

PHYTOCHEMICAL AND ANTI-PLASMODIAL SCREENING OF THREE SELECTED TROPICAL PLANTS USED FOR THE TREATMENT OF MALARIA IN OSOGBO, SOUTHWESTERN NIGERIA

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Abstract

The use of herbal remedy is featuring prominently as alternative to orthodox medicine but little is known on scientific validation of their efficacies in malaria treatment. Questionnaire survey was conducted in Osogbo metropolis to identify the frequently used antiplasmodial herbal remedies. The aqueous extracts of the three frequently used antimalaria herbs, *Mangifera indica* leaves, *Lawsonia inermis* leaves and *Enanthia chlorantha* stem bark were prepared as described by herbal vendors and subsequently analyzed for phytochemical constituents and antiplasmodial efficiencies using mice model. The qualitative phytochemical analysis of the extracts showed differences in the phytochemical constituents of the three plants. The comparison of the parasite load before and after treatment showed that the parasitemia level reduced significantly ($p < 0.05$) in the mice treated with *E. chlorantha* and *M. indica* but increased significantly ($p = 0.012$; $p < 0.05$) in the group treated with *L. inermis* while no parasite was detected in the group treated with chloroquine (antimalaria drug) after treatment. The treated groups had higher concentrations of creatinine, urea, bilirubin, Aspartate aminotransferase and Alkaline phosphate in comparison with the control, an indication of the plant extracts cyto-toxicity. The results therefore showed that the extracts of *E. chlorantha* and *M. indica* only possess chemosuppressive not curative antimalaria potential while *L. inermis* did not show any antiplasmodial effect. Further screening on antimalaria herbal remedies therefore becomes imperative so as to guide the policy on malaria treatment regime in Nigeria.

Key words: Phytochemistry, antiplasmodial, plant extracts, biochemical markers

1.0 Introduction

Malaria is a deadly disease and a major public health problem in tropical and sub-tropical regions (WHO, 2002). It is commonly associated with poverty and is a major hindrance to economic development (Mboera *et al*, 2007). The disease is caused by *Plasmodium* species and transmitted by female *Anopheles* mosquitoes (Adeleke *et al.*, 2010). Malaria is the number one cause of mortality and morbidity in Nigeria and accounts for 25 and 30% of infant and childhood death respectively (FMH, 2005).

Several strategies have been deployed to combat the threat of malaria in Nigeria. The current strategies rely on the use of insecticide treated bednet (ITN) to prevent man- mosquito contact and artemisin based therapy for the treatment of malaria in the infected individuals (FMH, 2005). In addition to these efforts in malaria control using conventional methods, herbal treatment has been used as an alternative in malaria endemic countries in Africa for a number of years (Ahmed *et al.*, 2010). The recent event of chemotherapeutic failure has made it to gain prominence as first choice of treatment in many rural and urban areas of Nigeria (Aijayeoba *et al.*, 2006). In Nigeria, several authors have documented the use of various plants for malaria herbal remedies (Aijayeoba *et al.*, 2003; Aijayeoba *et al.*, 2006; Idowu *et al.*, 2010; Onaku *et al*, 2011; Erinoso and Aworinde 2012). Despite the wide use of herbs, the scientific validation of their anti-plasmodial potency has not been thoroughly effected (WHO, 2002). Therefore adequate knowledge and scientific validation of the anti-malaria efficacy of these herbal medicines are necessary to guide policy makers in their formulation of guidelines for the use of herbal medicines in the treatment of malaria in the Nigeria. In this study, we document the phytochemical constituents and anti-plasmodial potency of three most frequently used plants for the treatment of malaria in Osogbo, southwestern Nigeria.

2.0 Materials and Methods

2.1 Study Area

2.1.1 Survey on Plants Used for the Treatment of Malaria in Osogbo Metropolis

The study was carried out in Osogbo, Osun State, Nigeria. Osogbo lies on the latitude 7°49'N and longitude 4°37'E. Ethnobotanical survey to identify the frequently used anti-malaria plants was carried out among the traditional herb vendors in three major herb selling markets, Oja- Oba, Oluode and Orisunmibare in the greater Osogbo Metropolis.

Structured questionnaires were administered to the traditional herb vendors to gather information on the types of plants used for the treatment of malaria, the method of preparation of the extracts and the doses administered. The responses of the vendors on the plants were ranked, and the three frequently mentioned plants were selected for this study.

2.2 Plant Materials

The plants and plant parts that were frequently mentioned by the vendors were *Mangifera indica* (leaf), *Lawsonia inermis* (leaf) and *Enanthia chlorantha* (stem bark). The plants were purchased and later authenticated at the Department of Biological Science, Osun State University, Osogbo, Nigeria.

2.3 Extracts Preparation

The crude extracts were prepared as mentioned by the vendors. Ten grammes of the stem bark of *E. chlorantha* were weighed and soaked in 500 ml of water and boiled for 20mins. This was repeated using the leaves of *M. indica* and *L. inermis*. A combination of the three extracts was also prepared.

2.4 Phytochemical Screening

The aqueous extracts of the plant parts of *E. chlorantha*, *L. inermis* and *M.indica* were subjected to phytochemical analysis using protocol previously described by Sofowora (1993). Tannins and flavonoids were determined by adding 2ml of sample with few drops of ferric chloride and hydroxide solution respectively. The brownish green or blue black colouration showed the presence of tannins while yellow colouration indicated the presence of flavonoids. Saponnins were determined by floatation method and the formation of emulsion indicated the presence of saponnins. Alkaloids were determined by dissolving 1 ml of extract in 2 ml of 0.1M hydrochloric acid in a test tube with few drops of Meyers reagent. The presence of turbid precipitate showed the presence of alkaloids.

2.5 Antiplasmodial Screening using Animal Model

The mice used for the study were obtained from the Nigeria Institute of Medical Research (NIMR) Yaba, Lagos Nigeria. The mice were maintained on food and water ad libitum in the animal house of the College of Health Sciences, Osun State University Osogbo in accordance with current ethical guidelines for the care of laboratory animals.

2.6 Malaria Parasite

The malaria parasite, *Plasmodium berghei*, was obtained from the Nigeria Institute of Medical Research, Yaba, Lagos, Nigeria. Parasitised erythrocytes were obtained by bleeding the eyes of donor mice and diluted using phosphate buffer saline (PBS) to a concentration of 1×10^7 (parasites/ml). The mice were inoculated intraperitoneally with 0.2ml of blood suspension containing the parasitized erythrocytes on Day 0.

2.7 Treatment Regime

Forty mice were randomly allocated into six groups, each group containing six mice, with the exception of Group 7 which had four mice. Post infection treatment was done on establishment of parasitemia in the mice after three days of inoculation. Treatment was based on the body weight of the mice. The mice were treated using the extract twice daily as per the information obtained from the traditional herb vendors. All animals were treated for five days. The animals were grouped as follows;

Group 1 were treated with 0.1 ml extract of *M. indica* leaves

Group 2 were treated with 0.11 ml extract of *E. chlorantha* stem bark

Group 3 were treated with 0.11 ml extract of *L. inermis* leaves

Group 4 were treated with 0.12 ml combination of the three extracts.

Group 5 were treated with 0.13 ml of chloroquine (65 mg/kg^{-1}) twice daily.

Group 6 were not infected with the parasite and not treated with extracts or drug

Group 7: Infected but not treated

2.8 Parasitemia Determination

Thick smears of blood films were prepared three days after inoculation of the mice and five days after the commencement of treatment using the peripheral blood collected from the tails of the mice. The films were stained with Giemsa and the number of parasitized erythrocytes was enumerated using 50 high-power objectives.

2.9 Kidney and Liver Function Tests

Some enzymes and biochemical markers associated with the kidney and liver function were determined. Aspartate aminotransferase (AST), bilirubin, alkaline phosphate (ALP), creatinine and urea were determined using the serum obtained from the mice upon sacrifice after five days of treatment. The parameters were determined using Randoxkits (England) following manufacturer's instructions and absorbances (nm) were read using UNISPEC SM7504UV Spectrophotometer, (UNISCOPE, England).

2.10 Statistical Analysis

Paired t-test was used to determine the significant difference in the number of parasitized erythrocytes before and after treatment. Analysis of variance was used to determine significant difference in the biochemical parameters determined. All analysis were done using SPSS version 16.0

3.0 Results

3.1 Phytochemical Screening

The results of the qualitative phytochemical analysis of the three extracts are presented in Table 1. The results showed that the stem bark of *E. chlorantha* contained tannins, flavonoids and saponins but not alkaloids, while the leaves of

M. indica and *L. inermis* contained tannins, saponins but not alkaloid and flavonoids respectively.

Table 1: Phytochemical analysis of Magnifera indica leaves, Lawsonia inermis leaves and Enanthi chlorantha stem bark

Tested parameters	<i>E. chlorantha</i>	<i>M. indica</i>	<i>L. inermis</i>
Tannins	±	++	++
Flavonoids	+	±	ND
Saponins	++	++	+
Alkaloids	ND	ND	++

++ Strongly positive, + positive, ± weakly positive, ND= not detected

3.2 Antiplasmodial Activities of the Plant Extracts

The comparison of the parasitemia infection before and after treatment showed that the parasitemia level reduced significantly ($p < 0.05$) in the mice treated with *E. Chlorantha* and *M. indica* extract. The group treated with the combination of the three extracts had reduced parasitemia but the difference in the number of parasitized erythrocytes before and after treatment was not significant ($p = 0.058$; $p > 0.05$). The parasitemia level however increased significantly ($p = 0.012$; $p < 0.05$) in the group treated with *L. inermis* extract while no parasite was detected in the group treated with chloroquine (conventional malaria drug). All the mice infected but not treated died between 3-5 days post inoculation (Table 2).

Table 2: The mean parasitemia of the Plasmodium berghei infected mice before and after treatment with aqueous plant extracts.

Treatment groups	Dose ml/kg	Mean parasitemia before treatment	Mean parasitemia after treatment	P value
G1	0.1	30.00±7.07	11.25±2.5	0.029
G2	0.11	31.25±9.46	13.75±2.5	0.027
G3	0.11	10.00±3.35	31.50±4.78	0.012
G4	0.12	10.65±3.14	6.87±5.6	0.058
G5	0.13	16.25±2.50	0.000	0.0001
G6	0.11 of water-	00	00	00
G7	0.12	30.25±8.34	ND	ND

The results are mean and standard deviation in 50 high power fields. Group 1= Mice treated with aqueous extract of *M. indicaleaves*, Group 2= Mice treated with aqueous extract of *E.chlorantha* stem bark, Group 3: mice treated with aqueous extract of *L. Inermis* leaves; Group 4: mice treated with the combination of the three aqueous extracts, G5= mice treated with chloroquine, Group 6= control (not

infected nor treated), G7= Group infected but not treated. ND= not determined because all the mice died before day 6 post inoculation.

P value <0.05 is significant.

3.3 Biochemical Analysis of the Liver and Kidney Function

Kidney function test: The concentrations of creatinine and urea determined in the sera of the treated mice are presented in Table 3.

Table 3: Biochemical analysis of the liver and kidney function tests

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Bilirubin (mg/dl)	4.50±0.1a	3.39±0.0a	3.62±0.7a	3.43±0.3a	3.95±0.6a	3.95±0.9a
ALP (µmol/l)	17.05±0.8a	14.30±3.1a	11.55±0.7a	9.85±1.5a	9.24±0.9a	9.35±3.9a
AST (µmol/l)	22.92±6.2a	39.99±3.4a	18.99±3.4a	30.73±18.8a	31.35±12.9a	39.07±12.2a
Creatinine (mg/dl)	0.845±0.1a	0.92±0.0a	0.73±0.2a	1.20±0.5a	1.27±0.2a	1.08±0.2a
Urea (µmol/l)	11.66±0.3b	6.77±1.0a	8.30±1.1a	13.55±3.2b	7.82±2.5a	8.29±0.9a

AST= Aspartate aminotransferase; ALP= Alkaline phosphate

Group 1= Mice treated with aqueous extract of *M. indica* leaves, Group 2= Mice treated with aqueous extract of *E. chlorantha* stem bark, Group 3: mice treated with aqueous extract of *L. Inermis* leaves; Group 4: mice treated with the combination of the three aqueous extracts, G5= mice treated with chloroquine, Group 6= control (not infected nor treated),

Values with same letter along the row are not significant at $p < 0.05$.

The results revealed that the concentration of creatinine was lower in the mice treated with the plant extracts when compared with the control (untreated) mice with the exception of mice treated with a combination of the extracts which showed higher values than that found in the control mice. However, the differences in the concentrations between the group were not significant ($p < 0.05$). The analysis of the urea levels showed that the mice treated with *E. chlorantha* extract and chloroquine had mean urea level lower than that in the control, while the concentration was significantly higher in other groups ($p < 0.05$) except for mice treated with aqueous of *L. inermis* extracts which did not show any statistical differences ($p > 0.05$).

Liver function Test: The concentrations of bilirubin and AST were lower in all the treatment groups when compared to the controls except in the group that was treated with a combination of *M. indica* and *E. chlorantha* extract which showed higher concentrations of bilirubin and AST respectively than in the control. However, the concentration of ALP was higher in all the groups than control except in group

treated with chloroquine. The difference in the concentrations of all the parameters determined was not significant among the groups ($p < 0.05$) (Table 3).

4.0 Discussion

The use of herbal medicine is becoming popular as an alternative to orthodox medicine (Ahmed *et al.*, 2010; Erinoso and Aworinde, 2012) but scientific validation of their efficacies in disease treatment was relatively unknown. *M. indica* and *L. inermis* extracts have a long history of being used as a cure for malaria in many rural communities in Nigeria (Idowu *et al.*, 2010). However there has been paucity of information on anti-plasmodial potency of *E. chlorantha*. The results obtained in the present study showed that aqueous leaf extracts of *M. indica*, aqueous stem bark extract of *E. chlorantha* and the combination of the three aqueous extracts could suppress but not completely kill the malaria parasite. The leaf extract of *L. inermis* did not show any anti-plasmodial effect since the parasite load increased significantly even after treatment. Nevertheless, some of the mice treated with *L. inermis* extract survived unlike the infected but not treated group that died between 3-5 days post inoculation. *L. inermis* extract has been known to contribute to replenishing of blood in the system (Idowuet *al.*, 2010) and it is not unlikely that more erythrocytes are produced as the infected ones are parasitized. However treatment of the mice using drug chloroquine showed a complete curative effect. The difference in the antiplasmodial potency of the extracts may be associated with variation in the phytochemical constituents. Aqueous extracts of *M. indica* leaves and *E. chlorantha* stem bark had saponnins and flavonoids which were almost absent or not found in the aqueous extract of *L. inermis*. These two phytochemical constituents have been known to possess anti-microbial and medicinal properties (Sofowora, 1993).

The extraction methods play major role in the pharmacologic properties of herbal remedies (Mathaura *et al.*, 2007). The chemo-suppressing abilities of the plants may be due to the extraction method normally used locally for the preparation of the herbs. In this study, the plant extracts were prepared as described by the traditional herbal vendors. It is likely that this method may not be suitable enough to extract the active anti-malaria agents from the plants. The suppressing but not curative potentials had also been reported on other plants used for the malaria treatment in Nigeria (Shittu *et al.*, 2011). This in-turn implies that people taking these herbs may not be cured until standard anti-malaria drugs are used. The suppressing effects of the extracts have epidemiological implications in the control of malaria as it can easily promote resistance to antiplasmodial drugs over time. Even though the thrust of the present study was not geared towards assessing the perception of the people/patients using these herbs for malaria treatment in the locality, further studies on this subject line would be of great benefit in educating people on the abuse of these extracts.

One paramount but often overlooked aspect of herbal treatments is the cytotoxicological effects in the system. The relatively high concentrations of the enzymes and biochemical markers used to test the kidney and liver functions may suggest the toxic effects of these herbal extracts in some treated groups as compared to the control. The possibility of cyto-toxicity of herbal remedies had also been reported in literature (Aijayeobaet *al.*, 2006; Idowu *et al.*, 2010). Albeit the concentrations of these enzymes and biochemical markers compared favourably with concentrations in the mice treated with chloroquine. The cyto-toxicity of chloroquine as a conventional malaria drug has been reported by Taylor and White (2004).

5.0 Conclusion

The results of this study showed that two out of the three frequently used plants for the treatment of malaria in Osogbo, Nigeria have chemo-suppressing but not curative potential. These observations thus provide impetus for further scientific screening of other herbal remedies used for the treatment of malaria in the endemic areas. Proper documentation and scientific validations of these herbs will go a long way to discourage the abuse of herbal remedies that may later escalate the spate of drug resistant Plasmodium in the endemic communities.

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