South African Journal of Botany 149 (2022) 290–296

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

In vitro antimalarial properties and chemical composition of *Diospyros chamaethamnus* extracts

Iwanette du Preez-Bruwer[a,*], Davis R. Mumbengegwi[a], Stefan Louw[b]

[a] Multidisciplinary Research Services, Centre for Research Services, University of Namibia, P/Bag 13301, Windhoek, Namibia
[b] Department of Physics, Chemistry and Material Science, School of Science, University of Namibia, P/Bag 13301, Windhoek, Namibia

ARTICLE INFO

Article History:
Received 22 November 2021
Revised 20 May 2022
Accepted 6 June 2022
Available online xxx

Edited by Dr. L. McGaw

Keywords:
Diospyros chamaethamnus
Traditional medicine
Namibia
Antimalarial
Antioxidant
Chemical composition

ABSTRACT

Diospyros chamaethamnus, a low woody perennial, which is endemic to Namibia, Botswana, Angola, and Zambia, is used in the management of malaria. The current study aimed to assess the effectiveness of this plant against malaria parasites and to identify some of the compounds, which may be responsible for the therapeutic properties of this plant. Dried roots were macerated in dichloromethane-methanol (1:1 v/v) and water to isolate phytoconstituents, which were then screened for antimalarial activity using the *P. falciparum* chloroquine-sensitive strain, D10. Furthermore, the antioxidant activities of the plant extracts were determined using the DPPH method. Lastly, the plant extracts were analyzed by gas chromatography-mass spectrometry. Moderate *in vitro* antiplasmodial activity (IC_{50} < 50 μg/ml) was observed for the *D. chamaethamnus* organic (19.51 μg/mL) and aqueous (18.30 μg/mL) extracts. Antioxidant activity based on DPPH scavenging potential showed that *D. chamaethamnus* aqueous and organic extracts exhibited significant antioxidant activities with IC_{50} values of 7.63 and 10.74 μg/mL, respectively. Some of the biologically active compounds found in the extracts of *D. chamaethamnus* include lupeol, palmitic acid, stigmasterol, and pyrogallol. These findings support the use of *D. chamaethamnus* against malaria and/or malaria-associated symptoms and provide a foundation or guide for further studies of this plant as an alternative treatment option for malaria.

1. Introduction

Secondary metabolites have been deemed therapeutic, and as a result, have played a prominent role in the development of chemotherapies (Hussein and El-Anssary, 2018). Secondary metabolites are produced by plants to serve as a protection mechanism against microorganisms, herbivores, and harsh environmental conditions (Bennett and Wallsgrove, 2006; Isah, 2019). They have produced drugs such as artemisinin from the Chinese herb *Artemisia annua* (Hill and Staunton, 2010), or provided precursors from which drugs can be synthesized organically such as quinine from Cinchona bark (*Cinchona officinalis*) (WHO, 2007). Interestingly, quinine was the first drug to treat malaria and served as a lead structure for the synthesis of several drugs such as chloroquine and mefloquine which are still used today (Wink, 2012). Many more compounds with antimalarial activity have been isolated from plants including phenolics (flavonoids, xanthones, coumarins, and curcumin), naphthopyrones, quinones, terpenoids (iridoids, sesquiterpenes, diterpenes, triterpenes), quassinoids, cucurbitacins, alkaloids (indolizidine, indole, isoquinoline) and polyacetylenes (Wink, 2012). For these reasons, plants remain a source of naturally derived medicines.

Plant-based traditional remedies are used as a first-line treatment for various diseases and disorders, especially in developing countries (Sofowora et al., 2013;Wachtel-Galor and Benzie, 2011). There are about 615 plant species used medicinally in Namibia (Cheikhyoussef et al., 2011), of which not much information exists, in terms of empirical evidence. *Diospyros chamaethamnus* (Fig. 1) is one such plant, and is confined to the northeastern parts of Namibia largely on sandy plains, dunes, and along dry river beds, and is also known as the Gingerbread plum, Sand apple, or dwarf Jackal-berry in English, or *Mundjongolo* in SiLozi (a Bantu language). Its roots are used in a polyherbal treatment for headaches (a prominent symptom of malaria) in the Zambezi region of Namibia, which is a malaria-endemic area. Furthermore, its roots and leaves have been reported to be used for treatment of malaria together with *Ochna pulchra, Burkea africana*, and *Combretum zeyheri* (Simon et al., 2012).

Today herbal or plant-based medicines are widely accepted and used. Knowledge about the composition of secondary metabolites in herbal preparations is essential for the quality control and standardization of these medicines. Despite the use of *D. chamaethamnus* in traditional medicine, information regarding their biological and phytochemical properties is limited. The objective of the present study

* Corresponding author.
E-mail address: idupreez@unam.na (I. du Preez-Bruwer).

https://doi.org/10.1016/j.sajb.2022.06.006
0254-6299/

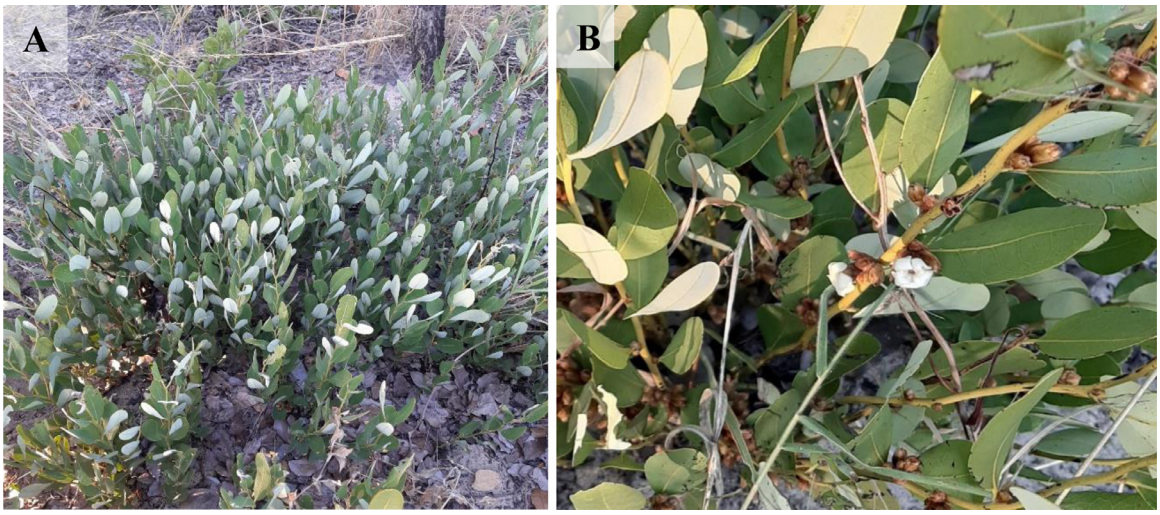

Fig. 1. Photographs of *D. chamaethamnus* – (A) a suffrutex plant with a height of less than one meter (B) with elongated, evergreen, spirally arranged leaves, and white flowers (original photos by I. du Preez).

was to investigate the biological properties of *D. chamaethamnus* as a remedy for malaria and to characterize the volatile and semi-volatile organic compounds in crude water and dichloromethane-methanol extracts of *D. chamaethamnus* roots.

Malaria continues to be a concern; in 2021, over 241 million cases of malaria were reported worldwide (World Health Organization, 2021). It is an infectious disease caused by Plasmodia parasites. Earlier research has found a relationship between malaria and oxidative stress; greater levels of oxidative stress has been detected in people and animals with malaria compared to those with no malaria (Vasquez et al., 2021). The immune system of the host creates reactive oxygen species (ROS) to eliminate the malaria parasites. This generation of excess free radicals is worsend by the parasites production of haem. This imbalance in oxidants and antioxidants can result in oxidative stress and inflammation, as well as cell and tissue damage and the onset and progression of diseasese including as diabetes, dementia, cancer, cardiovascular diseases, and metabolic syndromes (Sharifi-Rad et al., 2020; Tan et al., 2018). Fortunately, antioxidant supplements can help to mitigate these effects.

2. Experimental

2.1. Plant material

Fresh roots of *Diospyros chamaethamnus* (voucher no. CID12) were harvested during the flowering stages in the Zambezi region of Namibia. The plant material was dried at ambient temperature and ground to obtain a fine powder (particle size less than 1 mm), which was then stored in airtight containers at cool temperatures (-20°C). The identity of the plant was confirmed by botanists at the National Herbarium of the National Botanical Research Institute (NBRI) of Namibia.

2.2. Extraction procedure

Organic extracts were obtained by maceration of ground plant material using dichloromethane-methanol (1:1 v/v) at a sample-to-solvent ratio of 1:20 (w/v) for 48 hours at room temperature with intermittent shaking. Aqueous extracts were prepared by soaking ground plant material in distilled deionized water (1:20 w/v), which was placed in a water bath set at 60 °C for 2 hours with occasional shaking. The root suspension was spun down at 800 x G for 30 minutes and subjected to gravity filtration using Grade 1 Whatman filter paper. The extracts were concentrated and dried under reduced pressure using a rotary evaporator and subsequently a freeze-dryer. Dry extracts were obtained and stored at -20°C until subjected to further analysis.

2.3. Antiplasmodial activity

2.3.1. Parasite strain and maintenance of parasite cultures

Laboratory-adapted *P. falciparum* parasites were obtained from the Biodefense and Emerging Infections Research Resources Repository (BEI Resources), American Type Culture Collection (ATCC) (Manassas, VA, USA), which is a chloroquine-sensitive strain (D10). The parasite culture was grown in O^+ red blood cells and maintained in RPMI1640 media supplemented with 25 mM HEPES, L-glutamine, 2 mM NaOH, 4 % D-glucose, 0.02 mg/mL gentamicin, and 10 % serum. The culture was in a gas mixture of 90 % nitrogen, 5 % oxygen, and 5 % carbon dioxide, and incubated at 37 °C. This method is similar to the one described by Trager and Jensen (Trager and Jensen, 1976). The hematocrit and parasitemia of the parasites were maintained at 2 %.

2.3.2. Bioassay

Unsynchronized parasites, i.e. parasites in the asexual stages including rings, trophozoites and schizonts were used for this assay. Parasites in red blood cells (RBCs) were seeded in flat bottomed 96-well plates. Varying concentrations of plant extracts diluted in complete media were added to each well to obtain final concentrations of 5, 25, 50, and 100 μg/mL, and a total volume of 200 μL of parasitized RBCs with a parasitemia and hematocrit of 2 %. This was followed by incubation at 37°C for 48 hours. Wells with chloroquine diphosphate (25 μg/mL) and infected RBCs were used as the positive control, and wells with infected RBCs void of any treatment was used as the negative control. The experiment was done in triplicate with with all treatments in replicates of 3 for each experiment. The number of parasites observed for treatments (plant extracts), as well as for negative and positive controls were quantified using light microscopy (10 % Giemsa stain). Each smear was observed at 10 different fields and 3 different parts of the slide. Infected RBCs were counted against 1000 total RBCs. The mean percentage parasitemia and percentage growth inhibition were calculated. The latter was expressed as a percentage of the mean percentage parasitemia for each concentration compared with the mean percentage parasitemia for the untreated controls:

$$\%\ \text{Growth inhibition} = 100 - \frac{\text{Parasitemia of treatment}}{\text{Parasitemia of control}} \times 100.$$

2.4. Antioxidant activity

2,2-Diphenyl-1-picryl-hydrazyl (DPPH) method described by Mensor et al., (2001) was used to determine the antioxidant activity of the plant extracts. An equal volume of plant extract resuspended in methanol (100 μL) was added to 100 μL of 0.3 mM methanolic DPPH (Aldrich) solution to obtain final concentrations of 10, 20, 30, and 40 μg/mL. Ascorbic acid (Sigma) was used as the positive control. The scavenging activity of plant extracts was measured after 30 minutes of incubation in the dark using absorbance at a wavelength of 518 nm with a multimode plate reader. Three replicates of each sample were used for statistical analysis and the mean ± S.E.M. reported. The inhibitory effect of plant extracts of each parameter was calculated as:

$\%\text{Inhibition} = \left((A_C - A_E)\right)/A_C) \times 100$; where A_C is the absorbance of the fully oxidized control and A_E is the absorbance of the extract. The concentration required to scavenge 50 % of the DPPH radical (IC_{50}) was calculated using linear regression analysis by plotting the percentage inhibition against the concentrations of the plant extracts. The antioxidant activity of the plant extracts was also expressed as the ascorbic acid equivalent antioxidant capacity (AEAC) using the equation below:

$$\text{AEAC}(\text{mgAA}/100\text{g}) = (IC_{50}\text{ascorbicacid})/IC_{50}\text{extract}) \times 100.$$

2.5. GC-MS analyses

2.5.1. Apparatus

GC-MS analyses of plant extracts were performed on a Focus GC coupled to an ITQ 700 MS (Thermo Scientific, Italy). The injector temperature was maintained at 220°C, the MS transfer line at 250°C, and the ion source at 200°C. Helium was used as the carrier gas set at a constant flow of 1 mL per minute. An SGE BP5MS capillary GC column with dimensions of 30 m x 0.25 mm i.d., and a stationary phase coating thickness of 0.25 μm (Genmed, Windhoek, Namibia) was used for the separations. The oven temperature was programmed at 2°C per minute^{-1} from 40°C to 300°C and was held isothermal for 60 minutes. The electron ionization mass spectra were recorded with an ionization voltage of 70 eV and an *m/z* 25-515 scan range. Xcalibur software version 2.1 was used for data acquisition and processing. One microliter of either sample or the standard solution was injected with a split ratio of 1:10 using the hot-needle technique.

2.5.2. Sample and standard solution preparation

The dry extracts of the plants were reconstituted in methanol for GC-MS analysis. Dry extracts were suspended in methanol to obtain final concentrations of 10 mg/100 μL (organic extracts) and 2 mg/100 μL (aqueous extracts). The reference compounds, α-amyrin, palmitic acid, methyl palmitate, stigmasterol, oleic acid, and stearic acid, were purchased from Medlab Services CC (Windhoek, Namibia). Solutions of the reference compounds were prepared for GC-MS analysis at concentrations of *ca.* 1 mg/mL.

2.5.3. Identification of chemical constituents

The sample solutions and a mixture of C_{10}-C_{40} *n*-alkanes (even number of carbons only) (Sigma Aldrich, Germany) were analyzed using the same GC-MS conditions, and the Kováts retention indices (RIs) of the detected constituents were calculated. Subsequently, the compounds in the plant extracts were identified by comparing their mass spectra and RIs to those in the National Institute of Standards and Technology (NIST) version 11 MS and RI databases, as well as other RI values reported in the literature. The identities of several compounds were confirmed by retention time comparison using authentic reference standards.

2.6. Data analysis

A statistical software package GraphPad Prism 6 was used to analyze the antimalarial and antioxidant data. The results are reported as mean ± standard error (SEM) and were analyzed statistically using one-way and two-way ANOVA followed by post hoc tests, *i.e.* the Tukey Krammer (Tukey's) and Dunnett's multiple comparisons tests, to compare mean differences for all treatments and controls. Values of $P < 0.05$ were considered significant. Furthermore, a log dose-response curve was generated to determine IC_{50} values. The growth inhibition values were plotted against corresponding concentrations of the plant extracts at a 95 % confidence interval. The IC_{50} values were determined by nonlinear interpolation from each of the inhibition curves in GraphPad Prism 6.

3. Results and discussion

Antimalarial activity of the plant extracts was screened at concentrations of 5, 25, 50, and 100 μg/mL. The aqueous and organic extracts of *D. chamaethamnus* demonstrated antimalarial activity against the chloroquine-sensitive strain D10 (Fig. 2). At each of the

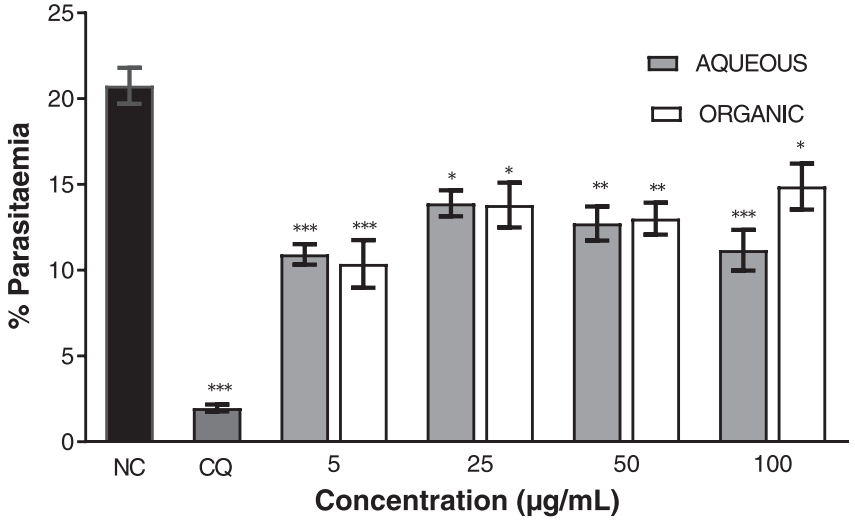

Fig. 2. Percentage parasitemia (i.e. percentage parasite survival) of *P. falciparum* D10 48 hours post-treatment with *D. chamaethamnus* aqueous and organic extracts at various concentrations. Data are presented as means ± SEM. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, compared to the negative control (one-way ANOVA followed by Tukey's post hoc test). NC: negative control or vehicle and CQ: chloroquine or positive control.

Table 1
In vitro antiplasmodial activity of aqueous and organic extracts from *D. chamaethamnus* against erythrocytic stages of the *P. falciparum* D10 strain at 48 hours.

Treatment	Extract	% Parasite growth inhibition				IC_{50} (μg/mL) [R^2]
		5 μg/mL	25 μg/mL	50 μg/mL	100 μg/mL	
D. chamaethamnus	***Aqueous***	47.36	33.01	38.68	46.18	**18.30** [0.7707]
	Organic	50.04	33.51	37.32	28.31	**19.51** [0.8247]
Chloroquine (25 μg/mL)	-	-	92.54	-	-	-

four concentrations for both extracts, there was a significant reduction in the number of parasite-infected RBCs relative to the control ($P<0.05$). Chloroquine showed a significant mean percentage suppression of 92.55 % compared to the aqueous (46.18 %) and organic (28.31 %) extracts of *D. chamaethamnus* at their highest concentration. There, however, was not a concentration-dependent effect for the percentage parasitemia with increasing concentrations of the plant extracts, nor was there a statistical difference between organic and aqueous extracts of the plant under investigation ($P>0.05$). As presented in Table 1 the extracts of *D. chamaethamnus* showed moderate activity with IC_{50} values ranging from 10 to 50 μg/ml (Dolabela et al., 2008). The aqueous extract exhibited higher antiplasmodial activity with an IC_{50} value of 18.30 μg/mL compared to the organic extract, which had an IC_{50} value of 19.51 μg/mL.

The biological activities for *D. chamaethamnus* have not yet been described, but for several other *Diospyros spp.*the antimalarial, antibacterial, antipyretic, and anticancer activities have been reported (Pinho et al., 2011). Some of the species that have been studied include *D. rubra* (Prachayasittikul et al., 2010), *D. quaesita* (Ma et al., 2008), *D. glandulsa,* and *D. rhodocalyx* (Theerachayanan et al., 2007). A reduction in parasitized RBCs was observed for these species using different solvents and Plasmodia strains. According to Prachayasittikul et al. (2010), antiplasmodial activities (IC_{50}) of 176.20 μg/mL, 23.95 μg/mL, 33.58 μg/mL, and 135.05 μg/mL were exhibited by hexane, dichloromethane, ethyl acetate, and methanol extracts, respectively for *D. rubra. D. mespiliformis,* while on the other hand displayed an IC_{50} of 2.91 μg/mL for its aqueous extract (Nafuka, 2014). For *D. quaesita*, betulinic acid was isolated and tested against *P. falciparum* D6 and W2 strains, which exhibited IC_{50} values of 0.9 and 0.6 μg/mL, respectively.

What's interesting is that in the present study, the antimalarial activities of the extracts at the highest concentrations were comparable to those of lower concentrations. It could be that the maximal response was not produced and that the compounds present in the extracts may be partial agonists. It has been shown that many pharmaceuticals produce therapeutic effects by binding to receptors causing either agonist or antagonist effects. At certain concentrations, the therapeutic response reaches a maximum due to the saturation of available receptors by a drug (Harro, 2004). Normally a drug produces a maximal response when all of the receptors are occupied and a half-maximal response when 50 % of the receptors are occupied. However, some drugs elicit a half-maximal response regardless of maximal occupancy of receptors by compounds. To the best of the knowledge of the authors, no previous data have been reported for the *in vitro* antimalarial activities of *D. chamaethamnus*, but for related plant species. However, Dushimemaria *et al.* (2017) recently described the cytotoxicity activities and classes of phytochemicals of this plant using TLC, as well as its potential as an antitumor agent.

IC_{50} values were determined by nonlinear interpolation from each of the inhibition curves in GraphPad Prism 6.

The antioxidant results are shown in Table 2. The extracts of *D. chamaethamnus,* both aqueous and organic, exhibited DPPH radical scavenging activity. These scavenging effects were concentration-dependent ($P < 0.05$). The results revealed that both extracts exhibited prominent antioxidant activity. At concentrations as low as 10 μg/mL, inhibition of DPPH formation was relatively high for the organic extract (90.4 %) and the aqueous extracts (89.9 %) at a concentration of 20 μg/mL. Furthermore, the IC_{50} values for the antioxidant activity of the aqueous (7.63 μg/mL), and organic (10.74 μg/mL) extracts were comparable to that of the ascorbic acid (5.97 μg/mL) ($P > 0.05$). Interestingly, the aqueous extract displayed higher antioxidant activity than the organic extract. The ascorbic acid equivalent antioxidant capacity (AEAC) was greater than 600 mg AA/100 g for the aqueous (78 244) and aqueous (55 587) plant extracts, which is rather high (Harro, 2004; Leong and Shui, 2002). The free radical scavenging activities of the plant under investigation have not been reported before, although reports on related species for *D. chamaethamnus* (Baravalia et al., 2009; Loizzo et al., 2009; Mondal et al., 2006) has been documented, and is consistent with the findings of this study further confirming the plant's antioxidant activities.

The antiplasmodial and antioxidant activities for the organic and aqueous extracts of *D. chamaethamnus* were comparable, suggesting that the active phytoconstituents responsible for the observed pharmacological effects are present in both the water and methanol-dichloromethane (1:1 v/v) extracts. The number and type of compounds identified in both extracts in this study also confirmed this. As a result, the use of water as an extractant in a traditional setting is supported or substantiated.

Percentage inhibition values in the table are expressed as means ± SEM of three replicates relative to control (vehicle), n=3. Nonlinear regression analysis was used to calculate IC_{50} values in GraphPad Prism 6. *Ascorbic acid equivalent antioxidant capacity.

Using GC-MS analysis, a total of 29 compounds were identified in the extracts of *D. chamaethamnus* roots (Table 3), 19 compounds in the organic extract (Fig. S1), and 17 in the aqueous extract (Fig. S2). Seven of these compounds were present in both of the extracts. The compounds found in the plant extracts were mostly alkanes, fatty acids, triterpenoids, and steroids. Compound **22** from the aqueous

Table 2
Antioxidant activity observed for the aqueous and organic extracts of *D. chamaethamnus* compared to ascorbic acid (positive control).

Concentration (μg/mL)	Aqueous extract			Organic extract			Ascorbic acid
	% Inhibition	IC_{50} (μg/mL) [R^2]	AEAC* (mg AA/100 g)	% Inhibition	IC_{50} (μg/mL) [R^2]	AEAC* (mg AA/100 g)	IC_{50} (μg/mL) [R^2]
10	90.40±1.33	7.63 [0.9748]	78244	39.92±2.12	10.74 [0.9971]	55587	5.97 [0.9968]
20	92.75±0.30			89.90±1.21			
30	93.27±0.62			94.28±0.36			
40	94.19±0.31			94.27±0.37			

Table 3
Compounds identified in the organic and aqueous extracts of *Diospyros chamaethamnus* roots.

Compound no.[a]	RI (exp)[b]	RI (lit)[c]	Name of compound	ID[d]	Aqueous extract[e]	Organic extract[e]
1	1147	1149	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	B	√	-
2	1382	1386	Pyrogallol	B	√	-
3	1400	1400	Tetradecane	A	√	√
4	1600	1600	Hexadecane	A	√	√
5	1707	1700	Heptadecane	B	-	√
6	1800	1800	Octadecane	A	√	√
7	1927	1926	Methyl palmitate	A	-	√
8	1963	1958	Palmitic acid	A	√	√
9	2132	2140	Linoleic acid	B	-	√
10	2138	2146	Oleic acid	A	-	√
11	2164	2162	Stearic acid	A	-	√
12	2323	-	Unknown	C	√	√
13	2386	2381	Hexyl palmitate	B	√	-
14	2500	2498	2-Palmitoylglycerol	B	√	-
15	2586	-	Unidentified heptadecanoic acid ester	C	√	-
16	2660	-	Unknown	C	√	-
17	2708	2697[f]	2-Stearoylglycerol	C,D	√	-
18	2757	-	Unidentified steroid	C	√	-
19	2808	2800	Octacosane	A	√	-
20	3113	3111	Vitamin E	B	√	√
21	3223	3249	Stigmasterol	A	√	√
22	3288	3290	γ-Sitosterol	B	√	√
23	3332	-	Lanosterol	C	-	√
24	3337	-	Unidentified triterpenoid	C	√	-
25	3362	3376	α-Amyrin	A	√	-
26	3366	-	Lupeol	C	-	√
27	3414	-	Unidentified sterol	C	-	√
28	3561	-	Unidentified triterpenoid	C	-	√
29	3596	-	Unidentified triterpenoid	C	-	√
30	3698	-	Unidentified triterpenoid	C	-	√
31	3820	-	Unidentified triterpenoid	C	-	√

[a] Compounds are numbered in order of elution from the GC column.
[b] RI values determined experimentally on an HP-5 equivalent column.
[c] Selected RI values determined on HP-5 equivalent columns listed in the NIST 11 RI database
[d] Identification: A – comparison of mass spectrum and retention time with those of an authentic reference compound; B – comparison of mass spectrum and RI with NIST MS and RI databases; C – comparison of the mass spectrum with NIST MS database (tentative identification); D – match predicted RI (tentative identification).
[e] Present: √; Absent: -
[f] Predicted RI value from the NIST MS database

and organic extracts was identified based only on the fragmentation patterns observed in its mass spectra. The MS data showed fragmentation patterns typical of steroids, with a prominent ion at m/z 329 (base peak) and accompanying ions at m/z 213, 255, 303, 381, and 414 similar to that of β-sitosterol (Bataglion et al., 2015). In addition, two compounds (**18** in the aqueous extract and **27** in the organic extract) displayed MS fragmentation patterns very similar to those of cholest-4-en-3-one and lanosterol with peaks at *m/z* 124 and 43, and 255 and 215, respectively (Louw et al., 2011) suggesting that these two compounds can be steroids, however, the identities of these phytoconstituents could not be confirmed.

Similarly, compounds **28-31** in the organic extract as well as compound **24** in the aqueous extract of *D. chamaethamnus* were identified as triterpenoids. The most important feature in the mass spectra of the unidentified triterpenoids was the base peak at *m/z* 189 followed by peaks at *m/z* at 203/204, which is characteristic of the mass spectra of 18-oleanenes (triterpenoids) (Assimopoulou and Papageorgiou, 2005). The mass spectrum of the unidentified triterpenoid in the aqueous extract (compound **24**) also contain intense fragment ion peaks at *m/z* 204, 175, and 137, which are ions consistent with those commonly found in the mass spectra of triterpenoids including those for lupenone, α-amyrinone, β-amyrinone, and lupeol (Hemmers et al., 1988). The RI data of the triterpenoids suggested by the NIST RI database, however, did not correspond with the experimentally determined RIs of these compounds. Contaminants, such as phthalate esters, were also identified in the extracts. These compounds were considered contaminants as they are industrial chemicals (plasticizers), and are usually found in plastics. The source of these compounds could be from the plastic Falcon tubes in which the extracts were stored (McDonald et al., 2008).

The presence of these phytochemicals are consistent with the compound classes previously identified by Dushimemaria et al., (2017) using qualitative preliminary phytochemical techniques such as thin-layer chromatography. In another study, 7-methyljuglone, a naphthoquinone, was identified in the root barks of *D. chamaethamnus*, together with several derivatives including diospyrin, isodiospyrin, diosquinone, mamegakinone, biramentaceone, xylospyrin, and 6-[2-(7-methyljuglonyl)]-isoxylospyrin) (Costa et al., 1998). Naphthoquinones, which are phenolic compounds, are known for their biological activities in Diospyros spp. (Mbaveng and Kuete, 2014). Notably, pyrogallol (1,2,3-benzenetriol) (**2**), a phenolic compound, was found in the aqueous extract of the *D. chamaethamnus* roots. Interestingly, pyrogallol (**2**) can be formed by the decarboxylation of gallic acid (Li and Wang, 2015). Since the aqueous extract was prepared using hot water, it is conceivable that this compound was produced from gallic acid during the extraction process and, that it is not necessarily produced by the plant. Reported biological activities of pyrogallol that are of interest to us include antioxidant and antibacterial activities (Kougan et al., 2013). Moreover, this molecule is being investigated as a potential antimalarial candidate (Alfaqih and Abu Bakar, 2020; Kougan et al., 2013).

Phenolic compounds are known to demonstrate a wide range of pharmacological activities, notably antioxidant activity. According to Bruins *et al.* (2006), these phytoconstituents have antimicrobial, anti-inflammatory, antiviral, antidiarrhoeal, and immune-modulatory activities. In addition, phenols have been shown to exhibit toxicity

towards *Plasmodia* parasites (Ramanandraibe et al., 2008). Hence, the reduction in the number of parasitized RBCs observed for the root extracts of *D. chamaethamnus* may be attributed to the presence of phenolic compounds, which may also be responsible for their antioxidant activities (Scalbert et al., 2005). Other compounds that may contribute to the antimalarial properties of the extracts include terpenoids (Amoa Onguéné et al., 2013) and steroids (Pabón et al., 2002). Furthermore, the high antioxidant activities of the plant extract observed in this study, suggests that the observed antimalarial activities could be a result of the scavenging of ROS that is involved in the pathogenesis of malaria (Gopalakrishnan and Kumar, 2015), and in turn prevent progression of malaria into severe malaria (Da et al., 2014). The antioxidant effects of the extracts of these plants may therefore represent a possible mechanism contributing to their antiplasmodial activity. Another interesting compound that was also only detected in the aqueous extract of the *D. chamaethamnus* roots, is 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (**1**). It has been reported that this compound actively inhibits the growth of microorganisms and mitigates inflammatory responses (Gopalakrishnan and Kumar, 2015).

Although fatty acids are present in all plant cells and together with lipids they act as membrane compounds, storage products, metabolites, and a source of energy (Wada et al., 1994), several are well-known for their antibacterial and antifungal properties (Li et al., 2004), including long-chain unsaturated fatty acids such as oleic acid (**10**) (McGaw et al., 2002). This compound was found to be active against *Mycobacterium aurum* and *M. phlei* (Agoramoorthy et al., 2007). It is also likely to be involved in the inhibition of FabI, an important drug target for antibiotics (Zheng et al., 2005). Furthermore, oleic acid has been shown to possess anti-inflammatory activity (Li et al., 2004) and activity against the *Plasmodium* parasites (Carballeira, 2008). Linoleic acid (**9**) has also been reported to possess anti-inflammatory activity (Maruthupandian and Mohan, 2011). Stearic acid (**11**), on the other hand, displayed similar activities as the other two compounds, including inhibitory activity against bacterial and fungal infections (Abou-Elela et al., 2009) and antimalarial activity (Zito et al., 2010). Palmitic acid (**8**), the most common fatty acid, and which is present in both extracts of the *D. chamaethamnus* roots is reported to have antifungal, antioxidant, antimalarial, antimicrobial activities (Hema, Kumaravel, & Alagusundaram, 2011; Hsouna *et al*., 2011). Fatty acid esters such as stearic acid esters exhibit antifungal, antimicrobial, antibacterial, hypocholesterolemic (Abou-Elela et al., 2009), and antioxidant activities (Syeda et al., 2011); and palmitic acid esters exhibit anti-inflammatory, antimicrobial, anticholesterolemic, hepatoprotective, antihistaminic properties (Kumar et al., 2010) as well as low antiplasmodial activity ($IC_{50} > 100\ \mu g/ml$) (Banzouzi et al., 2015). Previous studies have shown specifically that methyl palmitate (**7**) exerts antifungal, antioxidant, hypocholesterolemic, nematicidal, insecticidal, haemolytic and potent antimicrobial activities (Hema et al., 2011). Ethyl palmitate also exhibit hypocholesterolemic, antioxidant, nematicidal activities among others (Jargalsaikhan et al., 2013), as well as antimicrobial activities (Kumar et al., 2010).

It is important to note that many of the compounds found in the extracts of *D. chamaethamnus* have antibacterial properties, which is important in the prevention of microbial infections in malaria patients, as they are often are at risk of secondary bacterial infections such as septicemia urinary tract infections, meningitis, or bacterial pneumonia. Fever blisters caused by the Herpes simplex virus, are also an accompaniment of the disease (Berkley et al., 1999). It is probable that the reported antibacterial activity of some of the compounds present in the aqueous and organic extracts of the plants under investigation may act against some of the organisms causing these infections, whilst compounds implicated in antiplasmodial activities inhibit the growth of the *Plasmodia* parasites. This is a classic example of synergism in plant extracts. Thus, antimicrobial and anti-inflammatory compounds can be of therapeutic value for the prevention and treatment of secondary infections in malaria-infected persons.

5. Conclusion

The present study showed that the root extracts of *D. chamaethamnus* exhibit antimalarial and antioxidant activities. The study also demonstrated the presence of compounds with known biological activities including antimalarial and antioxidant activities, as well as antibacterial. These results are reported for the first time herein and support the medicinal applications of *D. chamaethamnus.* Its roots might have beneficial effects on malaria control. Further studies are required to confirm this, as well as to isolate and identify active components from the root extracts.

Author contributions

IDPB and DRM: Conceptualization. IDPB and DRM: Funding acquisition. IDPB: Validation. IDPB and SL: Formal analysis. DRM: Supervision. IDPB: writing, original draft. IDPB, DRM and SL: Review and editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

This study was supported financially by the Multidisciplinary Research Centre and the German Academic Exchange Service (DAAD).

References

Abou-Elela, G.M., Abd-Elnaby, H., Ibrahim, H.A.H., Okbah, M.A., 2009. Marine natural products and their potential applications as anti-infective agents. World Appl. Sci. J. 7, 872–880.

Agoramoorthy, G., Chandrasekaran, M., Venkatesalu, V., Hsu, M.J., 2007. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Braz. J. Microbiol. 38, 739–742.

Alfaqih, H., Abu Bakar, N., 2020. The potential of pyrogallol as a possible antimalarial. drug candidate 1, 2020.

Amoa Onguéné, P., Ntie-Kang, F., Lifongo, L.L., Ndom, J.C., Sippl, W., Mbaze, L.M., 2013. The potential of anti-malarial compounds derived from African medicinal plants, part I: a pharmacological evaluation of alkaloids and terpenoids. Malar. J. 12, 449. https://doi.org/10.1186/1475-2875-12-449.

Assimopoulou, A.N., Papageorgiou, V.P., 2005. GC-MS analysis of penta- and tetra-cyclic triterpenes from resin of Pistacia species. Part I. Pistacia lentiscus var. Chia. Biomed. Chromatogr. 19, 285–311. https://doi.org/10.1002/bmc.454.

Banzouzi, J.T., Njomnang Soh, P., Ramos, S., Toto, P., Cavé, A., Hemez, J., Benoit-Vical, F., 2015. Samvisterin, a new natural antiplasmodial betulin derivative From Uapaca paludosa (Euphorbiaceae). J. .Ethnopharmacol 173, 100–104. https://doi.org/10.1016/j.jep.2015.07.023.

Baravalia, Y., Kaneria, M., Vaghasiya, Y., Parekh, J., Chanda, S., 2009. Antioxidant and antibacterial activity of Diospyros ebenum Roxb. leaf extracts. Turk. J. Biol. 33, 159–164.

Bataglion, G.A., da Silva, F.M.A., Santos, J.M., Barcia, M.T., Godoy, H.T., Eberlin, M.N., Koolen, H.H.F., 2015. Integrative approach Using GC-MS and easy ambient sonic-dpray ionization mass spectrometry (EASI-MS) for comprehensive lipid characterization of Buriti (Mauritia flexuosa) oil. J. Braz. Chem. Soc. 26, 171–177. https://doi.org/10.5935/0103-5053.20140234.

Bennett, R., Wallsgrove, R., 2006. Secondary Metabolites in Plant Defense-Mechanisms. New Phytol. 127, 617–633. https://doi.org/10.1111/j.1469-8137.1994.tb02968.x.

Berkley, J., Mwarumba, S., Bramham, K., Lowe, B., Marsh, K., 1999. Bacteraemia complicating severe malaria in children. Trans. R. Soc. Trop. Med. Hyg. 93, 283–286. https://doi.org/10.1016/S0035-9203(99)90024-X.

Bruins, M.J., Cermak, R., Kiers, J.L., van der Meulen, J., van Amelsvoort, J.M.M., van Klinken, B.J.W., 2006. In vivo and in vitro effects of tea extracts on enterotoxigenic Escherichia coli-induced intestinal fluid loss in animal models. J. Pediatr. Gastroenterol. Nutr. 43, 459–469. https://doi.org/10.1097/01.mpg.0000239992.12646.df.

Carballeira, N.M., 2008. New advances in fatty acids as antimalarial, antimycobacterial and antifungal agents. Prog. Lipid Res. 47, 50–61. https://doi.org/10.1016/j.plipres.2007.10.002.

Cheikhyoussef, A., Mapaure, I., Shapi, M., 2011. The use of some indigenous plants for medicinal and other purposes by local vommunities in Namibia with emphasis on Oshikoto region: a review. Res. J. Med. Plant 5, 406–419. https://doi.org/10.3923/rjmp.2011.406.419.

Costa, M.A., Alves, A.C., Seabra, R.M., Andrade, P.B., 1998. Naphthoquinones of Diospyros chamaethamnus. Planta Med 64, 391. https://doi.org/10.1055/s-2006-957464.

Da, O., Traore/Coulibaly, M., Yerbanga, R.S., Koama, B., Ouedraogo, N., Tamboura, S., Dakuyo, M.P., Sekhoacha, M.P., Nikiema, J.B., Ouedraogo, G.A., Matsabisa, M.G., Ouedraogo, J.B., 2014. Antiplasmodial and antioxidant activities of Saye: a traditional herbal remedy for malaria. Am. J. Biochem. Mol. Biol. 4, 155–166. https://doi.org/10.3923/ajbmb.2014.155.166.

Dolabela, M.F., Oliveira, S.G., Nascimento, J.M., Peres, J.M., Wagner, H., Póvoa, M.M., de Oliveira, A.B., 2008. In vitro antiplasmodial activity of extract and constituents from Esenbeckia febrifuga, a plant traditionally used to treat malaria in the Brazilian Amazon. Phytomedicine 15, 367–372. https://doi.org/10.1016/j.phymed.2008.02.001.

Dushimemaria, F., Du Preez, C.I., Mumbengegwi, D.R., 2017. Randomized anticancer and cytotoxicity activities of Guibourtia coleosperma and Diospyros chamaethamnus. Afr. J. Tradit. Complement. .Altern. Med. 14, 1–7. https://doi.org/10.21010/ajtcam.v14i4.1.

Gopalakrishnan, A.M., Kumar, N., 2015. Antimalarial action of artesunate involves DNA damage mediated by reactive oxygen species. Antimicrob. Agents Chemother. 59, 317–325. https://doi.org/10.1128/AAC.03663-14.

Harro, J., 2004. Appendix A: Pharmacodynamics and Pharmacokinetics. In: Panksepp, J. (Ed.), Textbook of Biological Psychiatry. John Wiley & Sons, Inc., pp. 661–681.

Hema, R., Kumaravel, S., Alagusundaram, K., 2011. GC-MS determination of bioactive components of Murraya koenigii. J. Am. Sci. 7, 80–83.

Hemmers, H., Gulz, P., Marner, F., 1988. Triterpenoids in epicuticular waxes of three European Euphorbia species. ZNaturforsch 43c, 799–805.

Hill, A.M., Staunton, J., 2010. Type I modular PKS. In: Mander, L., Liu, H. (Eds.), Comprehensive Natural Products II: Chemistry and Biology. Elsevier, United Kingdom, pp. 385–452.

Hsouna, A.B., Trigui, M., Mansour, R.B., Jarraya, R.M., Damak, M., Jaoua, S., 2011. Chemical composition, cytotoxicity effect and antimicrobial activity of Ceratonia siliqua essential oil with preservative effects against Listeria inoculated in minced beef meat. Int. J. Food Microbiol. 148, 66–72. https://doi.org/10.1016/j.ijfoodmicro.2011.04.028.

Hussein, R.A., El-Anssary, A.A., 2018. Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. Herbal Medicine. Builders, P.F. (Ed.)IntechOpen.

Isah, T., 2019. Stress and defense responses in plant secondary metabolites production. Biol. Res. 52, 39. https://doi.org/10.1186/s40659-019-0246-3.

Jargalsaikhan, U., Javzan, S., Selenge, D., Nedelcheva, D., Philipov, S., Nadmid, J., 2013. Fatty acids and their esters from Cicuta virosa L. Mongol. J. Chem. 14, 71–74. https://doi.org/10.5564/mjc.v14i0.203.

Kougan, G.B., Tabopda, T., Kuete, V., Verpoorte, R., 2013. 6 - Simple Phenols, Phenolic Acids, and Related Esters from the Medicinal Plants of Africa. In: Kuete, V. (Ed.), Medicinal Plant Research in Africa. Elsevier, Oxford, pp. 225–249. https://doi.org/10.1016/B978-0-12-405927-6.00006-0.

Kumar, P.P., Kumaravel, S., Lalitha, C., 2010. Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. Afri. J. Biochem. Res 4, 191–195.

Leong, L.P., Shui, 2002. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem. 76, 69–75.

Li, R.W., Leach, D.N., Myers, S.P., Leach, G.J., Lin, G.D., Brushett, D.J., Waterman, P.G., 2004. Anti-inflammatory activity, cytotoxicity and active compounds of Tinospora smilacina Benth. Phytother. Res. 18, 78–83. https://doi.org/10.1002/ptr.1373.

Li, W., Wang, C., 2015. Biodegradation of Gallic Acid to Prepare Pyrogallol by Enterobacter aerogenes through Substrate Induction. BioResources 10. https://doi.org/10.15376/biores.10.2.3027-3044.

Loizzo, M., Said, A., Tundis, R., Hawas, U., Rashed, K., Menichini, F., Frega, N., Menichini, F., 2009. Antioxidant and Antiproliferative Activity of Diospyros lotus L. Extract and Isolated Compounds. Plant Foods Hum. Nutr. https://doi.org/10.1007/s11130-009-0133-0.

Louw, S., Burger, B.V., Le Roux, M., Van Wyk, J.H., 2011. Lizard epidermal gland secretions. II. Chemical characterization of the generation gland secretion of the sungazer, Cordylus giganteus. J. Nat. Prod. 74, 1364–1369. https://doi.org/10.1021/np1008366.

Ma, C.-Y., Musoke, S.F., Tan, G.T., Sydara, K., Bouamanivong, S., Southavong, B., Soejarto, D.D., Fong, H.H.S., Zhang, H.-J., 2008. Study of antimalarial activity of chemical constituents from Diospyros quaesita. Chem. Biodivers. 5, 2442–2448. https://doi.org/10.1002/cbdv.200890209.

Maruthupandian, A., Mohan, V.R., 2011. GC-MS analysis of some bioactive constituents of Pterocarpus marsupium Roxb. Int. J. Chem. Tech. Res. 3, 1652–1657.

Mbaveng, A.T., Kuete, V., 2014. Review of the chemistry and pharmacology of 7-Methyljugulone. Afr. Health Sci. 14, 201–205. https://doi.org/10.4314/ahs.v14i1.31.

McDonald, J.G., Cummins, C.L., Barkley, R.M., Thompson, B.M., Lincoln, H.A., 2008. Identification and quantitation of sorbitol-based nuclear clarifying agents extracted from common laboratory and consumer plasticware made of polypropylene. Anal. Chem. 80, 5532–5541. https://doi.org/10.1021/ac8005632.

McGaw, L.J., Jäger, A.K., van Staden, J., 2002. Isolation of antibacterial fatty acids from Schotia brachypetala. Fitoterapia 73, 431–433. https://doi.org/10.1016/S0367-326X(02)00120-X.

Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., dos Santos, T.C., Coube, C.S., Leitão, S.G., 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 15, 127–130.

Mondal, S.K., Chakraborty, G., Gupta, M., Mazumder, U.K., 2006. In vitro antioxidant activity of Diospyros malabarica Kostel bark. Indian J. Exp. Biol. 44, 39–44.

Nafuka, S.N., 2014. In vitro antiplasmodial activity and phytochemicals screening of ethnomedicinal plants used to treat Malaria associated symptoms. University of Namibia, Windhoek, Namibia. (Thesis).

Pabón, A., Carmona, J., Maestre, A., Camargo, M., Blair, S., 2002. Inhibition of P. falciparum by steroids isolated from Solanum nudum. Phytother. Res. 16, 59–62. https://doi.org/10.1002/ptr.1035.

Pinho, B.R., Sousa, C., Valentão, P., Andrade, P.B., 2011. Is nitric oxide decrease observed with naphthoquinones in LPS stimulated RAW 264.7 macrophages a beneficial property? PLoS One 6, e24098. https://doi.org/10.1371/journal.pone.0024098.

Prachayasittikul, S., Saraban, P., Cherdtrakulkiat, R., Ruchirawat, S., Prachayasittikul, V., 2010. New bioactive triterpenoids and antimalarial activity of Diospyros rubra Lec. EXCLI J. 9, 1–10.

Ramanandraibe, V., Grellier, P., Martin, M.-T., Deville, A., Joyeau, R., Ramanitrahasimbola, D., Mouray, E., Rasoanaivo, P., Mambu, L., 2008. Antiplasmodial phenolic compounds from Piptadenia pervillei. Planta Med. 74, 417–421. https://doi.org/10.1055/s-2008-1034328.

Scalbert, A., Johnson, I.T., Saltmarsh, M., 2005. Polyphenols: antioxidants and beyond. Am. J. Clin. Nutr. 81, 215S–217S.

Sharifi-Rad, M., Anil Kumar, N.V., Zucca, P., Varoni, E.M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P.V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M.E., Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A.O., Setzer, W.N., Calina, D., Cho, W.C., Sharifi-Rad, J., 2020. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Front. Physiol. 11.

Simon, J.E., Raskin, I., Juliani, R., Graf, B., Grazione, R., Linder, D., Fitzgerald, B., Wu, Q., Brendler, T., Mortensen, D., Freiter, U., Phillips, D., Mumbengegwi, D., Hedimbi, M., Simon, J.E., Raskin, I., Juliani, R., Graf, B., Grazione, R., Linder, D., Fitzgerald, B., Wu, Q., Brendler, T., Mortensen, D., Freiter, U., Phillips, D., Mumbengegwi, D.R., Hedimbi, M., 2012. Inventory and Rapid Throughput Screening of Indigenous Utilized Plant Species for Development of New Natural Products. (Quarterly Progress Report). Indigenous Natural Products Innovation Fund, New Jersey.

Sofowora, A., Ogunbodede, E., Onayade, A., 2013. The Role and Place of Medicinal Plants in the Strategies for Disease Prevention. Afr. J. Tradit. Complement. Altern. Med. 10, 210–229.

Syeda, F.A., Habib-ur-Rahman, Choudahry, Atta-ur-Rahman, M.I., 2011. Gas chromatography-mass spectrometry (GC-MS) analysis of petroleum ether extract (oil) and bioassays of crude extract of Iris germanica. Int. J. Genet. Mol. Biol. 3, 95–100.

Tan, B.L., Norhaizan, M.E., Liew, W.-P.-P., Sulaiman Rahman, H., 2018. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Front. Pharmacol. 9, 1162. https://doi.org/10.3389/fphar.2018.01162.

Theerachayanan, T., Sirithunyalug, B., Piyamongkol, S., 2007. Antimalarial and antimycobacterial activities of dimeric naphthoquinone from Diospyros glandulosa and Diospyros rhodocalyx. Chiang Mai Univ. J. Natl. Sci. (Thailand) 6, 253–259.

Trager, W., Jensen, J.B., 1976. Human malaria parasites in continuous culture. Science 193, 673–675. https://doi.org/10.1126/science.781840.

Vasquez, M., Zuniga, M., Rodriguez, A., 2021. Oxidative Stress and Pathogenesis in Malaria. Front. Cell Infect. Microbiol. 11, 768182. https://doi.org/10.3389/fcimb.2021.768182.

Wachtel-Galor, S., Benzie, I.F.F., 2011. Herbal Medicine: an Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs. In: Benzie, I.F.F., Wachtel-Galor, S. (Eds.), Herbal Medicine: Biomolecular and Clinical Aspects. CRC Press/Taylor & Francis, Boca RatonFL.

Wada, H., Gombos, Z., Murata, M., 1994. Contribution of membrane lipids to the ability of the photosynthetic machinery to tolerance temperature stress. In: Proc. Natl. Acad. Sci, USA.

WHO, 2007. TDR news.

Wink, M., 2012. Medicinal plants: a source of anti-parasitic secondary metabolites. Molecules 17, 12771–12791. https://doi.org/10.3390/molecules171112771.

World Health Organization, 2021. World malaria report 2021. World Health Organization, Geneva.

Zheng, C.J., Yoo, J.-S., Lee, T.-G., Cho, H.-Y., Kim, Y.-H., Kim, W.-G., 2005. Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Lett 579, 5157–5162. https://doi.org/10.1016/j.febslet.2005.08.028.

Zito, P., Sajeva, M., Bruno, M., Maggio, A., Rosselli, S., Formisano, C., Senatore, F., 2010. Essential oil composition of stems and fruits of Caralluma europaea N.E.Br. (Apocynaceae). Molecules 15, 627–638. https://doi.org/10.3390/molecules15020627.