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In *vivo* Antiplasmodial Studies on *Trichilia heudelotii* Planch ex. Oliver (Meliaceae) Leaf

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Abstract: The study evaluated the antimalarial activities of the methanolic extract of *Trichilia heudelotii* leaf in mice, identified the most active partitioned and chromatographic fractions with a view to providing information on the scientific basis of the ethnomedicinal uses of the plant in treatment of malaria. The air-dried leaf of *T. heudelotii* was milled into powder and extracted with methanol. The median lethal dose (LD_{50}) was determined according to Lorke's method. The extract was tested against chloroquine-sensitive strain of *Plasmodium bergheii bergheii* NK-65 at doses of 100–800 mg/kg using the chemosuppressive antimalarial model while distilled water (0.2 mL) and chloroquine (10 mg/kg) were used as the negative and positive controls, respectively. The methanol extract was successively partitioned to obtain n-hexane (THH), dichloromethane (THD), ethylacetate (THE), n-butanol (THB) and aqueous fractions (THA) which were each tested at 50, 100 and 200 mg/kg similarly. The most active THH was successively chromatographed and the most active fractions THH2B and THH3B identified. The percentage chemosuppression and percentage survivor in mice was used as a measure of the antiplasmodial activities of the extract, partitioned and column fractions. The LD_{50} of *T. heudelotii* leaf extract was greater than 5000 mg/kg. It gave the highest chemosuppression of 88.7% at 200mg/kg, the n-hexane partitioned fraction (THH) gave percentage chemosuppression of 66% at 50mg/kg while subsequent column fractions, THH2B and THH3B gave 77 and 74% respectively. The chemosuppressive activities of *T. heudelotii* leaf was obviously retained during subsequent purification with the fractions demonstrating good% chemosuppression and percentage survivor profile as to contain the antimalarial constituents of the plant. Gas Chromatography- Mass Spectroscopy of THH3B, the most active column fraction suggested 6, 10, 14-trimethyl-2-pentadecanone, methyl palmitate and 11-Octadecenoic acid, methyl ester that were the major compounds identified in the fraction as possible antimalarial compounds in the plant.

Keywords: *T. heudelotii*, *Plasmodium bergheii bergheii* NK-65, Meliaceae, Chemosuppressive, Antimalarial

1. Introduction

The malarial infection, caused by its vector, *Plasmodium*-carrying mosquitoes, is a common endemic disease in tropical zones of the world [1] especially in Africa, where children under the age of 5 years die every 2 minutes of malaria [2]. Also, in Africa, up to 80% of the African population use traditional medicines for primary health care [2] while plant extracts are still widely used in the treatment of malaria and other ailments. The search for antimalarial molecules has become a recurrent issue particularly, with the rate at which resistance develop to the limited number of antimalarial chemicals in clinical use, some of which also have serious side effects [3]. As already in vogue, plants have been a ready source of such chemicals, *Cinchona succirubra* stem bark and *Artemisia annua* herb being ready examples. The current major ACT component, Artemisinin was obtained from *Artemisia annua*, a medicinal plant that has

been used ethnomedicinally in China for about 2000 years while quinine from *Cinchona succirubra* stem bark has been in use for the treatment of malaria [4].

Africa is blessed with a rich flora, it is therefore not out of place to source for chemicals from this rich arsenal of medicinal plants. Many plants have been tested for antiplasmodial activities and at the same time yielded a variety of compounds that have been formulated as drugs of which some are currently on the shelves pharmacies for the treatment of malaria and other diseases. Such plants include *Azadirachta indica, Citrus medica, Anarcadium occidentale, Morinda lucida, Cymbopogon citratus, Nigella sativa* and *Enantia chlorantha,* among others [5-10]. Moreso, ethnomedicine has been shown to be a potential source of antiprotozoal compounds that can be used as template for the synthesis of novel molecules [11].

The leaf of *Trichilia* species are trifoliate, pinnate and measures 20 m to 30 m in height with 0.4 m girth [12]. It is mostly common found in the tropical rainforest in Africa [13]. The leaf apex ranges from acute to acuminate, margin entire, leaf opposite, the fruits have three locules of ovary and valves with light green flower [12].

T. heudelotii is known to possess diverse pharmacological activities. The articles [14, 15] reported that *T. heudelotii* leaf extract displayed both antibacterial and antifungal activities. It was also reported to be active against *Escherichia coli* NCTC 10418, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, *Trichophyton rubrum* and *Candida pseudotropicalis* (clinical isolate). The antimicrobial activities of ethylacetate fraction of this plant have also been reported [16].

The morphological parts of the various species of *Trichilia* have been reported to possess antimalarial activities. These include the bark of *T. monadelpha* [17], the leaf of *T. emetica* [18], stem bark, root bark and leaves of *T. megalantha* [19] and the root bark of *T. roka* [20].

The family Meliaceae contains, oxygenated triterpenes, known as limonoids. The genus Trichilia has afforded the insecticide, trichillin, a triterpenoid, limonoids, steroids and other terpenes derivatives [21-23] while *Trichilia heudelotii* has yielded heudelottin [24, 25].

Trichilia heudelotii was among the 98 plant species that were reported in an ethnobotanical survey to have been used singly or in combination for the treatment of malaria and/or fever [12, 26-28].

Currently, the antimalarial ethnomedical claims have not been scientifically verified, and till date, there has been no literature cited to show that this plant has been scientifically investigated to establish whether or not it possesses *in vivo* antimalarial activity neither has any compound being isolated, which showed antimalarial or antiplasmodial activities. The objective of this study was therefore to evaluate the antimalarial activities of the methanol extract of *T. heudelotii* leaf in mice and identify the most active partition and chromatographic fractions with a view to providing information on the scientific basis for its use in the treatment of malaria in Nigeria.

2. Materials and Methods

2.1. Plant Identification and Authentication

The leaf of *Trichilia heudelotii* was collected at the back of the Department of Botany, Obafemi Awolowo University, Ile –Ife in December, 2015 and identified by Mr. G. A. Ademoriyo, the curator of the Department of Botany, Obafemi Awolowo University, Ile –Ife. The voucher specimen with number IFE-17662 was deposited at the Obafemi Awolowo University, Department of Botany herbarium. Subsequently, a 5.0kg of air-dried and powdered leaf was exhaustively extracted with 100% methanol by maceration. The filtrate was concentrated to dryness *in vacuo* at 40°C to obtain 360 g (7.2% w/w) of its methanol extract.

2.2. Acute Toxicity Testing

The acute toxicity of the resulting methanol extract was carried out in two phases: In the first phase, three groups of three animals each were administered orally with doses of 10, 100, 1000 mg /kg body weight respectively. These were observed for a period of 24 hours for mortality. If no mortality was observed, then three groups consisting of one animal per group were given 1600, 2900 and 5000 mg/kg body weight of the extract respectively in the second phase and observed for mortality: The lowest dose that produced mortality (D_{100}) and the highest that produced no mortality (D_0) were recorded. The LD_{50} was calculated from the formula: $LD_{50} = \sqrt{(D_0 \times D_{100})}$ [29].

2.3. Animals and Parasites

The Swiss mice (30) weighing 18 – 22 g used for this research were purchased from the Multi-disciplinary Laboratory, College of Health Sciences, Obafemi Awolowo University, housed in aluminium cages with 12 hours day/night cycle, fed with growers' mesh obtained from Brand cereals and oil mills limited, Bukuru, Jos and fed with clean tap water *ad libitum*. The animals were acclimatized for 2 weeks before use and handled in accordance with National Institute of Health Guidelines [30].

2.4. In vivo Antimalarial Assays Studies

The mice were divided into 6 or 5 or 1 group(s) of five animals each and groups I to IV or I-III were administered with 100, 200, 400 and 800 mg/kg of the extract respectively or 50, 100 and 200 mg/kg of the partition fraction or 50mg of the column fractions while groups V and VI or IV and V or II and III were given 0.2 mL of distilled water and 10 mg/kg of chloroquine as negative and positive controls, respectively as the case may be. This was done two hours after inoculation with parasite on the first day, and then daily, subsequently for the next three days. Temperature were taken prior to the administration of the extract or fraction. The blood collected from the tail vein of the mice on the fifth day was smeared on a microscopic slide, fixed with methanol and stained in 10% dilution of a 4% Giemsa in tap water. Ten fields of the red blood cells were counted using the oil immersion objective of

the light microscope. The percentage ratio of the parasitized erythrocytes to the total number of the parasitized and un-parasitized erythrocytes was the percentage parasitaemia, the average of which gave the Average percentage parasitaemia (APP) for 5 mice. The percentage chemosuppression (PCM) was determined from the APP. The effective doses, ED_{50} and ED_{90} were forecast from PCM. The results were analysed using one-way analysis of variance and student's Newman Keul's t-test as post hoc test in a Vinstat Graphpad statistical programme [30].

2.5. Evaluation of Survival Times in the Mice

The number of days by which the drug extended the life of the animal after drug administration was determined by monitoring the mice from the day of drug administration till 28 days thereafter for mortality. The mice were monitored daily to record the date of death and observing the animals for signs of toxicity. The length of days that each mouse survived was recorded and presented as mean ± standard error of the mean [30].

2.6. In Vivo Antimalarial Assays of the Methanol Extract

The mice were divided into 6 group(s) of five animals each and groups I to IV were administered with 100, 200, 400 and 800 mg/kg of the extract respectively; groups V and VI were given 0.2 mL of distilled water and 10 mg/kg of chloroquine as negative and positive controls, respectively. This was done two hours after inoculation with parasite on the first day, and then daily, subsequently for the next three days. Temperature was taken prior to the administration of the extract or fraction. The blood collected from the tail vein of the mice on the fifth day was smeared on a microscopic slide, fixed with methanol and stained in 10 ml of 4% Giemsa dissolved in 90 mL of water.

2.7. Statistical Analysis

The antimalarial activities of the methanol extract of *T. heudelotti,* its partition fractions, column fractions of the most active n-hexane fraction and further column fractions were evaluated by comparing their percentage chemosuppression ED_{50}, and ED_{90} with those of the positive and negative control and with each other by subjecting the values to statistical analysis using ANOVA followed by Dunnett and Bonferroni t-test as the post-hoc tests. $P < 0.05$ was considered as significant.

2.8. Purification of the Leaf Extract

2.8.1. Partitioning of the Extract

The methanol extract (200g) was suspended in 100 mL distilled water and successively partitioned into *n*-hexane, dichloromethane, ethylacetate, *n*-butanol, to obtain the corresponding fractions THH, THD, THE THB, and the aqueous residue (THA). Each partitioned fraction was concentrated to dryness *in vacuo* at 40°C.

2.8.2. In Vivo Antimalarial Assays of the Partition Fractions

The mice were divided into 5 group(s) of five animals each and groups I to III were administered with 50, 100 and 200 mg/kg of each of the partition fraction THH respectively. Groups IV and V were given 0.2 mL of distilled water and 10 mg/kg of chloroquine as negative and positive controls, respectively. This was done two hours after inoculation with parasite on the first day, and then daily, subsequently for the next three days. Temperature was taken prior to the administration of the extract or fraction. The blood smears were taken from each mouse on the fifth day fixed with methanol and stained in 4% Giemsa (10ML) diluted to 10 mL volume of tap water. The other fractions, THD, THE, THB and THA were assayed in the same way as THH.

2.8.3. Column Chromatography of the n-Hexane Partition Fraction of T. Heudelotii

The most active *n*-hexane partition fraction (40 g) (THH) adsorbed with 10.0 g of silica gel (70 and 230 mesh size) was chromatographed with graded ratios of n-hexane, dichloromethane, ethylacetate and methanol as follows: The 38 eluates obtained were pooled based on their TLC pattern as follows: THH2A (1-13: n-hexane, 100% 12000mL, n-hexane-DCM, 8:2, 200mL; n-hexane- DCM, 7:3, 200mL; n-hexane- DCM, 6:4, 200mL; n-hexane-DCM, 1:1, 200mL; n-hexane- DCM, 4:6, 200mL; 6.2g) THH2B (14-24: n-hexane-DCM, 3:7, 200mL, n-hexane-DCM, 2:8, 200mL; n-hexane-DCM, 1:9, 200mL; n-hexane-DCM, 0.5:9.5, 200mL; n-hexane- DCM, 0:10, 200mL; DCM- EtOAc,, 9:1, 200mL; DCM-EtOAc, 8:2, 400mL; DCM-EtOAc, 7:3, 200mL; DCM-EtOAc, 6:4, 200mL; DCM-EtOAc, 1:1, 200mL; n-hexane-DCM-EtOAc, 4:6, 200mL; 7.7g) THH2C (25-34: DCM- EtOAc, 3:7, 200mL; DCM- EtOAc, 2:8, 200mL; DCM- EtOAc, 1:9, 200mL; DCM-EtOAc, 0:10, 200mL; EtOAc - MeOH 9.5:0.5, 200mL; EtOAc - MeOH 9:1, 200mL; EtOAc - MeOH 8:2, 200mL, 4.5g); THH2D (35-38: EtOAc - MeOH 7:3, 200mL; EtOAc - MeOH 1:1, 200mL; EtOAc - MeOH 3:7, 200mL; EtOAc - MeOH 0:10, 200mL, 11.1g) The pooled fractions 2A-2D were each tested for antiplasmodial activity. Most active Fraction THH2B was further subjected to column chromatography for further purification.

2.8.4. In Vivo Antimalarial Assays of the Column Fractions of the n-Hexane Partition Fraction (2A-2D)

The mice were divided into 6 group(s) of five animals each and groups I to IV were administered with 50mg/kg column fractions 2A, 2B, 2C and 2D while groups IV and V were given 0.2 mL of distilled water and 10 mg/kg of chloroquine as negative and positive controls, respectively. This was done two hours after inoculation with parasite on the first day, and then daily, subsequently for the next three days. Temperature was taken prior to the administration of the extract or fraction. The blood smears were taken from each mouse on the fifth day fixed with methanol and stained in 4% Giemsa diluted to 10 mL volume of tap water.

2.8.5. Column Chromatography of the Sub Fraction 2B of the n-Hexane Partition Fraction of T. Heudelotii

A 7.0 g of the most active sub fraction 2B of the n-hexane partition fraction (THH 2B) adsorbed with 10.0 g of silica gel (70 and 230 mesh size) and chromatographed with graded ratios of n-hexane, dichloromethane, ethylacetate and methanol as follows: The 39 eluates obtained were pooled based on their TLC pattern as follows: THH 3A (1-5: n-hexane, 100% 2000mL, n-hexane- DCM, 9.5:0.5, 200mL; n-hexane- DCM, 9:1, 200mL; n-hexane- DCM, 8.5:1.5, 200mL; n-hexane- DCM, 8:2, 200mL; 0.80 g) THH 3 B (6-9: n-hexane- DCM, 7.5:2.5, 200mL, n-hexane- DCM, 7:3, 200mL; n-hexane- DCM, 6.5:3.5, 200mL; n-hexane- DCM, 6:4, 200mL; 0.60g) THH 3C (10-13: n-hexane- DCM, 6:4, 200mL, n-hexane- DCM, 5.5:4.5, 200mL, n-hexane- DCM, 1:1, 200mL, n-hexane- DCM, 4.5:5.5200mL, n-hexane- DCM, 4:6 1.05g). THH 3D (14-20: n-hexane- DCM, 3.5:6.5, 200mL; n-hexane-DCM, 3:7200mL; n-hexane-DCM, 2.5:7.5, 200mL, n-hexane-DCM, 2:8, 200mL, n-hexane- DCM, 1.5:8.5, 200mL, DCM- EtOAc, 9:1, 200mL; DCM- EtOAc, 8.5:1.5, 200mL, 3.10g) THH 3E 21-27: DCM- EtOAc, 7:3, 400mL; DCM- EtOAc, 1:1, 200mL; DCM- EtOAc, 2:8, 200mL; DCM- EtOAc, 1:9, 200mL; DCM- EtOAc, 0:1, 400mL; EtOAc-MeOH, 1:1, 400mL; MeOH 100%, 400mL, 2.11g). The pooled fractions THH 3A-THH 3E were each tested for antiplasmodial activity.

2.8.6. In vivo Antimalarial Assays of the Column Fractions (THH 3A-THH 3E) from the n-Hexane Partition Fraction

The mice were divided into 5 group(s) of five animals each and groups I-V were administered with 50 mg/kg of the column fraction respectively while groups VI and VII were given 0.2 mL of distilled water and 10 mg/kg of chloroquine as negative and positive controls, respectively. Temperature was taken prior to the administration of the extract or fraction. The blood smears were taken from each mouse on the fifth day fixed with methanol and stained in 4% Giemsa diluted to 10 mL volume of tap water.

2.9. Gas Liquid Chromatographic Analysis

Gas Liquid Chromatographic (GLC) separation was performed on a Gas chromatography (Agilent, USA) hyphenated to a mass spectrophotometer (5957C) with triple axis detector equipped with an auto injector (10 µL syringe) with Helium gas as carrier. All chromatographic separation were performed on a capillary column, specification 19091S-413: 3516.15684, dimensions: 30 m x 320 µm x 0.25 µm, treated with 5% phenyl methyl siloxan and operated at a constant flow rate of 1.5 mL/min of helium gas with other conditions as follows: EI (ion source temperature), 300°C, interface temperature 300°C, pressure 3.2875 psi, out time 1.8 mins; 1.0ml injector in the split mode with a split ratio 1:50 and an injection temperature of 300°C. The oven temperature was held for 2.0 min at an initial temperature of 80°C and programmed to increase to 280°C at 3°C/min, held for 20 min and later increased to 250°C at 20°C/min and finally held isothermally for 5 minutes, giving a total run time of 88.667 mins. Transfer line temperature was set to 34°C and post run temperature was to 325°C for 10 min. The data solution software supplied was used to control the system and acquire the data. The separated constituents were passed to the detector which recorded the emergence of the constituents as peaks with a retention time. The percentage compositions of the compound in the entire sample were computed from the peak areas automatically generated by the machine. The results were recorded as retention time against percentage composition in the original sample.

Gas Chromatographic–Mass Spectrometric (GC-MS) Analysis of THH3B

GC-MS analysis was performed on THH3B as stated above. Samples were prepared and injected into the GC-MS machine and the result acquired as peaks with respective retention times. Data handling was done using GC-MS solution software. The identities of the components were assigned by comparing their retention times with those of the standard spectra from NIST.

3. Results

Table 1. *Percentage Chemosuppression, Survival time and Percentage Survivor (in parenthesis) of T. Heudelotii Leaf Methanolic Extract.*

Dosage (mg/kg)	Chemosuppression (%)	Survival time in days, (Percentage Survivor)
NC	0.00 ± 0.00^{a}	9.60 ± 0.00^{a} (20)
100	71.71 ± 3.61^{c}	17.60 ± 0.60^{e} (60)
200	88.08 ± 0.78^{d}	27.33 ± 0.88^{e} (80)
400	60.37 ± 3.35^{b}	25.80 ± 0.33^{e} (60)
800	$68.92 \pm 1.70^{b.c}$	17.60 ± 1.33^{b} (20)
PC	64.14 ± 3.10^{b}	26.40 ± 1.29^{d} (80)

Data are expressed as mean ± standard error of mean; n=5; values with different superscripts within columns are significantly different (P<0.05); while those with similar superscripts within columns are not significantly different (P>0.05); NC (Negative control): 0.2 mL distilled water; PC (Positive control): 10 mg/kg chloroquine.

Table 2. *The Percentage Chemosuppression and Effective Doses Elicited by Trichilia Heudelotii Partition Fractions on Plasmodium Berghei Infected Mice.*

Doses	% Chemosuppression by partitioned fractions ± SEM				
	THH	THD	THE	THB	THA
NC	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
50	65.58 ± 12.38^{c}	41.63 ± 7.16^{b}	38.87 ± 7.22^{b}	65.94 ± 3.63^{c}	44.10 ± 10.94^{b}

Doses	% Chemosuppression by partitioned fractions ± SEM				
	THH	THD	THE	THB	THA
100	35.57 ± 1.57 [b]	50.41 ± 15.08 [b]	44.77 ± 8.73 [b]	51.87 ± 3.10 [c]	63.19 ± 10.49 [b]
200	39.77 ± 10.10 [b]	43.31 ± 3.17 [b]	50.82 ± 7.62 [b]	35.81 ± 9.73 [b]	58.46 ± 11.98 [b]
PC	68.64 ± 4.99 [c]	68.64 ± 4.99 [b]	68.64 ± 4.99 [c]	68.64 ± 4.99 [c]	68.64 ± 4.99 [b]
ED_{50}	101.70 ± 6.56 [a]	138.13 ± 25.44 [a]	136.67 ± 11.14 [a]	96.14 ± 1.88[a]	105.30 ± 8.45 [a]
ED_{90}	173.86 ±16.36 [a]	245.13 ± 54.45 [a]	245.32 ± 19.40 [a]	130.62 ± 13.49[a]	199.36 ± 23.32 [a]

Data are expressed as mean ± standard error of mean; n=3; values with different superscripts within columns are significantly different (P<0.05); while those with similar superscripts within columns are not significantly different (P>0.05); NC (Negative control): 0.2 mL distilled water; PC (Positive control): 10 mg/kg chloroquine. ED_{50}: The dose that will cause 50% reduction in parasitaemia; ED_{90}: The dose that will cause 90% reduction in parasitaemia; Partition Fractions: THH: n-Hexane; THD: Dichloromethane; THA: Ethylacetate; THH: THB; Aqueous: THA.

Table 3. *The Survival Times and Percentage Survivor Elicited by of Trichilia Heudelotii Partition Fractions in Plasmodium Berghei Infected Mice.*

Doses	Survival times by partitioned fractions ± SEM (percentage survivor) in parenthesis				
	THH	THD	THE	THB	THA
NC	15.00±3.79 [a b](33.3)	15.00±3.79 [a] (33.3)	15.00±3.79 [a] (33.3)	15.00±3.79[a, b] (33.3)	15.00±3.79 [a, b] (33.3)
50	11.00 ± 1.16[a](67)	15.00 ± 1.73 [a](67)	7.00 ± 1.16 [a](67)	5.67 ± 0.67[a] (33.3)	5.67 ± 1.76 [a] (33.3)
100	6.3 ± 0.67 [a](67)	14.3 ± 2.40 [a](33.3)	10.67 ± 3.71 [a](33.3)	14.3± 1.76[a, b](33.3)	13.67± 0.88 [a,b](67)
200	11.6 ± 2.40 [a](67)	13.67 ± 2.6 [a](67)	21.6 ± 6.1 [a](67)	8.3 ± 2.4 [a](67)	5.33 ± 0.88 [a](33.3)
PC	21.66± 3.53 [b](67)	21.66± 3.53 [a] (67)	21.66± 3.53 [a] (67)	21.66± 3.53[b] (67)	21.66± 3.53 [b](67)

Data are expressed as mean ± standard error of mean; n=5 (extract); n=3 (partition fraction); values with different superscripts within columns are significantly different (P<0.05); while those with similar superscripts within columns are not significantly different (P>0.05); NC (Negative control): 0.2 mL distilled water; PC (Positive control): 10 mg/kg chloroquine. Partition Fractions: THH: n-Hexane; THD: Dichloromethane; THA: Ethylacetate; THH: THB; Aqueous: THA.

Table 4. *Antimalarial Activities of Sub-Column Fractions from n-Hexane Partition Fractions of T. Heudelotti.*

FRACTIONS	% CHEMOSUPPRESSION	SURVIVAL TIMES (PS) in parenthesis
NC	0.00± 0.00 [a]	12.00±0.82 [a] (20)
THH2A	72.34 ±4.75 [b]	8.00±2.83 [a] (33)
THH 2B	77.54±5.35 [b]	13.00±4.55 [a] (67)
THH 2C	68.70±9.53 [b]	12.67±1.70 [a] (33)
THH 2D	55.90±12.3 [b]	14.67±4.11 [a] (67)
PC	79.91±2.83 [b]	24.00±1.29 [a] (33)
THH	65.58±12.38 [b]	27.33±0.88 [a] (80)

Data are expressed as mean ± standard error of mean; n=5 (extract); n=3 (partition fraction); values with different superscripts within columns are significantly different (P<0.05); while those with similar superscripts within columns are not significantly different (P>0.05); NC (Negative control): 0.2 mL distilled water; PC (Positive control): 10 mg/kg chloroquine. Sub-Column Fractions: THH 2A, THH 2B, THH 2C, THH 2D; Partition Fraction: THH.

Table 5. *Antimalarial Activities of Sub-Column Fraction of THH 2B.*

FRACTION	% CHEMOSUPPERSSION	Survival time and% survivor (in parenthesis)
NC	0.00 ±0.00[a]	8.60±3.38 [a] (40)
THH3A	49.63 ±6.52 [b c]	10.20±1.94 [a] (60)
THH 3B	74.43±10.16 [c]	15.40±1.36 [a] (60)
THH 3C	70.72±4.76 [b c]	16.80±2.71 [a] (60)
THH 3D	68.16±9.04 [b c]	11.4±1.86 [a] (20)
THH 3E	37.00 ±9.53 [b]	6.60±1.63 [a] (40)
PC	57.25±4.12 [b c]	17.00±3.35 [a] (80)
THH	65.58±12.38 [b c]	27.33± 0.88 [b] (80)

Data are expressed as mean ± standard error of mean; n=5 (extract); n=3 (partition fraction); values with different superscripts within columns are significantly different (P<0.05); while those with similar superscripts within columns are not significantly different (P>0.05); NC (Negative control): 0.2 mL distilled water; PC (Positive control): 10 mg/kg chloroquine. Sub-Column Fractions: THH 3A, THH 3B, THH 3C, THH 3D, THH 3E; Partition Fraction: THH.

Table 6. *Gas Chromatographic Analysis of the Isolates from THH 3B.*

CODES	NAME OF COMPOUNDS	M/W	FORMULA	CAS NUMBER	Rt (min)	Peak area
THH 3B1	6, 10, 14-trimethyl 2--pentadecanone,	268.4778	$C_{18}H_{36}O$	000502-69-2	37.652	3.26
THH 3BIII	Methyl palmitate	270.5	$C_{17}H_{34}O_2$	000112-39-0	38.515	20.16
THH 3BVII	11-octadecenoic acid, methyl ester	296.5	$C_{19}H_{36}O_2$	052380-33-3	39.754	3.47
THH 3BVI	Methyl palmitate	270.5	$C_{17}H_{34}O_2$	000112-39-0	39.916	4.79

4. Discussion

An array of chemicals meant to ameliorate all the diseases of mankind abound, but most of them are hidden in the bush within the variety of medicinal plants in nature. It is a well-known fact that almost all the chemicals used in the management of various diseases are obtained majorly from

medicinal plants [31]. These chemicals, in most cases appear bound or combined with each other that they needed to be isolated in order to identify each of them. In most cases, activity–guided purification lent itself to the identification of the active constituents of plants. Most of the time, the plants chosen are ethnomedicinal plants that are in common uses and are effective for certain ailments in Traditional Medical practice [32].

As a preliminary effort to ascertaining the safety of extracts and a means of determining the doses for testing, toxicity tests are usually mounted, primarily to determine the LD50. The methanolic leaf extract of *T. heudelotii* was not toxic at 5000 mg/kg or higher doses, it was therefore considered non-toxic and safe since there was no morbidity or mortality based on Lorkes method [33, 34]. In earlier studies, the stem bark of this plant has been reported to have no antimalarial activity [35].

This study therefore concentrated on the leaf methanol extract. The highest chemosuppression of 88.08 ±0.78% was obtained at a dose of 200 mg/kg implying that the optimum activity occurred at that dose. Lower% chemosuppression obtained at higher doses may indicate that the activity of the extract is not dose-dependent. Also the highest survival time and% survivor at a similar dose of 200mg/kg and the% survivor similar to that of the standard drug in *T. heudelotii* is noteworthy. Also that the% chemosuppression of the positive control drug is lower than that of the optimum dose (Table 1) suggest that the leaf of *T. heudelotti* is an active antiplasmodial drug. An ED_{50} = 258.90 ± 3.83 elicited by the extract compared favourably with those of similarly active antimalarial plant drugs. The methanol extract of *Plumeria alba* in a similar test elicited ED_{50} and ED_{90} 305.82±9.99 and 389.74±9.59 respectively [36]. The implication is that the leaf extract of *T. heudelotti* is a very active antiplasmodial agent which is likely to contain antiplasmodial compounds that are in combination in the plant. A few methanol extracts of medicinal plants compare favourably in activity with chloroquine, a standard antimalarial drug [37]. With this background about this leaf extract, it was set out to purify it using activity guided methods. This method has the advantage of identifying most active fractions which are sure to contain the antiplasmodial compounds of the plant. The most active partitioned fractions obtained was also subjected to further methods of column chromatography with the intention of identifying the active column fraction(s) from which the active compound(s) can be isolated and identified.

4.1. The Partitioned Fractions

The partitioned fractions, THD and THA gave comparable values of% chemosuppression to the positive control at all the doses tested (Table 2), while THH and THB gave relatively higher% chemosuppression values that were comparable ($p<0.05$) to that of the positive control at 50mg/kg and 50 & 100mg/kg respectively (Table 2). THE elicited relatively lower% chemosuppression at all the tested doses except at 200mg/kg. THB seems to be the most active of the partitioned fractions by the percentage chemosuppression profile but the effective doses (ED_{50} & ED_{90}) showed that all the fractions have comparable ($p>0.05$) activities (Table 2). This implies that all the fractions have equal activities and have equal potential of suppressing the malarial parasites *in vivo*. It has been reported that the drug with a lower ED_{50} would be considered more potent when two drugs are tested in the same individual [38]. THH on the other hand elicited high percentage survivor similar to that given by chloroquine at all doses tested (Table 3) whereas THB gave the same values only at the highest dose tested. THE elicited the same survivor pattern as THD. The high percentage survivor profile of THH, however may suggest it as the fraction to choose for further purification. The n-hexane partition fraction, THH was therefore chosen for further purification based on the percentage survivor profile and the comparable% chemosuppression of the lowest dose tested with the positive control.

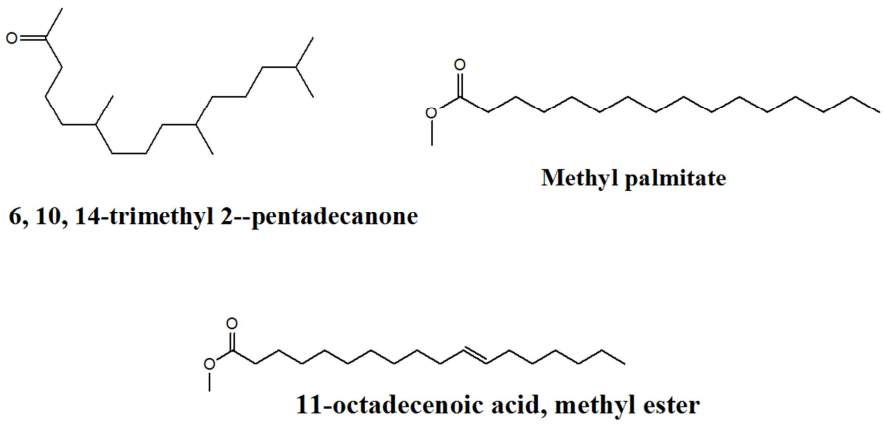

Figure 1. *Structures of Some Compounds Identified from THH 3B.*

4.2. The Column Fractions

The column fractions THH 2A-2D obtained from THH were further tested for antiplasmodial activities. All were comparable ($p>0.05$) in activities to each other with chemosuppression values as high as 78% and with the

positive control and including the mother n-hexane fraction (Table 4). The column fractions must have retained the activities of the n-hexane partition fraction and consequently that of the methanol extract. THH 2B with the highest value of% chemosuppression and relatively high percentage survivor was further chosen for purification to produce THH 3A-3E which on further antiplasmodial testing produced THH 3B as the most active column fraction with a chemosuppression of 74% and a relatively high percentage survivor (Table 5). The activity of the n-hexane partitioned fraction must have been concentrated in column fraction THH 3B and so most likely to have possessed the antimalarial constituents of the leaf extract of *T. heudelotii*. The column fraction THH 3B was therefore subjected to GC-MS in order to identify the possible antimalarial compounds.

4.3. GC-MS of THH 3B

GC in hyphenation with MS has proved to be a valuable tool in natural product research where it can separate and identify chemical compounds in a complex mixture especially volatile oil or organic extracts. It has especially been utilized in separating structurally similar mono and sesquiterpene that are the major constituents of some plants essential oils [39]. In these cases, isolation may not be mandatory if such constituents can be rightly identified and characterised unless it is a new compound. The MS fragmentation pattern is only compared automatically with stored EI spectra data in the computer library; this will suffice in identifying the separated components.

The GC-MS data obtained for THH 3B gave a total of 10 peaks identified by their retention times (Rt) and coded THH 3B (i-x). The peaks comprise six, THH 3B (i, ii, iii, vii, viii, ix) (Rt, 37.652, 37.921, 38.415, 39.754, 39.916, 40. 298). The THH 3B (v) (38.822) with peak area between 5 and 10% and only three THH 3B (iv, vi, x) (38.515, *39.072,* 42.350) above 10%.

Out of these, only four (THH 3B (i, iv, viii, vii) respectively with retention times, Rt, 37.652, 38.515 39.916 & 39.754) that were characterised were those found to possess biological properties in literature. THH 3B (i) was characterised as 2--pentadecanone, 6, 10, 14-trimethyl, with molecular formula $C_{18}H_{36}O$ and molecular weight, 268.4778. Two of these, THH 3B iv and THH 3B viii with retention times, Rt, 38.515, 39.916 and which possess similar structural and chemical identity when compared with library literature was characterised as methyl palmitate with molecular formula $C_{17}H_{34}O_2$ and molecular weight 270. 5 are probably isomers. Also, THH 3B (vii) with molecular formula $C_{19}H_{36}O_2$ and weight 296.5 was characterised as 11-octadecenoic acid, methyl ester. The compound, 11-octadecenoic acid, methyl ester which have been isolated from the seeds of *Acacia nilotica* possessed antidiarrhoeal activity [40].

Hexahydrofarnesyl acetone also known as 6, 10, 14-trimethyl-2-pentadecanone, is a celery, fat, and herbal tasting compound that can be found in a number of food items such as sweet basil, common oregano, roselle, and wild celery, which makes 6, 10, 14-trimethylpentadecan-2-one a potential biomarker for the consumption of these food products [41]. It is also a natural product found in *Thymus zygioides, Tilia tomentosa,* and other organisms. This sesquiterpene isolated as the major constituents of the essential oil from *Impatiens parviflora,* possesses antibacterial, anti-nociceptive and anti-inflammatory activities [42].

Methyl palmitate is a natural product found in *Zanthoxylum beecheyanum, Lonicera japonica*, and other organisms. It possesses anti-inflammatory and anti-fibrotic effect. It prevents bleomycin-induced lung inflammation and fibrosis in rats, by inhibiting NF-κB [43]. Methyl palmitate also prevents CCl4-induced liver fibrosis linked to abridge TGF-β [44]. Its cardioprotective activities through its antioxidant, anti-inflammatory, anti-apoptotic, anti-fibrotic, and vasodilatation properties have been reported [45]. These three compounds are likely to be the antimalarial constituents of this plant especially as fatty acids and their esters have been contemplated as possible compounds having activities against the malarial parasite [3, 46-48]. Fatty acids themselves have inhibited the fatty acid biosynthetic machinery of the parasite *P. falciparum* putting such in the fore as antimalarial agents [3]. Methyl palmitate alongside other fatty acids and their derivates have been reported to possess antiplasmodial activities [49, 50].

In summary, the study showed that the methanolic leaf extract of *T. heudelotii* demonstrated good antiplasmodial activity in mice model of antimalarial chemosuppressive test at an optimum dose of 200 mg/kg thereby establishing its use in Nigerian ethnomedicine as an antimalarial drug. The hexane partition fraction and the subsequent column fractions obtained through successive purification demonstrated good% chemosuppression and percentage survivor profile as to contain the antimalarial constituents of the plant. Also, the identification of fatty acid esters, which have been reported to possess antiplasmodial activities, in the most active column fraction is an indication that they are the antimalarial constituents of the plant.

5. Conclusion

The ethnomedicinal antimalarial claims of *Trichilia heudelotii* leaf was scientifically verified in this work thus establishing its antimalarial potential. The compounds that are likely to be responsible were identified as fatty acid esters and other complimentary constituents. This should pave a way for the isolation of these and other constituents and so justify its use in the treatment of malaria in ethnomedicine.

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