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Repository and curative antimalarial activities of *Artemisia absinthium* in mice experimentally infected with *P. berghei* (NK 65)

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Abstract
Malaria has claimed many lives, especially of children less than five years of age and pregnant mothers. Availability of vector breeding sites influence continuous transmission of the disease in developing African nations and other tropical regions of the world. Although, a new vaccine for malaria was recently approved for use in endemic regions, resistance to frontline antimalarial drugs in clinical use is apparently high. Poverty has necessitated many families to depend on herbal remedies for treatment of malaria and other infectious diseases. In this study, the prophylactic and curative antimalarial properties of *Artemisia absinthium* was assessed in Chloroquine sensitive *Plasmodium berghei* (NK 65) infected mice. Results obtained revealed the presence of Anthraquinones, Alkaloids, flavonoids and other essential phytochemicals in crude *A. absinthium* (aqueous) extract. Similarly, GCMS analysis of the extract showed the presence of Humulene, Aromadendrene Methyl chloroformate, Carbonic acid and Ethanamine among other important compounds. Findings revealed no sign of acute toxic reaction in mice, following the administration of 2000 mg/kg body weight of the extract. Similarly, results further revealed 52.72% and 89.12% repository and curative antimalarial activities respectively, when mice were dosed with 200 mg/kg body weight of the extract. These findings showed that *A. absinthium* has good antimalarial activity and substantiate the traditional claim for its use in the treatment of fever related illnesses suspected to be malaria by locals. Further fractionation of the extract and assessment of the antiplasmodial properties of individual fractions is essential, to underpin the exact compound responsible for the biological activity identified.

Keywords: *Artemisia absinthium*, repository, curative, *Plasmodium berghei*, Malaria.

Introduction
Malaria has remained the cause of death, especially of pregnant women and under five children (Okoyo *et al.*, 2021; Imboumy-Limoukou *et al.*, 2020) [26, 10]. Parasites of the genus plasmodium had since been implicated as the cause of Malaria in human and non-human primates (Sato, 2021; Yohanna, *et al.*, 2019) [32, 40]. The malaria parasite has a complicated life cycle, which alternates between mosquitoes and other vertebrate hosts (Cox, 2010; Service, 1980) [7, 35]. Natural and human activities, such as indiscriminate disposal of cans, tins, polythene bags, pots, containers, tree holes, hoof prints, ditches etc provides breeding sites for mosquitoes which later constitutes nuisance and transmits infectious diseases including malaria (Service, 2008) [34]. In developing African countries, Malaria was mentioned as one the major cause of school absenteeism, loss of man hours and economic loss (Sarma *et al.*, 2019; Lukwa *et al.*, 2019) [31, 16], thus, resulting in drastic decline in gross domestic products (GDP) and per capita of a given nation (Malaney *et al.*, 2004) [18]. Similarly, malaria results in the retardation of educational development in endemic African countries (W.H.O, 2020; Vitor-Silva *et al.*, 2009) [38, 27].
Despite the availability of Health care centers, provision of malaria medicines at cheaper rates or mass distribution of seasonal malaria chemo preventive drugs (to children under five free of charge), as well as other malaria preventive measures enforced/provided by authorities, some hard to reach communities in developing nations still rely on local medicinal plants to treat malaria (W.H.O., 2019; Mahomoodally, 2013; Sofowora *et al.*, 2013) [39, 17, 36]. Since time immemorial, plants remained the source of medication for poor and underprivileged populations in developing nations (Petrovska, 2012) [29].
In addition to lack of malaria vaccines in clinical use (Coelho *et al*, 2017) [6], resistance to most of the frontline antimalarial drugs currently in use was reported in most part of the world

(Kozlov, 2021; Kuehn, 2021) [13, 14]. This necessitates the need for search of alternative malaria drugs. The discovery of quinine from Cinchona tree in the early 18th century (Zhu, 2018; Permin *et al.*, 2016) [41, 28] as well as artemisinins from Sweet worm wood (*Artemisia annua*) following the Second World War, open up the room for search of antimalarial drugs from plant sources.

Records of ethnomedicinal studies (Babalola & Adelakun, 2018) [2] conducted in Yobe State, North East Nigeria revealed *Artemisia absinthium*, otherwise termed Wormwood in English or "Tazargade" in Hausa Language as one of the most frequently used medicinal plants to treat fever related illnesses suspected to be malaria.

To substantiate traditional claim and determine the antimalarial potential of *A. absinthe*, further studies was conducted in Swiss mice, experimentally infected with chloroquine resistant *Plasmodium berghei* (NK 65).

Material and Methods

Collection and Identification of Plant Material

Artemisia absinthe (Tazargade) was purchased from local traditional herbalist in Damaturu Sunday market. Sample was taken the herbarium, Department of Biological Sciences, Yobe State University for authentication by a Plant taxonomist.

Processing of Plant Sample

Plant material collected was ground to powder using grinding machine (IKA Werke M20) and stored in a screw cap vial until further use. Extraction of secondary metabolites from powdered plant sample was performed by cold Extraction procedures with distilled water. Powdered plant material 100g each was transferred into separate amber bottles and macerated in 500ml each of distilled water. The suspension was stirred, screw capped and rigorously shake in a shaker (IKAWERKE, Hs, 501) for 24hours at room temperature. Mixture was filtered using mucilin cloth and filtrate frozen. Extract was dried using a lyophilizer (FDL-10N-50-TD-MM) set at 40°C. Subsequent to concentration of plant extract to dryness, powdered material was stored clean sample containers at 4ºC until required for use.

Phytochemical Extraction

Extraction of secondary metabolites from powdered plant samples was performed in distilled water. Samples were weighed as previously described (Daskum, 2019). One hundred grams (100 g) each of powdered plant material was transferred into screw cap, wide mouth, clear sample bottles and macerated in 500 ml each of distilled water (i.e. 1:5 w/v). The suspension was then stirred, screws capped and shake for 24 hours in a shaker (IKA WERKE, HS 501) at room temperature. Mixture was filtered using mucilin cloth and extracts were freezed at -20ºC and lyophilized in a freeze drier (FDL-10N-50-TD-MM). Dried aqueos extracts were further grinded in a mortar and pestle, sieved using 0.015 MM standard test sieve (Supertek) to obtain fine powder and stored at 4ºC in a screw cap vial until required for use. Subsequent to extraction, extract was weighed and the percentage yield calculated using the following formula

$$\text{Percent (\%) yield} = \frac{\text{Weight of extract (g)}}{\text{Weight of Powdered Material (g)}} \text{ X } 100$$

Phytochemical Analysis

Following phytochemical extraction, crude extracts were subjected to phytochemical screening, with a view to determining the presence or otherwise of some active metabolites. For the analysis of phytochemicals, qualitative test was performed in accordance with the protocol described by Kumar *et al.* (2013) [15].

Test for Saponins: Two grams (2.0 g) of powdered plant material was transferred into a test tube and macerated in distilled water. The preparation was then boiled in a boiling water bath, then filtered. To 10 ml of the filtrate, 5 ml of distilled water was added and vigorously shake until a stable persistent froth is formed. Thereafter, 3 drops of olive oil was added to the froth and vigorously shake. Formation of an emulsion indicates the presence of saponin.

Test for Phenols: To 1 ml extract 2 ml distilled water was added followed by few drops of 10% aqueous ferric chloride. A Blue or green colouration indicates the presence of phenols.

Test for Anthraquinones: One (1 ml) of extract was treated separately with a few millilitres of alcoholic potassium hydroxide solution. Coloration ranging from red to blue indicates the presence of anthraquinones.

Test for Alkaloids: To 0.5g of extract, 8 ml of 1% HCl was added, warmed in a water bath and filtered. Two (2 ml) of the filtrate was treated separately with Mayer's and Dragendorff's reagents. Each tube was observed for the formation of turbidity or precipitate. Formation or otherwise of turbidity or precipitate indicates the presence or absence of alkaloids.

Test for Flavonoids: 0.5 g of extract was weighed and macerated in few drops of petroleum ether and shake to remove the fatty materials (lipid layer). The defatted residue was then dissolved in 20 ml of 80% ethanol and then filtered. The filtrate was later divided into three (3) portions and used for the following tests:

a) To 3 ml of the filtrate, 4 ml of 1% potassium hydroxide was added in a test tube. A dark yellow colouration indicates the presence of Flavonoids

GC-MS Analysis of extract

Powdered extract (0.5g each) was macerated in distilled water. Samples were filter sterilized using 0.22 µm pore size filter unit and analyzed (GC-MS Qp2010 ULTRA Shimadzu). Column temperature was set at 40 °c-300 °c with helium gas running as the carrier gas at a flow rate of 4.8 and run/post run time of 48 minutes. Compound produced we're compared with those in the National institute standard and technology (NIST) data base.

Experimental Animals

Swiss mice aged (9-13 weeks), weighing 26-35 g were purchased from the National Veterinary Research Institute (NVRI), Vom, Jos, Plateau State. Animals were acclimatized for two weeks in the Biology Research Laboratory, Department of Biological Sciences, Yobe State University, and Damaturu. Experimental animals were fed with poultry diets (Starter feed) purchased from Rico Gado Nutrition, Yola, Nigeria and served *water ad libitum*. Mice were maintained under standard 12 hour dark and 12 hour light cycles (Bains *et al.*, 2018) [3].

Donor Mice

Swiss mice experimentally infected with chloroquine resistant

Plasmodium berghei (NK 65) were obtained from the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. Tail tip of individual donor mice was amputated, blood samples collected on a clean, grease free slides and parasitaemia determined (Meyer, *et al*., 2020) [19]. Subsequent to determination of percent parasitaemia, donor mice were intraperitoneally injected with 0.2 mL Ketamine hydrochloride (Trittau, Germany), and *P. berghei* parasitized erythrocytes were collected by cardiac puncture and resuspended in normal saline (1:10 v/v). Clean mice were experimentally infected by intraperitoneal injection with blood suspension (0.2 mL i.e. $1x10^7$) as previously described (Alaribe *et al.,* 2020) [1].

Acute Toxicity Test

The limit test as described in the OECD 423 guideline for testing of chemicals in experimental animals was adopted. Three experimental mice were used per step, with a starting dose of 2000 mg/kg selected. On day 0, all animals (n=3) per group were fasted for 4 hours, with water *ad libitum* but not food served (OECD 423, 2001). During fasting period, animals were weighed and records noted. To each mice, 0.2 ml of 2000mg/kg of aqueous *A. absinthium* extract dissolved in normal saline was served by oral gavage (Alaribe *et al.*, 2020) [1]. Food was further withheld for another 1 hour as described (OECD 423).

All animals were monitored individually for signs of acute toxic reactions during the first 30 minutes after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours.

The procedure was repeated with group 2 (n=3) and monitored as described. All groups were then monitored for 14 days with normal diet and water served.

Repository Test

The prophylactic activity of crude extract was determined using the residual infection procedure described (Source?). Experimental mice were weighed and grouped into five groups (n=5) at random. Groups 2-4 were served different doses (200 mg/kg, 100 mg/kg ad 50 mg/kg respectively) of extract for three consecutive days, while groups 1 and 5 were served the reference antimalarial (Larydox®) and normal saline respectively, to serve as positive and placebo controls respectively. At this point, it should be noted that none of the experimental groups was infected. On the 4th day post treatment, *Plasmodium berghei* (NK 65) parasitized erythrocyte was obtained from donor mice by cardiac puncture and resuspended in normal saline (1:10 v/v). This was used to infect experimental groups by intraperitoneal injection as earlier described (Alaribe *et al.*, 2020) [1]. Following infection, none of experimental groups were further treated with either of extract or reference drug. Three days post-infection, the level of parasitaemia and change in body weight was obtained daily for 3 days.

Curative Antimalarial Activity (Rane's Test)

Here, experimental animals were infected with *P. berghei* (NK 65) blood suspension intraperitoneally on day 0 as described for repository test. Following infection, mice were grouped into 5 groups of 5 mice each as earlier described. Parasitaemia was allowed to build-up until 3rd day post infection. On the 4th day post infection, parasitaemia level was determined. Mice were then served different doses (200 mg/kg, 100 mg/kg ad 50 mg/kg respectively) of the plant extract and reference antimalarial for 4 consecutive days. Assessment of the level of parasitaemia and change in body weight was performed daily before treatment.

Blood collection for determination of percent parasitaemia

At the end of each experiment, the tail tip of individual mice was cleaned with alcohol treated swab, cut with scissors and two or more drops of blood was collected directly on a clean, grease free glass slide to make thin films (smear). To stop bleeding following blood collection, a finger pressure was applied to the site of collection before placing the mice back in the cage. Smears were labelled, air dried, fixed with absolute methanol and stained with 10% giemsa in PBS for 20 minutes (Moll *et al.*, 2008). Smears were then examined microscopically (Olympus CX22LED) at 100x magnification under oil immersion. Blood collection for thin smears and determination of percent parasitaemia was performed as earlier described. At the end of each experiment, all mice were euthanized by cervical dislocation (Carbone *et al.*, 2012) [5]. Percentage parasitaemia and antimalarial effect of extract was calculated as previously described (Daskum *et al.*, 2019).

Determination of Body Weight

The body weights of individual mice was taken daily in the morning before feeding and administration of crude extract or drug (day 0 to day 4 post infection). Subsequently, the change in body weight was determined by taking the mean weight of mice in respective groups and the weight gain or loss was obtained by subtracting the initial weight (Weight in day 0) from the final weight (weight in day 4) post infection.

Ethical Clearance

Ethical clearance was sought from the Institutional Animal Care and Use Committee (IACUC) of Yobe State University. Protocols adopted throughout this research complies strictly with the guidelines for the care and use of experimental animals, OECD 423 (guidelines for testing of chemicals) and follows the committee's approval

Statistical Analysis

Data obtained was analysed using Microsoft excel version 2010. Data obtained was expressed as percent parasitaemia (Mean ± SEM) and percent prophylactic and curative effect in relation to the control groups.

Results

Phytochemical analysis: Result of the phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, phenols and anthraquinones respectively, in the aqueous extract of *Artemisia absinthium* (Table 1).

Table 1: Phytochemical constituents of crude *Artemisia absinthium (aq.)* extract

Phytochemicals	Status
Alkaloids	+
Flavonoid	+
Saponins	+
Phenols	+
Anthraquinones	+

Key: Present= +; Absent= -

Yield of Extract: Following extraction, 14g of extract was obtained from 500g of powdered plant material macerated in distilled water. This reveals that 2.8% yield was obtained (Table 2)

Table 2: Percentage yield of crude *Artemisia absinthium* (*aq.*) extract

Plant part used	Weight of Powder	Extract yield	%Yield
Whole leaves	500g	14g	2.8%

GC-MS: Compounds in crude extracts from crude A. absinthium were identified by GC-MS. Results obtained revealed the presence of Terpenoids specifically, sesquiterpenes (Humulene and Aromadendrene) Similarly compounds such as Methyl chloroformate, Carbonic acid, Ethanamine, 10-Heneicosene, Tetradecanoic acid, α-Tocopheryl acetate were also identified. Other compounds identified, their chemical formulae, molecular weight as well as retention times are summarized in tables 3. Similarly, chromatograms obtained as functions of retention time for extracts are presented in Figure 1.

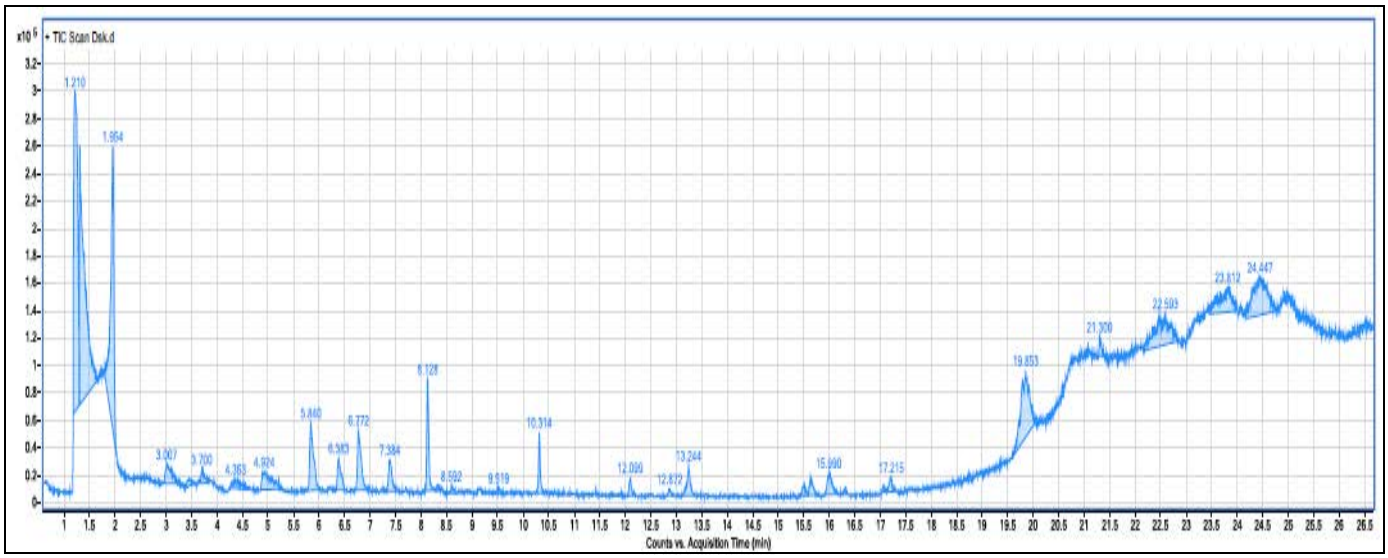

Fig 1: GC-MS chromatogram of crude *A. absinthium* extract

Table 3: Compounds in crude *A. absinthium* (Aqueous) extract as revealed by GC-MS analysis

Name of Compound	Chemical Formula	Mol. Weight (MW)	Retention Time (RT)	Peak Area	Peak Height
Methyl chloroformate	$C_2H_3ClO_2$	94	1.21	1209199.13	232823.17
Carbonic acid	$C_6H_{11}ClO_4$	182	1.308	1294079.41	188703.65
Ethanamine	$C_{11}H_{17}NO$	179	1.954	911509.59	206802.28
4-Nonene, 3-methyl	$C_{10}H_{20}$	140	3.007	115344.29	13683.58
Cis-1-Methyl-2-(2'-propenyl)cyclopropane	C_7H_{12}	96	3.448	26955.31	5118.6
6-Tridecene	$C_{13}H_{26}$	182	3.7	52272	10221.04
1,2-Benzenedimethanol	$C_8H_{10}O_2$	138	4.363	74847.29	6794.4
Aromadendrene	$C_{15}H_{24}$	204	6.383	110195.44	23175.89
Humulene	$C_{15}H_{24}$	204	6.772	213325.63	43803.87
Phenol 2,5-bis(1,1-dimethyl)	$C_{14}H_{22}O$	206	7.384	101866.56	22654.17
1-Nonadecene	$C_{19}H_{38}$	266	8.128	214054.6	81436.65
2-Methyl-6-methylene-octa-1,7-dien-3-ol	$C_{10}H_{16}O$	152	8.329	13028.64	4289.03
Cyclohexene,1-(1-propynyl)	$C_{93}H_{12}$	120	9.519	12990.15	4515.72
10-Heneicosene	$C_{21}H_{42}$	294	10.314	95902.08	44429.3
Tetradecanoic acid	$C_{16}H_{32}O_2$	256	12.099	35944.05	13012.5
1-Hexyl-2-nitrocyclohexane	$C_{12}H_{23}NO_2$	213	15.636	59738.25	12678.79
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	15.99	105621.33	15866.18
Cyclohexanecarboxyaldehyde	$C_8H_{14}O_2$		17.06	13224.14	4797.29
(2R,4R)-p-Mentha-[1(7),8]-diene,2-hydroperoxide	$C_{10}H_{16}O_2$	168	17.215	43387.98	10400.35
α-Tocopheryl acetate	$C_{31}H_{52}O_3$	472	19.853	553563	48515.94
Trimethyl[4-(1,1,3,3-tetramethylbutyl) phenoxy]silane	$C_{17}H_{30}OSi$	278	21.3	37404.2	13784.99
Phenol,4-(1,1,3,3-tetramethylbutyl)	$C_{14}H_{22}O$	206	22.593	429979.69	22614.65

Acute Toxicity: Results obtained revealed no mortality nor any sign of acute toxic reaction, following the admininstration of 2000 mg/kg of the plant extract. This implies that the acute lethal dose (LD_{50}) of crude A. absinthium (aq.) extract is above 2000 mg/kg body weight.

Repository antimalarial properties: The prophylactic antimalarial activities of *A. absinthium* are presented as mean ± Standard Error of Mean (SEM) (Table 4). Parasitaemia was observed to reduce over the period of observation, in experimental animal groups treated with the plant extracts. Similarly, a dose dependent repository activity was observed in extract treated groups, with the highest effect (26.49%) seen in experimental animal groups treated with 200mg/kg of extract and least (18.09%) in the 50mg/kg treated groups.

Table 4: Repository antimalarial properties of *A. absinthium*

Extract	Doses (mg/kg)	Percent Parasitaemia Post Infection			Percent Prophylactic effect on day 3 Post infection
***absinthium* (aq.)**		**Day 1 Mean ±SEM**	**Day 2 Mean ±SEM**	**Day 3 Mean ±SEM**	
	Larydox® (2mg/kg)	2.03±0.51	1.96±0.10	0.03±0.02	99
	200	4.06±0.29	4.03±0.04	2.52 ± 0.02	52.72
	100	4.68±0.37	4.66±0.38	4.00±0.06	25.37
	50	4.93±0.01	4.46±0.18	4.39±0.14	18.09
	Normal Saline	5.29±0.11	5.33±0.09	5.36±0.07	0.00

Curative antimalarial properties: Result of the curative antimalarial effect of *A. absinthium* are expressed as percent parasitaemia (Mean ± SEM) and percent cure in relation to the control groups. Reduction of parasitaemia was observed in all groups of mice treated with the plant extracts when compared to the negative control (Table 5). A relatively good curative effect was observed for all dose levels (50mg/kg, 100 mg/kg and 200mg/kg respectively), relative to the negative and positive control groups. Percent cure was in the range of 52.72% to 28.33% for the 200mg/kg to 50mg/kg treated groups. This activity was observed to be dose dependent in all groups treated with the plant extract.

Table 5: Curative antimalarial properties of crude *A. absinthium* aqueous extract

Extract	Doses (mg/kg)	Parasitaemia	Percent Parasitaemia Post Treatment			% Curative effect on day 3 Post Treatment
A. absinthium (aq.)		**Before Treatment (Day 0)** Mean± SEM	**Day 1 Mean± SEM**	**Day 2 Mean± SEM**	**Day 3 Mean ± SEM**	
	Artesunate (4mg/kg)	4.10±0.26	0.93±0.06	0.80±0.00	0.00±0.00	100
	200	4.05 ± 0.29	3.33±0.14	2.90 ± 0.12	0.58 ± 0.00	89.12
	100	4.99 ± 0.21	3.71±0.05	3.34 ± 0.13	2.60 ± 0.02	51.21
	50	4.16 ± 0.02	3.94±0.06	3.83± 0.12	3.82 ± 0.12	28.33
	Normal Saline	4.46 ± 0.18	4.82 ± 0.30	4.99 ± 0.21	5.33± 0.09	0.00

Effect of crude *Artemisia absinthium (aq.)* extracts on body weight: Results obtained (Table 6) revealed a decrease in body weight (-1.76% and -1.83%) for repository and curative tests in animals who were served the vehicle (negative control groups). This was expected since parasitaemia was allowed to build up, and treatment was not served. In the extract treated group, a gradual increase in body weight was observed throughout the period of the investigation. For repository test to be specific, the 200 mg/kg body weight treated group (Group 1) showed an increase (4.44%) in body weight relative to the 100 and 50mg/kg treated group which showed 3.76% and 1.97% increase in body weight, respectively (Table 6); For curative test on the other hand, the change in body weight is clearly pronounced in all extract treated groups.

Table 6: Effect of crude *Artemisia absinthium (aq.)* extracts on body weight of mice

Extract	Doses (mg/kg)	Repository Test			Curative Test		
A. absinthium(aq)		**Day 0 Mean ± SEM**	**Day 4 Mean ± SEM**	**Change in body weight (%)**	**Day 0 Mean ± SEM**	**Day 4 Mean ± SEM**	**Change in body weight (%)**
	Larydox®/ Artesunate	28.64±0.39	30.23±0.62	5.55	26.86±0.34	29.27±0.90	8.97
	200	25.70±0.62	26.84±0.96	4.44	25.78±0.59	27.75±0.90	7.64
	100	26.58±0.39	27.58±0.99	3.76	25.38±0.67	27.19±0.83	7.12
	50	28.26±0.55	28.82±0.85	1.97	25.70±0.55	27.52±0.59	7.10
	Normal Saline	26.86±0.48	27.33±0.47	-1.76	26.24±0.65	26.72±1.18	-1.83

Discussion

Malaria despite being one of the leading causes of death among children and pregnant mothers in sub Saharan Africa, eradication efforts is facing strong setbacks associated with resistance of the commonly available antimalarial drugs in clinical use, resistance of the vectors to synthetic insecticides used for impregnating bed nets as well as lack of available vaccines. In this study, results of the phytochemical analysis revealed the presence of bioactive metabolites such as Alkaloids, flavonoids, and anthraquinones among other biologically active phytochemicals, the presence of which may be an indication for the antimalarial properties observed. This finding is in consonance with those of Szopa *et al.* (2020) [37], who reported the presence of these essential metabolites in *A. absinthium*. Similar studies have attributed the scavenging of free radicals in response to infectious agents to Alkaloids and Flavonoids (Daskum, 2020) [9].

Further analysis of the crude plant extract by GC-MS revealed the presence of the Sesquiterpenoids, specifically Humulene and Aromadendrene. Other compounds identified include α-Tocopheryl acetate, Methyl chloroformate, Carbonic acid, Ethanamine. This finding was in agreement with that of Saunoriūtė *et al.* (2020) [33], who reported the presence of Aromadendrene in essential oils extracted from *Artemisia abrotanum* L. It is not surprising that this species (*A. absinthium*) does not contain artemisinin. This could simply be attributed to the finding of similar studies on Artemisia species who reported other compounds but not artemisinin (Mouton *et al.*, 2013) [21]. Similarly, this could be an indication that other compounds present exert antimalarial effect synergistically.

It is worthy of note that mortality or sign of acute toxic reactions such as pilo erection, moribund behaviour, tremors, convulsions, salivation, diarrhoea, lethargy and coma were not observed in all mice served with 2000mg/kg body weight of the extracts, throughout the follow-up period. This is an indication that the LD_{50} is above 2000mg/kg body weight and suggests that the extracts is safe for use in laboratory animal models.

Studies revealed that plant extract are classified as

biologically active in animal models when such extracts are capable of suppressing parasiteamia by 30% Mulisa *et al.* [49]. While many studies on medicianl plants focused primarily on the suppressive antimalarial activity of commonly availabe medicinal plants, only a handful of literature assessed the prophylactic activities of such plants. In this research however, the repository antimalarial properties revealed 52.72% prophylactic effect by crude *A. absinthium* extract (Table 4) at 200 mg/kg body weight relative to the positive control. This efficacy may be attributed to the presence of essential oils identified in the crude extract. Specifically, Aromadendrene was formerly reported to possess antimicrobial activity (Mulyaningsih *et al.,* 2010) [24]. Our finding further showed that mice treated with 200 mg/kg of crude *A. absinthium* extract are cured by 89.12%. This substantiate the results of Mojab, (2012) [20] who reported good antimalarial activity of *A. absinthium* on the Multidrug resistant and Multidrug sensitive strains of *P. falciparum in vitro*. Similarly, *A. absinthium* was proved to suppress parasitaemia in *Plasmodium berghei* infected mice by 94% when dosed with 200 mg/kg of the plant extract (Ramazani *et al.,* 2010; Mojab, 2012) [30, 20]. The plant was reported to possess other biological activities to include treatment of chronic fever, hepatitis and oedema in Indian Unani medicine, *as well as* cancer therapy in the traditional Chinese medicine (TCM) (Szopa *et al.,* 2020) [37]

The effect of *A. absinthium* on body weight revealed a steady change in body weight especially in all extract treated groups. This gradual change in body weight may not be unconnected to the initial body weight and survival of experimental animals until 4th day post infection. As reported (Table 6), an increase (4.44%) in body weight was observed in the 200 mg/kg treated group (Group 1) relative to the 100 and 50mg/kg treated group which showed 3.76% and 1.97% increase in body weight, respectively for repository test. On the other hand, the change in body weight is clearly pronounced in all extract treated groups for curative test. This is in agreement with the result of Daradka *et al.* (2014) [8] who reported that the loss of weight induced by alloxan treatment in alloxan-induced diabetic rats was reversed when *A. absinthium* extract was administered to the experimental animals. This study also validates the traditional use of *A. absinthium* in the treatment of malaria "suspected" fever.

Conclusion

Findings showed that *A. absinthium* has good antimalarial activity and substantiate the traditional claim for its use in the treatment of fever related illnesses suspected to be malaria by locals. Further fractionation of the extract and assessment of the antiplasmodial properties of individual fractions is essential, to underpin the exact compound responsible for the biological activity identified and develop novel antimalarial drugs from this plant.

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References

1. Alaribe CS, Oladipupo AR, Nani MO, Ijeoma IN, Olanipekun BD, Coker HA. Suppressive and Curative antiplasmodial properties of *Nauclea latifolia* root extract and fractions against erythrocytic stage of mice-infective chloroquine-sensitive Plasmodium berghei NK-65. Journal of Medicinal Plants for Economic Development. 2020;4(1):1-6.
2. Babalola IT, Adelakun EA. Compendium of medicinal plants for the ethnotherapeutic management of tuberculosis and other respiratory diseases. Journal of Pharmacognosy and Phytochemistry. 2018;7(3):1983-1994.
3. Bains RS, Wells S, Sillito RR, Armstrong J, Cater HL, Banks G, *et al*. Assessing mouse behaviour throughout the light/dark cycle using automated in-cage analysis tools. Journal of Neuroscience Methods. 2018;15(300):37-47. Doi:10.1016/j.jneumeth.2017.04.014
4. Batiha GES, Olatunde A, El-Mleeh A, Hetta HF, Al-Rejaie S, Alghamdi S *et al*. Bioactive Compounds, Pharmacological Actions, and Pharmacokinetics of Wormwood (*Artemisia absinthium*). Antibiotics. 2020;9(353):1-25.
5. Carbone L, Carbone ET, Yi EM, Bauer DB, Lindstrom KA, Parker JM *et al*. Assessing Cervical Dislocation as a Humane Euthanasia Method in Mice. Journal of the American Association of Laboratory Animal Science, 2012;51(3):352-356.
6. Coelho CH, Doritchamou JY, Zaidi I, Duffy PE. Advances in malaria vaccine development: report from the 2017 malaria vaccine symposium. NPJ Vaccines. 2017;2(34):1-5. doi:10.1038/s41541-017-0035-3
7. Cox FE. History of the discovery of the malaria parasites and their vectors. Parasites & Vectors. 2010;3(5):1-9. doi:https://doi.org/10.1186/1756-3305-3-5
8. Daradka HM, Abas MM, Mohammad MA, Jaffar MM. Antidiabetic effect of *Artemisia absinthium* extracts on alloxan-induced diabetic rats. Comparative Clinical Pathology. 2014;23:1733-1742.
9. Daskum AM. Ethnobotanical Survey and *In vivo* Assessment of the Antimalarial Activities of a Locally Used Medicinal Plant (*Senna occidentalis*) for "Malaria Suspected" Fever in Potiskum and Nangere Local Government Areas of Yobe State. Asian Journal of Research in Infectious Diseases. 2020;4(4):2582-3221.
10. Imboumy-Limoukou RK, Maghendji-Nzondo S, Sir-Ondo-Enguier PN, De Carvalho JN, Tsafack-Tegomo NP, Buekens J *et al*. Malaria in children and women of childbearing age: infection prevalence, knowledge and use of malaria prevention tools in the province of Nyanga, Gabon. Malaria Journal. 2020;19(387):1-8. doi:https://doi.org/10.1186/s12936-020-03411-5
11. Jimenez K, Kulnigg-Dabsch S, Gasche C. Management of Iron Deficiency Anemia. Gastroenterol Hepatol (N Y), 2015;11(4):241-250.
12. Kharoubia O, Slimania M, Kroufb D, Seddika L, Aouesa A. Role of Wormwood (*Artemisia absinthium*) extract on Oxidative Stress in Ameliorating Lead Induced Haematotoxicity. African Journal of Traditional Complementary and Alternative Medicine. 2008;5(3):263-270.
13. Kozlov M. Resistance To Key Malaria Drugs Confirmed in Africa. Nature, 2021;597:604.
14. Kuehn BM. Drug-Resistant Malaria Detected in Africa Will Require Monitoring. JAMA, 2021;325(23):2335-. doi:10.1001/jama.2021.9133
15. Kumar SR, Venkateshwar C, Samuel G, Rao GS. Phytochemical screening of some compounds from plant leaf extracts of *Holoptelea integrifolia* (Planch.) and *Celestrus emarginata* (Grah.) used by Gondu tribes at

Adilabad District, Andhra Pradesh, India. International Journal of Engineering Science Invention. 2013;2(8):65-70.
16. Lukwa AT, Mawoyo R, Zablon KN, Siya A, Alaba O. Effect of malaria on productivity in a workplace: the case of a banana plantation in Zimbabwe. Malaria Journal, 2019;18(390):1-8. Doi: https://doi.org/10.1186/s12936-019-3021-6
17. Mahomoodally FM. Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants. Evidence-Based Complementary and Alternative Medicine. 2013, 1-14.
18. Malaney P, Spielman A, Sachs J. The Malaria gap. American Journal of Tropical Medicine and Hygiene, 2004;71(2):141-146.
19. Meyer N, Kro¨ger M, Thu¨mmler J, Tietze L, Palme R, Touma C. Impact of three commonly used blood sampling techniques on the welfare of laboratory mice: Taking the animal's perspective. PLOS ONE. 2020, 1-23. doi:https://doi.org/10.1371/journal.pone.0238895
20. Mojab F. Antimalarial natural products: A review. Avicenna J Phytomed. 2012;*2*(2):52-62.
21. Mouton J, Jansen O, Frédérich M, Kooy FV. Is Artemisinin the Only Antiplasmodial Compound in the *Artemisia annua* Tea Infusion? An *in vitro* study. Planta Med. 2013;79:468-470.
22. Muangphrom P, Seki H, Fukushima EO, Muranaka T. Artemisinin-based antimalarial research: application of biotechnology to the production of artemisinin, its mode of action, and the mechanism of resistance of Plasmodium parasites. Nat Med, 2016;70:318-334.
23. Mulisa E, Girma B, Tesema S, Yohannes M, Zemene E, Amelo W. Evaluation of *In vivo* Antimalarial Activities of leaves of *Moringa oleifera* against *Plasmodium berghei* in Mice. Jundishapur Journal of Natural Pharmaceutical Products, 2018;13(1):1-5.
24. Mulyaningsih S, Sporer F, Zimmermann S, Reichling J, Wink M. Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. Phytomedicine. 2010; 17(13):1061-1066.
25. OECD 423. OECD Guideline for Testing of Chemicals. Organisation for Economic Coorporation and Development. 2001.
26. Okoyo C, Githinji E, Muia RW, Masaku J, Mwai J, Nyandieka L *et al*. Assessment of malaria infection among pregnant women and children below five years of age attending rural health facilities of Kenya: A cross-sectional survey in two counties of Kenya. PLOS ONE, 2021;16(9):1-19. doi:https://doi.org/10.1371/journal.pone.0257276
27. Vitor-Silva S, Reyes-Lecca RC, Pinheiro TR, Lacerda MV. Malaria is associated with poor school performance in an endemic area of the Brazilian Amazon. Malaria Journal, 2009;8(230):1-7. Doi:https://doi.org/10.1186/1475-2875-8-230
28. Permin H, Norn S, Kruse E, Kruse PR. On the history of Cinchona bark in the treatment of Malaria. Dan Medicinhist Arbog. 2016;44:9-30.
29. Petrovska BB. Historical review of medicinal plants' usage. Pharmacognsy Review, 2012;6(11):1-5. doi:10.4103/0973-7847.95849
30. Ramazani A, Sardari S, Zakeri S, Vaziri B. *In vitro* antiplasmodial and phytochemical study of five Artemisia species from Iran and *in vivo* activity of two species. Parasitology Research. 2010;107:593-599.
31. Sarma N, Patouillard E, Cibulskis RE, Arcand JL. The Economic Burden of Malaria: Revisiting the Evidence. American Journal of Tropical Medicine and Hygeine, 2019;101(6):1405-1415.
32. Sato S. Plasmodium—a brief introduction to the parasites causing human malaria and their basic biology. Journal of Physiological Anthropology. 2021;40(1):1-13. doi:https://doi.org/10.1186/s40101-020-00251-9
33. Saunoriūtė S, Ragažinskienė O, Ivanauskas L, Marksa M. Essential oil composition of *Artemisia abrotanum L*. during different vegetation stages in Lithuania. CHEMIJA. 2020;31(1):52-56.
34. Service M. *Medical Entomology for Students*. Cambridge: Cambridge University Press. 2008.
35. Service MW. *A Guide to Medical Entomology*. London: Macmillan Press. 1980.
36. Sofowora A, Ogunbodede E, Onayade A. Role and Place of Medicinal Plants in the Strategies For Disease Prevention. African Journal of Traditional Complementary and Alternative Medicine. 2013;10(5):210-229.
37. Szopa A, Pajor J, Klin P, Rzepiela A, Elansary HO, Al-Mana FA *et al*. *Artemisia absinthium L*. Importance in the History of Medicine, the Latest Advances in Phytochemistry and Therapeutical, Cosmetological and Culinary Uses. Plants. 2020;9(1063):1-33. doi:10.3390/plants9091063
38. WHO. World Malaria Report 2020. Geneva: World Health Organisation. 2020.
39. WHO. WHO global report on traditional and complementary medicine Geneva: World Health Organisation. 2019.
40. Yohanna JA, Gau PC, Adejoh VA. Prevalence of Malaria Parasites in Children at Vom Christian Hospital, Jos South, Plateau State, Nigeria. *Nigerian Journal of* Parasitology. 2019;40(2):152-157.
41. Zhu L. Products of the Empire: Cinchona: a short history. Cambridge: Cambridge University Library. 2018.