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In vivo Evaluation of the Antimalarial Activity of the Aqueous Ethanol Extract of *Monodora myristica* Seed in Albino Mice

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Abstract: ***Introduction*: *In vivo antimalarial activity of ethanol seed extract of Monodora myristica was evaluated in albino mice. Method: Dried Monodora myristica seeds were soxhlet extracted with 70% ethanol. Phytochemical analysis and median lethal dose (LD_{50}) were evaluated. Antimalarial evaluation was evaluated, using 20 mice grouped into 4. Blood was collected from donor mouse infected with Plasmodium berghei by occulo-puncture. The blood was diluted with normal saline and 0.2ml each was administered to the 20 mice. The animals were left for 72 hours to establish infection. The animals were given treatment by single oral administration for seven days. Groups 1- 4 received 10ml/kg distilled water, 0.78mg/kg of ACT, 250 and 500mg/kg of the extract respectively. AST, ALT and ALP were determined by enzyme kits method. Results: The seed yielded 48.78g (8.13%) extract. Monodora myristica seed contained alkaloids (28.4%), saponins (15.20%), and tannins (4.9%). Monodora myristica seed has high moisture content (22.67%) and low ash value (4.52%). The LD_{50} was >5000mg/kg. The significant decrease in parasitemia observed was dose dependent as the 250mg/kg and 500mg/kg of the extract caused 86.64% and 90.58% suppression in parasitaemia respectively, at day 7. The positive controls recorded 94.76% suppression in parasitaemia at day 7. The treatment groups recorded significant decrease ($p<0.05$) in percentage parasitaemia at day 7 compared to the infected untreated group which showed no significant decrease ($p>0.05$) in percentage parasitaemia. No significant change in ALT, AST and ALP was observed. Conclusion: Monodora myristica seed has antimalarial activity, which supports its antimalarial ethnomedicinal use.*

Keywords: *Monodora myristica*, antimalarial, antiplasmodial, biochemical parameters, *Plasmodium berghei*

1. Introduction

Malaria disease has remained a global leading cause of death and disability in which about 50% of the world population is estimated to be at risk, especially in low and middle income countries (1). There were an estimated 219 million cases of malaria and about 660 000 deaths in 2010 (1, 2). Malaria can cause a number of life- threatening complications. In most cases, malaria deaths are related to one or more serious complications (3- 5). Malaria could be detected through clinical diagnosis; microscopic diagnosis; antigen detection and detection of antibodies against malaria parasites (6- 10).

Resistance to antimalarial drugs is a major threat to the control and elimination of malaria. All geographical areas are affected, with the exception of Central America, and the worst affected is mainland South-East Asia, where parasites with reduced susceptibility to all the available antimalarial drugs are now prevalent. Resistance has developed to several antimalarial medications; for example, resistance to artemisinin has become a problem in some parts of Southeast Asia (11- 13). To date, parasite resistance to antimalarials has been documented in three of the five malaria species known that affect humans- *P. falciparum*, *P. vivax* and *P. malariae*. Parasite resistance results in a delayed or incomplete clearance of parasites from the patient's blood leading to treatment failure. Treatment failure is dangerous as it increases malaria transmission and fuels the emergence and spread of antimalarial drug resistance (11, 12, 14). The problem of antimalarial drug resistance is compounded by cross resistance. The continued use of oral artemisinin-based monotherapies is a major contributing factor to the development of resistance to artemisinin and its derivatives (11, 12, 14).

Monodora myristica is a large tropical deciduous tree with huge leaves and exotic, scented flowers; which can grow from 10 - 35 meters tall. The tree is particularly valued for its aromatic seed, which is used as a condiment and medicinally (15- 17). The bark is used to treat haemorrhoids, stomach-ache, fatigue and febrile pains. In Eastern Nigeria, *Monodora myristica* seeds are used for preparing special hot soups with *Piper guineense* for newborn mothers for easy control of uterine hemorrhage. It is also believed that adding these two spices in their soups helps the newborn mother's milk to start flowing normally immediately after childbirth. Basically, *Monodora myristica* seeds can be ground and used as postpartum tonic. When grounded to powder, the kernel is used to prepare soup as stimulant to relieve constipation and stomach ache. The berry also has diuretic properties and used for mild fever and antiseptic. *Monodora myristica* seeds can be used to make an ointment that can be used for treating rheumatism and arthritis. *Monodora myristica* seeds have been proven to possess anti-sickling properties and that the seeds contain cholesterol lowering ability thus can be used for treating individuals with high cholesterol level. *Monodora myristica* seeds can be decocted or infused, which is used for treating sores and wounds (16).

Herbal and natural remedies are commonly employed in developing countries for the treatment of various diseases, this practice being an alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy (15). There is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these remedies. These, in addition to the problem of toxicity with most of the present therapies; such as neurotoxicity seen mostly with the artemisinins, intense itching seen with chloroquine use, and so on; coupled with ineffective therapies has necessitated an urgent need for new highly efficacious, affordable, safer and scientifically validated antimalarial alternative medicine; hence this study was carried out to evaluate the antimalarial potentials of the seeds of the plant *Monodora myristica*, as a potential alternative medicine in malarial treatment.

2. Methods

Sample Collection and Preparation

The unpeeled *Monodora myristica* seeds samples were procured from Awka, Anambra, Nigeria. Using hands, the seeds were peeled and thoroughly screened to remove the bad ones and stones. These were then dried at room temperature. The African nutmeg flour was prepared by grinding the seeds using the local mechanical hand mill. The powdered sample obtained was stored in an air tight plastic container under room temperature prior to extraction.

Extraction

The powdered *Monodora myristica* seeds were soxhlet extracted using 70 % ethanol. 600 g of powdered *Monodora myristica* seeds were weighed into a filter paper and then wrapped, the wrapped filter paper was placed inside the thimble. The thimble was loaded into the main chamber of the Soxh let extractor. 70 % Ethanol was placed in a distillation flask. Then the flask was placed on the heating element. The Soxh let extractor was placed atop the flask and a reflux condenser was placed atop the extractor. The solvent was heated to reflux. The solvent vapour travelled up a distillation arm, and flooded into the chamber housing the thimble of solid. The condenser ensured that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material was slowly filled with warm solvent. Some of the desired compound dissolved in the warm solvent. When the Soxh let chamber was almost full, the chamber was emptied by the siphon. The solvent was returned to the distillation flask. The thimble ensured that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle was allowed to repeat many times, over days. During each cycle, a portion of the non- volatile compound dissolved in the solvent. After many cycles the desired compound was concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction the solvent was removed, typically by means of a rotary evaporator, which yielded the extracted compound. The non-soluble portion of the extracted solid remained in the thimble, and is usually discarded. The extract was then weighed and the percentage yield determined.

Phytochemical Tests on Crude M*onodora myristica* extract

The phytochemical analysis of powdered *Monodora myristica* seed was carried out using standard procedures to identify the constituents as described by (20- 22). Quantitative phytochemical analysis to quantify alkaloid, saponin, tannin and flavonoid were done using standard procedures (20, 23)

3. Physicochemical Analysis

Determination of water soluble extractives: 5 g of powdered *Monodora myristica* seed was added to 50 ml of distilled water at 80˚C in a stoppered flask. It was shaken well and was allowed to stand for 10 min, it was cooled and 2 g of kieselguhr was added and then it was filtered. Then 5 ml of the filtrate was transferred to a tarred evaporating dish 7.5cm in diameter. The solvent was evaporated on a water bath, drying was continued for 30 min, it was finally dried in a steam oven for 2 h and the residue was weighed. The percentage water-soluble extractive was calculated with reference with the air dried drug and experiments were carried in triplicates.

Total ash: About 2 g of accurately weighed *Monodora myristica* powder was placed into a nickel crucible that has been heated, cooled and stored in a desiccator (the powder was spread in an even layer). This was heated gently in the fume cupboard until all the moisture has been driven off and the material has been completely charred. The flame (450˚C) was gradually increased until the residue became white, an indication that it is free from carbon, it was then cooled and weighed. Heating and cooling was continued until a constant weight was achieved. The ash value was calculated with reference to air dried drug. Experiment was carried out in triplicate.

Acid insoluble ash: The ash obtained from method 1 above was boiled in a crucible with 25 ml of dilute hydrochloric acid (2 M) for 5 minutes the crucible was covered with a watch glass. Then filtration was done to collect the insoluble matter on an ashless filter paper. The wash glass and the crucible were washed with hot water and the washings passed through the filter paper. Washing of the insoluble matter was continued until it was free from acid (i.e. until the filtrate was neutral) and the solid was washed into the tip of the edge of the filter paper. The filter paper containing the insoluble matter was transferred into the original crucible, then dried on a hot late and ignited to constant weight. The residue was allowed to cool in a desiccator for 30 min and then weighed. The acid – insoluble ash was calculated in mg/g with reference to the air dried drug**.** Experiment was carried out in triplicate.

Water soluble ash: To the crucible containing total ash, 25 ml of distilled water was added and boiled for 5 min. The insoluble matter was collected on an ashless filter paper. Then it was washed with hot water and ignited in a crucible for 15 min at a temperature not exceeding 450˚C. The weight of this residue was subtracted from the weight of the total ash. The

content of water soluble ash in mg/g of the air dried material was calculated.

Loss on drying: To an evaporating dish which has been heated to constant weight and stored in a desiccator, 3 g of Monodora myristica powder was accurately weighed into the dish. Then it was placed in an oven at 100 - 105˚C for 5 h and the sample was weighed. The drying and weighing was continued at 1 h intervals till the difference between two successive weighing corresponded to not more than 0.25 %. The moisture content is the total weight lost expressed as percentage of the initial weight of sample.

Acute Toxicity Test

Ethanol extract (70%) was used for the acute toxicity studies. The method of Lorke (1983) was used to estimate the acute toxicity profile. Thirteen (13) mice were utilized in this study. The test involved two phases. Nine mice were divided into three groups of three each for the phase I study. Three dose levels of 10, 100 and 1000mg/kg were administered orally to group 1, 2, and 3 respectively. In the phase II study, doses of 4 sets of doses(2000, 3000,4000, and 5000mg/kg) corresponding to the outcome of phase1 study was adopted from the table provided by (24) and given to 4 groups of one mouse and the LD_{50} was estimated according to
Lorke's model: $LD_{50}= \sqrt{a} X \sqrt{b}$
Where, a = Lowest dose that is lethal; b = highest dose that is safe.

Curative Test
In this model of study, a total of 20 mice were selected and were grouped into 4 groups of 5 mice per group. Blood was collected from donor mouse infected with parasite (*Plasmodium berghei*) by occulo- puncture. The blood was diluted with normal saline such that 0.2ml of the blood contains approximately 1 x 107 infected red blood cells. 0.2ml of the diluted blood was administered to the 20 mice. All the animals in each group were infected with the parasite *Plasmodium berghei* (approximately 1 x 107 infected red blood cells) by single intra peritoneal administration of 0.2 ml of the diluted blood. The animals were then left for 72hours for the infection to be established. After 72 hours, the animals were given treatment as follows by single oral administration which started immediately for seven (7) days, starting from day 0, day 1, day 2, day3, day 4, day 5, day 6 and day 7.

Group 1 received 10ml/kg distilled water, Group 2 received 0.78mg/kg of act (Artemether and lumenfantrin), Group 3 received 250 mg/kg of *Monodora myristica* extract, and Group 4 received 500 mg/kg of *Monodora myristica* extract

A thin blood film was made from the tail blood, stained with Giemsa stain and was examined under an electron microscope for parasitemia levels on day 4 and day 7 post treatment respectively. The % curative was calculated using the formular D0 – DA divided by D0 * 100 divided by 1 where Do =day 0 mean parasitemia DA = Treated day mean parasitaemia

Biochemical parameters evaluation

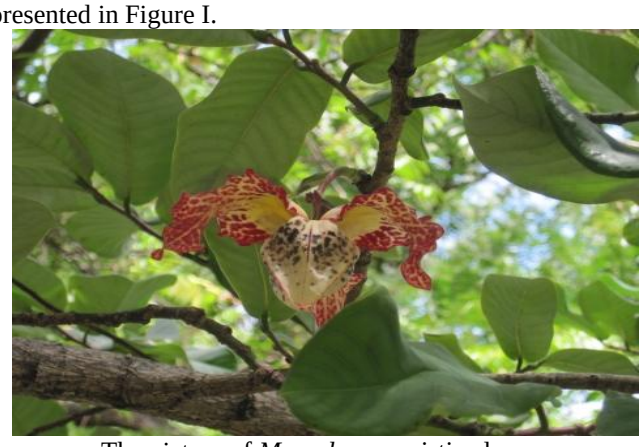
Determination of alkaline phosphatase (ALP): Fresh tubes were labeled "standard", "sample", and "blank" respectively. 0.5ml of alkaline phosphatase, substrate (in the kit) was dispensed in all the labeled tubes and was equilibrated to 37°C for 3 minutes using water bath. To the standard, 50UL of the standard reagent(in the kit) was dispensed, to the " sample", 50UL of the samples were dispensed and to the "blank" , 50UL of de- ionized water was dispensed. The whole tubes were mixed gently, and incubated for 10mins at 37˚C. Then 2.5 ml of alkaline phosphatase color developer (in the kit) was dispensed in all the tubes and was mixed properly. The absorbance was read at 600nm using Spectrophotometer. Then the concentration of ALP was calculated as follows:
Absorbance of Sample divided by absorbance of standard * concentration of standard

Determination of alanine aminotransferase (ALT): R1 and R2 (in the kit) was mixed at the ratio of 5:1 and was termed "working reagent". Fresh tubes were labeled "Samples", and "Blank". To all the tubes, 1.0ml of working reagent was dispensed and Incubated for 5minutes at 37˚C . To the samples, 100UL of each sample was added respectively, and to the blank, 100UL of distilled water was added. The absorbance was read at 1minute and at 3 minutes using Spectrophotometer at 340nm. Then the ALT concentration was calculated as follows: A/minute * 1768

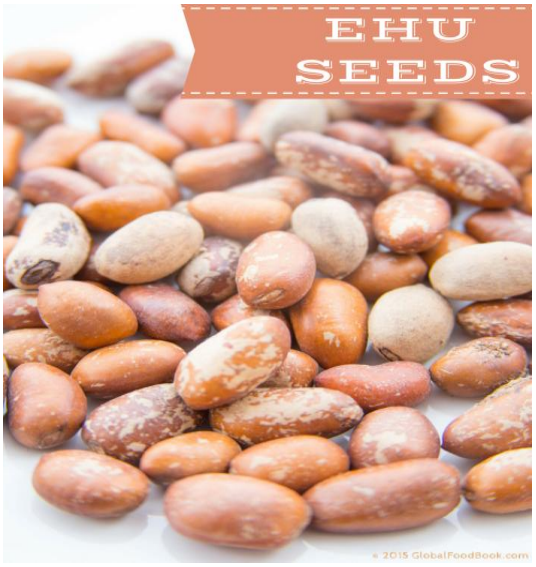
Determination of Aspartate Aminotransferase (AST): R1 and R2 (in the kit) was mixed at the ratio of 5:1 and was termed "working reagent". Fresh tubes were labeled "Samples", and "Blank". To all the tubes , 1.0ml of working reagent was dispensed and Incubated for 5minutes at 37˚C . To the samples, 100UL of each sample was added respectively, and to the blank, 100UL of distilled water was added. The absorbance was read at 1minute and at 3 minutes using Spectrophotometer at 340nm. Then the AST concentration was calculated as follows: A/minute * 1768.

4. Results

The picture of *Monodora myristica* leaves and seeds are presented in Figure I.



The picture of *Monodora myristica* leaves



The picture of *Monodora myristica* seeds

Figure 1: The picture of the leaves and seeds of *Monodora myristica*

The result of the phytochemical screening, proximate analysis and median lethal dose determination are presented in Tables 1- 4 respectively; showing abundance of alkaloids, low ash values and LD_{50} > 5000 mg/kg dose.

Table 1: Result of phytochemical analysis of *Monodora myristica* seeds

S/N	Analysis	Result
1	Alkaloids	+
2	Flavonoids	++
3	Cardiac glycosides	-
4	Tannins	-
5	Saponins	-
6	Proteins	++
7	Starch	-
8	Terpenoid	+
9	Steroid	-

Key: - = absent, + = trace / mildly present, ++ = moderately present, +++ = abundantly present

Table 2: Result of quantitative phytochemical analysis of *Monodora myristica* seeds

Parameters	% Phytochemical content
Alkaloid	28.40%
Flavonoid	0.16%
Saponin	15.20%
Tannin	4.90%

Table 3: Proximate compositions (%) of *Monodora myristica* seeds

Parameters	% Compositions
Moisture	22.67
Ash	4.52
Water soluble ash	2.5
Acid insoluble ash	0.5

Table 4: Acute toxicity test result

Phases	Dose(mg/kg)	Number of death
1	10	0/3
	100	0/3
	1000	0/3
2	2000	0/1
	3000	0/1
	4000	0/1
	5000	0/1

Toxicological studies established LD 50 of the crude to be > 5000mg/k

The results of the *in vivo* curative tests of the seed extract are presented on Tables 5- 6.

Table 5: Effect of the ethanol seed extract of *Monodora myristica* on malaria infected mice

Treatment (mg/kg)	Mean parasitemia Day 0 ± SEM	Mean parasitemia Day 4± SEM	Mean parasitemia Day 7 ± SEM	% Curative Day 4	% Curative Day 7
0.78 mg/kg of ACT	12.93±1.48	2.74±0.32	0.68±0.11	78.85	94.76
10mg/kg of distilled water	14.20±1.34	11.47±0.75	11.42±0.12	19.24	19.54
250mg/kg of ethanol extract	12.47±1.17	3.80±0.62	1.67±0.50	69.52	86.64
500mg/kg of ethanol extract	13.47±1.90	9.27±2.97	1.27±0.16	31.17	90.58

Table 6: Statistical comparison of results

Comparison	P value
0.78 mg/kg of ACT	
Control versus day 4	**
Control versus day 7	**
10ml/kg of distilled water	
Control versus day 4	NS
Control versus day 7	NS
250mg/kg of ethanol extract of crude drug	
Control versus day 4	**
Control versus day 7	**
500mg/kg of ethanol extract of crude drug	
Control versus day 4	**
Control versus day 7	**

Figures 2- 4, showing significant suppression

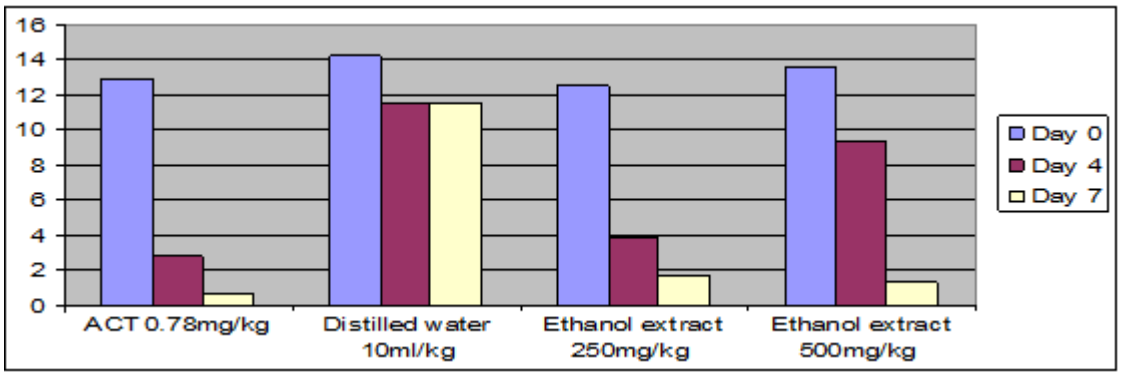


Figure 2: A bar chart representing mean percentage parasitemia

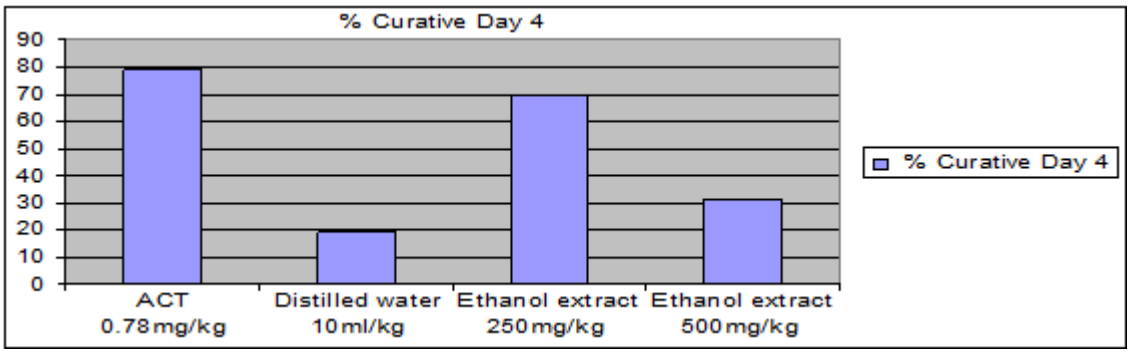


Figure 3: A bar chart representing day 4 percentage curative

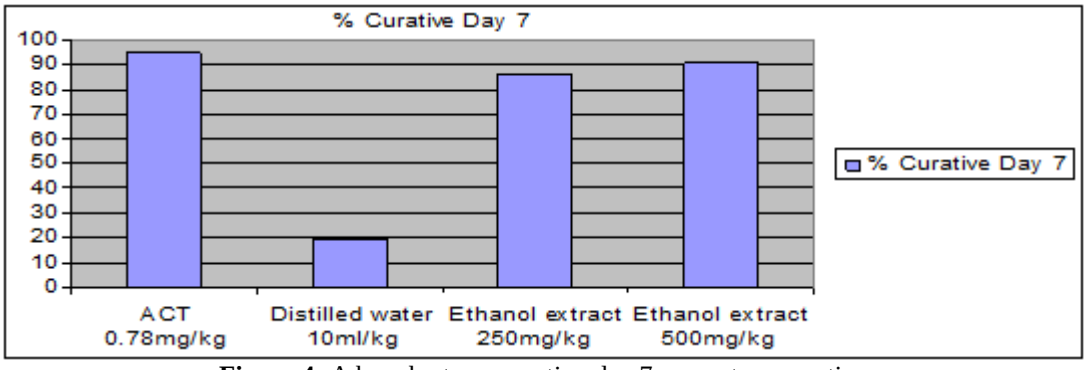


Figure 4: A bar chart representing day 7 percentage curative

Biochemical Analysis

The results of the biochemical evaluations are presented on Tables 7- 9.

Table 7: Effect of extract on Alkaline Phosphatase (ALP)

Groups	Pre- treatment (u/ml)	7^{th} day (u/ml)
Control	1.85 ± 0.45	1.91 ± 0.48
Ethanol extract(250mg/kg)	2.31± 0.45	2.38± 0.48
Ethanol extract(500mg/kg)	1.27± 0.07	1.31 ± 0.06

Table 8: Effect of extract on Alanine Aminotransferase (ALT)

Groups	Pre- treatment (u/ml)	7^{th} day (u/ml)
Control	53.00 ±12.12	53.56 ±19.24
Ethanol extract(250mg/kg)	51.93 ±13.98	58.0 ±116.30
Ethanol extract(500mg/kg)	66.13 ±11.18	65.69±15.34

Table 9: Effect of extract on Aspartate Aminotransferase (AST)

Groups	Pre- treatment (u/ml)	7^{th} day (u/ml)
Control	47.13±4.42	50.37±4.90
Ethanol extract(250mg/kg)	57.23±7.09	58.63±7.86
Ethanol extract(500mg/kg)	67.37±10.75	68.33±10.62

Figures 5- 7, showing no significant change in liver enzymes

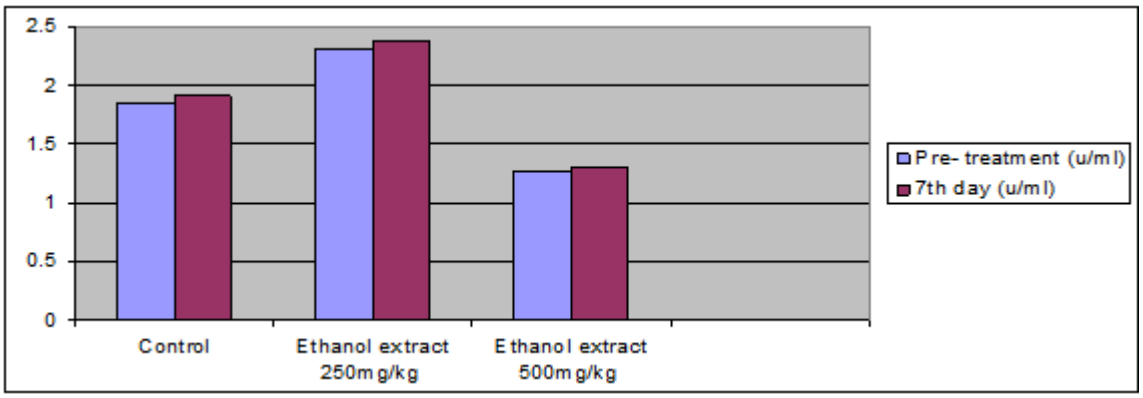


Figure 5: A bar chart representing the effect of extract on ALP

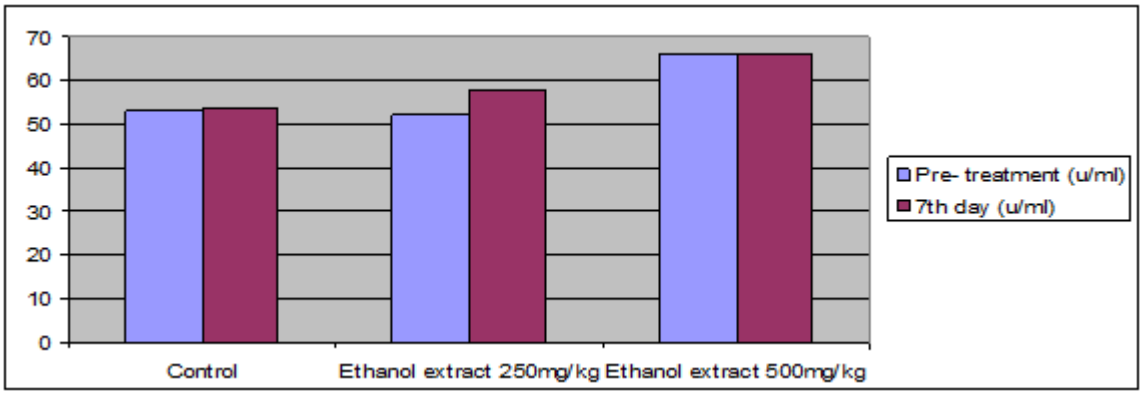


Figure 6: A bar chart representing the effect of extract on ALT

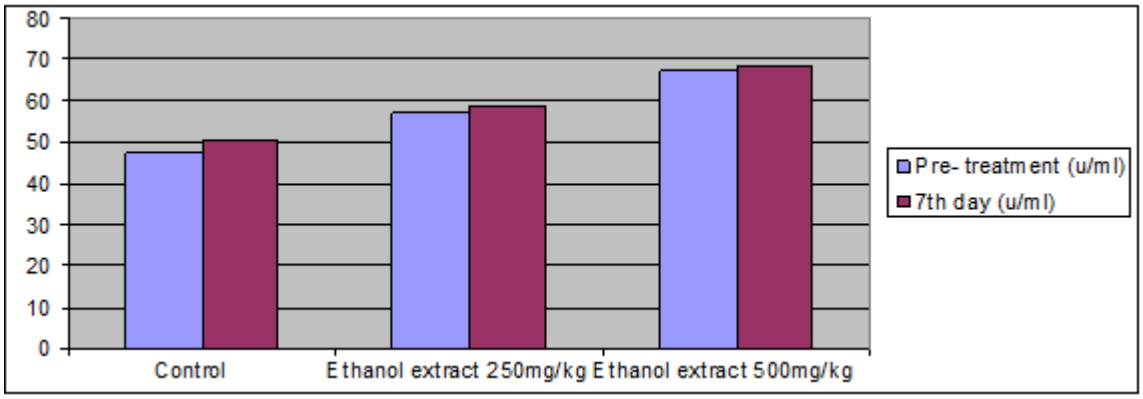


Figure 7: A bar chart representing the effect of extract on AST

5. Discussion

The percentage extraction yield was 8.13% which is considered to be low. This implies that a large quantity of the seed will be needed to obtain the requisite quantity of extract. The result of phytochemical analysis of the ethanol seed extract of *Monodora myristica* (Table 1) revealed the presence of saponins, tannins, terpenes, proteins, alkaloids and flavonoids and absence of carbohydrates, cardiac glycosides and steroids. Quantitatively, the plant contained alkaloids (28.4%), flavonoids (0.16%), saponins (15.20%), and tannins (4.9%) (Table 2). The results obtained showed that the seed of the plant is rich in phytochemicals implicated in many pharmacological activities. The presence of these secondary metabolites in *M. myristica* seed may be responsible for its anti-malarial activity. For instance, flavonoids are reported to chelate with nucleic acid base pairing of the parasite (25) and terpenoid are potent protein inhibitors (26). Antiplasmodial screening of plant substances have been shown to be caused by alkaloids, terpenoids and flavonoids (17, 27, 28). David and Okokon *et al*. (29, 30) showed clearly that saponin, flavonoids and tannins have been suggested to act as primary antioxidant or free radical scavengers that can counteract the oxidative damage induced by malaria parasite. These compounds could be acting singly or in synergy with one another to exert the anti-plasmodial activity observed in this study. These compounds, flavonoids, terpenoids, alkaloid and saponins, present in the plant extract may in part have contributed to the antimalarial activity of this extract. The

mechanism of action of aqueous ethanol seed extract of M. *myristica* has not been elucidated. The extract could have exerted its anti malarial action by causing elevation of red blood cell oxidation or by inhibiting protein synthesis. It is evident by these findings that *M. myristica* possessed anti-plasmodial activity.

From the result of the proximate composition (%) of *M. myristica* seed (Table 3), the moisture content of 22.67% is high. Moisture content of any food is an index of its water activity and is used as a measure of stability and susceptibility to microbial contamination (6, 31, 32). The high moisture content is indicative of the fact that the seeds of this plant cannot be stored for a long period without deterioration in quality or microbial spoilage since microbial activity may be increased. Most drugs may be stored safely if the moisture content is reduced to 6% or less. The seed of *M. myristica* has moderately high ash value of 4.52%. This means that it has good mineral content, and thus serves as a viable tool for nutritional evaluation (33). Ash represents the mineral matter left after feeds are burnt in oxygen (34). It is used as a measure of the mineral content in any sample (35). Also a high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the crude drug for marketing (36, 37).

The acute toxicity test result (Table 4) showed that no death occurred at all the dose level used which showed that the seed extract does not have any toxic effect that can lead to the death of the mice. Behavioral signs of toxicity like stretching, salivation, paw-licking, severe reduced activities and respiratory distress were not observed. The median lethal dose LD_{50} was thus estimated to be >5000mg/kg body weight, this indicates that the extract has a wide margin of safety and hence safe for consumption (38).

Plasmodium berghei parasite is used in predicting treatment outcomes of any suspected antimalarial agent due to its high sensitivity to Chloroquine, Arthemether and lumenfantrin, etc. making it the appropriate parasite for this study (2, 29). *Plasmodium berghei* has been used in studying the activity of potential antimalarials in mice (39). It produces diseases similar to those of human plasmodium infection (2, 7). It is also the parasite of choice because other species of the plasmodium parasite such as *P. vivax, P. falciparum* and *P. malariae* cannot survive in mice.

From the antimalarial test result (Table 5), the reduction in parasitemia level for the negative control (distilled water) was not significant as the mean percentage parasitemia level only reduced from 14.20 to 11.47 and 11.42 on day 4 and day 7 respectively. This shows that the negative control has no antimalarial activity. However, the slight reduction in parasitemia level seen on day 4 and day 7 reflects the effect of the immune system on the disease malaria. For the positive control (Arthemether and lumenfantrin), the mean percentage parasitemia reduced significantly from 12.93 to 2.74 and 0.68 on day 4 and day 7 respectively. This shows that the positive control has antimalarial activity. For the 250mg/kg of the ethanol extract of the crude *Monodora myristica* seed, the mean percentage parasitemia was reduced significantly from 12.47 to 3.80 and 1.67 on day 4 and day 7 respectively; while for the 500mg/kg of the ethanol extract of the crude *Monodora myristica* seed, the mean percentage parasitemia was reduced significantly from 13.47 to 9.27 and 1.27 on day 4 and day 7 respectively. Therefore, it is clear from the result (Table 4) that the *P. berghei*–infected mice treated with the aqueous ethanol seed extract of *M. myristica* reduced in percentage parasitaemia compared to those of the untreated control animals. This shows that the seed extract of *M. myristica* has antimalarial activity.

From the curative test result (Table 5), the positive control (Arthemether and lumenfantrin) has the highest antimalarial activity as it caused 78.85% and 94.76% suppression in parasitaemia of *P. berghei* infected mice at day 4 and day 7 respectively. Kiseko *et al*. (40) showed that when a standard antimalarial drug is used in mice infected with *P. berghei*, it suppresses the parasitemia to a non-detectable level which is in line with this study. It is worthy to note that the significant decrease in parasitaemia observed in this study was dose dependent as the 250mg/kg of *M. myristica* seed extract caused 69.52% and 86.64% suppression in parasitaemia of *P. berghei* infected mice at day 4 and day 7 respectively and 31.17%, and 90.58% suppression in parasitaemia of *P. berghei* infected mice at 500mg/kg body weight. The initial low percentage chemosuppression observed in the extract-treated group and the Arthemether and lumenfantrin -treated group on day 4 (Table 4) may be due to the fact that the extract at the dose administered had not accumulated sufficiently to bring about considerable chemosuppression (41). However, the prolonged administration of the extract led to the total clearance of the parasites. This result from accumulation of enough active compounds to effect an increased clearance of the parasites.

In the comparison test result (Table 5), the *p value* on day 0 versus day 4 and day 4 versus day 7 for Arthemether and lumenfantrin, 250mg/kg and 500mg/kg of *M. myristica* seed extract were significant ($p<0.05$) while for the negative control (distilled water), the *p value* on day 0 versus day 4 and day 4 versus day 7 was not significant ($p>0.05$). The results revealed that the treated groups (Groups 1, 3 and 4) recorded significant decrease ($p<0.05$) in percentage parasitaemia compared to the infected untreated group (Group 2) which showed no significant decrease ($p>0.05$) in percentage parasitaemia over the period of observation.

From the biochemical test result (Tables 6, 7 and 8), no significant change in ALT, AST and ALP was observed indicating that the *M. myristica* seed extract does not affect the functionality and cellular integrity of the liver. The plasma concentration of ALT, AST, and ALP determines the functionality and cellular integrity of the liver (42). ALT and AST are biomarkers of the hepatocytes. Under pathological conditions of the liver including, cirrhosis, adverse effects of some drugs (e.g., paracetamol), there is a leak of these enzymes in to the plasma, thus raising their levels and activity

(43). ALT is specific for the liver but AST is also found in other tissues including the red blood cells, the cardiac and the skeletal muscle. ALP is located in the biliary duct of the liver (44). Obstruction of this duct increases the level of the enzyme in the plasma. Increase in the serum levels of AST and ALT (especially ALT) are reported to be associated with liver damage (45).

6. Conclusion

Monodora myristica seeds have been proven to possess anti-malarial activity thus it could be exploited in the formulation of cheap alternative anti-malarial drug for the treatment of malaria. It is important to note that the anti-malarial effect observed was dose- dependent. The safety study carried out showed the plant is safe for administration in malaria treatment.

7. Competing Interests

There is no conflict of interest between the authors of this study. The authors consented to the publication of this research manuscript.

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