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 (LD₅₀) was determined according to Lorke’s method. The extract was tested against chloroquine-sensitive strain of *Plasmodium berghei* berghei 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 LD₅₀ 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 berghei* berghei NK-65, Meliaceae, Chemosuppressive, Antimalarial

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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 antimalarial 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*, *Anacardium 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, trichilin, a triterpenoid, limonoids, steroids and other terpenes derivatives [21-23] while *Trichilia heudelotii* has yielded heudelotin [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 (D₀) and the highest that produced no mortality (D₅₀) were recorded. The LD₅₀ 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 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, THB, and THA were assayed in the same way as THH.

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, 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, 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% 1200mL, n-hexane-DCM, 8:2, 200mL; n-hexane- DCM, 7:3, 200mL; n-hexane-DCM, 6:4, 200mL; n-hexane-DCM, 5:5, 200mL; n-hexane- DCM, 4:6, 200mL; n-hexane- DCM, 3:7, 200mL; n-hexane-DCM, 2:8, 200mL; n-hexane-DCM, 1:9, 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, 5:5, 200mL; DCM-EtOAc, 4:6, 200mL; DCM-EtOAc, 3:7, 200mL; DCM-EtOAc, 2:8, 200mL; DCM- EtOAc, 1:9, 100mL; DCM-EtOAc, 0:10, 200mL; EtOAc - MeOH 9:1, 200mL; EtOAc - MeOH 8:2, 200mL; EtOAc, 8:2, 400mL; THH2B (14-24: n-hexane-DCM, 3:7, 200mL; n-hexane- DCM, 2:8, 200mL; n-hexane-DCM, 1:9, 200mL; n- hexane-DCM, 0:10, 200mL; DCM- EtOAc, 9:1, 200mL; DCM-EtOAc, 8:2, 400mL; DCM-EtOAc, 7:3, 200mL; DCM-EtOAc, 6:4, 200mL; DCM-EtOAc, 5:5, 200mL; DCM-EtOAc, 4:6, 200mL; DCM-EtOAc, 3:7, 200mL; DCM-EtOAc, 2:8, 200mL; DCM- EtOAc, 1:9, 200mL; DCM-EtOAc, 0:10, 200mL; EtOAc - MeOH 9:1, 200mL; EtOAc - MeOH 8:2, 200mL; THH2D (35-38: EtOAc - MeOH 7:3, 200mL; EtOAc - MeOH 6:4, 200mL; EtOAc - MeOH 5:5, 200mL; EtOAc - MeOH 4:6, 200mL; EtOAc - MeOH 3:7, 200mL; EtOAc - MeOH 2:8, 200mL; EtOAc - MeOH 1:9, 200mL; EtOAc - MeOH 0:10, 200mL). 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.

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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 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% 200mL, 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 3B (14-20: n-hexane- DCM, 7:3, 200mL; n-hexane- DCM, 6.5:3.5, 200mL; 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, 3:7200mL; n-hexane- DCM, 2:8, 200mL, n-hexane- DCM, 1:1, 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, 0:1, 400mL; EtOAc-MeOH, 1:1, 400mL; MeOH 100%, 400mL, 2.11g). The pooled fractions THH 3A-THH 3E were each tested for antimalarial 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.

3. Results

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

<table>
<thead>
<tr>
<th>Dosage (mg/kg)</th>
<th>Chemosuppression (%)</th>
<th>Survival time in days, (Percentage Survivor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.00±0.00</td>
<td>9.60±0.00 (20)</td>
</tr>
<tr>
<td>100</td>
<td>71.71 ± 3.61</td>
<td>17.60 ± 0.60 (60)</td>
</tr>
<tr>
<td>200</td>
<td>88.08 ± 0.78</td>
<td>27.33 ± 0.88 (80)</td>
</tr>
<tr>
<td>400</td>
<td>60.37 ± 3.35</td>
<td>25.80 ± 0.33 (60)</td>
</tr>
<tr>
<td>800</td>
<td>68.92 ± 1.70</td>
<td>17.60 ± 1.33 (20)</td>
</tr>
<tr>
<td>PC</td>
<td>64.14 ± 3.10</td>
<td>26.40 ± 1.29 (80)</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Doses</th>
<th>% Chemosuppression by partitioned fractions ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>THH</td>
</tr>
<tr>
<td>NC</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>50</td>
<td>65.58 ± 12.38</td>
</tr>
</tbody>
</table>
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 known fact that almost all the chemicals used in the...
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. heudelotii* is an active antimalarial drug. An ED₅₀ = 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 ED₅₀ & ED₉₀ 305.82±9.99 and 389.74±9.59 respectively [36]. The implication is that the leaf extract of *T. heudelotii* is a very active antimalarial agent which is likely to contain antimalarial 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 antimalarial 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 (ED₅₀ & ED₉₀) 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 ED₅₀ 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.

4.2. The Column Fractions

The column fractions THH 2A-2D obtained from THH were further tested for antimalarial 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 3B 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-trimethylpentadecan-2-one a potential biomarker for the consumption of these food products [41]. It is also a natural product found in Thymus zygiodes, 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 CC14-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 derivatives 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.

References


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Sulaiman Muhammed Olatunji et al.: *In vivo* Antiplasmodial Studies on *Trichilia heudelotii* Planch ex. Oliver (Meliaceae) Leaf


