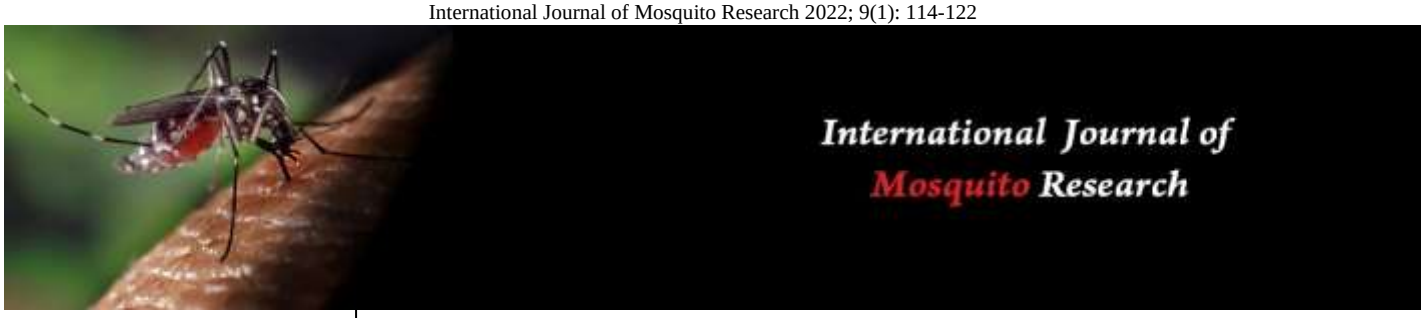

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In vitro antiplasmodial and *in vivo* toxicity potential of *Mentha piperita* and *Ocimum gratissimum* essential oils and their synergistic effect with conventional antimalarial drugs against *Plasmodium falciparum*

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Abstract
The study aimed to investigate the *in vitro* antiplasmodial and *in vivo* toxicity potential of essential oils from *Mentha piperita* and *Ocimum gratissimum* against *Plasmodium falciparum*, singly and in combination with antimalarial drugs. The essential oils (EOs) were obtained by steam distillation and phytochemical screening conducted using gas chromatography equipped with a flame ionization detector. Blood samples of malaria positive patients were cultured and *P. falciparum* identified microscopically. Micro-technique of schizont counting was used to determine the antiplasmodial baseline of the EOs and in combination with antimalarial drugs. Hematological and serum biochemical indices for *in vivo* toxicity assay using Wister rats were determined following WHO test procedures. Dose-response assay determined after 48 h for *in vitro* inhibition of maturation of trophozoites into schizonts showed that EO of *M. piperita* had a higher antiplasmodial activity of 6.62 ± 0.20, 10.74 ± 1.64 and 14.21 ± 0.45 µg values than *O. gratissimum* for IC_{30}, IC_{50} and IC_{90}, respectively against *P. falciparum*. When the EOs were combined with conventional antimalarial agents, *M. piperita* with Sulfadoxine/Pyrimethamine had the highest antiplasmodial activity with inhibitory concentration values of 1.92 ± 1.00, 4.07 ± 1.34 and 6.85 ± 0.75 μg/mL for IC_{30}, IC_{50} and IC_{90}, respectively than *O. gratissimum. In vivo* hematological and biochemical studies after 14 days oral treatment showed no toxicity as a result of treatment with the oil extracts and these were significantly different ($P < 0.05$). The study suggests that EOs of *M. piperita* and *O. gratissimum* could act individually or synergistically with conventional antimalaria drugs as effective alternative drug candidate in human malaria treatment.

Keywords: *Plasmodium falciparum*, malaria, essential oils, *M. piperita*, *O. gratissimum*, toxicity

Introduction
Malaria remains the most important tropical parasitic disease in the world, which kills a large number of people, thus causing greater public health burden after tuberculosis as stated by WHO (2018) [28]. It is a fatal disease that results from infection with a protozoan parasite, *Plasmodium*, transmitted by infected female *Anopheles* mosquito. Of the *Plasmodium* species responsible for malaria in humans, *Plasmodium falciparum* is the deadliest and it is responsible for roughly 50% of all malaria cases (C.D.C. 2018) [7]. Nigeria accounts for one-quarter of the burden of malaria in sub-Saharan Africa (WHO, 2019) [29]; and (WHO, 2020) [30] also declared mosquitoes as one of the deadliest animals in the world, causing millions of death every year, especially in the poorest countries due to diseases they transmit.
Vector control with insecticides and malaria treatment with conventional antimalarial drugs remains the primary strategy for the disease management. Unfortunately, development of insecticide and drug resistance, coupled with increasing concerns about environmental safety and exposure of humans to both antimalaria drugs and insecticides, limit their usage, thus, resulting in increased number of malaria morbidity and mortality.
P. falciparum is known to develope resistance to nearly all currently available antimalarial dru gs such as mefloquine, quinine among other quinine-based antimalaria drugs. Although drug resistance to these group of drugs are known to be less widespread, however, in some areas of

the world, especially Africa, the effect of multi-drug resistance in malaria is quite enormous. More importantly, reports abound in most parts of Southeast Asia and Africa on the cases of resistance to artemisinin-based combination therapy. This is known to have serious impact on the efficacy of this important antimalarial drugs and putting at risk all the effort made in malaria control as stated by (Tindana *et al*, 2021) [24].

With increased malaria incidences and rise in antimalarial drug resistance, which has become a serious global public health threat, there is an increasing demand for organic drugs made from natural sources than products made with synthetic additives. Thus, essential oils provide a good alternative as organic products with substantive potential in drug manufacture. They are recognized as safe natural eco-friendly substances with reduced capacity for resistance development by parasites and pathogenic microorganisms. Finding their safe inhibitory concentration as single therapy and in combination with recognized antimalarial drugs may improve health and consumer acceptance. A good example of such "herbal combo" used by the inhabitants of most Nigerian communities is made from clove, scent leaf, garlic, ginger, onions, lemon grass, unripe pawpaw, lime/lemon, and West African Black pepper, most of which are aromatic herbs with essential oils (Muanya, 2019) [17].

Mint leaf (*Mentha piperita*) of the family Lamiaceae is among important herbal tropical plants cultivated in West Africa and is known to contain huge quantities of complex chemical compounds with proven medicinal value. Essential oil of this plant has been reported by researchers for its insecticidal, antimicrobial, and antioxidant effects (Kumar *et al.,* 2011; Chraibi *et al.,* 2017 and Singh *et al.,* 2014) [12, 8, 22]. Similarly, scent leaf (*Ocimum gratissimum*), is a tropical plant species that belongs to the family Labiatae, also known to play significant role in treating a good number of ailments and diseases. It has also found wide application in traditional medicine for treatment of various protozoan intestinal infestations (Pessoa *et al.,* 2002; Ueda-Nakamura *et al.,* 2006 and Sandeep *et al.,* 2017) [19, 25, 21]. According to our knowledge, most previous studies on these two aromatic herbs have either emphasized the use crude solvent extracts or those that have used EOs usually test their efficacy on non-human *Plasmosium berghei* and their antimicrobial activity. Also, few of these studies have been conducted in Nigeria. Also, the use of herbal drug combinations has existed for ages but the efficacy and safe-dose of such combinations have sparsely been authenticated. In an attempt and effort to further identify new natural active antimalarial candidates against human malaria parasites, the present investigation evaluated the *in vitro* anti-plasmodia properties and *in-vivo* toxicity potentials of two selected plants, mint leaf (*M. piperita*) and scent leaf (*O. gratissimum*) based on Nigerian traditional medicinal beliefs.

Objective

The study aims to evaluate the *in vitro* antiplasmodial and *in vivo* toxicity potential of *Mentha piperita* and *Ocimum gratissimum* essential oils and their synergistic effect with conventional antimalarial drugs against *Plasmodium falciparum*. The specific objectives include the evaluation of *in vitro* inhibitory potentials and sensitivity of *M. piperita* and *O. gratissimum* singly and in combination with existing antimalarial drugs against *P. falciparum* isolate and the *in vivo* toxicity of *M. piperita* and *O. gratissimum* using animal model.

Materials and Methods

Ethical consideration

Informed consent was obtained from ten (10) individuals whose blood samples were used for the isolation of *P. falciparum*. The ethical approval for use of humans in the study was obtained from Health Research Ethics Committee of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Awka, Anambra State, Nigeria with protocol number COOUTH/CMAC/ETH.C/VOL.1/ FN: 04/0027. Also approval for the use of animal (Wistar Rat) was obtained from Ethics Committee for Animal Experimentation of the Veterinary Services Department, Anambra State Ministry of Agriculture and Rural Development protocol number VSD/R EC/A/R/026/2021.

Procurement of plant materials and conventional antimalarial drugs

M. piperita (mint leaf) and *O. gratissimum* (Scent leaf/ Basil) were bought from a local market in Awka, Anambra State, Nigeria. Authentication of both herbal leaves was done by a Taxonomist in the Herbarium section of the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria. Also, conventional antimalarial drugs Artemether/lumefantrine, sulfadoxine/pyrimethamine, and chloroquine phosphate were procured from government approved pharmacy shop in Awka.

Extraction of essential oil from leaves of *M. piperita* and *O. gratissimum*

The leaves of the two plants were cleaned in order to get rid of extraneous materials. The plants were dried under shade in the laboratory store for a week to reduce the moisture contents. Subsequently, they were pulverized and packaged in an airtight polyethene bag for extraction. Steam distillation method (Clevenger method) as described by (Majda *et al.,* 2019) [14], was adopted in the extraction of the essential oils. Hundred grammes (100 g) of each plant sample were added to 800 ml of distilled water in a 2-liter flask. The flask was heated using a balloon heater for 5 hours. When the distillation was completed, two phases were obtained, a liquid phase and an organic phase (essential oil), which is less dense than water. The essential oils were collected by the help of the separating funnel and stored at 4°C in amber bottles to circumvent photo-oxidation until when needed for analysis.

Phytochemical screening of the plant material

This was carried out at Spring Board Laboratory, Awka Anambra State. The method involved two standard procedures as described by (Kelly and Nelson, 2014) [11] with some modifications.

A. Pre-quantification procedures: One gram each of the plant materials was weighed and transferred into test tubes, and 15 milliliter (mL) of ethanol was added. The contents in the test tubes were heated up in a water bath at 60 ^{o}C for 60 minutes. At the end of the 60 minutes, the samples were transferred to a separatory funnel. The tubes were washed with 20 mL ethanol, 10 mL of hot water, and 3 mL of hexane, and were all transferred to the funnel. Extraction of the unsaponified matter was done five times with 25 mL of hexane. The extracts obtained were put together and washed

thrice with 10 mL of 10% v/v ethanol aqueous solution. Solution was then dried using anhydrous sodium sulfate to get rid of excess water and the solvent was lost by evaporation. The samples were solubilized in 1000 µL of pyridine and 200 µL of each sample were collected from the stock and put into a vial for analysis. A volume of 100 µL of MSTFA (derivatizing agent) was added to the vial.

B. Quantification by GC-FID: The analysis of phytochemical constituents was performed on a BUCK M910 Gas Chromatography equipped with a Flame Ionization Detector on two capillary columns of different polarities OV type: 101 (25 m x 0.22 mm x 0.25 mm) and Carbowax 20 M (25 m x 0.22 mm x 0.25 µm). The injector temperature was 280 ºC with split less injection of 2ul of sample and a linear velocity of 30cms^{-1}. The carrier gas was Helium 5.0pa.s which has a flow rate of 40 ml/min. The oven programming temperature was first operated at 200 °C and gradually extended to 330 ºC at a rate of 3 ºC/min with a gradient of 5 °C/min. The detector was operated at a temperature of 320ºC as outlined by (Majda *et al.*, 2019) [14]. The phytochemical constituents were determined by the ratio between the area and mass of internal standards and the area of the identified phytochemicals; and the different concentrations demonstrated in ug/g or ppm.

Collection of blood samples and determination of *P. falciparum* malaria infection

The chloroquine resistant strain of *P. falciparum* for this study was isolated from blood samples of patients from the hematology laboratory of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital who already tested positive microscopically for malaria infection. After confirmation, 2 mL of blood sample in triplicate were drawn from 10 participants by the use of venipuncture. Their blood types were type A and O positive. The blood was transferred to cryoprotectant vials, stored in liquid nitrogen and transported to the laboratory within 6 hours of sample collection for culturing.

Determination of *Plasmodium falciparum* infection by microscopy

Thick and thin smears of the medium containing the *Plasmodium* isolate were prepared on glass slides and fixed with 100% methanol. Smears were stained with filtered 1/10 dilution of Giemsa stain in phosphate buffered saline (PBS). Incubation was done for 10 minutes at room temperature. The slides were washed with distilled water, air dried and observed under an oil immersion lens (100x). The isolates were thereafter identified as described by (Anusha *et al.*, 2015) [3].

Susceptibility of *P. falciparum* to essential oils of *M. piperita* and *O. gratissimum* singly and in combination with antimalarial drugs

Cultivation of *P. falciparum*

Bioassay standard procedures outlined by (WHO, 1990 and Basilico *et al*., 1998) [27, 6] were adopted. Blood samples were centrifuged at 1500 r.p.m for 10 minutes in a centrifuge tube. The supernatant was removed and an equal volume of 3.5% sodium chloride (NaCl) was added. Second centrifugation was carried out on the suspension and the supernatant removed afterwards. Washing of the pellet was done twice with complete medium supplemented with 15% serum (Albumax), and the red cells obtained after centrifugation was used for the culture.

Preparation of the essential oils of *M. piperita, O. gratissimum* and antimalarial drugs

The EOs from the two plants dried under anhydrous sodium sulphate, were weighed and dissolved in normal saline to obtain a stock solution of 100mg/mL. The stock solutions were filter-sterilized through a 0.2μm Millipore filters and was then diluted with complete parasite media RPMI (Rosewell Park Memorial Institute) to a final working concentration of 10,000μg/ml (10mg/ml). Aliquots of 0.4, 0.8, 1.2, 1.6 and 2.0 mL were used to determine 30, 60 and 90% inhibitory concentrations of the EOs singly and in combination with antimalarial drugs. Concentrations of the antimalarial drugs (Artemether/lumefantrine, sulfadoxine/pyrimethamine, and chloroquine phosphate) were prepared as described by (WHO, 1990) [27] using *in vitro* microtest procedure in sterile distilled water picomole/litre (pM) quantities. Parasitaemia estimation and parasite visualization before incubation was done using Giemsa stained blood films on normal light microscopes.

Culturing of the red blood cells

The blood samples were cultured *in vitro* using a candle jar in RPMI medium at pH 7.4 and incubated at 37 ºC for 48hours for maturation of trophozoites to schizont. Subsequently, samples of the inoculum were collected from the different concentrations of the media and control in order to prepare a thick smear. The micro-technique of schizont counting based on simple microscopy as described by (Basilico *et al*., 1998) [6] was used to determine the baseline levels of malaria drug sensitivity of the extracts and in combination with existing antimalarial drugs. Control wells were maintained without the EOs or antimalarial drugs. Finally, *in vitro* activity was demonstrated as the percentage of the total counted schizonts at each drug concentrations, with regard to the total counted schizonts in drug-free controls.

***In vivo* toxicity of the extracts using mouse model**

In vivo toxicity assay was carried out using standard procedure described by (Andrade *et al*., 2014 and Chude *et al.*, 2018) [2, 9]. Forty (40) male Wistar rats with body weights of (120-150g) housed in metal cages were used for the study. The cages containing the rats were housed in a ventilated room at temperature of 25 ± 2°C with 12 h light/dark cycle. The rats were allowed for a minimum of 5 days to acclimatize to the laboratory conditions prior to the study and those that show symptoms of ill health were excluded from the study. The rats were shared into two sets (Set I and Set II for *O. gratissimum* and *M. piperita*, respectively) and each set contained four groups (n = 5 per group) for the analysis of each plant oil extracts. Each group of rats was administered with concentrations of the EOs (0.5, 1, 2, and 3 mg/mL) daily for 14-days. The rats were fed with chow during the experimental period with no restriction to food and water, and the EOs were administered through oral gavage. Control groups were administered with equal volume of normal saline with no EO.

Systematic behavioural observations

Systematic behavioural observations were undertaken to

provide a general measure of the effects of the EOs on the general disposition, consciousness state, activity, co-ordination and reflexes in the animal model as described by (Andrade *et al*., 2014) [2]. Parameters such as general activity, body weight, response to touch, tail squeeze response, respiration, corneal reflex, convulsions, hypnosis, anesthesia, urination, defecation, hypothermia, hyperemia, death were assessed at 15 min, 30 min, 1h, 2h, 4h and 8h post administration and then daily for 14 days. The animals were weighed on alternate days to monitor weight gain or loss.

Haematological and serum biochemical analysis

The rats were sacrificed in a sealed container containing diethyl ether and incision was made in the abdomen extended to the thorax. Blood was collected directly from the heart with a syringe and needle into two types of tubes, one with HB anticoagulant to determine the haematological parameters and the other, without anticoagulant, to obtain the serum to assess the biochemical parameters. Blood were taken from both treated and untreated groups. Haematological indices such as red blood cell count, packed cell volume (PCV), white blood cell count and haemoglobin estimation were determined as described by (Chude *et al*., 2018) [9]. Serum biochemical components such as the amount of creatinine, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, indirect bilirubin, direct bilirubin and total bilirubin were measured as described by (Mekonnen *et al*., 2019) [16].

Data analysis

Data generated from the *in vitro* antiplasmodial susceptibility profile of *M. piperita* and *O. gratissimum* singly and in combination with antimalarial drugs against *Plasmodium falciparum* were analyzed in triplicate and the values were then averaged and mean separation done by Duncan's new multiple range test and paired t-tests. Linear regression analysis was used to determine inhibitory concentrations (IC_{30}, IC_{50}, and IC_{90}). Significant difference was determined at $P< 0.05$.

Results and Discussion

Essential oil (EO) extracts of *M. piperita* and *O. gratissimum* were screened for the presence of phytochemical components and subsequently evaluated for their *in vitro* antiplasmodial and *in vivo* toxicity potentials. Thereafter, their synergistic activities with conventional antimalarial drugs were tested. The results of the qualitative and quantitative phytochemical screening of *M. piperita* EO revealed the presence of twenty compounds with Rutin (18.4335 ug/ml) and Phenol (16.6163 ppm) being the most abundant, followed by Sapogenin (9.9081 ug/ml) with Flavan-3-ol the least abundant (0.0361ppm) as presented in Table 1 and Figure 1. *Mentha* spp are known to have superfluity of biological traits such as antioxidant, insecticidal, antidiabetic, antimicrobial among others, and are as such widely used in the treatment of cold, fever, microbial, gastro-intestinal and cardiovascular disorders (Eftekhari, 2021) [10]. Also, results obtained from *O. gratissimum* EO showed that twenty-one compounds were present with Naringenin (45.4578 µg/mL) being the most abundant followed by Anthocyanin (25.3532 µg/mL) and Phenol (21.5943 ppm) with Rutin as the least abundant (0.3068 µg/mL) as shown in Table 2 and Figure 2. Previous studies have shown that flowers and leaves of *O. gratissimum* are used in preparation of teas and infusion and are known to be rich in essential oils with the presence of huge quantities of bioactive phytochemical compounds, the major reason for their pharmacological activities. It has also been reported that quite a good number of highly active monoterpene components of plants EOs display antiplasmodial activity in *Plasmodium falciparum* (Sandeep *et al*., 2017) [21].

Study by Megaly (2012) [15], is of the opinion that low molecular weight components of EOs, a very prominent class of antimalarial natural products, have biological activity toward *Plasmodium* parasites. Their study also showed that low molecular weight monoterpenes (limonene and linalool) and sesquiterpenes (farnesol and nerolidol), hinder the biosynthesis of metabolites in trophozoite and/ or schizont stages of *Plasmodium falciparum*. Most bioactive components of most herbs are known to cause various pharmacological effects and may be responsible for various actions in the two herbs used in our study. For instance, previous studies have reported that flavonoids possess prominent antioxidants activity as stated by (Pietta, 2009) while saponins have anti-inflammatory activities (Wang *et al*., 2008) [26], both of which were reported in the present study.

The *in vitro* antiplasmodial inhibitory action of essential oils from *M. piperita* and *O. gratissimum* singly and in combination with Artemether/lumefantrine, sulfadoxine/pyrimethamine, and chloroquine phosphate showed that all the treatments caused reasonable level of inhibition against *P. falciparum* (Table 3). It is a known fact that pure isolates of EOs are usually obtained from aromatic plants with proven high level of activity against organisms, however, they have at sometimes exhibited lesser activity and cases of resistance has been reported in them than in crude extracts using similar doses of active ingredients (WHO, 2018) [28]. There is also a general belief that drug combinations are absolutely necessary for effective control of malaria. This is because drugs when combined provide improved efficacy through synergistic activities. This practice has therefore been accepted as a standard in the management of *P. falciparum* drug resistance (Tarkang *et al.,* 2014) [23]. There are claims that herbal medications may either increase or decrease therapeutic outcome with over-the-counter malaria drugs when both are taken simultaneously (Eftekhari *et al*., 2021) [10]. However, the present study has shown increased activity after combination of EOs of *M. piperita* and *O. gratissimum* with conventional anti-malaria drugs. Also, synergism was observed in these herbs in combination with conventional antimalaria drugs indicating their potential use in combating drug resistance.

The inhibitory activities of the two herbs showed that *M. piperita* exhibited high *in vitro* antiplasmodial activity with inhibition concentrations of (6.62 ± 0.20, 10.74 ± 1.64 and 14.21 ± 0.45 µg/mL) for IC_{30}, IC_{50} and IC_{90} respectively thus, causing significant inhibition in the maturation of trophozoites into schizonts. This is comparable to inhibition concentrations of *O. gratissimum* with values of (4.14 ± 1.60, 4.97 ± 0.70 and 8.44 ± 1.00 µg/mL) for IC_{30}, IC_{50} and IC_{90}, respectively against chloroquine resistant *P. falciparium* (Table 3). The results obtained from the control group indicate that the trophozoites completed the replicative process of schizogony and there was 100% maturation to schizonts. Studies by (Kweyamba *et al*., 2019) [13] have shown that identification of locally used plant extracts with IC_{50} values of less than 15 µg/mL are important steps in the search for new anti-malarial

plant extracts. However, this claim is based on their investigation as other studies have reported values less or higher than 15 µg/mL. When the EO extracts were combined with conventional anti-malaria drugs, the results showed that the combination of *M. piperita* with Sulfadoxine/ Pyrimethamine had highest antiplasmodial activity with inhibitory concentration (IC) values of (1.92 ± 1.00, 4.07 ± 1.34) and (6. 85 ± 0.75) for IC_{30}, IC_{50} and IC_{90}, respectively, followed by EO of *O. gratissimum* in combination with chloroquine phosphate (1.69 ± 1.20, 2.84 ± 1.00 and 5.48 ± 0.60 μg/ml) for IC_{30}, IC_{50} and IC_{90}, respectively (Table 3).

The results of the *in vivo* toxicity activity of the EOs from *M. piperita* and *O. gratissimum* by oral route in Wister albino rats at concentrations of 0.5, 1, 2 and 3 mg/mL, did not show any abnormal weight-loss related toxicity signs in the Wister albino rats for both treated and control groups as shown in the Table 2. The body weight measurements showed minor percentage weight gain in *O. gratissimum* EO treated group (126.7±1.00) compared to *M. piperita* treated group (134.2±1.00) and control (133.8 ± 0.70) (Table 4). The results for haematological analysis (Table 5) indicate no significant difference ($P > 0.05$) when compared with the reference values for rat haematological indices and the values were all found to be within the reference values. Although the values were significantly different from each other ($P < 0.05$, 0.0001), the biochemical indices were also significantly different in both control and EO treated Wister albino rats. The amount of creatinine, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, indirect bilirubin, direct bilirubin and total bilirubin in both EOs treated Wister albino rats were comparable to the control groups and fall within the reference values (Table 6). Haematological values are essential for measuring the physiological and pathological status of both animals and humans (Adeneye *et al*., 2006) [1] and can be used to evaluate the degree of harmful effect of foreign compounds on the blood of rats (Odeyemi *et al*., 2009) [18]. Serum biochemical biomarkers like creatinine, alkaline phosphatase, aspartate aminotransferase, alanine amino transferase, indirect bilirubin, direct bilirubin and total bilirubin demonstrate that EOs of *M. piperita* and *O. gratissimum* did not induce any toxicological effect on the Wister albino rats. Measurements of creatinine levels in the blood are among the parameters used to evaluate kidney function (Arsad *et al*., 2013) [4] and in our study, creatinine levels between EO treated and untreated groups all fall within the reference level. These results point to the fact that the EOs of the two plants had no toxic effect on the animal model.

Table 1: Phytochemical screening of *M. piperita* essential oil

Component	Retention Time	Area	Height	Phyto-composition	units
Proanthocyanin	0.189	4760.4178	392.551	5.8898	ppm
Lunamarin	1.680	2707.9788	211.396	5.8012	ug/ml
Anthocyanin	8.000	3111.2627	244.086	6.9634	ug/ml
Naringenin	15.070	3335.9490	258.741	8.1864	ug/ml
Spartein	17.726	5801.8128	384.892	8.0358	ug/ml
Sapogenin	21.083	6981.8836	515.125	9.9081	ug/ml
Phenol	21.916	6505.2996	509.978	16.6163	ppm
Flavonones	28.890	4207.2472	330.404	9.4811	ppm
Steriods	31.973	3937.0518	305.767	6.3331	ppm
Kaempferol	36.456	6159.2316	469.868	7.3719	ug/ml
Flavone	37.726	5348.5161	418.085	6.8454	ug/ml
Epicatechin	40.243	3853.2248	276.485	2.3039	ug/g
Oxalate	42.083	3483.0057	250.664	6.8033	ug/ml
Phytate	42.950	8047.9474	565.256	3.9096	ug/ml
Quinine	44.216	199.5008	11.840	0.0748	ug/ml
Rutin	45.060	6842.5236	505.467	18.4335	ug/ml
Catachin	45.486	5229.3436	404.177	7.9083	ug/ml
Resveratol	47.676	3204.8954	251.636	2.7836	ppm
Tannin	50.010	4403.3232	344.646	5.9184	ug/ml
Flavan 3 ol	50.843	31.1666	0.745	0.0361	ppm
	Total	88151.5811		139.6039	

Table 2: Phytochemical screening of *O. gratissimum* essential oil

Component	Retention Time	Area	Height	Phyto-composition	units
Proanthocyanin	0.120	2704.1348	299.821	3.3457	ppm
Epihedrine	4.730	4791.2934	375.583	8.5789	ug/ml
Anthocyanin	9.513	11327.7878	882.322	25.3532	ug/ml
Ribalinidine	9.923	11599.4539	900.415	16.8352	ug/ml
Naringenin	15.536	18524.0420	912. 320	45.4578	ug/ml
Sapogenin	19.863	6068.5252	474.540	8.6119	ug/ml
Phenol	21.526	8454.1736	660.621	21.5943	ppm
Lunamarin	25.320	9486.9716	712.846	20.3234	ug/ml
Flavonones	26.710	5072.3662	348.674	11.4307	ppm
Steriods	32.606	5745.5714	405.005	9.2422	ppm
Spartein	34.163	6937.7727	501.355	9.6061	ug/ml
Kaempferol	36.340	7159.4727	523.102	8.5691	ug/ml
Flavone	37.590	182.5697	9.660	0.2337	ug/ml

Epicatechin	40.816	6971.3953	517.326	4.1682	ug/g
Oxalate	41.936	240.1318	5.538	0.4690	ug/ml
Phytate	42.956	219.7780	5.456	0.1068	ug/ml
Quinine	43.926	247.4416	5.151	0.0928	ug/ml
Rutin	45.196	113.8884	5.048	0.3068	ug/ml
Catechin	45.970	9892.7116	763.215	14.9606	ug/ml
Resveratol	48.340	9606.5926	742.664	8.3439	ppm
Flavan-3-ol	48.340	3890.8148	314.462	4.5033	ppm
	Total	129236.8891		222.1365	

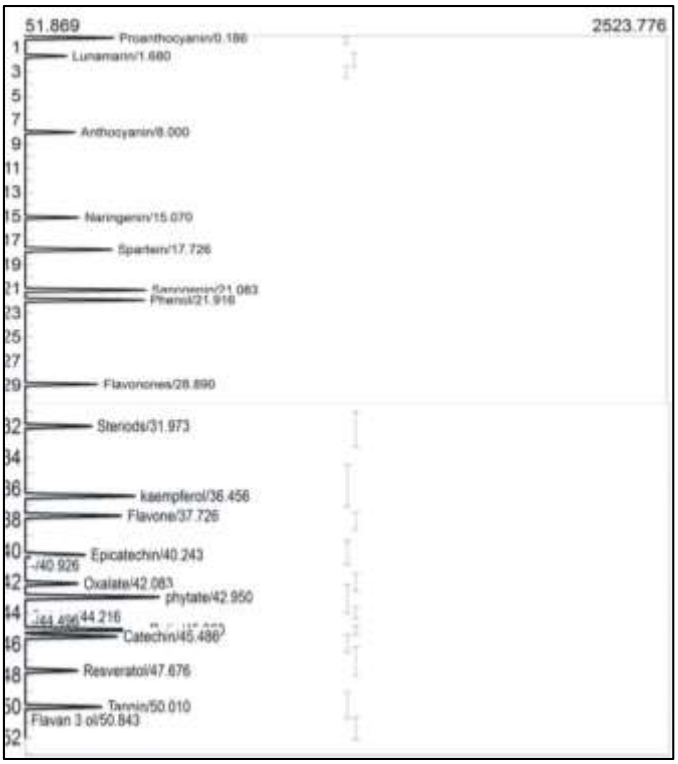

Fig 1: Chromatogram from GC-FID analysis of *M. piperita*

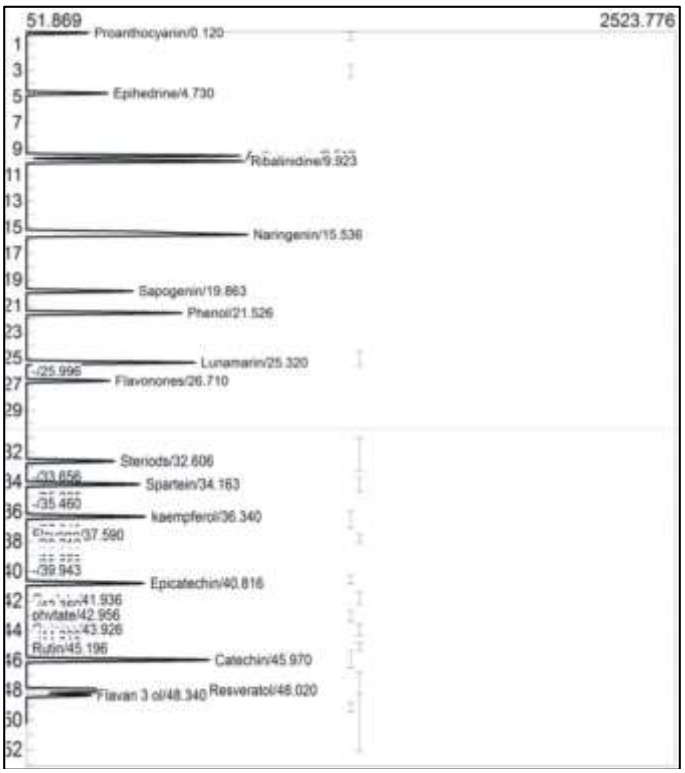

Fig 2: Chromatogram from GC-FID analysis of *O. gratissimum*

Table 3: Susceptibility *P. falciparum* against EOs of *M. piperita* and *O. gratissimum* singly and in combination with antimalarial drugs

	Inhibition of Maturation into Schizonts		
Treatment	**IC_{30} (µg/mL ± SD)**	**IC_{50} (µg/mL ± SD)**	**IC_{90} (µg/mL ± SD)**
M. piperita	6.62^{g} ± 0.20	10.74^{h} ± 1.64	14.21^{g} ± 0.45
O. gratissimum	4.14^{f} ± 1.60	4.97^{f} ± 0.70	8.44^{f} ± 1.00
EOMp + Chloroquine Phosphate	3.79^{e} ± 1.60	5.74^{g} ± 0.34	7.08^{e} ± 0.88
EOMp + Artemether/Lumefantrine	1.37^{bc} ± 0.40	3.14^{c} ± 0.10	6.11^{c} ± 0.25
EOMp + Sulfadoxine/Pyrimethamine	1.92d ± 1.00	4.07^{e} ± 1.34	6.85^{d} ± 0.75
EOOg + Chloroquine Phosphate	1.69^{c} ± 1.20	2.84^{b} ± 1.00	5.48^{bc} ± 0.60
EOOg + Artemether/Lumefantrine	0.89^{a} ± 0.10	2.20^{a} ± 1.64	4.67^{a} ± 1.00
EOOg + Sulfadoxine/Pyrimethamine	1.20^{b} ± 1.00	3.56^{d} ± 1.74	5.12^{b} ± 0.10

Values are mean of three independent determinations. Columns sharing different superscripts are significantly different *(P >0.05, 0.7129)*
EOMp: Essential oil of *M. piperita*
EOOg: Essential oil of *O. gratissimum*

Table 4: Body weight of *M. piperita* and *O. gratissimum* EO treated rats

	Initial Body Weight (g)	**Final Body Weight (g)**
M. piperita	117.5^{c}±1.40	134.2^{b}±1.00
O. gratissimum	108.8^{a}±1.00	126.7^{a}±1.10
Control	114.7^{b}±1.25	133.8^{b}±0.70

Values are mean determinations. Columns with different superscripts are significantly different (p> 0.05, 0.0001).

Table 5: Haematological indices of *M. piperita* and *O. gratissimum* EO treated rats

Parameter	***M. piperita***	***O. gratissimum***	**Control Group**	**Haematologic Reference Values for Male Rats***
Red Blood Cells ($x10^6$/µL)	9.05^{a}± 0.40	9.28^{ab}± 0.30	8.44^{b} ± 0.50	7.74 to 9.72
White Blood Cells ($x10^3$/µL)	6.60^{bc}± 0.10	6.75^{ab}± 0.40	6.45^{a}± 0.20	5.29 to 10.80
Packed Cell Volume (%)	43^{a}± 0.40	42^{b}± 0.10	39^{c}± 0.10	39 to 48
Haemoglobin (g/dL)	17.15^{a}± 0.20	17.40^{a}± 0.40	16.80^{b}± 0.20	14.7 to 18.0

Values are mean of three independent determinations. Rows sharing different superscripts are significantly different (p > 0.05, 0.0001)
*Charles River Laboratories (1998)

Table 6: Biochemical parameters of *M. piperita* and *O. gratissimum* EOs treated rats

Parameters	***M. piperita***	***O. gratissimum***	**Control Group**	**Biochemical Reference Range for Male Rats***
Creatinine (mg/dL)	0.8^{a} ± 0.05	0.7^{b} ± 0.05	0.8^{a} ± 0.01	0.4 - 2.3
Alkaline phosphatase (IU/L)	65.4^{ab} ± 1.00	63.8^{a} ± 0.40	97.5^{c} ± 0.20	30 - 130
Aspartate aminotransferase (U/L)	78.5^{ab} ± 0.70	94.2^{b} ± 0.50	77.5^{a} ± 0.10	74 - 143
Alanine aminotransferase (U/L)	28.2^{b} ± 0.95	22.10^{a} ± 0.65	36.8^{c} ± 0.25	18 - 45
Indirect bilirubin (mg/dL)	0.09^{c} ± 0.03	0.07^{b} ± 0.01	0.05^{a} ± 0.25	0.01 – 0.12
Direct bilirubin (mg/dL)	0.04^{b} ± 0.02	0.04^{b} ± 0.03	0.03^{a} ± 0.03	0.03 – 0.05
Total bilirubin (mg/dL)	0.13^{c} ± 0.01	0.11^{b} ± 0.01	0.08^{a} ± 0.03	0.05 – 0.15

Values are mean of three independent determinations. Rows sharing different superscripts are significantly different (p > 0.05, 0.0001)
*Charles River Laboratories (1998)

Conclusion

Plants biodiversity is still considered as one of the leading approach in the development of novel anti-parasitic drug molecules and this may account for their rich chemical diversity, ethnopharmacological claims and diverse pharmacological reports available in the literature. Among their benefits is that they are environmentally friendly, less expensive and less toxic systematically compared to synthetic antimalarial drugs. It is a known fact that the therapeutic value of most herbs is dependent on the relationship that exit between the chemical structure of the herbal phyto-components and pharmaco-activities on the reactive elements of the body. Therefore, the present study have shown that *Mentha piperita* and *Ocimum gratissimum* possess these phyto-compounds and hence, are able to exert antiplasmodial activity both *in vitro* against chloroquine resistant *Plasmodium falciparum* and non-toxic *in vivo* as demonstrated in mouse model studies. Thus, these two plants could be explored as effective drug candidates singly and in combination with existing antimalarial drugs for the development of therapies for use in multidrug-resistant *Plasmodium* parasite in human host. Also, the findings from this study will open up possibilities for formulation, development and standardization of herbal-conventional anti-malaria therapy, thus, may provide information on ways to optimize their doses to ensure safety of the people.

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Declaration of competing interest

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Author contribution

Chude Chidimma Florence: contributed in conceptualization, methodology, performing laboratory work and writing the original draft of the manuscript.

Nwankwo Edith Nonye: contributed in the analysis, writing of reviews and provided significant editorial assistance and supervision.

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