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Original Research Article

Phytochemical and *In-vivo* Antimalarial Investigations of *Dichrostachys cinerea* (L.) Wight & Arn. (Fabaceae) Root Bark

Labake A. Fadipe[1]*, Chinedu Ajemba[1], Bilqis A. Lawal[2], Augustine A Ahmadu[3], Gabriel F. Ibikunle[1]

[1]*Department of Chemistry, School of Physical Sciences, Federal University of Technology, Minna, Nigeria*
[2]*Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Nigeria*
[3]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna, Nigeria*

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ABSTRACT

The various parts of *Dichrostachys cinerea* (L.) are ethnomedicinally useful in the management of malaria. This study investigates the *in-vivo* antimalarial potentials of the ethanol extract of *D. cinerea* root bark and its fractions, as well as, the isolation and characterization of a phytoconstituent from one of the active fractions. Phytochemical, acute toxicity and antimalarial potentials of the crude ethanol extract of *D. cinerea* (E_D) its chloroform- (E_DC), ethyl acetate- (E_DE) and butanol- (E_DB) fractions were evaluated. The extract, E_D and its fractions revealed no toxicity in mice, up to 2000 mg/kg. *In-vivo* antimalarial testing of extract and fractions against *P. berghei* using the 4-day suppressive testing revealed that fractions E_DC and E_DB at 600 mg/kg significantly ($p < 0.05$) suppressed the level of parasitemia, prolonged the mean survival time ($p < 0.05$) and protected infected mice against reductions in rectal temperature ($p > 0.05$), body weights ($p > 0.05$) and packed cell volume ($p < 0.05$); all in comparison with Chloroquine at 25 mg/kg/day. Application of different chromatographic and spectroscopic techniques to fraction E_DB led to the isolation and characterization of Methyl 2, 3-dihydroxy-4-methoxybenzoate (a derivative of methyl gallate). These findings revealed that the strong presence of phenolic constituents, either acting singly or synergistically with other bioactive compounds probably contributed to the antimalarial activity of factions E_DC and E_DB and hence, the folkloric use of *D. cinerea* root bark as an antimalarial agent; an indication that the plant might be a potential source of novel antimalarial agent(s).

Keywords: Antimalarial, *Dichrostachys cinerea*, Parasitemia, Phenolic derivative, Root bark.

Introduction

Dichrostachys cinerea (Linnaeus) Wight and Arn (family: Fabaceae/Leguminoseae/Mimosaceae) is a deciduous tree, commonly known as Sickle bush (English), Dundu (Hausa), Ami-ogwu (Igbo) and Kara (Yoruba). Traditionally, decoctions of the roots of the plant are used to make mouth washes for tooth decays in Northern part of Cote D'Ivoire [1] as well as in Nigeria.[2] They are used in Zimbabwe for the treatment of sexually transmitted infections,[3] while, in India, they are used as anti-snake venom, ophthalmic remedies, [4] paralysis, [5] antimicrobial, leprosy, anthelminthic, purgative and as a strong diuretic. [6] Also, it is used by the Ivoirians and Gabonese in the management of asthma, [7-9] while, in Kenya,[10-11] Tanzania [12] and some other parts of Africa, various parts of the plant are used in the treatment of malaria. Scientifically, the antibacterial,[13-14] anti-snake venom,[4] neuropharmacological,[5] antidiarrhoeal,[15] antimicrobial,[6, 16] antioxidant, anti-asthmatic, anticonvulsant, [8, 9, 17] anti-inflammatory and analgesic[18] activities of the root bark of the plant have been reported.

*Corresponding author. E mail: labake.fadipe@futminna.edu.ng; labsfad@yahoo.com
Tel: +2348034064217

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Malaria is a major tropical parasitic health problem that causes unwholesomeness and sometimes, leads to death, especially in young children and pregnant women in developing countries.[19] Though, through the years, remarkable progress has been made in malaria treatment by the use of artemisinin combination therapies (ACTs), factors, such as, increase in resistance of the malarial parasite to commonly sold insecticides, complications and side effects that arise from the use of orthodox antimalarial agents, as well as, drug resistance could threaten the future effectiveness of such therapies.[20] This has made it pertinent to continuously search for the discovery of safe, cheap, easily available and effective novel antimalarial agents from natural products, such as plants. Medicinal plants, because they are made up of several bioactive compounds, such as; alkaloids, saponins, phenolic compounds and anthraquinones, have reportedly exhibited good antimalarial potentials and have in no small measures contributed to the development of discoveries of potent antimalarial agents.[21]

Phytochemical screening of the roots and root bark of the plant revealed significant amounts of alkaloids, saponins, steroids, terpenoids and phenolic compounds.[7, 16, 18, 22] Steroidal and triterpenoidal sapogenins, as well as their saponins, [23-24] pyrrolidine alkaloids and their glycosides [22, 25-26] as well as, flavonoids [27] have been isolated and characterized from the roots of the plant, while tannins have also been detected. [14] Although, it was revealed that the root bark extract of the plant did not display anti-infective potentials *in-vitro*, it was suggested that the strong presence of some phytochemicals in the plant could open perspectives for the exploration of the plant for its antimalarial potentials.[8] This study, therefore, aimed at investigating the ethanol extract of the root bark of *D. cinerea* and its fractions for their *in-vivo* antimalarial potentials

using the 4-day suppressive test. Furthermore, an attempt was made at isolating and characterizing a phenolic derivative from one of the active fractions.

Materials and Methods

Plant material

The roots of *D. cinerea* were collected in January, 2018 from a farm in Maikunkele town, Minna, Nigeria and authenticated by a Taxonomist in the Department of Medicinal Plant Research and Development, National Institute for Pharmaceutical and Research Development, NIPRD, Idu, Abuja, Nigeria (Voucher number: NIPRD/H/7183). The barks were then separated from the roots, washed with distilled water and dried at room temperature for 2 weeks till a constant weight was obtained.

Extraction and partitioning of extract

Air-dried root bark (2 kg) was crushed and cold macerated exhaustively with 70% ethanol (7 L) at 28^{o}C with frequent manual agitation for a week. Resulting mixture was filtered and concentrated under reduced pressure using a rotatory evaporator and finally dried over a water bath at 40^{o}C to yield a dark brown gummy mass (52 g, coded E_D). Extract was screened qualitatively and quantitatively using standard methods.[28,29] The crude extract, E_D (40 g) was solubilized in 400 mL of distilled water, and the resulting mixture was filtered and the filtrate partitioned exhaustively and successively with chloroform (100 mL x 10), ethyl acetate (100 mL x 15) and n-butanol (100 mL x 20) to give their respective soluble portions which were concentrated *in-vacuo* and finally dried over a water bath to afford fractions labeled as the chloroform- (E_DC, dark brown gummy mass, 7.92%), ethyl acetate- (E_DE, brown gummy mass, 8.53%) and butanol- (E_DB, dark brown gummy mass, 24.8%) fractions.

Fractionation and isolation

The n-butanol fraction (E_DB), because of its good yield, promising spots on TLC and high phenolic content was further fractionated. Seven grams (7 g) of the fraction was applied to the surface of a prepared flash column packed with silica gel (200 - 400 mesh, 200 g, wet method) and eluted sequentially with varying proportions of increasing polarity of $CHCl_3$: MeOH (100:0 to 0:100). Similar fractions of eluents were pooled based on their TLC profile and concentrated *in-vacuo* to yield five (5) major sub-fractions, E_DB1 – E_DB5. Sub-fraction E_DB3 (obtained from $CHCl_3$: MeOH 4:1, two (2) major spots on TLC, 1.92 g) was further fractionated over a column (silica gel, mesh 230 - 400 nm, 60 g, increasing polarity of $CHCl_3$: EtOAc) to afford four (4) major sub-fractions, E_DB3a – E_DB3d. Further purification of sub-fraction E_DB3c (obtained from $CHCl_3$: EtOAc 1:1, one (1) major and two (2) minor spots on TLC, 0.21 g) over sephadex LH - 20 using 100% MeOH afforded a compound, coded, E_DB3ci (single spot on TLC, CH_2Cl_2: MeOH, 3:2).

Spectroscopic instrumentation

^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ on Bruker DRX NMR spectrophotometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C) respectively, while, mass spectra was recorded on Shimadzu LC-MS 8040 quadrupole mass spectrometer.

In-vivo antimalarial studies

Experimental animals

Male and female healthy albino mice (23 ± 5.0 g) were obtained and maintained at the Animal house, Department of Life Sciences, Federal University of Technology, Minna, Niger state, Nigeria. The mice were housed in well-ventilated cages at 25 ± 2^{o}C, having the normal exposure of 12 h light and 12 h dark and fed *ad libitum* with animal pellet diet and water. They were allowed a week to adapt to the conditions of the animal house before commencement of the study. The animals were treated and bred in accordance with the National Guidelines for handling laboratory animals.[30] Also, ethical clearance was obtained from the ethical review board (CUERB) of the Federal University of Technology, Minna, Nigeria (reference number: CUERB/ASN/2018/037).

Acute oral toxicity

Acute oral toxicity of crude ethanol extract of *D. cinerea* root bark, E_D and its fractions were determined by adopting the Organization of Economic Co-operation and Development (OECD - 425) guidelines. [31] Twenty-one (21) healthy, non-pregnant female mice (23 - 27 g, mean age 7 weeks) were randomly selected and divided into 5 mice per cage (labeled groups I - IV) and were denied access to food, but allowed water *ad libitum* for 3 h prior to the experiment. After 3 h, weight of each mice was noted and used to calculate the dose of the treatment to be given to each mice. Each mouse in Groups I – IV were administered orally a single dose of 0.2 mL each of 10, 100, 1000, 1600 and 2000 mg/kg body weight of E_D, E_DC, E_DE and E_DB respectively, while the last mouse received 0.2 mL of 7% tween 80 and 3% ethanol (extract vehicle) as the negative control. All mice were observed initially for 1 h, followed by 4 h and then 24 h for diarrhea, rigidity, hair erection, weight loss, behavioral changes, reduction in movement and feeding activities, as well as mortality and other signs of acute toxicity. Follow up continued for 14 days post experiment.[21, 32, 38]

Parasite strain and inoculation

Chloroquine-sensitive strain of *P. berghei* NK - 65 obtained from College of Medicine, University of Ibadan, Oyo state, Nigeria was maintained by serial blood passage from infected to non-infected mice. Donor mice with parasitaemia levels between 30 - 35% were used for the experiment. Mice were anaesthetized with diethyl ether and 0.5 mL of blood collected by cardiac puncture into heparinized tubes. Blood was then diluted with 0.9% normal saline based on parasitemia of the donor mice and the red blood cells (RBC) count of normal mice (4.5×10^9 RBC/mL) [38], so that 1 mL of blood contained 5×10^7 *P. berghei* infected RBCs. Each mouse was then injected intraperitoneally with an aliquot of 0.2 mL of the diluted blood which now contained approximately 1×10^7 *P. berghei* infected RBCs. [33]

Four-day suppressive test

The Peters' 4-day suppressive test against *P. berghei* infection was used for the evaluation of the schizontocidal activities of extract and fractions of *D. cinerea*. [34] Twenty mice of both sexes that were earlier inoculated with 0.2 mL infected blood from above (day 0) were further divided into four treatment groups (labeled A – D) of 5 mice per group. After three hours post-infection, groups A and B were given 300 and 600 mg/kg body weight of extract E_D respectively, while groups C and D were given 25 mg/kg of Chloroquine (positive control) [3, 32] and 0.2 mL of a mixture of 7% tween 80 and 3% ethanol as placebo (negative control) respectively. All doses were administered orally at 0.2 mL once daily for four consecutive days from day 0 to day 3 (24, 48 and 72 h post-infection).

The same procedure was repeated for fractions E_DC, E_DE and E_DB.

On the fifth day (24 h after the last dose), thin blood smears collected from the tail of each mouse was prepared on slides and fixed with methanol. Slides were stained with 10% Giemsa's solution and examined under the microscope using 100x magnification power. The level of parasitemia was determined by counting the number of parasitized RBCs per 100 RBCs in random 8 fields under the microscope. The % parasitemia for each group of mice treated and the average % parasitemia suppression for each dose of test compounds were calculated using equations 1 and 2 respectively.[33]

$$\% \text{ Parasitemia} = \frac{\text{Number of parasitized RBCs}}{\text{Total number of RBCs counted}} \times 100 \quad (1)$$

$$\% \text{ Parasitemia suppression} = \frac{\text{Parasitemia in control group (negative)} - \text{Parasitemia in the test group}}{\text{Parasitemia in control group (negative)}} \times 100 \quad (2)$$

Determination of mean survival time (MST)

During the 4-day suppressive test against *P. berghei* in mice, total number of mice that survived for groups A – D for each treatment (E_D,

E_DC, E_DE, E_DB and controls) were monitored for a period of 30 days. The MST for each group was calculated using equation 3.[3,32]

$$MST = \frac{\text{Total number of days mice in a group survived}}{\text{Total number of mice in the group}} \quad (3)$$

Changes in rectal temperature and body weights

The temperature of each infected mouse from groups A – D for each treatment (E_D, E_DC, E_DE, E_DB and controls) was determined before infection on day 0 and after treatment on day 4 using a rectal thermometer. Body weights were also determined pre- and post-infection using a sensitive digital weighing balance [32] (Radwag balance, Poland).

Determination of packed cell volume (PCV)

PCV was determined by drawing blood from the tail of each infected mouse from groups A – D for each treatment (E_D, E_DC, E_DE, E_DB and controls) using heparinized micro-hematocrit capillary tubes (Globe Scientific Inc., Paramus, NJ, USA) pre-infection (day 0) and post-infection (day 4). The tubes were filled with blood up to ¾th of their volume and sealed at one end with crystal seal. The tubes were then placed in a micro-hematocrit centrifuge (Hawskley and Sons Ltd, England) with the sealed end outwards and centrifuged at 12,000 rpm for 5 min. This separated the blood into 3 main layers, so that PCV was measured using the formula as shown in equation 4.[3, 32]

$$PCV = \frac{\text{Volume of total RBCs in a given volume of blood}}{\text{Total volume of blood (Plasma)}} \; x\; 100 \quad (4)$$

Statistical analysis

Data was expressed as mean ± standard error of the mean (Mean ± SEM). Statistical significance was determined by a one-way analysis of variance (ANOVA) and 2-tailed student's t-test using SPSS version 22 (SPSS IBM, Chicago, IL. USA). The Tukey multiple comparison tests was used to compare level of parasitemia, parasitemia suppression, weight, rectal temperature, and survival time of the infected mice between the control groups and the treated groups at a fixed time. All data were analyzed at a 95% confidence interval and $p \leq 0.05$ was considered to be statistically significant and $p \geq 0.05$ not significantly different.

Results and Discussion

Qualitative and quantitative screening of crude ethanol extract of *Dichrostachys cinerea* root bark, E_D and its fractions; chloroform, E_DC, ethyl acetate, E_DE and n-butanol, E_DB all revealed a strong presence of phenolic compounds (phenols, flavonoids, tannins) with fractions E_DB and E_DC possessing higher phenolic contents than the crude extract, E_D. Saponins and their sapogenins (triterpenoidal and steroidal), as well as alkaloids were also detected in good quantities. Fraction E_DB, because of its higher phenolic content (302.014 ± 0.55 mg/100 g) and promising spots on TLC was fractionated severally and purified to afford a compound, labeled E_DB3ci. The compound was recrystallized from CH_2Cl_2 as brown flakes, 12 mg, soluble in MeOH and EtOH, partially soluble in Et_2O, $CHCl_3$ and EtOAc and insoluble in H_2O; suggestive of a mid-polar compound. The TLC using the solvent system: CH_2Cl_2: MeOH (3: 2) gave a single spot, R_f 0.68, bluish-green color on spaying with freshly prepared $FeCl_3$ solution, indicative of a phenolic compound. Its ^{13}C-NMR (δ ppm) as shown in Table 1, displayed nine proton-decoupled peaks of different intensities. The most de-shielded peak at δ 168.7 assigned to a carbonyl group indicates the compound is an ester derivative, while, the other less de-shielded peaks indicate the compound possesses carbon atoms bearing – OH and – OCH_3 groups. The most shielded peaks at δ 55.0 and δ 55.4 confirmed the presence of – OCH_3 group of an ester and another on a phenol ring respectively.[34-35] Its proton NMR (δ ppm) as shown in Table 1, revealed the presence of highly de-shielded weak doublets at δ 7.90 and δ 7.88, indicating the presence of a proton on a carbon atom of a phenol ring. A sharp singlet at δ 4.88 was assigned to two – OH protons in a similar environment, while sharp singlets at δ 3.91 and δ 3.33 were attributed to methyl protons each attached to carbon atoms bearing electronegative oxygen atoms respectively.[35-36] The mass spectra revealed fragment ions with m/z values at 184 $[C_8H_8O_5]^+$, 170 $[C_8H_{10}O_4^-]^+$, 59 $[^-COOCH_3]$, base peak; 78 $[C_6H_6]^+$ and 77 $[C_6H_5]^+$. A peak at m/z 170 is a common cleavage of an aryl-oxygen bond, while, a base peak at m/z 59 suggests a loss of acetate radical confirming the compound is an ester derivative, though its molecular ion peak, M^+ was not discernible. Generally, esters give prominent base peaks as a result of elimination of COOR radical fragment. Peaks at m/z 78 and 77 are typical of six-membered aromatic compounds.[35-36] Therefore, based on physical, chemical and spectral values obtained, the name of compound E_DB3ci in comparison with literature was proposed as Methyl 2,3-dihydroxy-4-methoxybenzoate or 2,3-Dihydroxy-4-methoxybenzoic acid methyl ester, $C_9H_{10}O_5$ (Figure 1). It is a derivative of methyl gallate (Methyl 3, 4, 5-trihydroxybenzoate, $C_8H_8O_5$), a plant phenolic that possesses lots of biological properties. It is found in various species of some medicinal plants.[37]

In-vivo studies

Acute oral toxicity

In-vivo acute toxicity investigations of the crude extract of *D. cinerea*, E_D and its fractions revealed no changes in appearance or behavioral patterns, such as, rigidity, vomiting, diarrhea, depression, hair erection of mice at concentrations ranging from 10 - 2000 mg/kg/body weight. Absence of mortality even up to the end of the 14 days, indicates the safety of such test compounds [31] and therefore, suggesting an LD_{50} greater than 2000 mg/kg. [31, 38] This probably explains the routine use of such a medicinal plant for traditional management of malaria and other diseases. [39] The extract and fractions of the plant can be regarded as good candidates for further evaluation, since their LD_{50} is at least 6 times more than the minimum effective dose tested (300 mg/kg).[40]

Four-day suppressive test

The four-day suppressive test was used to evaluate the schizonticidal activities of the crude ethanol extract of *D. cinerea* root bark, E_D and its fractions against *Plasmodium berghei*, a rodent malarial parasite [33]. The chloroform fraction, E_DC displayed the highest level of suppression of parasitemia ($p < 0.05$) at 74.71 ± 0.42% followed by the butanol fraction, E_DB at 70.38 ± 1.08% both at a dose of 600 mg/kg. Also, suppression at 59.66 ± 0.25% and the lowest inhibition at 23.41 ± 1.31% were produced by the crude ethanol extract, E_D and ethyl acetate fraction, E_DE at a dose of 600 mg/kg/day respectively (Table 2). Although, the activity of the extract and its fractions were significantly ($p < 0.05$) lower than that of Chloroquine (99.19 ± 0.01%), they all dose-dependently suppressed the level of parasitemia at both 300 and 600 mg/kg in comparison with the negative control group ($p < 0.05$). Usually, compounds are considered active when % parasitemia reduction is ≥ 30%. [41, 43] An *in-vivo* antiplasmodial activity can be classified as moderate, good and very good, if an extract or its fraction displayed a % parasite suppression ≥ 50% at a dose of 500, 250 and 100 mg/kg body weight per day respectively.[41-42] Consequently, the extract, E_D and fractions E_DC and E_DB can be considered as active and therefore, endowed with antimalarial potentials.

Administration of fractions E_DC and E_DB at 600 mg/kg to infected mice significantly ($p < 0.05$) increased their mean survival time by 21.50 ± 0.43 and 17.20 ± 0.51 days respectively (Table 2). The MST of both fractions were significantly different ($p < 0.05$) from that of extract E_D and fraction E_DE, which were significantly not different ($p > 0.05$) from one another. Similarly, the MST of mice treated with Chloroquine was significantly ($p < 0.05$) prolonged by 29.60 ± 0.13 days as compared to the negative control group (6.86 ± 1.26 days) and extract/fraction-treated groups. Generally, it was observed that the higher the parasitemia inhibition, the longer the MST of the mice for all treated groups on day 4 as compared to day 0. A plant material that can prolong the survival time of infected mice in comparison with the negative control is considered an active antimalarial agent. [38] Also, a compound that prolong survival time of infected mice beyond twelve (12) days is considered as active.[44] The MST is an important

parameter used to assess the antiplasmodial effect of a plant extract and its fractions.[38]

Analysis of rectal temperature of infected mice from day 0 to day 4 revealed that extract E_D, fractions E_DC and E_DB at 600 mg/kg moderately protected the infected mice against a reduction in temperature by -1.38 ± 0.50%, -0.28 ± 0.49% and -0.55 ± 0.28% respectively. The activity of both fractions were not significantly different ($p > 0.05$) from one another and from Chloroquine (0.55 ± 0.24%), while, that of the extract, E_D was statistically different ($p < 0.05$) from Chloroquine, but not different ($p > 0.05$) from that of the fractions. Also, statistical differences were not observed in temperature effect ($p > 0.05$) among the test doses of extract and fractions, except, for fraction E_DB where dose-dependent effect was observed ($p < 0.05$). All treatment groups managed to protect the mice against a reduction in temperature in comparison with the negative control group (-6.57 ± 0.23%). The ability of the extract and fractions of *D. cinerea* to prevent a reduction in temperature might be associated with the antipyretic effect that has been reported for the plant [45] coupled with its antimalarial efficacy. It has been suggested that the antimalarial and antipyretic effects of a plant plays a role in prevention of decline of temperature during malaria infection. [46] The lack of protection against reduction in temperature of mice treated with a plant material, might be due to inability of such a material to improve some pathological processes of malaria that are implicated in reduction of rectal temperature. [47] Often, compounds that possess antimalarial activity could prevent a rapid reduction in rectal temperature. [48]

The result of changes in body weights of infected mice from day 0 to day 4 revealed that by day 4, fractions E_DC and E_DB at a dose of 600 mg/kg displayed significant ($p < 0.05$) maximal protection against reduction in body weights associated with increasing parasitemia by 1.46 ± 0.43% and 1.14 ± 0.27% respectively (Table 4). The fractions in a non-dose dependent manner ($p > 0.05$) significantly ($p < 0.05$) averted the loss in weights in mice in comparison with Chloroquine (3.16 ± 1.82%). There were no significant ($p > 0.05$) differences between the activities and doses of extract E_D, fraction E_DE and the negative control in prevention of weight loss. Extracts and fractions with antimalarial activity are expected to prevent weight loss in infected mice as treatment commences from day 0 to day 4.[32]

Fractions E_DC and E_DB both displayed a non-dose dependent ($p > 0.05$) difference at 600 mg/kg in protecting the infected mice against a reduction in PCV on day 4 by 1.14 ± 0.46% and 0.38 ± 0.08% respectively. The activity of the fractions were significantly ($p < 0.05$) different from that of Chloroquine (3.30 ± 0.14%). Only the crude extract, E_D (-5.15 ± 0.07%) demonstrated an activity that was dose dependent ($p < 0.05$), an activity that was more significant ($p < 0.05$) than that of fraction E_DE (-9.16 ± 0.28%) and the negative control (-10.44 ± 0.27%) in preventing PCV reduction (Table 5). PCV which was also measured from day 0 to day 4 is used to predict the effectiveness of an extract and its fractions in preventing hemolysis resulting from increased parasitaemia.[32, 50] The ability of fractions E_DC and E_DB to prevent a reduction in PCV might be as a result of their destructive antiplasmodial effects against parasitized erythrocytes and the sustainability of the available new erythrocytes.[50]

Generally, reduction in body weights, rectal temperature and packed cell volume are parameters that are manifested in *P. berghei*-infected mice as a result of increasing level of parasitemia from day 0 to day 4, [48]. Usually, a promising antimalarial agent is expected to protect such infected mice against a reduction of these parameters.[38, 48, 50]

The significant activity of fractions E_DC and E_DB may be attributed to the rich presence of phytochemicals, such as, the phenolic compounds, saponins, sapogenins and alkaloids. These bioactive compounds, by acting either singly or in combination synergistically [51] at the stated doses probably accounted for the blood schizonticidal activity of the fractions and therefore, antimalarial activity of the plant. [52] Also, the relative variation in the chemo-suppressive activity of the extract/fractions might be due to the difference in their phytochemical content (both qualitatively and quantitatively) in which E_DC and E_DB revealed higher phenolic contents than E_D and E_DE.

The findings of this study are in agreement with similar antiplasmodial investigations of some other plants[38, 40-56] and also corroborates with other *in-vitro* and *in-vivo* studies conducted on antimalarial effectiveness of other parts of the plant, such as, the roots, [10-11] stem bark and whole stems [12] of *D. cinerea*.

Table 1: ^{1}H- and ^{13}C- NMR spectral data for compound E_DB3ci in comparison with literature values*

Position	δ H (ppm)	δ H (ppm)*	δ C (ppm)	δ C (ppm)*
1	-	-	168.7 (-C=O)	169.7
2	5.33 (ss, 1H)	4.88 (ss)	147.3 (-C-OH)	147.3
3	5.33 (ss, 1H)	4.88 (ss)	131.6 (C-OH)	134.3
4	-	-	151.2 (-C-OMe)	153.6
5	6.86, 6.82 (sd, 1H)	6.60 (d)	104.7 (-C-H)	104.7
6	7.90, 7.88 (wd, 1H)	7.33 (wd)	123.8 (-C-H)	124.9
7	-	-	112.4 (-C-)	106.0
8	3.91 (ss, 3H)	3.89 (ss)	55.0 (-O-Me)	51.5
9	3.33 (ss, 3H)	3.83 (ss)	55.4 (-O-Me)	56.1

-: no peak observed, ss: sharp singlet, d: doublet, sd: sharp doublet, wd: weak doublet *ACD/ChemDraw (Product version 15)

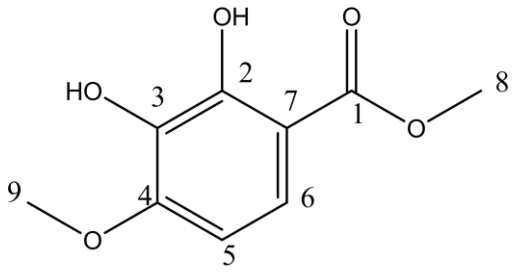

Figure 1: Proposed structure of compound

Table 2: Effect of crude ethanol extract of *D. cinerea* root bark, E_D and its fractions on parasitemia and survival of mice infected with *P. berghei*

Treatment	% Parasitemia			% Suppression			MST (days/mice)		
	Dose (mg/kg/day)			Dose (mg/kg/day)			Dose (mg/kg/day)		
	300	600	t-value	300	600	t-value	300	600	t-value
E_D	35.66 ± 0.38^{c}	34.00 ± 0.33^{c}	3.297*	57.69 ± 0.22^{c}	59.66 ± 0.25^{d}	-5.828*	9.72 ± 0.59^{cd}	9.67 ± 0.89^{d}	0.047^{NS}
E_DC	28.33 ± 1.57^{e}	21.33 ± 0.69^{d}	4.078*	66.44 ± 1.33^{b}	74.71 ± 0.42^{b}	-5.944*	15.80 ± 0.77^{b}	21.50 ± 0.43^{b}	-06.466*
E_DE	73.30 ± 2.14^{b}	64.60 ± 2.14^{b}	2.876*	13.09 ± 1.15^{d}	23.41 ± 1.31^{e}	-5.908*	7.67 ± 0.57^{d}	8.45 ± 1.41^{d}	-0.513^{NS}
E_DB	29.50 ± 0.55^{e}	25.00 ± 1.30^{d}	3.174*	65.01 ± 0.10^{b}	70.38 ± 1.08^{c}	-4.971*	12.90 ± 0.32^{bc}	17.20 ± 0.51^{c}	-7.083*
CQ (25mg/kg)	0.68 ± 0.00^{f}	0.68 ± 0.00^{e}	-	99.19 ± 0.01^{a}	99.19 ± 0.01^{a}	-	29.60 ± 0.13^{a}	29.60 ± 0.13^{a}	-
Tw (0.2 mL)	84.30 ± 1.35^{a}	84.30 ± 1.35^{a}	-	0.00^{e}	0.00^{f}	-	6.86 ± 1.26^{d}	6.86 ± 1.26^{d}	-

E_D = crude ethanol extract of *D. cinerea* root bark; E_DC = chloroform fraction of extract E_D; E_DE = ethyl acetate fraction of extract E_D; E_DB = butanol fraction of extract E_D; CQ = Chloroquine phosphate; Tw = mixture of 7% tween 80 and 3% ethanol; MST = Mean Survival Time.
Mean ± standard errors on the same column with different superscripts are significantly different ($p \leq 0.05$)
*Significantly different ($p \leq 0.05$). NS: Not significantly different ($p \geq 0.05$)

Table 3: Effect of crude ethanol extract of *D. cinerea* root bark, E_D and its fractions on rectal temperature of mice infected with *P. berghei*

Treatment	Dose (mg/kg/day)						
	300	300	600	600	300	600	
	Temperature (ºC)				% Change in Temperature		t-value
	Day 0	Day 4	Day 0	Day 4			
E_D	36.80 ± 0.44	36.00 ± 0.55	36.40 ± 0.22	35.90 ± 0.40	-2.18 ± 0.31^{b}	-1.38 ± 0.50^{b}	-1.364^{NS}
E_DC	36.70 ± 0.31	36.50 ± 0.57	36.50 ± 0.25	36.40 ± 0.43	-0.56 ± 0.73^{ab}	-0.28 ± 0.49^{ab}	-0.314^{NS}
E_DE	36.50 ± 0.58	34.80 ± 0.24	36.70 ± 0.72	35.40 ± 0.53	-4.63 ± 0.86^{c}	-3.53 ± 0.43^{c}	-1.147^{NS}
E_DB	36.20 ± 0.41	36.40 ± 0.32	36.90 ± 0.23	36.70 ± 0.33	0.56 ± 0.26^{a}	-0.55 ± 0.28^{ab}	2.854*
CQ (25 mg/kg)	36.60 ± 0.10	36.80 ± 0.19	36.60 ± 0.10	36.80 ± 0.19	0.55 ± 0.24^{a}	0.55 ± 0.24^{a}	-
Tw (0.2 mL)	36.60 ± 0.66	34.20 ± 0.70	36.60 ± 0.66	34.20 ± 0.70	-6.57 ± 0.23^{c}	-6.57 ± 0.23^{d}	-

E_D = crude ethanol extract of *D. cinerea* root bark; E_DC = chloroform fraction of extract E_D E_DE = ethyl acetate fraction of extract E_D; E_DB = butanol fraction of extract E_D; CQ = Chloroquine phosphate; Tw = mixture of 7% tween 80 and 3% ethanol. Mean ± standard errors on the same column with different superscripts are significantly different ($p \leq 0.05$)
*Significantly different ($p \leq 0.05$). NS: Not significantly different ($p \geq 0.05$)

Table 4: Effect of crude ethanol extract of *D. cinerea* root bark, E_D and its fractions on body weights of mice infected with *P. berghei*

Treatment	Dose (mg/kg/day)						
	300	300	600	600	300	600	
	Weight (g)				% Change in weight		t-value
	Day 0	Day 4	Day 0	Day 4			
E_D	26.10 ± 0.31	24.80 ± 0.57	27.50 ± 0.42	26.30 ± 0.58	-5.01 ± 1.06^{b}	-4.38 ± 0.68^{b}	-0.496^{NS}
E_DC	26.90 ± 0.38	27.00 ± 0.18	27.20 ± 0.39	27.60 ± 0.51	0.39 ± 0.74^{b}	1.46 ± 0.43^{a}	-1.255^{NS}
E_DE	26.10 ± 0.51	24.50 ± 0.62	26.20 ± 0.56	24.90 ± 0.30	-6.15 ± 0.52^{b}	-4.92 ± 0.89^{b}	-1.193^{NS}
E_DB	28.20 ± 0.31	28.40 ± 0.36	26.20 ± 0.28	26.50 ± 0.35	0.71 ± 0.16^{a}	1.14 ± 0.27^{a}	-1.373^{NS}
CQ (25 mg/kg)	26.50 ± 1.00	27.30 ± 0.55	26.50 ± 1.00	27.30 ± 0.55	3.16 ± 1.82^{a}	3.16 ± 1.82^{a}	-
Tw (0.2 mL)	26.10 ± 0.77	24.30 ± 0.74	26.10 ± 0.77	24.30 ± 0.74	-6.90 ± 0.09^{b}	-6.90 ± 0.09^{b}	-

E_D = crude ethanol extract of *D. cinerea* root bark; E_DC = chloroform fraction of extract E_D E_DE = ethyl acetate fraction of extract E_D; E_DB = butanol fraction of extract E_D; CQ = Chloroquine phosphate; Tw = mixture of 7% tween 80 and 3% ethanol; MST = Mean Survival Time.
Mean ± standard errors on the same column with different superscripts are significantly different ($p \leq 0.05$)
*Significantly different ($p \leq 0.05$). NS: Not significantly different ($p \geq 0.05$)

Table 5: Effect of crude ethanol extract of *D. cinerea* root bark, E_D and its fractions on packed cell volume (PCV) of mice infected with *P. berghei*

Treatment	Dose (mg/kg/day)						
	300	300	600	600	300	600	
	PCV				% Change in PCV		t - value
	Day 0	Day 4	Day 0	Day 4			
E_D	53.70 ± 0.30	49.90 ± 0.44	52.40 ± 0.25	49.70 ± 0.20	-7.08 ± 0.31^c	-5.15 ± 0.07^c	-6.078*
E_DC	52.40 ± 0.50	52.30 ± 0.32	52.30 ± 0.16	52.90 ± 0.40	-0.18 ± 0.34^b	1.14 ± 0.46^b	-2.323^{NS}
E_DE	53.00 ± 0.61	48.30 ± 0.53	53.50 ± 0.48	48.60 ± 0.58	-8.87 ± 0.05^d	-9.16 ± 0.28^d	1.058^{NS}
E_DB	52.80 ± 0.34	52.90 ± 0.26	53.20 ± 0.41	53.40 ± 0.37	0.19 ± 0.15^b	0.38 ± 0.08^b	-1.072^{NS}
CQ (25 mg/kg)	54.60 ± 0.10	56.40 ± 0.18	54.60 ± 0.10	56.40 ± 0.18	3.30 ± 0.14^a	3.30 ± 0.14 ^{a}	-
Tw (0.2 mL)	51.70 ± 0.72	46.30 ± 0.50	51.70 ± 0.72	46.30 ± 0.50	-10.44 ± 0.27^e	-10.44 ± 0.27^e	-

E_D = crude ethanol extract of *D. cinerea* root bark; E_DC = chloroform fraction of extract E_D E_DE = ethyl acetate fraction of extract E_D; E_DB = butanol fraction of extract E_D; CQ = Chloroquine phosphate; Tw = mixture of 7% tween 80 and 3% ethanol. Mean ± standard errors on the same column with different superscripts are significantly different ($p \leq 0.05$)
*Significantly different ($p \leq 0.05$). NS: Not significantly different ($p \geq 0.05$)

Conclusion

From the findings of this study, it can be concluded that the root bark of *D. cinerea* is relatively a safe plant that possesses moderate antimalarial potentials. The chloroform-, E_DC and butanol-, E_DB fractions of the plant displayed highest parasitemia suppression in *P. berghei* infected mice. The phenolic derivative isolated from the active butanol fraction, E_DB, may either, acting singly or synergistically with other constituents, serve as a potential source of antimalarial agents. This study, therefore, supports the folkloric use of the plant as an antimalarial agent. It is recommended that more constituents should be isolated, characterized and their antimalarial potentials evaluated.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article

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