ANTI-PLASMODIAL ACTIVITIES OF COMBINED EXTRACTS OF *Morinda morindiodes, Morinda lucida* AND *Vernonia amygdalina* IN *Plasmodium berghei* INFECTED-MICE

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Abstract

The development of resistance to a number of current drugs by *Plasmodium species* necessitated the search for herbs which have been traditionally employed in treatment of malaria. In this study, extracts of *Morinda morindiodes* (Mm) root, *Morinda lucida* (ML) leaf and *Vernonia amygdalina* (Va) (leaf) were combined and assessed for anti-malarial activities in *Plasmodium berghei* infected-mice. Infection of each mouse was initiated with 1×10^4 infected erythrocytes. Plant materials were extracted with water for three days and were dehydrated; 0.1mg/g body weight of the extract was given to each infected mouse in a six-day treatment. At the end the sixth day, Mm root extract, ML and Va leaf extract produced 86.87%, 84.73% and 47.81% suppression of parasitaemia respectively. Combination of *M. lucida* with *V. amygdalina* (ML+Va) *and Morinda morindiodes* (ML+Mm) produced 40.4% and 70% suppression respectively. The combination of *M. morindiodes* with *V. amygdalina* (Mm+Va) produced 50.63% suppression on day six of treatment. The combination of the three plants extract produced 53.21% suppression compared to the untreated group which recorded no suppression at the end of the sixth day ($p<0.05$). These results were significantly different ($p<0.05$) when compared with the infected non-treated group. The results obtained from this study revealed that though, the extracts of *Morinda morindiodes* and *Morinda lucida*, possess antiplasmodial effect when administered singly, the combination of these extracts reduced their chemosupression.

Keywords: malaria, herbal, treatment, parasitaemia, antimalarial, mice.

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Introduction

Malaria is a global disease that is predominant in the tropics and caused by blood parasites of *Plasmodium species* which includes *Plasmodium falciparium, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium vivax.* Malaria remains the world most devastating human parasitic infection (WHO, 2013a). Anti-malaria drugs have been used in various ways to prevent or treat malaria infections in malaria endemic areas for many years. The resistance of malaria parasite to anti-malaria drugs has motivated scientists into an intensive search for more effective agents against the parasites. Currently, there are reports of parasite resistance to the newly developed Artemisinin in some regions of sub-Saharan Africa (WHO, 2013b).

In recent times, natural products of plant sources have been the centre of focus as the main source of new, safer and more effective bio-active compounds with medicinal properties (Dike *et al,* 2012). Medicinal plants have been used in the treatment and prevention of malaria in various parts of the world. Quinine extracted from bark of the Cinchona tree was used as

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an anti-malarial agent as early as 1632 (Baird *et al,* 1996). Studies have documented over 1,200 plant species from 160 families used in the treatment of malaria or fever (Wilcox and Boderker, 2001). In fact, it is believed strongly that if the herbs used to treat malaria by our ancestors were not effective, malaria would have destroyed Africa. More so, missionaries that came to Africa would not have met a single person on the continent of Africa (Elujoba, 2005).

In Nigeria, malaria has become a household name, and the use of herbs to treat its symptoms is not new to all and sundry. Alaribe (2008) opined that about 80% of Nigerian homes, maintain some sort of private family traditional medicine practitioner. Furthermore, recent surveys conducted in different parts of Nigeria revealed many plants that had been identified by users to be potent against malaria (Nwachukwu *et al,* 2010; Idowu, *et al,* 2010).

Vernonia amygdalina (*bitter-leaf*) commonly found in West and Central African countries and *Morinda lucida* commonly found in northern and southern Nigeria, Fernadopo and over the Congo basin (Keay, 1989) have been proven to have anti-plasmodial or anti-malarial activities against drug sensitive *Plasmodium berghei* in mice (Abosi and Raseroke, 2003; Bello *et al,* 2009; Ebiloma *et al,* 2011; Paula *et al,* 2011; Lawal *et al,* 2012). A recent study has demonstrated that *Morinda lucida* possesses antimalarial properties attributed to anthraquinones (Idowu *et al,* 2010). The stem bark extract as well as the aqueous leaf extract of *M. lucida* have been reported to have chemosuppression properties with 96.4% and 85.05% respectively (Obih *et al,* 1985; Ebiloma *et al,* 2011) in *P. berghei* infected mice.

M. morindiodes (Rubiaceae) enjoys considerable reputations in traditional medicine in some African countries. It's use against malaria, diarrhoea, amoebiasis, gonorrhoea and rheumatic pains is frequent (Kambu, 1990). The root extract of *M. morindiodes* have been reported to have anti-plasmodial properties with 70% chemosuppression in *P. berghei* infected mice (Soniran *et al,* 2011).

In traditional medicine, the use of herbal plants for treatment of ailments is either singly or a combination of different plants/plant parts (Idowu *et al,* 2010; Rasoanaivo *et al,* 2011). There is a dearth of information on the anti-malarial activities of combined plant/plant parts which individually posses anti-malarial properties. It is against this background that this study was carried out to evaluate the anti-plasmodial activities of combination of extracts of *M. morindiodes*, *M. lucida* and *V. amygdalina* in *P. berghei* infected mice.

Materials and method

Plant materials

The plants were collected from locations around Odeda Local Government, Abeokuta. Visual Identification and authentication were done by Mr. Ekundayo of the Forestry Research Institute of Nigeria (FRIN) and were also validated by Dr. Aworinde of Botany Department, Federal University of Agriculture, Abeokuta.

Preparation of crude extract

The leaves and bark were washed, dried at room temperature, and then pulverised using plant grinder. The root was washed and air dried at room temperature and was powdered using mortar and pestle. These were soaked separately in distilled water for 72 hours and later filtered. The filtrates were afterward evaporated to dryness at 55^0C and 0.232 g each of the filtrates were dissolved in 10 mls of distilled water to give a concentration of 23.2 mg/ml.

Animals

A total 45 mice (weighing between 23.0 g-23.2 g) with mean weight of 23.2±0.2 g obtained from Nigerian Institute of Medical Research (NIMR), Yaba, Lagos were used in this study. The mice were kept in cages at room temperature (27^0C) and were fed with standard ration (Vital Feeds Limited, Ibadan) and clean water in the animal house.

Parasites

Sample of *P. berghei* (NK-65), obtained from National Institute of Medical Research (NIMR), Lagos, Nigeria, was used for the research to evaluate the anti-malarial activity of the plant materials used in this study.

Inoculation of experimental mice

Malaria parasite inoculums were prepared by collecting blood samples from donor mouse. The blood collected from the donor mouse was then diluted with normal saline such that 0.1 ml contained 10^4 of the parasite. Forty-five mice divided into nine groups of five mice each; all the mice were infected with the parasites by inoculating them intraperitonially with 0.1 ml of the prepared blood solution.

Blood smear were made and observed under the microscope on daily basis, to confirm the establishment of the parasite infection in the experimental mice. Treatment began when parasitaemia was established in the infected mice.

Treatment of infected mice

Eight out of the nine groups were treated with the extracts using oral administration with a single dose per day. A dosage of 0.1mg/g was administered to each mouse for treatment with single plant extract. A dosage of 0.05mg/g each of the combining plant extracts was added in the combined treatment. The untreated group served as the negative control while the group treated with Artesunate at a dose of 0.1mg/g served as the positive control for the experiment. The treatment continued daily for six consecutive days. *M. morindiodes* (Mm), *V, amygdalina*(Va) and *M. lucida* (Ml) extracts were administered as single therapy, while (Mm +ML), (Mm + Va), (Ml + Va) and (Mm + ML + Va) extracts were administered as combined therapy and Artesunate (Ar) was administered to the control group.

Estimation of parasitaemia in experimental mice (treated and untreated)

Each day, blood samples were taken from the caudal vein of each mouse on a clean glass slide, thin films were prepared and stained with 10% Giemsa solution. The parasitaemia was estimated by careful examination of the well-stained thin blood film. This was accomplished by counting the number of parasitized red blood cells (RBC) seen in 10^3 red blood cells using the ×100 oil immersion lens of a light microscope (Olympus, Japan). The number of parasitized red blood was then divided by the total number of red blood cells and then multiplied by 100 to express it as a percentage. (Mohd *et al,* 2007).

Estimation of percentage chemo suppression

Percentage chemo-suppression was estimated by subtracting the average percentage parasitaemia for untreated group, from the average percentage parasitaemia for the treated group and then divided by the average percentage parasitaemia for untreated group and multiplied by 100 (Mohd *et al,* 2007).

Statistical analysis

The results were analyzed using the ANOVA test. This was used to compare the results at a 95% confidence level. Values of $p<0.05$ were considered significant, using SPSS 16.0 Version.

Results

There was a systematic increase in chemo-suppression by all plant extracts from day 1 to day 6, although the extent of chemo-suppression varies with each plant extract. The root extract of *M. morindiodes* was found to produce a high reduction in parasitaemia (Table 1) with chemo-suppression of 86.9% on day 6 (Table 2). Extract of *M. morindiodes* demonstrated the highest level of chemosuppression when compared with the other plant extracts used in this study and even recorded a significantly higher day 1 ($p<0.05$) chemo-suppression than the positive control (Artesunate) (Table 2).

Table 1. Mean percentage parasitaemia of mice treated with single therapy.

Treatment Groups	Day 1 (%)	Day 2 (%)	Day 3 (%)	Day 4 (%)	Day 5 (%)	Day 6 (%)
M. morindiodes	23.40	21.98	20.53	11.87	10.40	4.30
M.lucida	27.75	26.75	25.00	18.20	11.00	7.00
V. amygdalina	29.08	28.65	23.50	18.84	18.03	17.09
Artesunate	26.30	20.83	9.97	3.18	0.00	0.00
Untreated	29.80	30.50	30.63	31.21	32.98	32.74

Table 2. Percentage Chemosuppression of Parasitaemia of infected mice treated with single therapy.

Treatments	Day 1 (%)	Day 2 (%)	Day 3 (%)	Day 4 (%)	Day 5 (%)	Day 6 (%)
M. morindiodes	21.48	27.93	32.84	61.97	68.52	86.87
M.lucida	6.88	12.30	18.38	41.69	66.65	84.73
V. amygdalina	2.42	6.07	23.28	39.63	45.32	47.81
Artesunate	11.75	31.71	67.45	89.84	100.00	100.00
Untreated	0.00	0.00	0.00	0.00	0.00	0.00

Chemo-suppression of *M. lucida* on Day 1 was 6.48% compared with 21.48% recorded in *M. morindiodes*. *V. amygdalina* produced a significantly ($p<0.05$) lower chemo-suppression than *M. morindiodes* and *M. lucida* on day 6 of the experiment. The differences observed in chemo-suppression of *M. morindiodes* and *M. lucida* was observed to be statistically ($p<0.05$) significant only for the first four days of the experiments. All the plant extracts show significant differences ($p<0.05$) when compared with the untreated group (Table 2).

Combined treatment

The combination of *M. morindiodes* and *M. lucida* for treatment of infected mice treatment was found to produce a high reduction in parasitaemia (Table 3) with chemo-suppression of 70.50% at day 6 (Table 4). It

produced the highest level of chemo-suppression among all the combinations of plant extracts used in the study, with a higher day one chemo-suppression compared with that of the positive control (Artesunate). Despite the strength of *M. morindiodes* and *M. lucida* in monotherapy, administration of combination of these extracts in infected mice was unable to achieve the chemosuppression level recorded when administered singly (Table 4).

Of all the plant extract combined in the study, combination of *M. lucida* and *V. amygdalina* (Ml + Va) recorded the lowest chemo-suppression at Day six. Chemo-suppression was observed to peak on Day five and declined on Day 6 in the combination treatment with Ml+Va and Mm+Ml+Va but increased from Day 1 to 6 in the other combinations (Table 4). None of the combined plant extracts was able to attain the chemo-suppression observed in the single treatment with *M. morindiodes* and *M. lucida* (Table 4).

Table 3. Mean percentage parasitaemia of mice treated with combined therapy.

Treatment Groups	Day 1 (%)	Day 2 (%)	Day 3 (%)	Day 4 (%)	Day 5 (%)	Day 6 (%)
Mm + Ml	22.45	22.60	20.24	12.83	10.78	9.66
Mm + Va	19.87	20.5	21.75	22.16	19.01	16.16
Ml + Va	25.98	26.03	24.03	19.96	15.23	19.63
Mm + Ml + Va	23.10	23.68	21.75	13.75	12.60	15.35
Artesunate	26.30	20.83	9.97	3.18	0.00	0.00
Untreated	29.80	30.50	30.63	31.21	32.98	32.74

Table 4. Percentage chemo-suppression of parasitaemia of infected mice treated with different combinations of plant extracts.

Treatment	Day 1 (%)	Day 2 (%)	Day 3 (%)	Day 4 (%)	Day 5 (%)	Day 6 (%)
Mm + Ml	24.66	25.90	33.92	58.87	67.30	70.50
Mm + Va	33.29	32.79	28.99	38.60	42.35	50.63
Ml + Va	12.82	14.66	21.55	36.05	53.82	40.04
Mm + Ml + Va	22.48	22.36	28.99	55.94	61.76	53.21
Artesunate	11.75	31.71	67.45	89.84	100.00	100.00
Untreated	0.00	0.00	0.00	0.00	0.00	0.00

Discussion

In this study, the anti-plasmodial effects of three plant extracts both in single therapy and combined therapy was observed in a six day treatment of *P. berghei* infected-mice.

M. morindiodes initiated a very early chemo-suppression than Artesunate and other plant extracts when administered singly or in combination (it recorded a significantly higher chemo-suppression than artesunate on the first day of treatment). This is an indication that *M. morindiodes* is able to respond quickly as an anti-plasmodial agent, although the threshold was not maintained in this study. Soniran *et al* (2011) also reported a similar performance in water extract of the root of *M. morindiodes* in which a significant reduction in parasitaemia (70%) was reported in comparison to the activities of other plant parts in *P. berghei* infected mice.

Leaf extract of *M. lucida* in this study demonstrated a chemo-suppression of 84.7% in a six-day treatment. This supports the findings of Ebiloma *et al* (2011), who reported that the *in vivo* anti-plasmodial activity of aqueous leaf extract of *M. lucida* carried out in *P. berghei* NK-65 parasitized mice showed a significant chemo-suppression of up to 85.05%. Studies from the phytochemical screening of the aqeous leaf extract of *M. lucida* revealed the presence of alkaloids and flavonoid as the predominant secondary metabolite (Ebiloma *et al,* 2011). Therefore, the observed anti-malaria activity in the group treated with *M. lucida* may be attributed to its high alkaloid and flovonoid contents. Previous works have also shown the anti-malaria activity of alkaloids and flavonoids in plants (Balogun *et al,* 2009; Okokon *et al,* 2005)

Leaf extract of *V. amygdalina* demonstrated a percentage chemo-suppression of 47.81% in a six-days treatment. Leaf extract of *V. amygdalina* assessed against the rodent-malaria parasite *P. berghei* by Abosi and Raseroka (2003) produced 67% suppression of parasitaemia. The low suppression rate observed in the mice treated with *V. amygdalina* in this study could be attributed to the dose dependent nature of its crude extract (Abosi and Raseroka, 2003; Njan *et al,* 2008; Paula *et al,* 2011) as many of the studies that reported high suppression, administered the plant extract at higher dose (>200 mg/kg compared with 0.1 mg/g employed in this study).

Studies have also shown that *V. amygdalina* produces varieties of flavonoids and bitter sesquiterpene lactones which contribute to their bio activities (Favi *et al,* 2008). Furthermore, the taxonomy of *V. amygdalina* from different geographical area could be different, hence, possessing variable bio-activities (Austin, 2000). Thus, the low suppression of parasitaemia observed in the mice treated with *V. amygdalina* compared with some other studies

(Abosi and Raseroka, 2003; Njan *et al,* 2008) could also be attributed to difference in geographical locations in which the plants were collected.

The combination of *M. lucida* and *V. amygdalina* (Ml + Va) showed 53.82% on day five which later decreased to 40.04% on day six. This showed that the combination of plant extracts did not show any synergy in this study. This might be due to the fact that the doses of each plant extracts were halved (0.05 mg/g each) when combined, during the experiment.

The standard drug Artesunate produced the highest efficacy, when compared with the activities of the plant extracts. This might be due to the fact that the plant extracts were in their crude forms with the active ingredients not having been isolated and compressed into active drugs (Adzu and Haruna, 2007; Ebiloma *et al,* 2011).

All the extracts showed chemo-suppression property which was significantly different when compared to the infected non-treated group (untreated). A complete clearance was observed in the positive control group treated with Artesunate, in the six-day treatment while the plant extracts showed ability to suppress parasitaemia but none either as monotherapy or combined therapy was able to achieve complete parasite clearance in the six-day treatment.

The results obtained from this study revealed that though, the extracts of *M. morindiodes* and *M. Lucida,* possess anti-plasmodial effect, the combination of these extracts reduced their chemo-supression.

References

Abosi, A. O. and Raseroke, B. H. 2003. *In vivo* anti-malarial activity of *Vernonia amygdalina. British journal of Biomedical science. 60(2):* 89-91.

Adzu, B. and Haruna, A. 2007. Studies on the use of Zizyphus Spina-Christi against pain in rats and mice. *African. Journal of Biotechnology. 6:* 1317-1324.

Alaribe, S. I. 2008. A Survey of the importance and problems of traditional health-care medicine. A case study of Ezinihitte Mbaise L.G.A. Imo State. Unpublished B.Sc. project, A.I.F.C.E. Owerri, Imo State.

Austin, D. F. 2000. Book Reviews. *Economic botany. 54:* 234-245.

Avwioro, O. G.A., Awoyemi, F. A. and Oduola, T. B. 2003. A novel natural collagen and muscle strain from *Morinda lucida* extracts. *Medical Journal 42:* 44-48.

Baird, J. K., Caneta, E. M., Marba, S., Bustos, D. G., Abrencia, J. A., Layawan, A. V., Calulut, J. M., Leksana, B. and Wignall, F. S. 1996. Survey of resistance to chloroquine of *falciparium* and *vivax* malaria in Palawan. *The Philippines Transaction Royal. Social. Tropical Medicine and Hygiene. 90:* 413-414.

Bello, I. S., Oduola, T., Adeosun, O. G., Omisore, N. O., Raheem, G. O. and Ademosun, A. A. 2009. Evaluation of anti-malarial activity of various fractions of *Morinda lucida* leaf extract and *Alstonia boonei* stem bark. *Global Journal of Pharmacology, 3(3):* 163-165.

Dike, I. P., Obembe, O. O. and Adebiyi, F. E. 2012. Ethno-botanical survey for potential anti-malarial plants in south-western Nigeria. *Journal of Ethno-pharmacology, 144(3):* 618-26.

Ebiloma, G. U., Omale, J. and Aminu, R. O. 2011. Suppressive, curative and prophylactic potentials of *Morinda lucida* (Benth) against erythrocytic stage of mice infective *Plasmodium berghei* NK-65. *British Journal of Applied Science and Technology. 1(3)*: 131-140.

Elujoba, T. 2005. Book review "Traditional medicinal plants and malaria. *African. Journal. of Traditional. Complement Alternative. Medicine. Vol. 2(2):* 206-220.

Favi, F., Cantrell, C. L., Mebrahtu, T., Kraemer M. E. 2008. Leaf peltate grandular trichomes of *V. amygdalina spp.* Galamensis var ethiopica gilbart: Development, ultrastructure and chemical composition. *International Journal of Plant Science. 169:* 605-614.

Idowu, O. A., Soniran O. T., Ajana, O. and Aworinde, D. O. 2010. Ethno-botanical survey anti-malarial plants used in Ogun State, South West. Nigeria. *African Journal of pharmacy and pharmacology. 4(2):* 55-60.

Kambu, K. 1990. Elements de phytotherapic compare. *Plants Medicinales Africanus, CRP-Kinshaska.* 20-22.

Keay, R. W. J. 1989. *Trees of Nigeria.* Oxford Science Publishers, pp. 418-437.

Lawal, H. O., Etatuvie, S. O. and Fawehinmi, A. B. 2012. Ethno-medicinal and pharmacological properties of *Morinda lucida. Journal of Natural Products, 5(2012)*: 93-99.

Mabry, T. T., Abdel-Bazet, Z., Pavolina, W. A., Jones, S. B. 1975. Systematic implications of flavonoids and sesquitoterpene lactones in species of Vernonia. *Biochem. Systematics Ecol. 2:* 185-192.

Mohd Ridzuan, M. A. R., Sow, A., Noor Rain, A., Mohd Ilham, A.and Zakiah, I. 2007. *Eurycoma longifolia* extract-artemisinin combination: Parasitemia suppression of *Plasmodium yoelii*-infectedmice. *Tropical Biomedicine. 24(1):* 111-118.

Njan, A. A., Adzu, B., Agaba, A. G., Byarugaba, D., Diaz-Llera, S. and Bangsberg, D. R. 2008. The analgesic and anti-plasmodial activities and toxicology of *Vernonia amygdalina. Journal of Medicine and Food, 11:* 574-581.

Nwachukwu, C. U., Umeh, C. N., Kalu, I. G., Okere, S. and Nwoko, M. C. 2010. Identification and traditional uses of some common medicinal plants in Ezinihitte Mbaise LGA, of Imo State, Nigeria. *Report and Opinion, 2(6):* 1-8.

Obih, P. O., Makinde, J. M. and Laoye, J. V. 1985. Investigation of various extracts of *Morinda lucida* for anti-malarial actions on *Plasmodium berghei* in mice. *African Journal of Medical Science, 14:* 45-49.

Paula, M., William, C., Paschal, E. and Peter, S. 2011. *In vitro* and *in vivo* anti-plasmodial activities of extracts of

Cymbopogon citratus Staph and *Vernonia amygdalina Delile* leaves. *Journal of Natural Products 4 (2011):* 0974-5211.

Rasoanaivo, P., Wright, C. W., Willcox, M. L. and Gilbert, B. 2011. Whole plant extracts versus single compounds for the treatment of malaria: Synergy and positive interactions. *Malaria Journal. 10(1):* 4.

Soniran, O. T., Idowu, O. A., Idowu, A. B., and Ajana, O. 2011. Evaluation of *in vivo* Anti-plasmodial activities of extracts of *Morinda morindiodes* (Bak.) in the treatment of malaria in Ogun State. *International Journal of Biomedical and Health Sciences. 7(4):* 299-306.

WHO. 2013a. Water-related diseases. World Health Organization, Geneva, Switzerland. Retrieved from: http://www.who.int/water_sanitation_ health/diseases/ malaria/en/.

WHO. 2013b. Artemisinin Resistance, World Health Organization, Geneva, Switzerland. Retrievedfrom:http://www.who.int/malaria/media/artemisinin_resistance_qa/en/index.html

Willcox, M.L. and Bodeker, G. 2001. Traditional herbal medicines for malaria. *British Medical Journal. 329:* 1156-1159.

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