



# GC-MS Profiling, *In Vitro* Antimalarial, and Antimicrobial activity of *Ricinodendron heudelotii* Seed Extracts

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**Keywords:** Cytotoxicity, Chloroquine resistant, Phytoconstituents.

**Abstract:** Malaria remains a significant public health challenge, particularly in sub-Saharan Africa, where the emergence of *Plasmodium falciparum* strains resistant to artemisinin-based combination therapies (ACTs) exacerbates the situation. This underscores the urgent need to identify novel, less toxic antimalarial compounds, particularly from natural sources. This study evaluated the *in vitro* antimalarial and antimicrobial activities of *Ricinodendron heudelotii* (RHD) seed extracts against *Plasmodium falciparum* (*P. falciparum*) and select pathogenic microorganisms using standard protocols. Seeds (250 g) were dried, ground, and extracted with n-hexane and dichloromethane, yielding RHD-HEX and RHD-DCM extracts, respectively. The oily extract (RHD-HEX) was analyzed via gas chromatography-mass spectrometry (GC-MS), revealing 26 phytoconstituents, including monoterpenes, fatty acids, and phytosterols. Antimalarial assays showed that RHD-HEX and RHD-DCM exhibited moderate activity (>200 µg/mL) against the chloroquine-sensitive *P. falciparum* (D6 strain). Both extracts demonstrated stronger activity against the chloroquine-resistant W2 strain, with IC<sub>50</sub> values of 30.29 and 33.48 µg/mL, respectively. Antimicrobial screening indicated moderate activity against tested pathogenic fungi and bacteria (IC<sub>50</sub> > 200 µg/mL) compared to fluconazole and cefotaxime controls. Cytotoxicity against VERO cell lines was also assessed. The findings suggest that the phytoconstituents in RHD extracts may contribute to the observed antimalarial and antimicrobial effects, warranting further investigation.

## Introduction

Malaria and other infectious diseases remain major global health challenges, particularly in sub-Saharan Africa. According to the WHO 2022 global report, malaria caused an estimated 608,000 deaths across 85 countries, with 95% (580,000 death) of these fatalities occurring in Africa, predominantly among children under five years of age and pregnant women (1). The burden of malaria in this region is further compounded by the emergence of resistance in *P. falciparum* to current antimalarials, including artemisinin-based combination therapies (ACTs) (2). Additionally, resistance in *Anopheles* mosquito vectors to commonly used insecticides exacerbates the challenge of malaria control and eradication (3). This alarming scenario underscores the urgent need to discover novel

antimalarial agents, particularly from natural sources, to address these resistance issues.

Medicinal plants have historically been a major source of bioactive compounds, serving as scaffolds for the development of potent drugs. Plant-derived phytochemicals exhibit diverse biological activities, including antibacterial, antimalarial, antidiabetic, and anticancer properties (4-6). In Africa, traditional medicine practices have extensively utilized medicinal plants for the treatment of malaria, bacterial infections, and other diseases. Examples include *Enantia chlorantha*, *Azadirachta indica*, *Vernonia amygdalina*, *Picralima nitida*, and *Alchornea cordifolia*, which are well-documented for their antimalarial potential (4, 8-11).

*Ricinodendron heudelotii* (Baill.) Pierre ex Heckel, belonging to the Euphorbiaceae family, is commonly referred to as the African oil-nut tree. It has a long history of use in traditional medicine, with its various parts (stem bark, roots, and fruits) employed for treating ailments such as cough, poisoning, stomach pain, fever, dysentery, and malaria (12). The seeds and fruits of *R. heudelotii* are rich in proteins and are widely consumed in West African countries, including Nigeria, as a food flavoring in soups and stews (13). Previous studies have highlighted the antioxidant and anti-inflammatory activities of *R. heudelotii* extracts, including its inhibition of nitric oxide production and its antioxidant activity comparable to vitamin C (14). Additionally, the seed oil has demonstrated anti-breast cancer properties by modulating TNF- $\alpha$  and INF- $\gamma$  levels (15). Synergistic effects of its crude leaf extract with artemisinin in enhancing antioxidant markers and liver biomarkers have also been reported (14).

The rising incidence of malaria, coupled with the emergence of resistant strains of *P. falciparum* and insecticide-resistant mosquito vectors, presents a significant obstacle to malaria eradication efforts in endemic regions. Given the traditional use of *R. heudelotii* in managing malaria and related conditions, it is crucial to scientifically evaluate its efficacy and phytochemical basis. This study aims to investigate the phytochemical composition and assess the in vitro antimalarial and antimicrobial potential of the essential oil and extracts of *R. heudelotii*, providing insights into its ethnomedicinal applications as a food and medicinal plant in southern Nigeria.

## Methodology or Experimental Section

### Materials

All the solvents and reagents used in this study were of analytical grade. Rotary evaporator (Stuart RE300, England), DMSO (Sigma-Aldrich, Germany). All microorganisms were obtained from the American Type Culture Collection (ATCC), Manassas, VA.

### Collection, Identification, and Preparation of Plant Simplicia

Dried seeds of *R. heudelotii* were purchased from the Ikpoba Hill spices market, Edo state, on 19th January 2023. The seeds were identified by Prof Aigbokhan Emmanuel Izaka at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, and a voucher specimen number UBH-R623 was assigned.

### Extraction

The seeds of *R. heudelotii* were ground into powder using a mechanical blender. The powdered sample (250 g) was successively extracted with 500 mL each of n-hexane and dichloromethane, respectively, for 72

h. The macerates were filtered using Whatman No. 1 filter paper and concentrated in vacuo using a rotary evaporator under reduced pressure at 40 °C. The extracts were stored in airtight bottles and kept in a refrigerator at 4 °C until further use.

### GC-MS Analysis of Extracted Oil

The sample was analyzed by GC-MS-QP2010 Plus (Shimadzu Japan) comprising Shimadzu QP-2010 GC with QP-2010 Mass Selective Detector (MSD), operated in the EI mode, electron energy of 70 eV, scan range of 45-700 amu, and Shimadzu GCMS solution data system. The gas chromatography column was Agilent HP-5 MS fused silica capillary with 5% phenyl methylpolysiloxane stationary phase, with a length of 30 m, internal diameter of 0.25 mm, and film thickness of 0.25  $\mu$ m. The carrier gas was helium 99.999%, with a flow rate of 1.61 mL/min. The gas chromatography oven temperature was 60 - 180 °C adjusted at a rate of 10 °C/min, then held at 180 °C for 2 min, followed by 180 - 280 °C at a rate of 15 °C/min, and then held at 280 °C for 2 min. The injection port temperature was 250 °C, the interface temperature was 250 °C while ion source temperature was 200 °C. The sample was dissolved in dichloromethane (ratio 1:9 v/v), filtered through 0.45  $\mu$ m and 1.0  $\mu$ L was injected using an autosampler and the split mode with a ratio of 25:75. Individual constituents were identified by comparing their mass spectra with known compounds and NIST Mass Spectral Library. The percentage composition of each chemical constituent is reported as a raw percentage based on the peak area of the total ion current (16).

### Antimalarial Assay

The in vitro antimalarial activity was determined using an assay protocol based on a colorimetric method that determines the parasite lactate dehydrogenase (pLDH) activity (17, 18). The assay was performed in a 96-well microtiter plate and included two *P. falciparum* strains [Sierra Leone D6 (chloroquine sensitive) and Indochina W2 (chloroquine-resistant)]. DMSO (0.25%) was used as a vehicle, while artemisinin and chloroquine were included in each assay as positive drug controls.

### In vitro Cytotoxicity Test

Cytotoxicity assay was performed in 96-well microtiter plates using the neutral red uptake method as described by (19). The cytotoxicity of the plant extracts was assessed against the VERO cell line (monkey kidney fibroblast) cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, and 0.2% NaHCO<sub>3</sub> at 37 °C in an atmosphere of 95% humidity, 5% CO<sub>2</sub>. Concentration ranges tested were between 0.19 - 48  $\mu$ g/mL for crude extracts. IC<sub>50</sub> was calculated from the dose-response curve. This assay was done to assess the cytotoxicity of the plant extracts against the VERO cell line (monkey kidney fibroblast) and their

inherent activity against *Plasmodium falciparum* to establish their selectivity indices (SI), which is the ratio of IC<sub>50</sub> values of test samples against Plasmodium falciparum to that of Vero cell line.

Antimicrobial Assay

The extracts were subjected to in vitro susceptibility testing against a panel of pathogenic organisms: the fungi include *Candida albicans* (ATCC 90028), *Cryptococcus neoformans* (ATCC 90113), *Aspergillus fumigatus* (ATCC 90906), while the bacteria include methicillin-resistant bacterium *Staphylococcus aureus* (MRSA; ATCC 43300), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 43816), vancomycin-resistance *Enterococcus faecium* (ATCC 49532) using a modified version of the NCCLS methods (20). The fungi and bacteria used in this experiment were obtained from the American Type Culture Collection (ATCC), Manassas, VA. All the test samples were dissolved in DMSO (0.25%), acting as a negative control agent. They were all diluted with 0.9% saline serially and transferred in duplicate to the 96-well microtitre plates. The final microbial inoculums were prepared after comparison of the absorbance at 630 nm of cell

suspensions to the 0.5 McFarland standard and diluting the suspensions in broth (Sabouraud dextrose and cation-adjusted Müller- Hinton (DIFCO) for the fungi and bacteria, respectively, and 5% Alamar Blue (BioSource International, US) in Middle brook 7H9 broth to afford recommended inocula. Microbial inocula were added to the diluted samples to obtain a final volume of 200 µL. The microtitre plates were read at 630 nm or 544ex/590em before and after incubation. IC<sub>50</sub> values relative to controls were obtained using XL fit 4.2 software (IDBS, Alameda, CA).

Statistical Analysis

Data are reported as mean ± SD of three treatments. All the statistical analyses were computed with IBM SPSS statistics software version 23.

Result

Yield of Hexane extract

Oily plant extracts are non-polar organic extracts of plants implicated in the characteristics and odor of the plant and are utilized in fragrances, food spices, and the treatment of various diseases. RHD oil obtained by hexane extraction gave a yield of 1.5% (Table 1).

Table 1. Percentage yield of hexane extract (n-hexane (RHD-HEX) and dichloromethane (RHD-DCM) extracts from the seeds of *Ricinodendron heudelotii*.

Extract	Amount of material extracted (g)	Volume of oil extracted (ml)	Percentage Yield(%)
RHD-HEX	250	3.75	1.5
RHD-DCM	250	2.75	1.1

**Note:** RHD = *Ricinodendron heudelotii*, HEX = n-Hexane extract, DCM = Dichloromethane extract.

Table 2. Chemical components (%) identified in the hexane extract of *Ricinodendron heudelotii*.

S/N	Compounds names	Mol Formula	M.W (g/mol)	RT	AREA (%)
1	5-Hydroxy-1-isopropyl-6,6-dimethyl-5-phenyl-piperidin-2-one	C <sub>16</sub> H <sub>23</sub> NO <sub>2</sub>	261.36	6.705	0.41
2	2,4-Nonadienal, (E,E)-	C <sub>9</sub> H <sub>14</sub> O	138.21	28.258	0.86
3	Sulfurous acid, butyl hexyl ester	C <sub>10</sub> H <sub>22</sub> O <sub>3</sub> S	222.35	40.681	1.36
4	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	156.42	57.92	8.32
5	Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.5	63.337	18.04
6	9,12-Octadecadienoic acid, ethyl ester	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308.5	63.99	1.76
7	Octadecadienoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284.5	64.094	9.20
8	9,12-Octadecadienoic acid (Z,Z)-	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	280.4	66.173	2.30
9	9,12,15-Octadecadienoic acid (Z,Z,Z)-	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278.4	66.93	7.04
10	Methyl 8,11,14-heptadecatrienoate	C <sub>15</sub> H <sub>24</sub>	278.4	67.294	0.90
11	Cyclopropaneoctanal, 2-octyl-	C <sub>19</sub> H <sub>36</sub> O	280.5	67.432	1.01
12	Hexadecanoic acid, pentyl ester	C <sub>21</sub> H <sub>42</sub> O <sub>2</sub>	236.6	67.6	2.23
13	Methyl 8,11,14-heptadecatrienoate	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	204.35	67.831	1.43
14	Butyl 9,12-octadecadienoate	C <sub>22</sub> H <sub>40</sub> O <sub>2</sub>	336.6	69.436	0.44
14	Linoelaidic acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280.4	72.024	3.05

16	14-Pentadecenoic acid	C <sub>15</sub> H <sub>28</sub> O <sub>2</sub>	240.3	72.22	1.48
17	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	C <sub>21</sub> H <sub>25</sub> NO <sub>4</sub>	355.4	72.318	0.30
18	Octadecanoic acid, pentyl ester	C <sub>23</sub> H <sub>46</sub> O <sub>2</sub>	354.6	72.959	1.50
19	Vanadium, (η <sup>7</sup> -cycloheptatrienylum)(η <sup>5</sup> -2,4-cyclopentadien-1-yl)-	V <sub>2</sub> O <sub>5</sub>	181.88	73.04	1.05
20	1,2-Bis(trimethylsilyl)benzene	C <sub>12</sub> H <sub>22</sub> Si <sub>2</sub>	222.47	74.524	0.37
21	5,8,11-Heptadecatrienoic acid, methyl ester	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278.4	75.379	2.60
22	1,2-Benzisothiazol-3-amine, TBDMS derivative	C <sub>13</sub> H <sub>20</sub> N <sub>2</sub> SSi	264.46	75.639	1.30
23	Silicic acid, diethyl bis(trimethylsilyl) ester	C <sub>10</sub> H <sub>28</sub> O <sub>4</sub> Si <sub>3</sub>	296.58	77.73	1.62
24	Tris(tert-butyldimethylsilyloxy)arsane	C <sub>18</sub> H <sub>45</sub> AsO <sub>3</sub> Si <sub>3</sub>	468.7	77.989	0.19
25	Acetamide, N-[4-(trimethylsilyl)phenyl]-	C <sub>11</sub> H <sub>17</sub> NOSi	207.34	79.543	3.14
26	γ-Tocopherol	C <sub>28</sub> H <sub>48</sub> O <sub>2</sub>	416.7	88.119	3.01

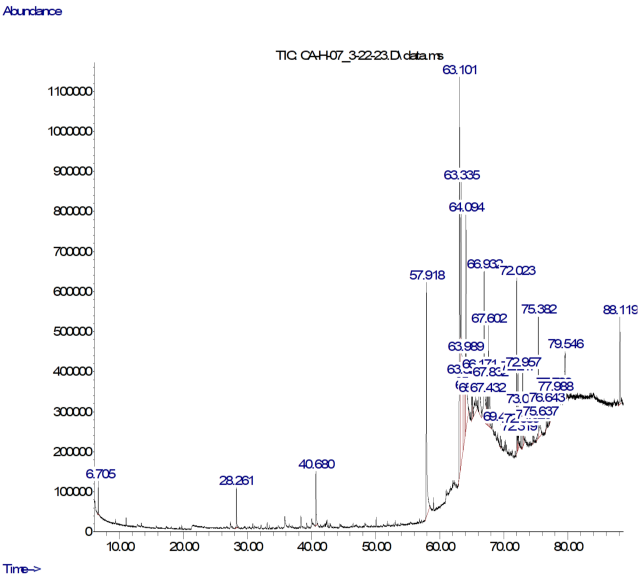


Figure 1. GC-MS chromatogram of *Ricinodendron heudelotii* oil extract.

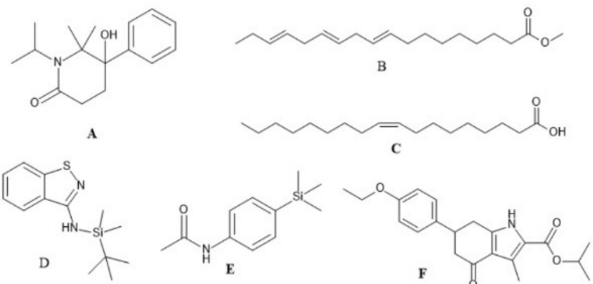


Figure 2. Selected chemical structures of phytoconstituents of *Ricinodendron heudelotii* oil extract.

Chemical Composition of Seed Oils

The maceration of the grounded seeds of RHD with n-hexane gave a cream color oil with a greasy texture and a characteristic pleasant odor. The phytochemicals in the oil of RHD from the GC-MS analysis are presented in **Table 2**, which shows compound names, percentage area, retention times, molecular formula, and weights in g/mol. The major constituents in RHD oil were Oleic acid (18.04%), Octadecadienoic acid (9.2%),

and n-hexadecanoic acid (8.32%). The oil comprises monoterpenes, fatty acids, and small amounts of other organic compounds. Some structures of the bioactive compounds listed in **Table 2** are shown in **Figure 2**.

In Vitro Antimalarial Activity

Both the oil (RHD-HEX) and dichloromethane extract (RHD-DCM) of *R. heudelotii* were evaluated against two strains of *Plasmodium falciparum* (D6 chloroquine-sensitive and W2 chloroquine-resistant) using the parasite lactate dehydrogenase (pLDH) assay method to determine their half maximal inhibitory (IC<sub>50</sub>) potentials in comparison with two standard antimalarial drugs, chloroquine and artemisinin. The results are presented in **Table 3** with their selectivity indices assessed from their cytotoxicity against the VERO cell line.

Table 3. Antimalarial activity of n-hexane and DCM extract of the seeds of *Ricinodendron heudelotii* against *Plasmodium falciparum* (Test Conc. 47.60-5.29 µg/mL).

Extracts	<i>P.falciparum</i> D6 IC <sub>50</sub> (µg/mL)	S.I	<i>P.falciparum</i> W2 IC <sub>50</sub> (µg/mL)	S.I	VERO IC <sub>50</sub> (µg/mL/mL)
RHD-HEX	>47.60	1	30.29	>1.6	>47.60
RHD-DCM	>47.60	1	33.48	1.4	>47.60
CQ	<0.026	>9	0.091	>2.6	NC
ART	<0.026	>9	<0.026	>9	NC

Also, the extracts were assessed for potential antimicrobial activities against a panel of pathogenic microorganisms (bacterial and fungi) of crude extracts of *R. heudelotii*. The results revealed that both RHD-HEX and RHD-DCM extracts exhibited weak antimicrobial activity, with IC<sub>50</sub> values exceeding 200 µg/mL across all tested organisms. In contrast, the positive controls demonstrated significantly lower IC<sub>50</sub> values, indicating stronger inhibitory effects (see **Table 4**). The weak activity suggests limited efficacy against the tested pathogens.



**Table 4.** Antimicrobial activity of n-hexane (RHD-HEX) and dichloromethane (RHD-DCM) extracts of the seeds of *Ricinodendron heudelotii* at concentrations of 200-8 µg/mL.

Microorganisms	RHD-HEX (IC <sub>50</sub> )	RHD-DCM (IC <sub>50</sub> )	FLU(IC <sub>50</sub> )	CEFO(IC <sub>50</sub> )
<i>C. albicans</i>	>200	>200	1.076	>100
<i>A. Fumigatus</i>	>200	>200	>100	>100
<i>C. neoformans</i>	>200	>200	3.933	>100
MRS	>200	>200	>100	>100
<i>E. coli</i>	>200	>200	>100	>100
<i>P. aeruginosa</i>	>200	>200	>100	12.518
<i>K. pneumoniae</i>	>200	>200	>100	>100
VRE	>200	>200	>100	>100

Discussion

Plants' secondary metabolites have shown potent pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antimalarial, and anticancer properties (12). Essential oils contain diverse groups of complex phytochemicals (hydrocarbons), mostly monoterpenes, sesquiterpenes, and oxygenated derivatives such as ketones, aldehydes, hydroperoxides, etc. They have been used in different traditional medicines to treat various ailments, including cancers and malarial (13). They have great uses as nutraceuticals, pesticides, insecticides, and acaricides (21-23) and for treating respiratory and skin infections, including inflammations (24).

GC-MS analysis is a powerful tool that is useful for identifying the chemical constituents of plants. In the current study, the GC-MS characterization of the various chemical compounds present in the n-hexane extracts of *R. heudelotii* was carried out and explores their retention times, percentage areas, molecular formulas, molecular weights (Table 2 and Figure 1). Bioactive compounds of essential oils have garnered significant attention due to their diverse properties and potential therapeutic uses (12). The GC-MS analysis of the oil of the seed of *R. heudelotii* in this study revealed 26 compounds with different retention times and percentage compositions. 5-Hydroxy-1-isopropyl-6,6-dimethyl-5-phenyl-piperidin-2-one (a quaternary ammonium compound), 2,4-Nonadienal (an alpha-unsaturated aldehyde), and Gamma-tocopherol (vitamin E) with percentage areas of 0.41%, 0.86% and 3.01%, respectively, were identified from the GC-MS analysis of *R. heudelotii* (Table 2). Quaternary ammonium compounds, such as 5-hydroxy-1-isopropyl-6,6-dimethyl-5-phenyl-piperidin-2-one, are known to have antimicrobial activity against a wide range of bacteria and fungi. They are thought to work by disrupting the cell membranes of these organisms, leading to cell death. Alpha-unsaturated aldehydes, such as 2,4-nonadienal, have also been shown to have

antimicrobial activity against a variety of bacteria and fungi. They inhibit the growth of these organisms by disrupting their metabolism. Gamma-tocopherol, or vitamin E, is a fat-soluble vitamin that has antioxidant and anti-inflammatory properties. Its antimicrobial activity has also been reported. The most prominent phytochemicals in RHD oil is oleic acid (18.04%), which has been reported for its antifungal and insect-repellant activities (25). However, other phytochemicals from this plant have been found to have various activities and are used in different industries, such as flavoring agents, antioxidants, anti-inflammatory agents, perfumery, etc. (26-28).

Malaria is a disease caused by Plasmodium parasites, mainly *Plasmodium falciparum*, *ovale*, *malariae*, *vivax*, and *knowlesi* species. It is transmitted by a bite of the female anopheles mosquito during a blood meal. Malaria is prevalent in tropical and sub-tropical countries, especially in Africa. This research studied the antimalarial activity of the n-hexane and dichloromethane extracts of *R. heudelotii* on the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. The experiment was conducted within the test concentration range of 5.29 – 47.60 µg/mL. Chloroquine and Artemisinin were used as reference standards. The selectivity index (SI) is a measure of investigational agent cytotoxicity, and it defines the degree to which plant extracts can inhibit the cells of *Plasmodium falciparum* while sparing human cells. The higher the SI, the lower the cytotoxicity, and vice versa. Therefore, plant extracts with notable antimalarial activity and high SI are preferred candidates for further investigation as potential antimalarial agents. In this study, both extracts of *R. heudelotii* and the RHD-DCM showed antimalarial activity in the range >200 µg/mL above the test concentrations range against the chloroquine-sensitive (D6) strain of *Plasmodium falciparum*. However, the extracts (RHD-HX and RHD-DCM) inhibited the growth of the chloroquine-resistant (D2) strain with IC<sub>50</sub> values of 30.29 and 33.48 µg/mL, respectively. In several studies, the antimalarial activity of RHD has been reported (29). Hervé et al. inferred the antimalarial potential of RHD and drew a correlation between the antioxidant activity of the plant and its antimalarial activity (30). A study on different fractions of the seed extract of *P. macrophylla* showed that the extracts exhibited potent and concentration-dependent chemo-suppression of parasitemia in *Plasmodium berghei*-infected mice. The extract achieved 61.36% chemo-suppression at a concentration of 100 mg/kg (31). In another study, the extracts and fractions of avocado pear (*P. americana*) and *D. edulis* showed potent antiplasmodial activity and suppressed parasitemia by 64.01 ± 0.08% and 71.99 ± 0.06%, respectively (32). Also, another study that investigated the *in vitro* and *in*

*vivo* activity of some plant extracts showed that the DCM extract of *Commiphora africana* and *Dichrostachys cinerea* exhibited potent antimalarial activity in the pLDH assay at an  $IC_{50}$  value of 29.44  $\mu\text{g/mL}$  with 64.24 and 53.12% parasites suppression, respectively (17).

Traditional plants are known to contain chemical compounds with antimicrobial capabilities. The efficacy of an antimicrobial agent depends on its capacity to prevent the growth or viability of any microorganisms in the affected body system. The extracts of *R. heudelotii* were screened against a panel of pathogenic microorganisms *C. albicans*, *A. fumigatus*, *C. neoformans*, Methicillin-resistant *Staphylococcus aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and Vancomycin-resistant enterococci (Table 4). The oil and dichloromethane extract of RHD showed moderate activity against the tested organisms with  $IC_{50}$  values >200  $\mu\text{g/mL}$  compared to the positive control agents fluconazole and cefotaxime which were active against *C. albicans* ( $IC_{50}$  = 1.076  $\mu\text{g/mL}$ ), *C. neoformans* ( $IC_{50}$  = 3.933  $\mu\text{g/mL}$ ) and *P. aeruginosa* ( $IC_{50}$  = 12.518  $\mu\text{g/mL}$ ). In some studies, the methanol extract of *R. heudelotii* leaves showed excellent antifungal activity against *Cryptococcus neoformans* with an  $IC_{50}$  value of 31.73  $\mu\text{g/mL}$  (33). Several studies have highlighted the antimicrobial potency of plant crude extracts, such as garlic, cinnamon, ginger, and basil, which have been shown to exhibit antimicrobial activity against a wide range of pathogenic microbes (20). Other plant extracts have potential activity against some organisms, such as *Escherichia coli*, *C. albicans*, and *P. aeruginosa* (34, 35). The leaf extracts of RHD have been reported to show antimicrobial activity against *E. coli* W3110 and *Enterobacter aerogenes* EA3 with a MIC = 256  $\mu\text{g/mL}$  (34). The pharmacological and medicinal potential of plant extracts in the development of new therapeutics cannot be overemphasized, leading to an increase in research activities geared towards the isolation and development of potent bioactive molecules as leads in drug discovery.

## Conclusion

The GC-MS analysis of the oil of RHD revealed 26 phytoconstituents, with oleic acid being the most predominant. The antimalarial potential of the oil and the dichloromethane extract have been evaluated against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. The results show moderate antimalarial activity of RHD-HEX and RHD-DCM against the D6 strain, while they significantly inhibited the chloroquine-resistant (D2) strain with  $IC_{50}$  values of 30.29 and 33.49  $\mu\text{g/mL}$ , respectively. The extracts showed moderate activity against the pathogenic fungi and bacteria tested with  $IC_{50}$  = >200  $\mu\text{g/mL}$  compared to the controls. This study provides a framework for future explorative

studies on different parts of the plant using different organic solvents for extraction and isolation of pure compounds, which can be investigated against different diseases, including malaria and other infectious diseases.

## Abbreviations

vRE = Vancomycin resistance enterococci, MRS = Methicillin-resistant *Staphylococcus aureus*, FLU = Fluconazole, CEFO = Cefotaxime, CQ = Chloroquine, ART = artemisinin, SI = Selectivity index, RHD = *R. heudelotii*.

## Declarations

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## Conflict of Interest

The authors declare no conflicting interest.

## Data Availability

The unpublished data is available upon request to the corresponding author.

## Ethics Statement

Not applicable.

## Funding Information

Not applicable.

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