ANTIMALARIAL POTENTIAL OF METHANOL EXTRACT OF *Stigmaphyllonovatum* IN *Plasmodium falciparum* INFECTED MICE

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O. Iyekowa[1]*, M. O. Edema[2] and I. Igbe[3]
[1]Department of Chemistry, University of Benin, Benin City, Edo State, Nigeria
[2]College of Education, Warri, Deita State, Nigeria
[3]Department of Pharmacology, University of Benin, Benin City, Edo State, Nigeria
*Corresponding author: osaro.iyekowa@uniben

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Abstract: *Stigmaphyllonovatum*, family- Malpighiaceae is a medicinal plant used in the traditional treatment of fever and malaria in rural areas in Benin City, Edo State. The research was conducted to determine the phytochemical screening and antimalarial activity of methanol extract of the plant leaf using Balb/c mice infected with Plasmodium falciparum. The plant leaves were collected, dried, pulverised and extracted with methanol solvent in a soxhlet extractor for eight hours. The extract was concentrated with rotary evaporator and a portion was used for phytochemical screening. The acute toxicity test was determined to estimate the lethal dose (LD_{50}) value of the extract inbalb/c albino mice while malaria chemo suppressive activities were conducted along with quinine to determine the antimalarial potency of the plant extract in *Plasmodium* –infected balb/c albino mice. Alkaloids, glycosides, phenolics, flavonoids and terpenoids were present while tannin was absent. The antimalarial activities revealed that the methanol extract exhibit significant suppression ($P<0.05$) of *Plasmodium falciparum* which was evident by the 32.44% mean chemo suppression of malaria parasite at day 4 of treatment of *P. falciparum* –infected mice with 84 mg/kg/day. This work supports the local use of the plant for the treatment of malaria.

Keywords: *Stigmaphyllonovatum*, antimalarial potential, phytochemical screening, *Plasmodium falciparum*

Introduction

Malaria is a life-threatening blood disease caused by a parasite that is transmitted to human by the Anopheles mosquito. For centuries and till date, it has been responsible for the decline of nations, prevented economic development and it continues to be a huge social, economic and health problem, particularly in the tropical countries where Africa and indeed the whole of Nigeria belongs (Carter and Mendis, 2002).Consequences of malaria include childhood deaths, anaemia, low birth-weight, epilepsy, and neurological problems. These consequences of malaria, compromise the health and development of millions of children throughout the tropical world (Roll Back Malaria, (RBM) 2006). It is estimated that nearly half of the world's population in 104 countries live at risk of the disease (WHO, 2012). Ninety percent of malaria cases in the world are estimated to be in Africa where the disease is endemic (WHO, 2008), particularly West and Central Africa, are attributed to a combination of very high transmission and weaker health services. In Nigeria in the past decades, malaria cases have been aggravated by the increasing spread of drug- resistant *Plasmodium falciparum* strains and this continuing threat to Africans (the Black people) by this tropical disease lends urgency to the need to expand the systematic exploration of medicinal plants in the search of new bioactive molecules (drugs) or their precursors.

Stigmaphyllonovatum (Amazon vine) belongs to the family *Malpighiaceae*. Its duration is perenial, growth habit - vine and locally called Ebeosanofua (Binis), orokofor (Yoruba), Ijiwere (Ifon). Phytochemical screening of *S. ovatum* showed it contains alkaloids, terpenoids, eugenol, saponins, flavonoids and phenolics with potential antibacterial, antifungal and antiplasmodial activities (Edema and Iyekowa, 2007). Sainty *et al.* (1981) had isolated galiosid, monotropein and geniposidic acid from *Stigmaphyllonsagittatum* which are irinoids. Consultation with herbal medicine practitioners in Benin City and its environ indicated that extract of *S. ovatum* leaves which have a slight bitter taste is used in the treatment of various feverish conditions including malaria, stomach-ache and in reduction of very high temperature (Edegbe, 2007). The leaf decoction is drunk (2 – 3 glasses a day) to treat malaria and inhalation of steam from the decoction is used as a febrifuge (fever-reducing) (Rasoanaivo *et al*. 1992).

Antimalarial drug resistance has emerged as one of the greatest challenges facing malaria control today. There is need for identification of new versatile drug to curtail the spread of malaria to new areas and re-emergence in areas where the disease had been eradicated. Thus, the research is aimed at investigating the antimalarial potential of methanol extract of *Stigmaphylonovatum* in *Plasmodim falciparum* infected balb/c mice.

Materials and Methods

Chemicals and reagents

All chemicals used in this work were of analytical grade from Merck and Sigma Aldrich and they include methanol, n-hexane, ethanol and dimethyl sulphoxide (DMSO).

Sample collection and treatment

The fresh leaves of *S. ovatum* were collected from the bush in Ekiadolor community in Ovia North East Local Government Area of Edo State, Nigeria. The plant was identified and authenticated by a taxonomist Prof. J. F. Bamidele, with herbarium voucher number (UBHm 0192) deposited in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The plant leaves were air-dried under shade in the laboratory for six weeks and pulverized to a powdered form. Four hundred grams (400 g) of the powdered leaves were extracted with methanol (Merck, Germany) in a Soxhlet apparatus for 8 h. The crude extract was dried with Na_2SO_4 (Vickers, England) and then concentrated in a rotary evaporator at 50^0C. A portion of the dried residue (crude extracts) were used for phytochemical screening.

Phytochemical screening

Phytochemical screening was carried out to find the presence of the active chemical constituents such as alkaloids, glycosides, steroids, flavonoids, saponins, terpenoids, phenolics, tannins and eugenols by using the standard procedures (Kokate *et al*, 2009) and (Evans and Trease, 2002).

Thin layer chromatography

Thin layer chromatography was carried out on the methanol extract of *S. ovatum* extract at different solvent ratios (100% methanol, hexane/methanol-1:1 and hexane/methanol-9:1) using the precoated TLC plates.

Acute toxicity test

The acute toxicity assay was performed according to standard procedure (Lorke, 1983) to estimate the lethal dose, LD_{50} values of the methanol extract using Balb/c albino mice before the antimalarial analysis (using *P. falciparum*-infected mice).

Antimalarial test

Development of experimental humanized mouse model

Mice: Balb/c mice weighing 20 – 33 g were used. They were housed in standard mosquito-netted metal cages under standard conditions of light and temperature and were maintained on a standard mice diet and water ad libitum. They were acclimatized for 14 days and were treated in accordance with guidelines for animal care approved by the Animal Ethics Committee of the University of Benin, Benin City, Nigeria. The mice were certified medically fit for the experiment by Dr. J. Danjuma, a veterinary Doctor.

Modification of mice: The innate immune responses of the Balb/c mice were retarded by modification using pharmacological compounds (4 mg aspirin/kg body weight and 4mg anhydrous doxycycline eq./kg body weight) (Javeed *et al.,* 2011; Bellahsene and Forgren, 1985) followed by engraftment of human blood (Bellahsene and Forgren, 1985) and (Imade *et al.,* 2012). Infection of experimental humanized mouse model with *Plasmodium falciparum* were done according to the method of (Bellahsene and Forgren, 1985; Imade *et al.,* 2012).

Antimalarial testing of the P. falciparum-infected mouse model with methanol extract of S. ovatum

Plasmodim falciparum-infected humanized immuneosuppressed mice were employed to verify the antimalarial principles of each of the test samples. The mice were divided into 5 independent experimental groups (5 mice per group). The negative control drug –DMSO (4 mg/kg body weight administered three time daily for four days) and positive control drug- quinine (73 mg/kg body weight administered three times daily for four days) were used to treat mice in two of the experimental groups (antimalarial validation). The mice received these drugs orally via a gastric cannular. Varying doses (21, 42, 84 mg/kg/day each) of the methanol extract were used to treat the mice in the other three experimental groups. The test samples were administered, by taking into consideration their LD_{50} values.

Upon administration of quinine, DMSO, and the plant extract, the net mean malarial chemo suppression (WHO, 2009) due to drug/extracts administration was calculated according to the formula:

$$Net\ mean\ malarial\ chemosuppression\ (\%) = \frac{100\,(A-B)}{A} - MC0$$

Where: A = Percentage of parasitaemia in the mice administered with DMSO; B = Percentage of parasitaemia in mice treated with quinine/plant extracts

$\frac{100\,(A-B)}{A}$ = Mean malarial chemosuppression

MC0 = Mean malarial chemosuppression obtained from mice at day 0 of treatment

Statistical analysis

Values were expressed as mean and standard error of the mean, as well as in percentages. Chi-square test and regression analysis, where appropriate, were used to determine the level of significance, and P-value less than 0.05 ($P < 0.05$) were considered significant. The software, SPSS version 16, was employed for the statistical analysis.

Results and Discussion

The results of the phytochemical screening of the methanol extract of the leaves of *S. ovatum* and malarial chemosuppresive activities are shown in Tables 1 and 2.

The result indicated that the methanol extract constitutes important phytochemicals like terpenenoids, steroids, saponins, phenolics and alkaloids (Table 1) which have physiological effects on man. Alkaloids have been reported to have anti- inflammatory, antiprotozoal and anti-microbial properties (Kumar and Tandon, 1979). Cardiac glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure (Yukari et al., 1995). Steroids have been reported to have antibacterial properties (Raquel, 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001)

Table 1: Phytochemical constituents of methanol extract of *S. ovatum* leaves

S/N	Phytochemical	Methanol extract
1	Glycosides	+
2	Saponin	+
3	Phenolics	+
4	Flavonoids	+
5	Tannins	-
6	Terpenoids	+
7	Eugenols	+
8	Steroids	+
9	Alkaloids	+

+ = Present - = Absent

Thin layer chromatography

The pre coated thin layer chromatography results of methanol extracts of *S. ovatum* are shown in the following Table 2. The retention factors of 0.52, 0.77 and 0.81 indicated that constituents of the extract can be isolated when subjected to further isolation techniques.

Table 2: Retention factor (Rf) values and colour reaction of *S. ovatum*

Extracts	Solvent system	Colour of spots (under UV lamp)	Rf values
*S.ovatum*M	100% methanol	Yellow	0.81
*S.ovatum*M	100% chloroform	Blurred yellow	0.52
*S.ovatum*M	Hexane: chloroform (1:1)	Blurred light yellow	0.46
*S.ovatum*M	Hexane: methanol (9:1)	Yellow	0.77

*S. ovatum*M = *Stigmaphyllonovatom* methanol extract

Table 3: Malarial chemosuppressive activities of *S. ovatum* against *P. falciparum*

Days of Therapy	Experimental groups of mice	Dose of the rapeuticagents given (mg/kg/day)	Mean parasite counts ($\times10^3$ cells/µl)	(%)	MCS (%)	NCS(%)
0	NC group	12	77.05±3.18	1.54±0.06	-	-
	PC group	219	76.14±4.45	1.52±0.09	1.18	0
	T1 *S. ovatum*	21	75.46±1.51	1.51±0.03	2.06	
	T2 *S. ovatum*	42	77.90±2.14	1.62±0.03	{1.11}	0
	T3 *S. ovatum*	84	80.64±1.49	1.61±0.03	{4.66}	0
2	NC group	12	80.79±2.60	1.62±0.05	-	-
	PC group	219	31.78±1.28	0.64±0.03	60.67	59.49
	T1 *S. ovatum*	21	73.45±0.71	1.47±0.01	9.09	
	T2 *S. ovatum*	42	74.34±0.46	1.49±0.01	7.99	
	T3 *S. ovatum*	84	77.79±2.91	1.56±0.06	3.72	
4	NC group	12	83.58±15.94	1.67±0.32	-	-
	PC group	219	13.52±0.57	0.27±0.01	83.82	82.65
	T1 *S. ovatum*	21	85.44±14.84	1.72±0.29	{2.23}	{4.29}
	T2 *S. ovatum*	42	68.32±0.71	1.37±0.01	18.26	19.37
	T3 *S. ovatum*	84	56.47±2.10	1.13±0.04	32.44	37.10

Antimalarial potential

Table 3 represents the malarial chemo suppressive activities obtained during therapy of *P. falciparum*-infected mice with the test sample (methanol extract) and positive control (quinine). Quinine-treated mice had the lowest parasite counts (mean parasite counts at day 4 of therapy) with 0.27±0.01%, while the lowest parasite count for methanol extract of *S. ovatum* was recorded as 1.13±0.04 (day 4, 84 mg/kg/day) and in the mice treated with DMSO as 1.67±0.32%, thus indicating that the methanol extract has suppressive effect on *P. falciparum* parasite more than the negative control group but less than quinine treated group. Mean chemo suppression of quinine treated *P .falciparum* –infected mice increased successively from day 0, 2 and 4 with 1.18, 60.67 and 83.82%, respectively while the methanol extract at day 0, 2 and 4 (84 mg/kg/day) had mean chemo suppression of 4.66, 3.72, and 32.44%, respectively. All the doses of the methanol extract exhibited significant suppression ($P < 0.05$).

MCS indicates mean malarial chemosuppression; NCS indicates net mean malarial chemosuppression; NC indicates the negative control group of *P. falciparum*-infected mice which received dimethyl sulfoxide; PC indicates the positive control group of *P. falciparum*-infected mice which were administered with quinine; T1 *S. ovatum*, T2 *S. ovatum*, T3 *S. ovatum*, indicate the experimental groups of *P. falciparum*-infected mice which were administered with varying doses of *S. ovatum* methanol extracts.

Table 3 represents the malarial chemo suppressive activities obtained during therapy of *P. falciparum*-infected mice with the test sample (methanol extract) and positive control (quinine). Quinine-treated mice had the lowest parasite counts (mean parasite counts at day 4 of therapy) with 0.27±0.01%, while the lowest parasite count for methanol extract of *S. ovatum* was recorded as 1.13±0.04 (day 4, 84 mg/kg/day) and in the mice treated with DMSO as 1.67±0.32%, thus indicating that the methanol extract has suppressive effect on *P. falciparum* parasite more than the negative control group but less than quinine treated group. Mean chemo suppression of quinine treated *P. falciparum* – infected mice increased successively from day 0, 2 and 4 with 1.18, 60.67 and 83.82%, respectively while the methanol extract at day 0, 2 and 4 (84 mg/kg/day) had mean chemo suppression of 4.66, 3.72, and 32.44%, respectively. All the doses of the methanol extract exhibited significant suppression ($P < 0.05$).

The mean malaria chemo suppression of the extract of *S. ovatum* is further represented below graphically in Fig. 1 while the net mean malaria chemo suppression between *S. ovatum* and standard drug quinine is shown in Fig. 2.

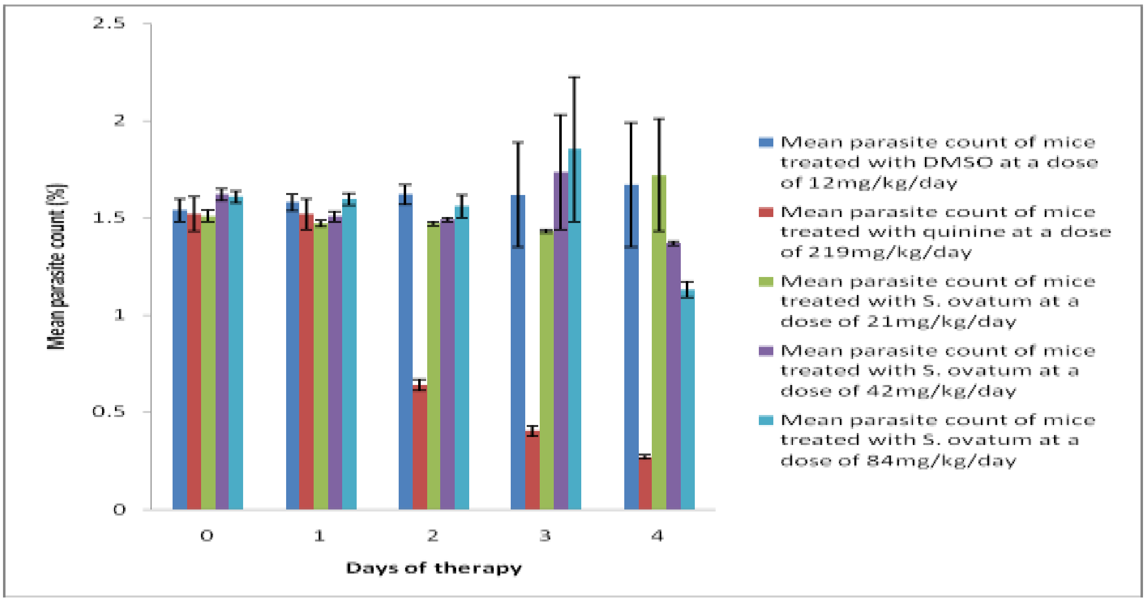

Fig. 1: *P. falciparum* densities obtained from quinine-validatedinfected mice treated with methanolic extract of *S. ovatum*

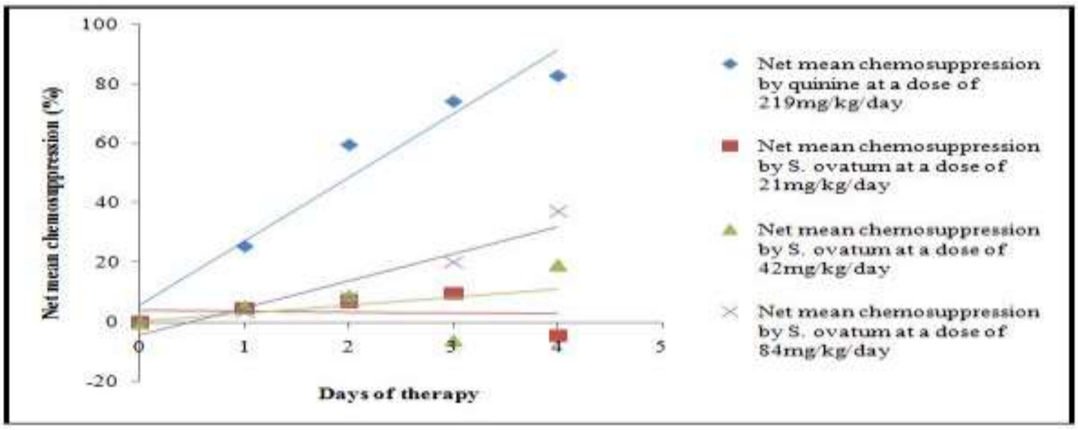

Fig. 2: Effects of treatment with methanol extracts of *S. ovatum* and quinine on *P. falciparum* suppression in the *P. falciparum*-infected mice

In Fig. 1 above, the degree of chemosuppression of *P. falciparum* upon treatment with varying doses of the methanol extracts and control were compared. Chemosuppression of *P. falciparum* parasites by the methanol extract was dose dependent. Parasite suppression was highest in the infected mice treated with 84 mg dose of *S. ovatum* extracts/kg body weight/day and lower in the mice treated with 21 mg dose of *S. ovatum* extracts/kg body weight/day.

In Fig. 2 above, the net mean chemosuppression of *P. falciparum* parasites by the 84 mg dose of *S. ovatum* extracts/kg body weight/day as well as the 219 mg dose of quinine/kg body weight/day were estimated at 37.10, and 82.65%, respectively. The degree of chemosuppression of *P. falciparum* offered by methanol was dose dependent. Parasite suppression was highest in the infected mice treated with 84 mg dose of *S. ovatum* extracts/kg body weight/day and lowest in the mice treated with 21 mg dose of *S. ovatum* extracts/kg body weight/day.

Conclusion

The phytochemical screening had shown that methanol extract of *S. ovatum* contains different essential bioactive phytochemicals. The antimalarial activity (malarial chemo suppressive activity) indicated that methanol extract of the plant have suitable antimalarial agent which when isolated can be an antimalarial drug precursor or drug.

Conflict of Interest

Authors have declared that there is no conflict of interest reported in this work.

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