chloroquine sensitive (3D7) *Plasmodium falciparum* parasite with IC_{50} value of 19.52. The cold ethanolic extract had moderate activity ($IC_{50} = 33.35$) whereas the soxhlet extracted ethanol extract showed the highest antimalarial activity ($IC_{50} = 11.41$). The difference in activity observed, between the hot and cold ethanolic extract may be attributed to the more exhaustive extraction of the active metabolites in CPE₁ (yield= 8.81%) than in the cold macerated extracts (yield= 6.51%) (Table 1).

The bitter taste and antimalarial activity of *Carapa* species have been attributed to a group of terpene chemicals called meliacins, which are very similar to the bitter antimalarial chemicals found in tropical plants. One of these meliacins, named gedunin, has recently been documented to have antiparasitic properties and antimalarial effect equal to that of quinine [20]. Chemical analysis of the oil obtained from the seeds of closely related species (*C. guianensis*) has also identified the presence of another group of chemicals called limonoids. The anti-inflammatory and insect repellent properties of *C. guianensis* seed oil, are attributed to the presence of these limonoids [21], including a novel one which has been named *andirobin*. Another limonoid called *epoxyazadiradione* is found in *C. guianensis* oil. The three chemicals present in *C. guianensis* have been found to have antiparasitic and insecticidal actions [22]. Thus the antimalarial activity exhibited by the pet-ether extract of *C. procera* may be due to the presence of one or more of such compounds. The results shown by the soxhlet extracted stem bark extract of *C. procera*, underlined the interest to fractionate and investigate it further for antiparasitic activity and phytochemistry.

The IC_{50} values obtained with these fractions on the chloroquine sensitive *Plasmodium falciparum* (3D7) are summarized in Table 4. Fractions H2A, H2C and H2D showed the highest antimalarial activities ($IC_{50} < 10 \mu\text{g/ml}$) H2B showed moderate antimalarial activity with ($IC_{50} < 50 \mu\text{g/ml}$). The activities of fractions H2A and H2C ($IC_{50} < 1 \mu\text{g/ml}$) were comparable to the standard antimalarial drug artesunate ($IC_{50} < 0.01 \mu\text{g/ml}$). TLC analysis of the fractions described in the present work revealed the presence of steroids and terpenoids (fraction H2A) which showed the highest activity. Flavonoids, catechins and proanthocyanidins were found in (H2B, H2C and H2D).

Representatives of these phytochemical classes have already been reported to inhibit *Plasmodium falciparum* growth *in vitro* and *in vivo* [23]. Terpenoids and steroids [24-25] saponins [26-27], tannins, anthocyanins and flavonoids

especially methoxylated flavonoids [28] have been reported to exhibit antiplasmodial activity. Stigmasterol was found in fraction H2A by co-chromatography with standard stigmasterol. Thus the high antiplasmodial activity shown by fraction H2A may be due to its steroidal and more importantly terpenoidal contents. The antiplasmodial activity of fractions H2B, H2C and H2D may be attributed to the catechin and the polymeric flavonoid proanthocyanidins. The exact mechanism of antimalarial action of flavonoids is unclear but some flavonoids are shown to inhibit the influx of L-glutamine and myoinositol into infected erythrocytes [29]. It is reported that catechins are potent inhibitors of mammalian facilitative glucose transporter 1 (GLUT1)-mediated d-glucose transport in human erythrocytes [30]. It is hypothesized that catechins exhibit antimalarial activity by a similar inhibition of d-glucose uptake via *P. falciparum* hexose transporter (PfHT), a parasite plasma membrane-localised protein that is a major route for parasite d-glucose and d-fructose uptake [31-32]. Thus these secondary metabolites may be responsible for the antimalarial activity of *Carapa procera*.

CONCLUSION

The present study has revealed that the leaves and stem bark of *A. boonei* show weak antiplasmodial activity. Extracting the constituents of *A. boonei* by cold maceration retains considerable antiplasmodial activity. The stem bark of *C. procera* however, showed significant antiplasmodial activity. Chromatographic fractionation afforded more potent antiplasmodial fractions.

REFERENCES

- [1] WHO; World Health Report, **1999**.
- [2] S Makalo. Malaria cripples Ghana's health budget, AFROL NEWS, **2016**, <http://www.afrol.com/articles/27317>
- [3] IA Agyapong. Malaria. *SocSci Med*, **1992**, 35(2): 131 - 7.
- [4] K Asenso-Okyere; JADzator. *SocSci Med*, **1997**, 45(5): 659 – 667.
- [5] MoH. Information for Action; A Bulletin of Health Information. MoH, Accra, **2002**.
- [6] MDR Camacho; SL Croft; JD Phillipson. *Curr Opin Investig Drugs*, **2000**, 2, 47–62
- [7] WHO. *Trans R Soc Trop Med Hyg*. **2000**;94(Suppl 1):1–90.
- [8] S Hout; A Chea; B Sok-Siya; B Elias; R Gasquet; M Timon-David; P Balansard; N Azas. *J Ethnopharmacol*, **2006**, 107, 12–18
- [9] KR Markham (Ed.). Techniques of flavonoid identification. Academic Press, INC LTD . London pp 14-25, **1982**.
- [10] JB Harbone. Phytochemical Methods. Chapman and Hall limited, London, **1976**.
- [11] AC Ene; SE Atawodia; DA Ameh; GI Ndukwe; HO Kwanashie. *Acta Tropica*, **2009**, 112, 288–294.
- [12] JR Miller. Graph Pad prism version 4.0 step-by step examples. San Diego, CA, Graph pad software Inc, **2003**.
- [13] HJ Motulsky. Prism 4 statistics guide. Statistical analysis for laboratory and clinical researchers, San Diego CA USA, **2003**.
- [14] P Rowe. Essential Statistics for the Pharmaceutical Sciences. John Wiley and Sons Ltd, England, **2007**.
- [15] DA Fidock; PJ Rosenthal; SL Croft; R Brun; S Nwaka. *Nature Reviews*, **2004**, 3, 509-518.
- [16] L Basco; S Mitaku; AL Skaltsounis; N Ravelomanantsoa; F Tillequin; M Koch; JL Bras. *Antimicrob. Agents Chemother*, **1994**, 38, 1169–1171.
- [17] J Addae-Kyereme; SL Croft; H Kendrick; CW Wright. *J Ethnopharmacol*, **2001**, 76, 99–103.
- [18] NG Zirihi; F Guédé-Guina; LB Bodob; GP Guedie. *J Ethnopharmacol*, **2005**, 98, 281–285.
- [19] S Schwikkard; FV Heerden. *Nat. Prod. Rep.*, **2002**, 19, 675–692.
- [20] BS Nayak; J Kanhai; DM Milne; LP Pereira; WH Swanston. *Evid. Based Complementary Altern. Med.*, **2009**, 1-7.
- [21] A Roy; S Saraf. *Biol Pharm Bull*, **2006**, 29: 191– 201
- [22] OS Silva; PR Romao; RD Blazius; JS Prohiro. *J Am Mosq Control Assoc*, **2004**, 20: 456-7.
- [23] CW Wright; JD Phillipson. *Phytother. Res*, **1990**, 4, 127–139.
- [24] C Krafi; K Jennett-Siems; K Siems; J Jakupovic; S Mavi; U Bienzle; EEich. *Phytother. Res.*, **2003**, 17, 123–128.
- [25] JD Phillipson; CW Wright. Antiprotozoal agents from plant sources. *Planta Medica*, **1991**, 57, S53–S59.
- [26] HA Oketch-Raban; SF Dossaji. *J. Nat. Prod.*, **1997**, 60, 1017–1022.
- [27] F Traore; R Faure; E Olivier; M Gasquet; N Azas; L Debrauwer; A Keita; P Timon-David; G Balansard. *Planta Medica*, **2000**, 66, 368–371.
- [28] B Oliver-Bever. Medicinal Plants in Tropical West Africa. Cambridge University Press, London. pp.148, 163, **1986**.
- [29] K Kaur; M Jain; T Kaur; R Jain. *Bioorg. Med. Chem*, **2009**, 17, 3229–3256
- [30] RJ Naftalin; I Afzal; P Cunningham; M Halai; C Ross. *Br J Pharmacol.*, **2003**, 140:487–499.
- [31] CJ Woodrow; RJ Burchmore; S Krishna. *Proc Natl Acad Sci USA*, **2000**, 97:9931–9936.
- [32] CJ Woodrow; JI Penny; S Krishna. *J Biol Chem*, **1999**, 274:7272–7277.