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In-vitro Antimalarial Activity of Ethanoic and Aqueous Extracts of Atamono (*Millettia aboensis*) Leaves

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Abstract The *in-vitro* antimalarial activity of ethanoic and aqueous extracts of *Millettia aboensis* leaves locally called Atamono was investigated. Organic and aqueous extracts of *Millettia Aboensis* leaves extracts were tested for their antimalarial activity against *Plasmodium falciparum* parasite infected whole blood samples with ++ parasite count. The ethanoic and aqueous leaves extracts exhibit high chemosupression of the *P. falciparum* infected blood samples. The presence of phytochemcials especially alkaloids were responsible for the antimalarial activity of the plant. The result showed that both ethanoic and aqueous leaves extracts of *Millettia aboensis* demonstrated promising antimalarial activity and there is potential for isolation of lead compounds from their extracts.

Keywords Antimalaria, Chemosupression, Phytochemicals, Alkanoids, Atamono

Introduction

Medicinal plants offer a wide range of phytochemicals which have been harnessed in traditional herbal medicinal as well as orthodox medicine for the formulation of herbal remedies and drugs respectively. *Millettia aboensis* commonly called Atamono in Ahoada-West local government of Nigeria contain a number of phytochemical which contribute to its wide use in Nigeria in the treatment of several ailments such as gastro-intestinal disturbance, ulcer, veneral diseases e.g. gonorrhoea, syphilis as well as malaria [1].

Millettia aboensis is a small tree of 30-40 feet high and up to 2 feet in girth but usually 12 feet high will show reddish-brown pubescence on the petioles, branches, inflorescence and fruits. They are found commonly in low land rain forest. The flowers are purple in erect woody racemes up to 18 inches long. It has conspicuously rusty-hairy leaves and handsome purple flowers in erect terminal racemes at branch [2]. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plant lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals [3].

Almost all the plant parts of *Millettia aboensis* have medicinal properties. The leaf is used by traditional herbalist for general healing including ulcer and laxatives while the root is used in treating gastro intestinal disturbance and liver disease. Also the stem, leaf and root mixed with other plant materials (herbs) is used to cure veneral diseases such as gonorrhoea, syphilis etc.

Phytochemicals are non-nutritive, naturally occurring chemicals present in a plant that possess disease preventive and/or curative properties. They are also the source of many modern pharmaceutical drugs. The most important of these phytochemicals are tannins, alkaloids, flavonoids and phenolic compounds [4].

Alkaloids a very common phytochemical, are well known for their characteristic bitter taste, and are a major component of chloroquine, a common antimalarial drug. According to [5] a species of Milletia, Millettia usaramensis has been discovered to exhibit antiplasmodial activities. Current research has also shown that polyphenols contribute to the prevention of cardiovascular diseases [6], cancer, and osteoporosis and antioxidant

character with potential health benefit. They are known to have beneficial effect on cardiovascular system and have a role in the prevention of neurodegenerative disease and diabetes mellitus [7].

In many population affected by malaria, conventional drugs are often unaffordable or inaccessible. Following the resistance of malaria parasite to existing malaria drugs, the need for research to discover new drugs to combat malaria is essential. As an alternative, medicinal plants are traditionally used to treat malaria. This study aims to determine antimalarial activity of the leaves of *Millettia aboensis* in ethanol and aqueous extracts.

Figure 1: Millettia aboensis (hook.f.) Baker

Table 1: Phtochemical Composition of Leaves of *Millettia aboensis*

Phytoconstituents	Presence of Phytochemicals	
	ES	AS
Flavonoids	+++	+
Saponins	+	+++
Alkaloids	+++	+++
Carbohydrates	++	+++
Steroids	++	+
Proteins	+	+++
Terpenoids	+++	++
Glycosides	+++	+++

[9]

The hepatoprotective effect of *millettia aboensis* was studied and shown the presence of a protection against injurious effect of CCl_4 and paracetamol resulting in the hindrance of formation of hepatic free radicals and also the significant hepatoprotective activity at dose dependent level [2].

The effect of ethanol leaf extract of *millettia aboensis* on some selected haematological indices of Wister albino rat was studied and showed the presence of reducing sugar, alkaloids and the absence of cyanogenic glycoside and anthraquinone [8]. Antimicrobial properties of ethanol leaf extract of *millettia aboensis* was evaluated on some selected clinical isolates and showed the presence of reference drug inhibited [1].

Phytochemical analysis of ethanol and water extract of *Millettia Aboensis*, *Cuscuta Reflexa*, *Daniella Oliveri* and *Synclisia Scabrida* was carried out and the presence of phytoconstituents with good medical qualities and showed that the medicinal value of this plants lies in those component to produce a definite physiological action on the human body [9]. The photochemical analysis of *Mangifera Indica* and showed the presence of bioactive compound like phenols, saponin, steroid, tannis, flavonoid [10]. The antimalaria activity of some plants traditionally used in Mozambique and showed the presence of moderate or no significant activity [11].

Materials and Methods

Collection and Identification of Plant Material

Fresh leaves of *Millettia Aboensis* were collected from Ahoada-west, Rivers state. The plant was identified and authenticated by a Biotechnologist of the department of plant science (Green house), university of Port Harcourt.

Sample Collection

Fresh blood samples of malaria- infected individuals were collected from St Matthew's hospital stadium road haematology laboratory in EDTA bottles. Samples were further screened to confirm the presence of plasmodium parasite as represented in Fig. 3 and 4.

Extraction of Plant Material

The leaves of *Millettia aboensis* were dried under room temperature after which they were homogenised to a uniform powder using a manually operated mill. The ethanoic extract were prepared by soaking 300 g of the dry powdered plant material in 2.4 L ethanol at room temperature for 72 hrs with intermittent shaking. The aqueous extracts were prepared by soaking 300 g of the dry powdered plant material in 3.2 L distilled water at room temperature for 72 hrs with intermittent shaking. The soaked plant material were filtered after 72 hrs with cotton wool. The ethanoic extract was concentrated using rotary evaporated at 65 °C. The aqueous extract was evaporated using water bath at 99 °C.

Antimalarial Activity

The ethanoic and aqueous extract of *Millettia aboensis* leaves were diluted to various concentrations and added to malaria parasite infected blood samples in the test tubes as represented in Table 3 and 4 below. Smears were fixed with Leishman stain and field strain A & B The slides were observed under compound microscope with oil immersion at x1000 magnification to determine the number of parasitized cell per given magnification field. For each blood smear specific for a given blood sample, four magnification fields were observed and the numbers of parasitized cells were recorded.

Results and Discussion

The results for the ethanoic and aqueous extracts of *Millettia aboensis* are represented in Table 2 below. The percentage yields were calculated as shown below and the aqueous (35.86%) extract had a better percentage yield than the ethanoic extract (33.79%).

Table 2: Percentage (%) Yield of Ethanoic and Aqueous Leaf Extract of *Millettia aboensis*

Millettia aboensis	**Weight (g)**	
	Ethanoic	**Aqueous**
Leaf powder	145	145
Extract	49	52
Extract yield (%)	33.79	35.86

The results for the in-vitro antimalarial analysis of aqueous and ethanoic leaves extracts of *Milletia aboensis* are presented. Leishman stain Field stain A and B were used to in fixing the slides, the features of the *plasmodium* parasite infected blood cells were very distinct when viewed under the oil immersion x1000 magnification. Figures 2 and 3 show the images of parasitized blood cells with Leishman stain and field stain respectively.

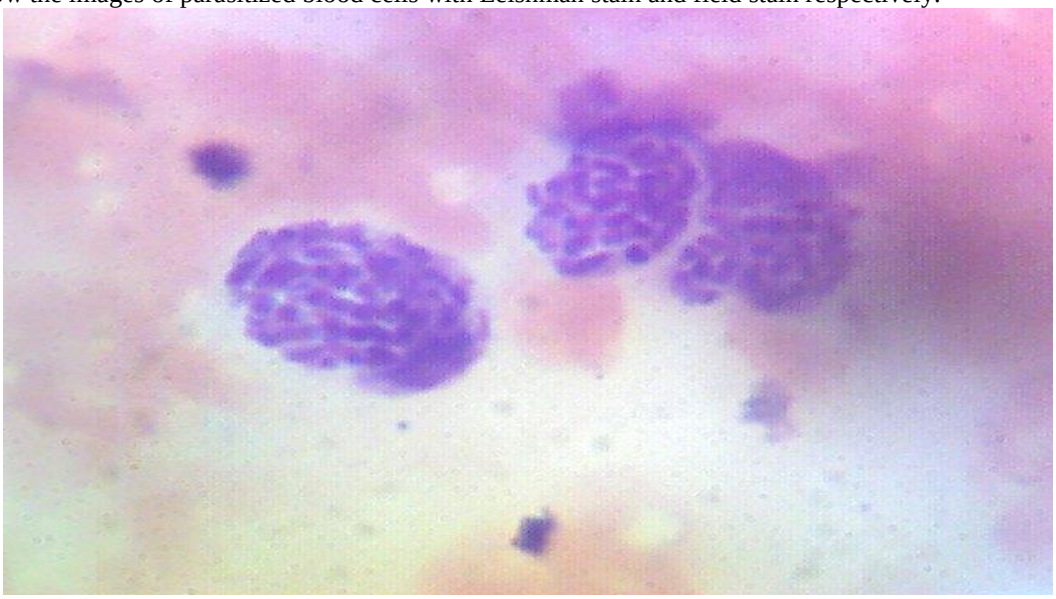


Figure 2: P. falciparium parasitized blood cell fixed with Leishman stain

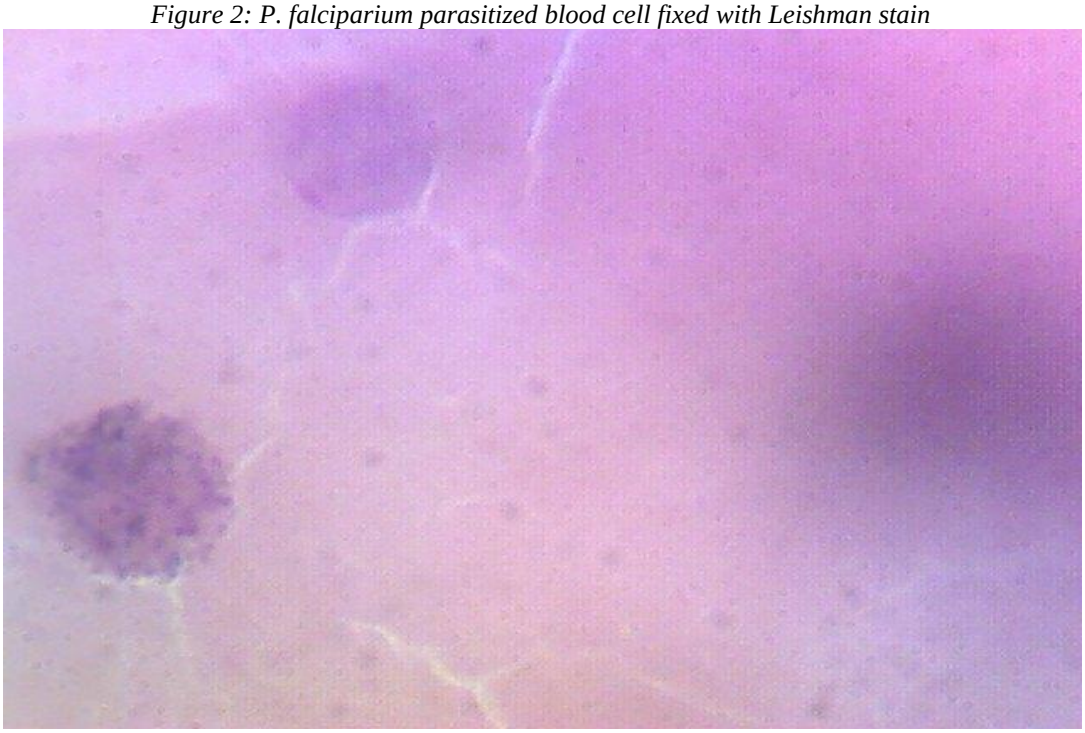


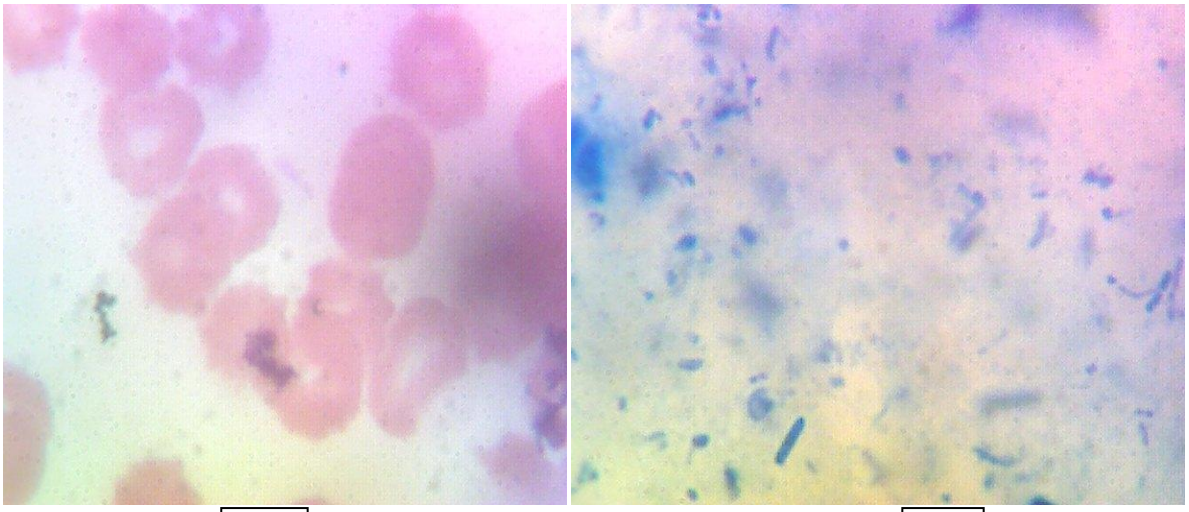
Figure 3: P. falciparium parasitized blood cell fixed with field stain A and B

The field stain lyses the red blood cells and projects the parasites. On the other hand, the Leishman stain keeps the walls of the red blood cell intact still allowing us to see the red parasitized cells

All blood samples used were confirmed to be *Plasmodium falciparum* infected up to ++. Sample A was used as the blank and control with 0.05ml normal saline added to the sample for the aqueous extract. Water could not be added because the presence of water may lyse the red blood cells before smearing and render the sample invalid. Table 3 shows that the *Plasmodium* parasite count was cleared and negative (-) upon the addition of 0.05 ml of aqueous extracts to 0.5ml to each of samples 1A, 2A, and 3A. When a lesser volume was added, (0.02 ml) the parasite count was not properly cleared (+). This indicates that the aqueous extract at 0.5ml was effective in destroying the parasites present in the blood sample.

Table 3: The Result of Aqueous Extract on the *Plasmodium falciparum* Parasite

Sample	Whole blood (ml)	Aqueous extract (ml)	Reaction
1A	0.5	0.05	_
2A	0.5	0.05	_
3A	0.5	0.05	_
4A	0.5	0.05	_
A	0.5	0.05	_



LS **FS**

Figure 4: Sample IA

Sample E was used as the blank with 0.05 ml ethanol added to the blood sample. The addition of 0.05 ml of ethanol did not lyse or destroy the plasmodium parasites present in the blood sample this indicates that the solvent ethanol did not any effect on the parasite. The plasmodium parasite count was also cleared and negative (-) upon the addition of 0.5 ml of ethanoic extracts to 0.5 ml of sample 1E, 2E, and 3E. When a lesser volume was added to sample 4E, (0.02 ml) the parasite count was not properly cleared as such the parasite count reduced to +. This indicates that the ethanoic extract at 0.05 ml was effective in killing the parasites present in the blood sample. Table 4 shows the result of ethanoic extract on the *Plasmodium falciparum* parasite.

Table 4: Ethanoic Extract on *Plasmodium falciparum* Parasite

Sample	Whole blood (ml)	Ethanol extract (ml)	Reaction
1E	0.5	0.05	_
2E	0.5	0.05	_
3E	0.5	0.05	_
4E	0.5	0.02	+
E	0.5	0.00	++

Conclusion

This study has shown that ethanoic and aqueous extracts of millettia aboensis killed the *Plasmodium falciparum* in the blood samples. Indeed, aqueous and ethanoic extracts of *Millettia aboensis* had chemosuppression on the

plasmodium parasites indicating a good antimalarial activity. The presence of phytochemicals especially alkaloid affords its antimalarial activities (which is present in chloroquine, an antimalarial drug), tannins, saponin their active constituents may be potential candidates with therapeutic value in the treatment of malaria. It is recommended that further research for the isolation of the actual alkaloid responsible for the antimalarial activity should be carried out.

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