In vitro anti-plasmodial activity of three herbal remedies for malaria in Ghana: *Adenia cissampeloides* (Planch.) Harms., *Termina liaivorensis* A. Chev, and *Elaeis guineensis* Jacq

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ABSTRACT

Background: Herbal remedies of *Adenia cissampeloides, Terminalia ivorensis,* and *Elaeis guineensis* among others have been used in Ghana for the treatment of various ailments including malaria. However, most of these remedies have not been scientifically investigated. **Objective:** This study, therefore, seeks to investigate the anti-plasmodial activity of these plants. **Materials and Methods:** The ethanolic extracts of *A. cissampeloides stem, T. ivorensis* stem bark, and *E. guineensis* leaves were tested for *in vitro* anti-plasmodial activity against chloroquine-resistant strains of *Plasmodium falciparum*. Thin blood films were used to assess the level of parasitemia and growth inhibition of the extracts. **Results:** The IC_{50} of *A. cissampeloides, T. ivorensis,* and *E. guineensis* were 8.521, 6.949, and 1.195 µg/ml, respectively, compared to artesunate with IC_{50} of 0.031 µg/ml. **Conclusion:** The result of this study appears to confirm the folkloric anti-malarial use these plants.

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INTRODUCTION

Malaria has been a threat to humans for many years. Despite the many advances in both preventive and curative treatments in the 20th Century, malaria is still a major killer today, and deaths from the disease have increased in the past three decades.[1] There are 350 to 500 million clinical episodes of malaria each year. It causes over 1 million deaths and is the eighth most important disease in terms of lost disability-adjusted life years.[2]

Indigenous plants play an important role in the treatment of a variety of diseases. About 80% of the population in many developing countries including Ghana, depend on plants as accessible, first line, and cost-effective therapy for malaria. However, many of these plants have not been systematically investigated as anti-malarials. Among the many species of plants employed in malaria remedies[3] in Ghana are *Adenia cissampeloides* (Planch.) Harms, *Termina liaivorensis* A. Chev., and *Elaeis guineensis* Jacq.[4] *A. cissampeloides*is a climber. Other common uses of the plant include the treatment of dysentery, rheumatic pain relief,[5] hypertension, and numbness.[6] *T. ivorensis* is appreciated mainly for its timber. However,the stem bark is also employed in wound healing and as a remedy for rheumatism.[7] Other folklore uses of *E. guineensis* include treatment of gonorrhea, headache, rheumatism diuretic and an aphrodisiac[8] and wound healing.[9,10] The dried leaves have been reported to possess hemostatic properties.[11]

This study, therefore, seeks to verify the anti-plasmodial properties of these plants in order to justify their use in malaria remedies.

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MATERIALS AND METHODS

Collection of plant materials

The plant materials were collected in February, 2011 from Kumasi in the Ashanti Region of Ghana and authenticated

at the Department of Pharmacognosy, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Voucher specimen of each plant (*A. cissampeloides*: FP/094/10; *T. ivorensis*: FP/095/10; *E. guineensis*: FP/096/10) have been deposited at the Herbarium of the same department.

Phytochemical screening

The presence of secondary metabolites in the powdered samples (saponins, reducing sugars, flavonoids, glycosides, steroids, terpenoids, and alkaloids) was investigated following simple qualitative methods.[14]

Preparation of plant extracts

The stem bark of *T. ivorensis*, whole plant of *A. cissampeloides,* and leaves of *E. guineensis* were washed and air-dried under room temperature for 7 days. The dried samples were ground to coarse powder, and each plant material (500 g) cold macerated with 70% ethanol (2 liters) at room temperature for 3 days with intermittent shaking. The percolate was evaporated into a syrupy mass under reduced pressure using a rotary evaporator and vacuum-dried for 24 hours to give a yield of 15.6%, 19.4%, and 12.8%$^{w}/_{w}$, respectively, for *T. ivorensis*, *A. cissampeloides,* and *E. guineensis*. The crude extracts were kept in a desicator until required for use.

Plasmodium falciparum culture and maintenance

In vitro anti-plasmodial activity of the plant extracts were assessed at the Immunology Department of the Noguchi Memorial Institute for Medical Research, Legon, Ghana. *Plasmodium* parasites were grown and maintained in culture using the Trager and Jensen method with some modifications[12] using *P. falciparum* chloroquine resistant strain, 3D7. The parasites were maintained *in vitro* in human O-positive red blood cells using RPMI 1640 medium supplemented with 25mM HEPES and 25 Mm $NaHCO_3$ and were complemented with 2% human O-positive serum and 10 mg/ml Gentamycin. The cultures were gassed with 5.5% CO_2, 2% O_2, and 92.5% N_2, and incubated at 37° C. Culture flask with 3D7 parasite strains was maintained by the daily change of culture media and preparation of thin RBC smears to monitor parasite growth.

Preparation of sterile extracts

A stock solution of 100 µg/ml was prepared from the crude ethanolic extract of each plant material and filtered with millipore filter of size 0.2 µg (Carrigtwohill, Ireland) under sterile conditions. Four-fold serial dilutions were prepared from each stock solution using Complete Parasite Medium, resulting in concentrations ranging from 100 - 0.0976562 µg/ ml. Artesunate (Sigma-Aldrich, USA; 98% purity), a known standard anti-malarial drug, was used as a positive control.

Establishment of anti-plasmodial activities of extracts

The *in vitro* inhibition assay used was a modification of the semi-automatic microdilution technique developed by Desjardins *et al.*[13] The cultures were treated with the selected concentrations of the extracts in two 24-well microtitre plates (Greiner Bio-One, Belgium) and incubated for 72 hours at 37°C. Thin blood smears were prepared on properly labeled slides. The blood smears were air-dried and fixed in methanol. The dried slides were Giemsa stained and observed with 100x microscopic lens in immersion oil using a light microscope.

Statistical analysis of data

Growth inhibition due to each extract defined as the difference between the % parasitemia of each treatment group, and the corresponding positive control was calculated as follows:[14]

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

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

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

Total parasitemia 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 (IC_{50}) for each drug/extract were determined using an iterative computer least squares method, with the following non-linear regression (three-parameter logistic) equation.

$$Y = \frac{a + (b - a)}{\left(1 + 10^{(LogIC50 - X)}\right)}$$

Where, X is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to b (the top) with a sigmoid shape.[15,16] Using regression equations of best fit of plotted growth inhibition versus concentration curves, the IC_{50} 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 analyzes. $P < 0.05$ was considered statistically significant.

RESULTS

The phytochemical analysis of the extracts revealed the presence of some secondary metabolites as elaborated in Table 1.

The ethanol extracts of *A. cissampeloides*, *T. ivorensis,* and *E. guineensis* manifested significant activities against the chloroquine-resistant strain of *P. falciparum*. The percentage growth inhibition was determined using different concentrations. Maximum growth inhibition of 73.95% was obtained for *A. cissampeloides* at a concentration of 1.562 µg/ml. The concentrations above this (25 and 6.25µg/ ml) exhibited similar percentage inhibitions. *T. ivorensis* also exhibited a maximum percentage inhibition of 83.34% at a concentration of 25 µg/ml while *E. guineensis* showed a maximum percentage inhibition of 83.72% at a concentration of 25 µg/ml. The IC_{50} values of these plants were calculated and found to be 8.52, 6.95, and 1.20 µg/ml for *A. cissampeloides*, *T. ivorensis,* and *E. guineensis,* respectively. That of artesunate control was found to be 0.03 µg/ml. The respective IC_{50} values are shown in Table 2.

DISCUSSION

Malaria still poses as a major health problem in the developing world. It is the number one cause of morbidity, accounting for over 40% of outpatient attendance in public health facilities.[17] Pregnant women and children are the most vulnerable to the disease.[18] When malaria is not properly treated in pregnant women, it can cause anemia and can also lead to miscarriages, stillbirths, underweight babies, and maternal death.[17] Malaria is caused by the unicellular protozoan of the genus *Plasmodium*. Four species are infectious to man, with *P. falciparum* being the deadliest[19] and accounts for up to 80% of all malaria cases in the world.[20]

In many parts of the world, the parasites have developed resistance to a number of anti-malarials such as chloroquine, sulphadoxine-pyrimethamine, and other conventional drugs.[21] Resistance to these drugs has been reported to be as high as 40 to 60% in some African and Asian countries.[22] Further studies indicate that resistance to chloroquine, which was the most widely used treatment for malaria, may be responsible for the increase in malaria-specific mortality in many malaria-endemic countries.[23,24] There is, therefore, an urgent need to discover new compounds with different modes of action. However, the use of plant-derived drugs for the treatment of malaria has a long and successful tradition. For example, quinine isolated from *Cinchona succirubra* and artemisinin from *Artemisia annua* illustrate the potential value of investigating traditionally used malaria herbal remedies for anti-malarial drugs or lead compounds for drug development agenda.[25]

The present study assessed the anti-plasmodial activity of *A. cissampeloides, T. ivorensis, and E. guineensis. E. guineensis* exhibited the highest activity with an IC_{50} value 1.195 µg/ ml.This was followed *T. ivorensis* ($IC_{50:}$ 6.949 µg/ml) and *A. cissampeloides* showed the least activity ($IC_{50:}$ 8.521 µg/ml). However, artesunate, a known anti-malarial used as a positive control in this experiment, exhibited a much higher activity ($IC_{50:}$ 0.031 µg/ml) than the plant extracts under. According to Zofou *et al.*, [26], an anti-plasmodial agent is very active when its IC_{50} value is less than 10 µg/ml, moderately active when its IC_{50} falls between 10 and 25 µg/ml, weakly active when its IC_{50} falls between 25 and 50 µg/ml, and very weakly active when the IC_{50} exceeds 50 µg/ml. This indicates that all 3 plants were highly active against *P. falciparum*.

Different classes of compounds in plants such as alkaloids[27], quassinoids, sesquiterpene lactones[23], and flavonoids[28] have been found to exhibit anti-plasmodial activity through several mechanisms. For instance, some alkaloids exhibit their action by binding with the haem within the erythrocytes, thus preventing its detoxification into its polymeric form (hemozoin) and thus preventing consumption by the parasite,[29,30] while some flavonoids have also been known to chelate metals such as iron and

Table 1: Phytochemical constituents of the plants extracts

Phytochemicals	Plant material		
	***A. cissampeloides* stem**	***T. ivorensis* stem back**	***E. guineensis* leaves**
Saponins	-	+	-
Tannins	+	+	+
Flavonoids	-	+	-
Glycosides	+	-	-
Steroids	-	-	-
Terpenoids	-	-	-
Alkaloids	+	+	+

Key: - (absent) + (present)

Table 2: IC_{50} values for the effect of plant extracts on chloroquine resistant strain of *P. falciparum*

Plant extract/drug	IC_{50} value (µg/ml)
A. cissampeloides	8.521
T. ivorensis	6.949
E. guineensis	1.195
Artesunate	0.031

copper as part of their antioxidant effects and that iron chelating therapies have been recommended for malaria patients.[31] Further studies to determine the stage–specific anti-plasmodial activities of the alkaloids and flavonoids from our plant samples are on-going in our laboratories.

CONCLUSION

The results of this study show that the crude extracts of *A. cissampeloides*, *T. ivorensis*, and *E. guineensis* possess *in vitro* anti-plasmodial activity against *P. falciparum*. These findings put them in the limelight as potential candidates for further studies.

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REFERENCES

1. Carter R, Mendis KN. Evolutionary and historical aspects of the burden of malaria. Clin Microbiol Rev 2002;15:564-94.
2. Breman J, Mills A, Snow R, Steketee R, White N, Mendis K. Conquering malaria. In: Jamison DT, Breman JG, Maesham AR, *et al*., editors. Disease Control Priorities in Developing Countries. New York: Oxford University Press; 2006. p. 413.
3. Asase A, Oteng-Yeboah AA, Odamtten GT, Simmonds MS. Ethnobotanical study of some Ghanaian anti-malarial plants. J Ethnopharmacol 2005;99:273-9.
4. Asase A, Akwetey GA, Achel DG. Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. J Ethnopharmacol 2010;129:367-76.
5. Schmelzer, G.H. & Gurib-Fakim, A. (Editors). Plant Resources of Tropical Africa 11(1). Medicinal plants 1. PROTA Foundation, Backhuys Publishers, Leiden, Netherlands / CTA, Wageningen 2008. pp. 34-37.
6. Nyarko AA, Addy ME. Effect of aqueous extract of Adenia cissampeoides on blood pressure and serum analytes of hypertensive patients. Phytother. Res. 2006; 4: 25-8. Available at http://onlinelibrary.wiley.com/doi/10.1002/ptr.2650040107/abstract.
7. Burkhill HM. The useful plants of West Tropical Africa, vol. 1: A-D. Kew: Royal Botanic Gardens. 1985.
8. Irvine TT. Wound Healing. Arch Emerg Med 1985;2:3-10.
9. Mshana NR, Abbiw DK, Addae-Mensah I, Adjanouhoun E, Ahyi RA, Ekpere JA, *et al*., Contribution to the revision of ethnobotanic and floristic studies in Ghana. Traditional Medicine and Pharmacopoeia. Organisation of African Unity/ Scientific, Technical and Research Commission 2000. pp. 465-466.
10. Keay RWJ. Trees in nigeria. London: Oxford Science publishers; 1989. p. 476.
11. Idu M, Onyibe HI. Medicinal Plants of Edo State, Nigeria. Res. J. Med. Plants. 2007; 1(2): 32-41. Available at http://www.scialert.net/abstract/?doi=rjmp.2007.32.41.
12. Trager W, Jensen JB. Human malaria parasites in continuous culture. 1976. J Parasitol 2005;91:484-6.
13. Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. Antimicrob Agents Chemother 1979;16:710-8.
14. Ene AC, Atawodi SE, Ameh DA, Ndukwe GI, Kwanashie HO. Bioassay-guided fractionation and *in vivo* antiplasmodial effect of fractions of chloroform extract of Artemisia maciverae Linn. Acta Trop 2009;112:288-94.
15. Miller JR. Graphpad prism version 4.0, step by step examples. San Diego, CAA: Graphpad Software Inc; 2003.
16. Motulski H. Prism for statistical guide. San Diego, CA, USA:Statistical analysis for laboratory and clinical researchers; 2003.
17. Asenso-Okyere K, Asante FA. Economic burden of malaria in Ghana. Ghana: WHO/AFRO. 2003. Technical report.
18. Bloom DE, Bloom RL, Weston M. Business and Malaria: A neglected? World Economic Forum, Global Health Initiative, Geneva, Switzerland. 2006. p. 1-53. Available at http://www.weforum.org/pdf/MalariaReport.pdf.
19. Ashley E, McGready R, Proux S, Nosten F. Malaria. Travel Med Infect Dis 2006;4:159-73.
20. Wongsrichanalai C, Pickard AL, Wernsdofer WH, Meshnick SR. Epidemiology of Drug-Resistant Malaria. Lancet infectious disease. 2002; 2: 209-18. Available at http://www.ncbi.nlm.nih.gov/pubmed/11937421.
21. Kassim OO, Loyevsky M, Amonoo H, Lashley L, Ako-Nai KA, Gordeuk VR. Inhibition of *in vitro* growth of *Plasmodium falciparum* by Pseudocedrela kotschyi extract alone and in combination with Fagara zanthoxyloides extract. Trans R Soc Trop Med Hyg 2009;103:698-702.
22. Trape JF, Pison G, Spiegel A, Enel C, Rogier C. Combating malaria in Africa. Trends Parasitol 2002;18:224-30.
23. Bero J, Frederich M, Quetin-Leclerq J. Antimalarial compounds isolated from plants used in traditional medicine. J. Pharm. and Pharmacol. 2009; 61: 1401-33. Available at http://www.ncbi.nlm.nih.gov/pubmed/19903367.
24. Trape JF. The public health impact of chloroquine resistance in Africa. Am J Trop Med Hyg 2001;64:12-7.
25. Srisilam K, Veersham C. Antimalarials of plant origin. In: Khanum, A Khan IA editors. Role of Biotechnology in Medicinal and aromatic plants. Hyderabad, India: Ukaaz Publishers; 2003;7:17-47.
26. Zofou D, Tene M, Ngemenya MN, Tane P, Titanji VP. *In vitro* antiplasmodial activity and cytotoxicity of extracts of selected medicinal plants used by traditional healers of Western cameroon. Malar Res Treat 2011;2011:561342.
27. Saxena S, Pant N, Jain DC, Bhakuni RS. Antimalarial agents from plant sources. Current Sci. 2003; 85(9): 1315-29. Article available at http://www.iisc.ernet.in/currsci/nov102003/1314.pdf
28. Kaur K, Jain M, Kaur J, Jain R. Antimalarials from nature. Bioorg Med Chem 2009;17:3229-56.
29. Paulo A, Gomes ET, Steele J, Warhurst DC, Houghton PJ.

Antiplasmodial activity of Cryptolepis sanguinolenta from leaves and roots. Planta Medica (2000) 66:30-34. http://www.ff.ul.pt/FCT/PTDC/SAU-FAR/114864/2009/ref_8.pdf.

30. Wright CW. Antiprotozoal natural products. In: Evans WC, Evans D, editors. Trease and Evans Pharmacognosy. India: Elsevier; 2009. p. 408-13.
31. Pagola S, Stephens PW, Bohle DS, Kosar AD, Madsen SK. The structure of malaria pigment beta-haematin. Nature 2000;404:307-10.

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