Bulletin of the National Research Centre

RESEARCH Open Access

Oral acute toxicity and antimalarial potentials of aqueous and methanolic extracts of roots, leaves and stem of *Dictyandra arborescens* (Welw.) on *Plasmodium berghei* infected mice

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Abstract

Background: Malaria is one of the tropical diseases of universal concern particularly with continuous appearance of resistant strains of *P.falciparum*. This calls for continous screening of traditional plants such that new and effective antimalarial agents will be developed. This study therefore explored the oral acute toxicity and antimalarial potentials of aqueous and methanolic extracts of roots, leaves and stem of *Dictyandra arborescens* on *Plasmodium berghei* infected mice.

Results: No mortality was recorded in any of the experimental animal groups even at the highest tested dose (5000 mg/kg b.wt) of the extract after monitoring them for 4hrs and subsequently for 7 days. Out of the six extracts, methanolic extracts of the roots and leaves exhibited more antimalarial activity than others. A significant difference ($P < 0.05$) was statistically observed in the parasite count of groups that received methanol extracts of roots and leaves of *D. arborescens*. This observation was made when these two extracts were compared with other groups as well as the negative control. However, activity of the standard antimalarial drug (artesunate) was higher ($p^{<}0.05$) than those of the extracts. Phytochemicals such as tannins, alkaloids, saponins, terpenoids, flavonoids etc. were present in the extracts in varying quantities. GC–MS analysis of methanol extract of the root of this plant showed different chemical compounds.

Conclusion: Administration of aqueous and methanol extracts of roots, leaves and stem of *D. arborescens* in mice is not harmful at any dose less than or equal to 5000 mg/kg. Methanol extracts exhibited more antimalarial activity than aqueous extracts suggesting that antimalarial activity of the plant parts could be affected by the solvent used for extraction and antimalarial activity may be more in a particular part of a plant. The presence of different bioactive compounds identified in phytochemical and GC–MS analysis could be the fundamental scientific evidence for the antimalarial activity exhibited by this plant especially in the root.

Keywords: *Dictyandra arborescens*, Acute toxicity, Antimalarial potentials, Gas chromatography-mass spectrometry, *Plasmodium berghei*

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Background

Malaria is one of the oldest diseases of man which causes more sufferings and deaths when compared to other infectious diseases. It is more prevalent in developing countries and is more severe in expectant mothers and young children (WHO 2017). Various studies reported that the disease causing agent transmitted by mosquitoes is a unicellular parasite called *Plasmodium* whose infective sporozoite stage is carried in the mosquito's salivary glands (Kulihya et al. 2016, Udoh et al. 2016). There are four species of this disease causing agent in humans namely *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium falciparum*. Most incidence of malaria in the tropical regions are due to *Plasmodium falciparum* (Udoh et al. 2016). This specie is equally accountable for most fatal and severe cases of malaria in Africa (Ani 2004). Malaria has been implicated as a major cause of loss of many children per annum (Okafor and Oko-Ose 2012). A report by the Nigerian malaria fact sheet (2011) and World Health Organization (WHO 2016) stated that approximately 97% of Nigerians live in high malaria transmission areas, while the remaining few live in low malaria transmission areas. This makes her the highest bearer of the disease burden in the African region (WHO 2016). This high prevalence has been attributed to rainfall, numerous stagnant water, warm temperature, type and distribution of vegetation cover, poor sanitary conditions etc. which favour the breeding and multiplication of mosquitoes (Ukaegbu et al. 2014). Yet, (Patz and Olson 2006) and (Stresmann 2010) have projected an increase in this figure as a result of climate change. The disease is not only detrimental to the health and lives of Africans, and Nigerians in particular, it equally drains the economy. This is due to reduction in manpower as well as cost of treatment and high death rates related to the disease, which has made malaria a major setback in African development (Ekpenyong and Eyo 2008).

Presently treatment of this disease still relies on the administration of drugs whose effectiveness is always countered by the presence of stubborn strains of the parasite, high cost of orthodox drugs and unavailability of these drugs. Therefore, many caregivers have resorted to using available native plants in the management and treatment of malaria. Medicinal plants which are sources of many potent drugs have been continuously used by various countries. Researchers have developed great interest in screening medicinal plants for their therapeutic activities. These therapeutic activities are conferred on these plants by the active principles of many secondary metabolites found in these plants. Medicinal plants and herbs are used in treating many ailments and infections (Shrivastava et al. 2014; Tomar et al. 2014). Consequently, there is increased interest in research on traditional medicinal and aromatic plants because plant-derived drugs are safe without side effects (Ogidi et al. 2019).

Despite increased threat of malaria to life, particularly in Africa, there is still hope in curbing this disease (White et al. 2004). Such approaches include scientific investigation of traditionally used medicinal plants and herbs (Wright 2005). *Dictyandra arborescens* is a member of flowering plants called the *rubiaceae*. Rubiaceae derived its name from the madder genus *Rubia*. Other plants in this family include cinchona, gambier, ixora, nauclaceae, and theligonaceae. About 630 genera and more than 13,000 species make up the rubiaceae. This positioned rubiaceae as one of the six largest families of angiosperms. Species in this family are usually found in warm and tropical climates (Dalziel 1997). Members of this family are mainly shrubs, although trees and herbs are also present. Although plants in the rubiaceae family are pharmacologically important, very little is known about the medicinal properties of *Dictyandra arborescens* especially its toxic and antimalarial activities. The aim of this study is therefore to evaluate the acute toxicity and in vivo antimalarial potentials of aqueous and methanolic extracts of roots, leaves and stem of *Dictyandra arborescens* (a traditional plant used for the treatment of malaria in Ahiazu Mbaise L.G.A, Imo State, South-East, Nigeria) (Figs. 1, 2).

Methods

Gathering and identification of samples

Fresh roots, leaves and stems of *Dictyandra arborescens* were collected from a fallow farmland at Ahiazu Mbaise L.G.A. Samples were identified by a taxonomist-Dr. M.C Duru in the Department of Biological Sciences, Federal University of Technology, Owerri, Nigeria. They were sliced into pieces, dried at room temperature and ground using a laboratory milling machine. The coarse powder obtained was used to prepare the extracts.

Preparation of extracts

Exactly 200 g of the dry powdered plant samples was soaked in 1000 ml of distilled water (cold maceration) at room temperature with frequent shaking for fourty-eight (48) hours (Ene et al. 2013). Methanol extract was obtained as described by Dejen et al. (2018). Exactly 200 g of the plant parts were extracted with 1000 ml of 80%v/v methanol solvent using soxhlet extraction. Filtrates were obtained using a piece of clean muslin cloth, then with whatman number 1 filter paper. Extracts were dried using a rotary evaporator (RE- 52A, Searchtech Instruments) set at a temperature of 50 °C. Subsequently, extracts were reconstituted in distilled water to obtain the required volume given to the experimental animals.

Fig. 1 *Dictyandra arborescens*

Experimental animals

Only male Swiss albino mice, weighing 18–25 g (7 to 8 weeks old), were used in this study; they were obtained from animal house of Veterinary medicine, University of Nigeria, Nsukka and transferred to animal house of the Department of Biochemistry, University of Nigeria, Nsukka, where the study was carried out. These animals were acclamatized for 14 days with free access to feed (rat pellets) and water ad libitum and monitored under 12: 12hour, light:dark cycles, in well aerated cages. After acclamatization, the experimental animals were then separated into their respective groups according to their body weights and the cages were randomly placed in the room before treatment commenced. The cages were then randomized within the treatment group.

For antimalarial test, animals were included in the study if the percentage parasitemia before treatment was up to 60%. Animals were excluded if their percentage parasitemia before treatment was below 60%. Animals whose body weights were below 18 g or above 25 g were also excluded from the present study. This study was conducted after obtaining ethical approval from the Animal Research and Ethics committee of the Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. A written consent was obtained whereby all the participants/authors filled and signed an informed consent form. No reference number was issued.

Phytochemical screening

Crude aqueous and methanolic extracts of roots, leaves and stem of *D. arborescens* were screened for phytochemicals using standard methods (Trease and Evans 1989, Harborne 1973, 1998, Sofowora 1993, Raboy et al. 2000, Kgosana 2019) in order to identify biological components.

Oral Acute toxicity evaluation

Oral acute toxicity (LD_{50}) of roots, leaves and stem of *Dictyandra arborescens* was determined using modified method of Lorke (1983) based on the description of Khan et al. (2015). This method involved two (2) phases with a total of 108 male Swiss albino mice. In the first phase, for each of the six extracts, nine male Swiss albino mice were randomly divided into 3 groups of 3 mice each ($n=3$) and increasing doses of 10, 100 and 1000 mg/kg body weight of the extracts were administered orally to groups 1, 2 and 3 respectively. These were observed for the first 4 h and subsequently for 7 days for any signs of toxicity and mortality. These signs include pains, loss of appetite, difficulty in respiration, paw licking, weakness and death. In the second phase (which was carried out when no death was recorded in the first phase), for each of the six (6) extracts, high doses of 1600, 2900 and 5000 mg/kg body weight of the extracts were administered to another 3 random groups of 3 ($n=3$) fresh male Swiss albino mice through the same route. They were observed for 4 h for signs of toxicity and mortality and subsequently for 7 days as in the first phase. This test was used to determine the safe dose of the extract that was administered to the experimental animals during the in vivo antimalarial test. Acute toxicity was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 x D_{100}}$$

where, D_0 = Highest dose that gave no mortality, D_{100} = Lowest dose that produced mortality

Animal grouping and administration of extracts and standard antimalarial drug for antimalarial test

Fifty-four (54) male Swiss albino mice weighing 18–25 g (7–8 weeks old) used for the study were sourced from animal house of Veterinary Medicine, University of Nigeria, Nsukka. The mice were accommodated in well aerated aluminum cages and acclimatized to laboratory conditions for a 2–week period under naturally illuminated environment with regular feeding (rat pellets) and water ad libitum, and monitored under 12:12 h dark/light cycle. These animals were used according to NIH guide

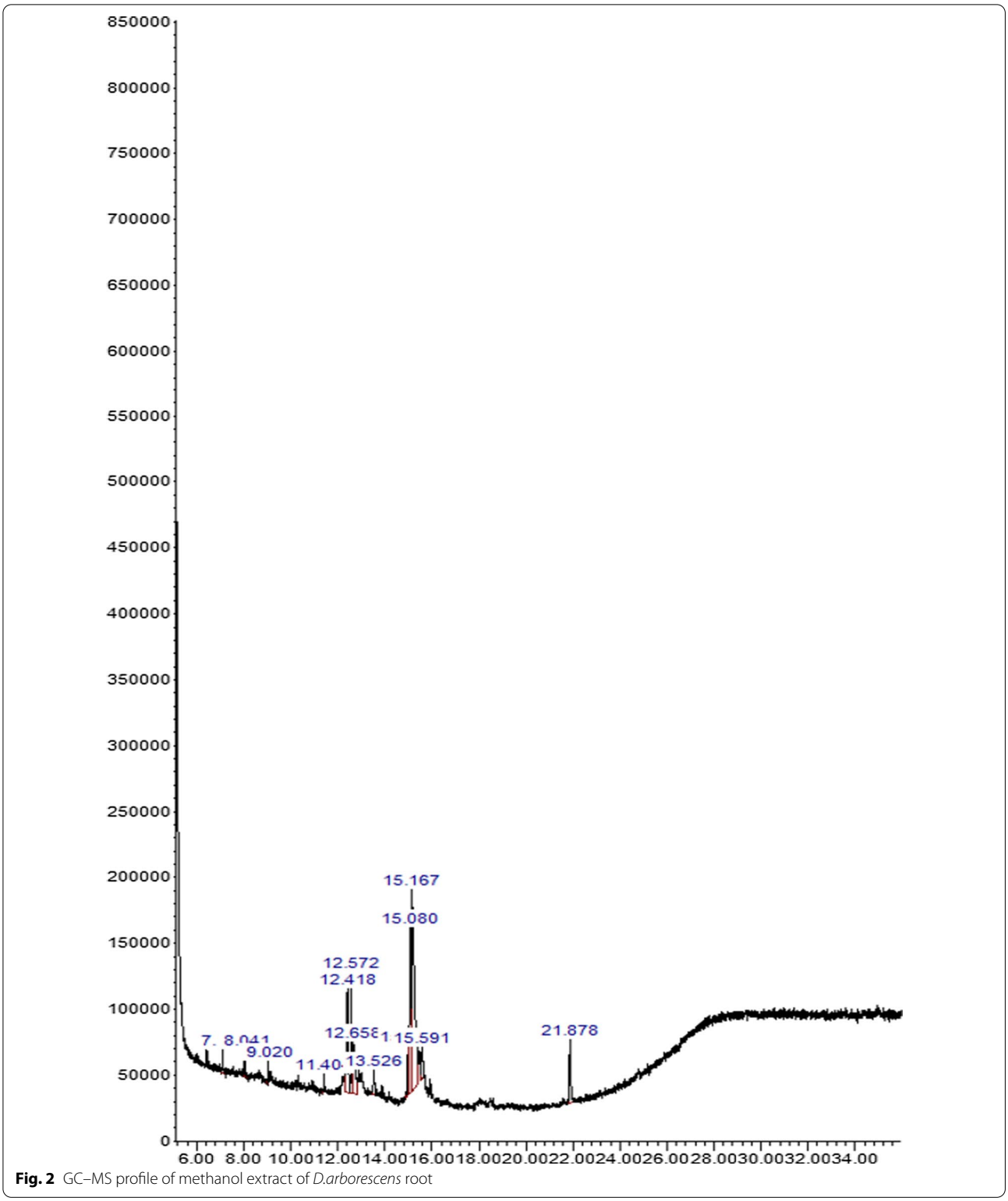


Fig. 2 GC–MS profile of methanol extract of *D.arborescens* root

for caring and usage of laboratory animals (NIH 1985) Standard operating manual of NIPRD. They were separated into 9 groups of 6 mice each ($n=6$) according to their body weights. The sample size ($n=6$) was selected based on information extracted from literature. A small sample size was selected because antimalarial potentials of aqueous and methanol extracts of *D. arborescens* roots, stem and leaves were tested in vivo for the first time in

the present study. Therefore, the initial intention was to gather basic evidence regarding the use of these extracts in the treatment of malaria.

Therefore, the initial intention was to gather basic evidence regarding the use of these extracts in the treatment of malaria. On the basis of their position on the rack, the cages were given numerical designation. For each treatment group, a cage was selected randomly from the pool of cages. The cages were then randomized within the treatment group.

Grouping of animals were as follows:

Group 1—feed + water (normal control).

Group 2—infected with *P.berghi* + 200 mg/kg b.w of artesunate (standard control group).

Group 3—infected with *P.berghei* + no treatment (negative control).

Group 4—infected with *P.berghei* + 200 mg/kg b.w methanolic extract of *D. arborescens* root.

Group 5—infected with *P.berghei* + 200 mg/kg b.w methanolic extract of *D. arborescens* leaves.

Group 6—infected with *P.berghei* + 200 mg/kg b.w methanolic extract of *D. arborescens* stem.

Group 7—infected with *P.berghei* + 200 mg/kg b.w aqueous extract of *D. arborescens* root.

Group 8—infected with *P.berghei* + 200 mg/kg b.w aqueous extract of *D. arborescens* leaves.

Group 9—infected with *P.berghei* + 200 mg/kg b.w aqueous extract of *D. arborescens* stem.

In vivo *culture of Plasmodium berghei*

Plasmodium berghei infected blood from a donor mice was used to infect the animals used for experiment. Parasitized blood was first diluted with normal saline as described by Kabiru et al. (2012). This innoculum was prepared such that each 0.2 ml contained approximately 1×10^7 parasitized erythrocytes. The animals were then injected with 0.2 ml of this suspension on day 0 through intraperitoneal route and were allowed to stand for seventy-two hours (72 h) so that infection will be fully established. *P. berghei* was maintained by weekly serial blood passage from infected animals to non infected ones.

In vivo treatment of infected animals

A 4-day curative test described by David et al. (2004), Peter and Anatoli (1998) was used for the in vivo anitimalarial test. Only animals whose percentage parasitemia was up to 60% or more were included in the treatment groups. Animals whose percentage parasitemia was below 60% were excluded in the treatment groups. Treatment commenced 72 h (day 3) later, after establishment of infection. All animals in the treatment groups orally received 200 mg/kg body weight of a known antimalarial drug (artesunate) and 200 mg/kg body weight of extracts once a day for four consecutive days. A dose of 200 mg/kg body weight was administered based on earlier results from acute toxicity test which was up to 5000 mg/kg body weight for the aqueous and methanol extracts of the roots, leaves and stem of *D. arborescens*. Treatment was administered based on the average body weight of experimental animals.

Parasite count was observed in all the groups on day 7 (4 days after treatment) and on day 14 (7 days post treatment) as described by Arrey et al. (2014) using blood smears made from a cut on the tail tip of each mice. Blood smears were prepared by fixing with methanol, stained with 10% Geimsa for 25 min, washed with phosphate buffer (pH 7.2) and dried. Parasitemia was examined microscopically (magnification = X100) under a drop of immersion oil. Percentage parasitemia was calculated as outlined by Iwalewa et al. (1997):

$$\% \text{ Parasitemia} = \frac{\text{No of Parasitemia in Treated}}{\text{No of Parasitemia in control}} \times \frac{100}{1}$$

Final body weight and percentage survival rates were also monitored after treatment with the extracts.

Euthanization of animals

At the end of the experiment, animals were euthanized as described by Moody et al. (2014). The animals were placed in a transparent perspex chamber and euthanized with slow rising concentrations of carbon dioxide using a gradual fill (30% chamber volume per minute) technique. Animals were monitored until they lost consciousness.

In this experiment, three different investigators were involved as follows: the first investigator administered the treatment based on the randomization table. This investigator was the only person aware of the treatment allocation. A second investigator unaware of the treatment was responsible for checking the toxicity and parasitemia level, while a third investigator was responsible for the euthanization procedure.

Gas chromatography-mass spectrometry (GC–MS) analysis of methanol extract of D. arborescens roots

The sample was analyzed as described by Pakkirisamy et al. (2017) using agilent technologies 7890A GC and 5977B MSD with experimental conditions of GC–MS system as follows: Hp 5-MS capillary standard non-polar column, dimension: 30 M, ID: 0.25 mm, Film thickness: 0.25 µm. Flow rate of mobile phase (carrier gas—Helium) was set at 1.0 ml/min. in the gas chromatography part, temperature programme (oven temperature) was 40^0C raised to 250 °C at 5^0C/min and injection volume was 1 µl. Samples dissolved in methanol were run fully scan at a range of 40–650 m/z and the results were compared and interpreted using National Institute Standard and

Technology (NIST) Mass Spectral library database search programme with over 62,000 patterns for identifying chemical components.

Statistical analysis

Data obtained were presented as mean of three replicates ± standard error of mean (SEM). Analysis was done using one-way analysis of Variance (ANOVA) procedure using statistical analysis system (SAS) package version 20 software (Betty and Stern 2003). Mean differences were subjected to Duncan's multiple range test (DMRT) and significance level was set at $P < 0.05$.

Table 1 Yield of extracts (%) from the leaves, roots and stem of *Dictyandra arborescens*

Plant parts	Solvent (ml)	Percentage yield (% w/w)
D.arborescens leaves	Methanol	24.9
D.arborescens leaves	Aqueous	21.3
D.arborescens root	Methanol	20.6
D.arborescens root	Aqueous	16.7
D.arborescens stem	Methanol	12.3
D.arborescens stem	Aqueous	8.1

Results

Yield of extracts

Exactly 200 g of each plant part (roots, leaves and stem) of *D. arborescens* were used for extraction but different yield of extract was obtained from the two solvents. As seen on Table 1, the highest yield (24.9%) of extract was obtained from methanolic extract of *D. arborescens* leaves, followed by aqueous extract of the same plant part whose yield was 21.3%. The least yield (8.1%) was obtained from aqueous extract of the stem of this plant. Comparatively, the yield was higher in the methanolic extracts than in the aqueous extracts.

Acute toxicity test

No death was recorded in the first phase of the experiment where doses of 10, 100 and 1000 mg/kg body weight of the extracts were administered to the experimental animals for the different extracts (Tables 2). In the second phase, where high doses of the extracts (1600, 2900, and 5000 mg/kg body weight) were administered to the experimental animals, no sign of toxicity or death was

Table 2 Acute toxicity test of the aqueous and methanolic extracts of the roots, leaves, and stem of *Dictyandra arborescens* at doses of 10, 100 and 1000 mg/kg body weight

Extracts	Number of animals per group	Dose of extract/weight of mice (10 mg/kg)	Signsof toxicity	Number of animals that survived
DARM	3	22 g	x	3/3
DARA	3	20 g	x	3/3
DALM	3	18 g	x	3/3
DALA	3	25 g	x	3/3
DASM	3	24 g	x	3/3
DASA	3	23 g	x	3/3
100 mg/kg				
DARM	3	24 g	x	3/3
DARA	3	20 g	x	3/3
DALM	3	25 g	x	3/3
DALA	3	19 g	x	3/3
DASM	3	21 g	x	3/3
DASA	3	24 g	x	3/3
1000 mg/kg				
DARM	3	21 g	x	3/3
DARA	3	19 g	x	3/3
DALM	3	23 g	x	3/3
DALA	3	22 g	x	3/3
DASM	3	19 g	x	3/3
DASA	3	21 g	x	3/3

DARM = *D. arborescens* root methanol, DARA = *D.arborescens* root aqueous, DALM = *D.arborescens* leaves methanol, DALA = *D.arborescens* leaves aqueous, DASM = D.*arborescens* stem methanol, DASA = *D.arborescens* stem aqueous, x = no sign of toxicity

also recorded in the albino mice for aqueous and methanolic extracts of roots, leaves and stem of *Dictyandra arborescens*. This indicated that these extracts were safe, even at a very high dose of 5000 mg/kg body weight (Table 3).

Table 3 Acute toxicity test (LD_{50}) of the aqueous and methanolic extracts of the roots, leaves, and stem of *Dictyandra arborescens* at high doses of 1600, 2900 and 5000 mg/kg body weight

Extracts	Number of animals per group	Doseof extract/ Weight of mice (1600 mg/kg)	Signs of toxicity	Number of animals that survived
DARM	3	19 g	x	3/3
DARA	3	22 g	x	3/3
DALM	3	20 g	x	3/3
DALA	3	18 g	x	3/3
DASM	3	20 g	x	3/3
DASA	3	20 g	x	3/3
2900 mg/kg				
DARM	3	20 g	x	3/3
DARA	3	21 g	x	3/3
DALM	3	21 g	x	3/3
DALA	3	21 g	x	3/3
DASM	3	23 g	x	3/3
DASA	3	22 g	x	3/3
5000 mg/kg				
DARM	3	24 g	x	3/3
DARA	3	19 g	x	3/3
DALM	3	23 g	x	3/3
DALA	3	20 g	x	3/3
DASM	3	22 g	x	3/3
DASA	3	24 g	x	3/3

DARM = *D. arborescens* root methanol, DARA = *D.arborescens* root aqueous, DALM = *D.arborescens* leaves methanol, DALA = *D.arborescens* leaves aqueous, DASM = *D.arborescens* stem methanol, DASA = *D.arborescens* stem aqueous, x = no sign of toxicity

Effects of extracts on established infection

Aqueous and methanolic extracts of roots, leaves and stem of *D. arborescens* exhibited a significant ($P<0.05$) decrease in parasitemia across the treatment groups unlike the infected and untreated group (group 3) where there was consistent increase in parasitemia. Table 4 showed the effects of crude aqueous and methanolic extracts of the roots, leaves and stem of *D. arborescens* on parasite count of *Plasmodium berghi* infected albino mice. Parasitemia was established in the groups infected with *P. berghei* after 72 h. Parasite count/percentage parasitemia in the group that received no treatment (Group 3) significantly ($P<0.05$) increased on day 7 while it significantly ($P<0.05$) reduced in all groups that received treatment. The experimental animals were further monitored till the 14^{th} day (7 days post treatment); results obtained revealed that parasite count/percentage parasitemia in all treated animals further reduced significantly ($p^{<}0.05$) but increased in the negative control group (Tables 4 and 5). When compared with the known antimalarial drug (artesunate), results showed that the plant extracts recorded a significant difference ($P<0.05$). Unlike the extracts, the standard antimalarial drug (artesunate) cleared all the parasites by the 14^{th} day. The significant ($P<0.05$) decrease in parasite count was more evident in groups 4 and 5 which received methanol extract of *D. arborescens* root and methanol extract of *D. arborescens* leaves, respectively. Increase in body weight as well as higher survival rate were also observed in animals across the experimental groups except the infected

Table 4 Activities of crude aqueous and methanolic extracts of roots, leaves and stem of *D.arborescens* on parasite count of male Swiss albino mice infected with *Plasmodium berghi*

Groups/extracts	Parasite count in days			
	Before inoculation (Day 0)	Before treatment (Day 3)	After treatment (Day 7)	After treatment (Day 14)
1. NC	0.00	$0^{b} \pm 0.00$	$0^{f} \pm 0.00$	$0^{f} \pm 0.00$
2. STD.C	0.00	$59^{a} \pm 2.00$	$4.0^{e} \pm 0.04$	$0.00^{f} \pm 0.00$
3. NEG.C	0.00	$59^{a} \pm 2.00$	$74^{a} \pm 2.39$	$99^{a} \pm 3.90$
4. DARM	0.00	$63^{a} \pm 3.40$	$33^{d} \pm 1.30$	$14^{e} \pm 0.85$
5. DALM	0.00	$61^{a} \pm 2.41$	$35^{d} \pm 1.33$	$18^{e} \pm 0.93$
6. DASM	0.00	$61^{a} \pm 2.48$	$42^{c} \pm 1.43$	$40^{b} \pm 1.41$
7. DARA	0.00	$60^{a} \pm 2.39$	$37^{c} \pm 1.35$	$25^{d} \pm 1.23$
8. DALA	0.00	$62^{a} \pm 2.45$	$38^{c} \pm 1.37$	$27^{d} \pm 1.26$
9. DASA	0.00	$62^{a} \pm 2.45$	$50^{b} \pm 1.52$	$42^{b} \pm 1.43$

Values are average parasite count ± SEM of 6 replicates. Mean values with different superscripts along the same column are significantly different ($P<0.05$)

NC = Normal control, STD.C = Standard control, NEG.C = Negative control, DARM = *D. arborescens* root methanol, DALM = *D.arborescens* leaves methanol, DASM = *D. arborescens* stem methanol, DARA = *D.arborescens* root aqueous, DALA = *D.arborescens* leaves aqueous, DASA = *D.arborescens* stem aqueous,

Table 5 Activities of crude aqueous and methanolic extracts of roots, leaves and stem of *D. arborescens* on percentage parasitemia of *Plasmodium berghei* infected male Swiss albino mice

Groups/extracts	Average % Parasitemia			
	Before inoculation (Day 0)	Before treatment (Day 3)	After treatment (Day 7)	After treatment (Day 14)
1. NC	0.00	0.00	0.00	0.00
2. STD.C	0.00	69.0	4.6	0.00
3. NEG.C	0.00	63.0	72.4	79.2
4. DARM	0.00	75.2	39.8	16.8
5. DALM	0.00	72.8	41.4	21.2
6. DASM	0.00	73.2	51.4	48.4
7. DARA	0.00	72.0	43.4	31.0
8. DALA	0.00	74.6	45.2	32.0
9. DASA	0.00	77.4	60.0	49.8

NC = Normal control, STD.C = Standard control, NEG.C = Negative control, DARM = *D. arborescens* root methanol, DALM = *D.arborescens* leaves methanol, DASM = *D.arborescens* stem methanol, DARA = *D.arborescens* root aqueous, DALA = *D.arborescens* leaves aqueous, DASA = *D.arborescens stem aqueous*

but untreated group (group 3) whose body weight and survival rate decreased (Table 6).

Discussion

Aqueous (water) and methanol were used as extracting solvents in this study is in line with folklore practice. Extraction yield (% w/w) measures the efficiency of a solvent to extract particular components from original plant materials. It refers to the amount of solid extract recovered in mass compared to the initial amount of plant material. Percentage yield of extract from methanol solvent was more than that of the aqueous solvent (Table 1). This result agrees with report by Wilcox et al. (2014) that phytochemicals are more soluble in organic solvents. Polarity of the various extracted compounds may be responsible for the difference in yield of extracts (Pareck et al. 2015). Low polarity of methanol could be accountable for the high yield of extracts because both polar and non polar compounds were extracted by the solvent. Methanol extracted more of the phytochemicals than the aqueous solvent implying that crude extracts obtained from *D. arborescens* could be affected by solvents used for extraction. This is in agreement with (Peace et al. 2011), who reported that aqueous methanol gave more yield of extractable solids in barley and flax seed respectively. Findings in this study suggested that most of the compounds in this plant are dissolveable in methanol and a mixture of 20% (v/v) water with methanol was a good choice for obtaining better extractable solids from this plant. Maceration used in this study has been reported to give low yield of extract when compared with other methods of extraction (Peace et al. 2011). It was selected as an extraction method in this study because heating is not required, thus preserving the phyto-constituents.

Africa is gifted with a myriad of medicinal plants. Indigenous people acquire this knowledge, preserve it and pass to their generations. Pharmacological activities of medicinal plants are attributed to their phytochemical contents (Ihekwereme et al. 2016). Results obtained in this study showed that *D. arborescens* is rich in various phytochemicals (Table 8). Tannins are major active components of medicinal plants (Haslam 2016). Tannins have antioxidant, anti-inflammatory, antibacterial, antiviral, antimicrobial, antimalarial properties (Vattern et al. 2015 and Mori et al. 2017). Terpenoids have biological activities and are used in fighting diseases such as malaria, inflammation, bacterial infections, cancer

Table 6 Activities of crude aqueous and methanol extracts of roots, leaves and stem of *D.arborescens* on percentage body weight and survival rate of male Swiss albino mice *infected with Plasmodium berghi*

Groups/extracts	Number of animals	Initial body weight of animals (g)	Final body weight of animals (g)	% body weight of animals	% survival rate
1: NC	6	23.0	26.7	16.1	100
2: STD.C	6	18.0	20.8	15.6	100
3:NEG.C	6	20.0	17.5	12.5	33.3
4: DARM	6	21.0	23.6	12.4	100
5:DALM	6	19.0	21.2	11.6	100
6:DASM	6	21.0	22.8	8.6	66.7
7:DARA	6	19.0	21.0	10.5	100
8:DALA	6	20.0	22.0	11.0	100
9:DASA	6	23.0	24.5	6.5	50

NC = Normal control, NEG.C = Negative control, STD.C = Standard control, DARM = *D. arborescens* root methanol, DALM = *D.arborescens* leaves methanol, DASM = *D. arborescens* stem methanol, DARA = *D.arborescens* root aqueous, DALA = *D.arborescens* leaves aqueous, DASA = *D.arborescens* stem aqueous

etc. (Ibrahim et al. 2005, Abdul-Fadl et al. 2011; Sunita et al. 2017). Alkaloids are significant biologically active compounds found in plants. The presence of this class of compounds brings about certain physiological changes in organisms such as antimalarial, antifungal, antibacterial (Stary 1998). Flavonoids have been reported to have significant in vitro antimalarial activity against *P. falciparum* (Chanphen et al. 2018). They also have effects against free radical, microbes, and tumour (Farquar 1996). Cardiac glycosides were present in the aqueous and methanol extracts of *D. arborescens*. This class of phytochemicals are referred to as 'natural drugs' because of their actions in the treatment of heart related problems (Radford et al. 2016). Activities of phenols also include antioxidant, anti-inflammatory, and antimalarial activities (Ovenden et al. 2011; Han et al. 2017). Steroids which were also present in the plant extracts have been reported to have anti-inflammation effects (Savithramma and Linga 2011). Saponins have anti-hyper cholesterol and haemolytic effects (Sodipo et al. 2010). Some medicinal herbs with high oxalate contents are used in the treatment of bronchitis, ringworm, skin diseases etc. (Huang and Liebman 2015). Consumption of phytate prevents formation of kidney stone, offers protection against atherosclerosis, cancer etc. (Navarro et al. 2016; Zheng et al. 2014). Since many of these phytochemicals have antimalarial potentials, their synergistic effect could have been responsible for the antimalarial properties exhibited by this plant.

Results showed that aqueous and methanolic extracts of *D. arborescens* roots, leaves and stem have negligible toxicity as recorded in their LD_{50} value of 5000 mg/kg b.wt. These extracts did not produce any physical signs of toxicity such as paw licking, salivation, stretching, weakness or death. Their LD_{50} was thus estimated to be ≥ 5000 mg kg^{-1} because no mortality was recorded even at a high dose of 5000 mg kg^{-1} body weight. Absence of mortality following oral administration of extracts at 5000 mg kg-1 body weight observed in the experimental animals indicated that the extracts were not toxic acutely. These results are in consonance with the reports of Onwusonye et al. (2014) and Ma et al. (2006) who reported no mortality in Swiss albino mice treated with 10 to 5000 mg kg^{-1} body weights of leaf extract of *Aspilia africana* (Pers), methanol leaf extract of *Annona senegalensis* and hydroethanolic stem extract of *Baphia pubescens* respectively. Testing for antimalarial properties of extracts from a particular plant is a preliminary stage in isolating novel compounds with potent activities (Ma et al. 2006; Njoroge and Bussmann 2006). Crude aqueous and methanolic extracts of roots, leaves and stem of *D. arborescens* showed significant antimalarial activities against *P. berghei* infection in male Swiss albino mice which was evident in the reduction of parasite count and decrease in percentage parasitemia (Tables 4 and 5). Results from this study clearly showed that treatment with aqueous and methanol root, leaf and stem extracts of *D. arborecsens*, significantly ($P<0.05$) reduced parasite count in infected mice and improved their survival and body weight. The differences between the antimalarial activities of methanol extracts of the roots and leaves of *D. arborescens* differed significantly ($p^{<}0.05$) from their aqueous extracts (Table 2). This gave an indication that the bioactive constituents of this plant were more soluble in methanol than the aqueous medium (Peace et al. 2011). Water is denser than methanol which may diffuse more in the same medium than water. Another possibility is that the bioactive compounds were more soluble in methanol than water which gave methanol extracts the benefit of having more of the bioactive compounds. This may be accountable for the higher antimalarial activity recorded for methanol extracts against the aqueous extracts. This corroborates the report of Ezeokeke et al. (2015). However, activity of the standard antimalarial drug (artesunate) was higher ($P<0.05$) than activities of the extracts.

The antimalarial activities of *D. arborescens* could be credited to the synergy between many phytochemicals present in the extracts as seen in Tables 7 and 8. Although the exact phytochemical responsible for the antimalarial activity is not known, studies have shown that tannins, saponins, flavonoids, alkaloids etc. inhibit malaria parasites in infected animals (Igile et al. 1994; Udensi et al. 2002). Saponins have also been reported to have antiprotozoal activity (Wallace et al. 1994; Newbold et al. 1997).

Aqueous and methanol extracts of the stem of *D. arborescens* exhibited the least antimalarial activities on *Plasmodium berghei* infected mice. This could be attributed to the fact that the stem of this plant is not woody. This corroborates earlier reports by Mathaura et al. (2017), that plants whose stem extracts have significant antimalarial activities are trees with stem bark. Survival rate of mice treated with the various extracts supported the prolonged effects of the antimalarial compounds in these two plants. Although aqueous and methanol extracts of the stem exhibited post-treatment antimalarial activities, they had the least weight gain and survival rate. This may be attributed to the type of antimalarial compound present in the stems and their quantities. These results are in line with the report of Soniran et al. (2011), who evaluated in vivo antiplasmodial activities of extracts *Morinda morindiodes* (Bak.) in the treatment of malaria. The decrease in body weight observed in animals in the infected and untreated animals was attributed to parasite feeding on blood cells of the animals which reduced their body weight. This corroborates reports of Ene et al. (2013). Comparatively, survival rate was higher in

Table 7 Phytochemicals present in aqueous and methanolic extracts of roots, leaves and stem of *D.arborescens*

Phytochemical	Specific test	DALM	DASM	DARM	DALA	DASA	DARA
Saponins	Frothing test	+	+	+	+	+	+
Alkaloids	Wagner's test	+	+	+	+	+	+
	Dragendorff's test						
Cardiac glycosides	Keller-Kilani test	+	+	+	+	+	+
Terpenoids	Salkowski test	+	+	+	+	+	+
Steroids	Liebermann-Burchard test	+	+	+	+	+	+
Phenols	Ferricchloride test	+	+	+	+	AB	+
Flavonoids	Sodium Hydroxide test	+	+	+	+	+	+
Oxalate	Oxalate test	+	+	AB	+	+	AB
Phytate	Colorimetric assay	+	+	+	+	+	+
Tanins	Ferricchloride test	+	+	+	+	+	+

+ = present, AB = Absent

DALM = *D.arborescens* leaves methanol, DASM = *D.arborescens* stem methanol, DARM = *D. arborescens* root methanol, DALA = *D.arborescens* leaves aqueous, DASA = *D.arborescens* stem aqueous, DARA = *D.arborescens* root aqueous

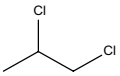
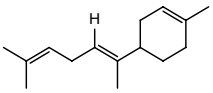
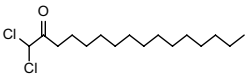
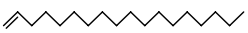
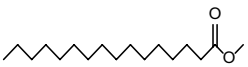
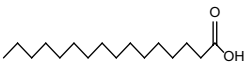
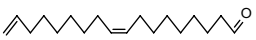
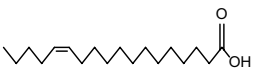
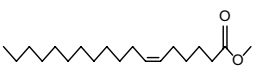
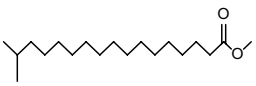
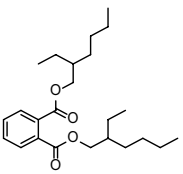
Table 8 Quantitative determination of phytochemicals in the methanolic and aqueous extracts of the roots, leaves, and stems of *Dictyandra arborescens*

Phytochemicals	Methanolic	Extracts		Aqueous	Extracts		References
	DAL	DAS	DAR	DAL	DAS	DAR	
Tanins (%)	31.24 ± 0.03	12.83 ± 0.03	21.15 ± 0.03	16.10 ± 0.13	10.60 ± 0.07	14.74 ± 0.22	Uyotismita and Arindan (2015)
Saponins (%)	20.60 ± 0.05	13.23 ± 0.02	15.65 ± 0.09	18.90 ± 0.05	3.80 ± 0.05	3.10 ± 0.03	Harbourne et al.(1998)
Alkaloids(%)	3.16 ± 0.02	1.66 ± 0.03	8.95 ± 0.05	1.89 ± 0.11	1.20 ± 0.01	4.00 ± 0.22	Harbourne et al. (1998)
Cardiac glycosides (%)	27.0 ± 2.02	11.79 ± 0.13	14.79 ± 0.05	15.80 ± 0.20	9.87 ± 0.16	11.92 ± 0.09	Osagie (1998)
Terpenoids (mg/100 g)	386.80 ± 0.05	50.40 ± 0.12	148.16 ± 0.03	359.41 ± 0.13	48.39 ± 0.18	148.20 ± 0.21	Ejikeme et al. (2014)
Steroids (mg/100 g)	31.04 ± 0.05	129.92 ± 0.65	50.94 ± 0.06	29.22 ± 0.27	127.80 ± 0.14	42.43 ± 0.27	Owiredu et al. (2013)
Phenols (mg/g)	11.26 ± 0.08	0.66 ± 0.11	11.16 ± 0.07	7.16 ± 0.02	_	8.02 ± 0.05	Ezeonu and Ejikeme (2016)
Flavonoids (%)	28.71 ± 0.29	3.22 ± 0.10	22.99 ± 0.39	7.25 ± 0.31	3.08 ± 0.06	5.61 ± 0.13	Ejikeme et al. (2014)
Oxalate (mg/100 g)	$1.9 \pm 0.06 \times 10^4$	$5.2 \pm 0.06 \times 10^4$	_	$1.20 \pm 0.09 \times 10^4$	$3.8 \pm 0.02 \times 10^4$	_	Osagie (1998)
Phytate (%)	2.47 ± 0.18	6.50 ± 0.14	3.25 ± 0.14	1.17 ± 0.12	3.05 ± 0.23	2.20 ± 0.09	Unuofin et al. (2017)

animals in the treatment groups than the infected and untreated group, suggesting that extracts used in this study were not toxic to the animals at the administered doses (Table 6). Observed deaths may be due to effects of the parasites. This agreed with the report of Adeyemo-Salami et al. 2014.

Several chemical compounds were identified in the GC–MS analysis of methanol extract of *D. arborescens root* as seen in Table 9. 1-Octadecene has been reported to have therapeutic activities such as antioxidant, antibacterial (Mishra and Sree 2007). n-Hexadecanoic acid has anti-inflammatory, antioxidant, mosquito larvicide, nematicide effects (Rahuman et al. 2000; Aparna et al. 2012). The identified 9,17 Octadecadienal, (Z)-has antimicrobial activity (Rajeswari et al. 2013) while 6-Octadecenoic acid methyl ester (Z)- has been reported to have anti-inflammatory, antioxidant, anticancer activities (Geetha and Varalakshmi 2001). Hexadecanoic acid is a methyl ester with antioxidant, antimicrobial, hypocholesterolemic, nematicide, pesticide, antiandrogenic, insecticide effects (Akpuaka et al. 2013). Heptadecanoic acid, 16 methyl-, methyl ester has activities such as Protein, anticancer (Elaiyaraja and Chandramohan 2018). Bis (2-ethylhexyl) phthalate has been reported to have antimicrobial, antifungal (Habib and Karim 2009), and antitumor potentials (Habib and Karim 2012). Therefore, the occurrence of these compounds in *D. arborescens* root could be responsible for the afore-mentioned activity exhibited by this plant.

Table 9 Compounds identified in GC–MS analysis of methanol extract of *D.arborescens* roots

PK	RT	Name of compound	Molecular structure	Molecular formular	Molecular weight (g/mol)	Percentage (%)
1	7.10	1,2-dichloropropane		$C_3H_6C_{12}$	112.98	1.14
2	8.04	Cis-alpha-bisabolene		$C_{12}H_{24}$	204.35	1.32
3	9.02	Dichloroacetic acid tridecyl ester		$C_{15}H_{28}Cl_2O_2$	311.3	0.91
4	11.40	1-Octadecene		$C_{18}H_{36}$	252.5	0.61
5	12.42	Hexadecanoic acid methyl ester		$C_{17}H_{34}O_2$	270.45	28.20
6	13.53	n-hexadecanoic acid		$C_{16}H_{32}O_2$	256.43	2.30
7	15.00	9,17-Octadecadienal, (Z)-		$C_{19}H_{34}O_2$	294.47	3.55
8	15.08	Cis-13-Octadecenoic acid		$C_{21}H_{40}O_2$	324.5	15.77
9	16.17	6-Octadecenoic acid methyl ester, (Z)-		$C_{19}H_{36}O_2$	282.47	36.80
10	15.59	Heptadecanoic acid, 16 methyl-, methyl ester		$C_{19}H_{38}O_2$	298.5	3.61
11	21.88	Bis(2-ethylhexyl) phtalate		$C_{24}H_{38}O_4$	390.56	5.79

Conclusion

Results obtained in this study revealed that aqueous and methanol extracts of roots, leaves and stem of *D. arborescens* were not harmful even at a high dose of 5000 mg/kg b.wt. The extracts equally posses antimalarial activities. This justifies the belief and use of this plant in ethnomedicine for the treatment of malaria. The presence of different bioactive compounds identified in phytochemical and GC–MS analysis could be the fundamental scientific evidence for the antimalarial activity exhibited by this plant especially in the root.

Dictyandra arborescens could therefore represent a prospective source of antimalarial drug development. Since many existing antimalarial drugs are natural product-based, there is hope that perharps, new antimalarial drugs may emerge from our tropical plants if properly harnessed. Findings in this study therefore justified the rationale behind the pharmacological usage of this plant in traditional setting especially in the treatment of malaria.

Abbreviations

mg/kg: Milligram/kilogram; b/wt: Body weight; L_D: Lethal dose; NIH: National institute of health; *P.berghei*: *Plasmodium berghei*; GC–MS: Gas chromatography-mass spectrometry.

Acknowledgments

Authors are grateful to Dr. M.C Duru of the Department of Biological Sciences, Federal University of Technology, Owerri, Mr Rex Ezeh of Department of Biochemistry, University of Nigeria, Nsukka, Engr. Clement Obot of New Concepts Laboratories Ltd, Owerri, Nigeria, as well as staff of ChemSolvers Research and Computational Laboratories, Owerri, Nigeria for their technical assistance.

Authors' contributions

Author UEE collected the plant samples, carried out the research work and wrote the manuscript, Author NCU, ICM, and HCN designed and supervised

the laboratory work, Author CKE analyzed the data, Author CED interpreted the GC–MS result while Author OIO helped in the literature search. All authors read and approved the manuscript and gave permission to submit the manuscript for publication. All authors read and approved the final manuscript.

Funding
Authors received no funding for this study.

Availability of data and materials
All the data generated or analyzed during the study are included in this published article.

Declarations

Ethics approval and consent to participate
This study was conducted after obtaining ethical approval from the Animal Research and Ethics committee of the Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. A written consent was obtained whereby all the participants/authors filled and signed an informed consent form. No reference number was issued.

Competing interests
The authors declare that they have no competing interests.

Consent for Publication
Not applicable.

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Received: 26 December 2020 Accepted: 22 March 2021
Published online: 14 April 2021

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