Original Article

Antiplasmodial, antinociceptive and antipyretic potential of the stem bark extract of *Burkea africana* and identification of its antiplasmodial-active fraction

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ABSTRACT

Background and aim: *Burkea africana* stem bark is used as a remedy for malaria in north-central and southern Nigeria. Based on its traditional use, this study was conducted to investigate the antiplasmodial, antinociceptive and antipyretic potential of an extract of *B. africana* stem bark.
Experimental procedure: A 70% v/v ethanol extract of stem bark of *B. africana* was prepared by cold maceration. Fractions (dichloromethane, ethyl acetate, and residual) were also prepared. The extract was screened for hemolytic, cytotoxic and antiplasmodial activity effects. The effect of the extract and fractions against chloroquine-sensitive (3D7) and multi-drug resistant (W2mef) *P. falciparum* was assessed. Acute toxicity test, acetic acid-induced abdominal writhing in mice, and lipopolysaccharide-induced fever in rats were also employed to screen the extract. Chromatographic fingerprints of the extract and active fraction were obtained.
Results: *B. africana* extract showed no cytotoxic or significant hemolytic effects and did not cause acute toxicity or mortality. The ethanol extract exhibited moderate antiplasmodial activity while the dichloromethane fraction showed high activity against *P. falciparum* 3D7 ($IC_{50} = 6.44$ μg/ml) and W2mef ($IC_{50} = 6.30$ μg/ml) respectively. The extract elicited significant ($p < 0.05$) attenuation of acetic acid-induced writhing and significantly ($p < 0.05$) ameliorated lipopolysaccharide-induced pyrexia at 300 mg/kg. The HPLC profile of the dichloromethane fraction showed peaks with retention times that corresponded with those of rutin and caffeic acid.
Conclusion: *Burkea africana* extract has antiplasmodial, antinociceptive and antipyretic potential and its antiplasmodial constituents are concentrated in its dichloromethane fraction.

1. Introduction

Malaria is a major parasitic protozoan tropical disease, responsible for high morbidity and mortality in sub-Saharan Africa.[1] In 2019, there were about 229 million new cases of malaria and about 409,000 deaths resulting from the disease.[1] It is caused by the single-celled protozoa *Plasmodium* which is transmitted by the female Anopheles mosquito. Four species of *Plasmodium* are known to cause the majority of human malaria cases and include *P. falciparum, P. vivax, P. ovale* and *P. malariae*; of which *P. falciparum* causes the most severe form of the disease. Drug resistance to conventional antimalarials is an ongoing challenge in the treatment of malaria. This ability of the parasite to develop resistance and the difficulty of creating efficient vaccines presents an urgent need for new antimalarial drugs. This will help to curb malaria-associated mortality in vulnerable people, especially pregnant women and children under five years of age.[2]

Plants have been and remain a vital source of therapy for various ailments and diseases all around the world. They have been used to

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treat health conditions such as pain, inflammation, malaria and typhoid fevers, eye problems, cognitive deficits among others. About 88% of 194 member states of the World Health Organization acknowledged the use of traditional and complementary medicine by their people to meet their different health needs.[3] For some people, herbal medicines are readily available and affordable, whereas conventional drugs though available in many instances, may not be affordable. Medicinal plants also play an important role in the discovery of novel therapeutics. They serve as a reservoir of chemically diverse molecules and constitute prototype molecules employed in the development of new active substances.[4,5] Knowledge of the use of such plants in different civilizations is continuously being exploited for drug discovery and modest successes have been recorded using this approach.[6,7]

Studies on *Cinchona officinalis* and *Artemisia annua* necessitated by their long history of use against fevers led to the discovery of quinine and artemisinin respectively, which remain very useful antimalarials to date. *Burkea africana* Hook. is a deciduous shrub with a flat top and spreading crown. It belongs to the family Leguminosae and grows well in tropical African countries to which it is indigenous such as Senegal, Namibia, South Africa and Nigeria.[8] The bark is a rich source of tannins which makes it useful in tanning leather. The bark when dried and powdered is used to paralyze fish.[8] The stem bark is used as an antidote for venomous stings and bites, cutaneous and subcutaneous parasitic infection, convulsion and pulmonary troubles. In a recent survey of Nigerian antimalarial plants, a stem bark decoction of *B. africana* was reported to be used to treat malaria.[9,10] Previous studies reveal that the stem bark extract of the plant possesses pharmacological properties which include antioxidant activity, as the polyphenolic-rich stem bark fractions were found to attenuate oxidative stress.[11,12] It has also been reported to possess antidiarrhoeal activity.[13] The plant has been utilized in wound healing, for relief of headache and to treat gastrointestinal disturbances and these effects have been attributed to the tyramine content of the bark, while its anti-inflammatory activity has been linked to its antioxidative effects.[14]

Given its widespread medicinal use and its remedial use for malaria in Nigeria, a systematic literature search was conducted to determine whether this claim has been scientifically validated. Our search revealed that the acclaimed use of the plant as an antimalarial remedy has not been scientifically substantiated. Therefore, this study was aimed at evaluating the effects of 70% v/v ethanol extract of *Burkea africana* stem bark and its fractions on peripheral pain, fever and against *Plasmodium falciparum* to ascertain if it has antiplasmodial potential.

2. Materials and methods

2.1. Plant material

The stem bark of *Burkea africana* was collected from Suleja, Niger State, Nigeria in April 2019. The plant material was identified by an ethnobotanist and authenticated at NIPRD herbarium, where a voucher specimen (NIPRD/H/7017) was prepared and deposited. Immediately after collection, the plant material was washed clean with water, cut into smaller pieces, dried under shade for 1 week and pulverized to a coarse powder.

2.2. Extraction and preparation of fractions

A 200 g quantity of the pulverized *B. africana* stem bark was extracted by cold maceration in 70% v/v ethanol (500 ml) for 48 h with intermittent agitation. The resulting mixture was filtered first with a muslin cloth and finally with Whatman filter paper (grade 3, 125 mm). The filtrate was concentrated over a water bath

maintained at 55 °C and the dry ethanol extract (12 g) was transferred to an air-tight glass vial. A 3 g portion of the extract was separated into fractions by successive liquid-liquid partitioning in a 500 ml separating funnel using sequentially, 100 ml each of dichloromethane and ethyl acetate. the fractions were evaporated to dryness on a hot water bath to give the dichloromethane (0.211 g) and ethyl acetate (0.589 g) fractions. The last liquid portion left was also evaporated to dryness to give the residual fraction (2.2 g), yielding a total of 3 fractions. The extract and fractions were refrigerated at 4 °C and freshly reconstituted (in distilled water for *in vivo* tests or dimethylsulfoxide (DMSO) for *in vitro* studies) before use.

2.3. Animals

Healthy, adult mice (18–25 g) and Wistar rats (100–120g) of either sex were obtained from the Animal Facility Center of the National Institute for Pharmaceutical Research and Development (NIPRD). The mice were housed in plastic cages (6 mice per cage) with softwood shavings as bedding material and the rats were kept in stainless steel cages. They were kept in a well-ventilated room with 12 h - light/dark cycle. The animals were fed with standard rodent feed and allowed free access to drinking water. The animals were acclimatized to laboratory conditions for two weeks before studies.

All animal experiments were carried out following Institutional standard operating procedures and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.[15]

2.4. Plasmodium falciparum growth inhibition assay

2.4.1. Sourcing of parasites and secondary cell line

Chloroquine-sensitive (3D7) and multi-drug resistant (W2mef) *P. falciparum* were sourced from the West African Center for Cell Biology of Infectious Pathogens (WACCBIP), Ghana. Human embryonic kidney cells (HEK 293) were sourced from the Special Centre for Molecular Medicine, Jawaharlal Nehru University, New Delhi, India.

2.4.2. Plasmodium falciparum in vitro culture

Chloroquine-sensitive (3D7) and multidrug-resistant (W2mef) *P. falciparum* strains were used for *in vitro* antiplasmodial assays. Parasites were cultured according to the Trager and Jensen method as modified by Ayuko et al.[16,17] Parasites were cultivated in O+ erythrocytes and complete culture medium (Roswell Park Memorial Institute, RPMI-1640) modified by containing sodium bicarbonate 2 g/L, hypoxanthine 50 mg/L, gentamycin 10 μg/ml, and enriched with Albumax I. Cultures were also maintained at 37 °C under an atmosphere of 4.9% carbon dioxide, 5.0% oxygen, and 90.1% nitrogen gas. Monitoring of parasite growth was done every 24 h by refreshing the culture medium, adding gas mixture and calculating parasitemia. Parasitemia was calculated as a percentage based on the total number of parasites counted in five fields with at least 200 erythrocytes per field.

2.4.3. Plasmodium falciparum growth inhibition assay

A suspension of sorbitol-synchronized, infected red blood cells was adjusted to 1% parasitemia and 2% hematocrit in complete RPMI-1640 medium and seeded in a 96-well microplate (100 μL per well). The extract was dissolved in DMSO to provide a stock solution of 10 mg/ml. The test solution was serially diluted with RPMI-1640 culture medium to give 0.0032–250 μg/ml concentrations. A 10 μl of each concentration was introduced into the wells of a 96-well plate containing 90 μl infected red blood cells in complete media. Stock solutions of 10 mM chloroquine and 1 mM artesunate

solutions in DMSO were prepared and used as positive controls at final concentrations between 0.0064 nM and 25 μM and 0.00248 nM–32 nM respectively. RPMI containing 0.1% DMSO was used as negative control whilst uninfected erythrocytes at 2% hematocrit were used as background control. Test plates were incubated at 37 °C for 48 h. After this period, 100 μl of lysis buffer (20 mM Tris pH 7.5, 5 mM EDTA, 0.008% saponin, 0.08% Triton X-100) was prepared and 0.2 μl SYBR green from ×10,000 concentrate added per ml of lysis buffer. A 100 μl volume of this solution was added to each well and the plates were kept frozen for 24 h. After 24 h, the plates were thawed and fluorescence was measured at excitation/emission wavelengths of 485/530 nm. Parasite inhibition was expressed as a percentage, relative to the negative control. In this study, the antiplasmodial activity of the extract or fraction was considered high when $IC_{50} < 10$ μg/ml, moderate for IC_{50} between 11 and 50 μg/ml, mild between 51 and 100 μg/ml and inactive when $IC_{50} > 100$ μg/ml.[18]

2.5. In vitro cytotoxicity test

A standard MTT assay was used to evaluate the effect of the extract on cellular proliferation.[19] Adherent human embryonic kidney cells (HEK 293) were seeded into a 96-well plate (5×10^3 cells per well) and exposed to the extract (125–1000 μg/ml) for 48 h. Etoposide 100 nM was used as a positive control for cell toxicity. After incubation, spent media was decanted and 100 μl of fresh media added. To each well, 10 μl of a 5 mg/ml stock solution of MTT prepared in autoclaved water was added and the cells incubated for 3 h at 37 °C. Afterward, culture media were removed and the crystals formed were dissolved by adding 100 μl DMSO to each well. After shaking the microplates at 200 rpm on an orbital shaker for 1 h, absorbance was read at 595 nm wavelength.

2.6. Hemolysis assay

Cytotoxicity of the extract on erythrocytes was evaluated by a hemolysis assay as described previously.[20] Fresh erythrocytes were washed thrice with three volumes of phosphate-buffered saline (PBS, pH 7.4). The assay was performed at 2% hematocrit with 0.125 - 1 mg/ml of extract in a final volume of 100 μl, followed by incubation at 37 °C for 5 h. Triton X-100 (0.1 %v/v) was used as a positive control, while PBS was used as a negative control. After incubation, the tubes were centrifuged at 1000×g for 10 min and the absorbance of the supernatants read at 540 nm to determine the amount of hemoglobin released due to hemolysis. Results were expressed as a percentage relative to 100% hemolysis produced by the positive control.

2.7. Acute toxicity test

An acute oral toxicity study was performed according to the Organization for Economic Cooperation and Development guidelines 423 on limit toxicity testing.[21] Five mice were used for the study with one of the mice serving as the control. The animals were fasted overnight with access to drinking water before the test. Each mouse received 2000 mg/kg of the ethanol extract, or for the control, distilled water (10 ml/kg). The mice were continuously observed during the first 4 h. Afterward, they were observed periodically for up to 24 h for mortality and other signs of toxicity and observed daily thereafter for 14 days.

2.8. Acetic acid writhing test in mice

This test was conducted as described by Koster et al. and adapted by Gupta et al., with slight modification.[22,23] All treatments

were administered orally in this study, 1 h before intraperitoneal injection of acetic acid. Twenty-five Swiss mice were randomly divided into five groups of five mice per group. Group I was treated with distilled water (10 ml/kg) and served as the negative control. Groups II to IV were administered 100, 300 and 900 mg/kg of the extract while group V received aspirin (150 mg/kg). After 1 h, each mouse was administered 10 ml/kg of 0.6 %v/v acetic acid intraperitoneally. After 5 min, the number of abdominal writhes displayed by each mouse was counted for 10 min.

2.9. Effect on lipopolysaccharide-induced pyrexia in rats

The method of Santos as adapted by Chomchuen et al. was used for the assay.[24,25] The experiment was carried out between 08:00 h and 17:00 h on the day of the study. Rats were randomized into 6 groups of six rats per group. Groups 1 and 2 served as normothermic and hyperthermic controls and received distilled water (10 ml/kg). Rats in groups 3–5 were treated with the extract (100, 300, 900 mg/kg) administered orally, while paracetamol (300 mg/kg), injected intraperitoneally served as the positive control. Fever was induced with 100 μg/kg of lipopolysaccharide (LPS, from *Escherichia coli*) injected subcutaneously into the right thigh of the rats. An equal volume of sterile phosphate-buffered saline was used in place of LPS in the normothermic control group. Rectal temperature was measured before the treatment and at 1 h intervals for 5 h after the administration of the endotoxin with a digital thermometer inserted 2 cm into the rectum of the rats.

2.10. High-performance liquid chromatography analysis of extract and dichloromethane (DCM) fraction

The chromatographic system included an HPLC system consisting of Ultra-Fast LC-20AB prominence, equipped with SIL- 20AC autosampler; DGU-20A3 degasser; SPD-M20A UV diode array detector (UV-DAD) at a wavelength range of 190–800 nm; column oven CTO-20AC, system controller CBM- 20 Alite and Windows LC solution software (Shimadzu Corporation, Kyoto Japan). A VP-ODS column, (5 μm; 150 × 4.6 mm) was used and chromatographic conditions included mobile phase solvent A: 0.2% v/v formic acid and solvent B: acetonitrile; mode: isocratic; (mobile phase solvent A and B in the ratio 80:20); flow rate 0.6 ml/min and column oven temperature of 40 °C. The total run time was 15 min. An injection volume of 20 μl of 20 mg/ml solution of extract or fraction in methanol was used and sample elution was detection was at 254 nm. Reference standards rutin, quercetin, caffeic acid, ferulic acid and apigenin (Fluka, Germany) prepared as 50 μg/ml solutions in methanol were analyzed separately under the same conditions, for comparison with analytes in the extract and fraction.

2.11. Statistical analysis

All *in vitro* assays were performed in duplicate. In the antiplasmodial assay, concentrations producing 50% inhibitory effect (IC_{50}) were obtained by non-linear regression from plotted graphs using GraphPad PRISM version 6.0 (Intuitive software for science, San Diego, CA, USA). Results of *in vivo* tests were analyzed by one-way analysis of variance (ANOVA) for acetic acid-induced abdominal writhing test or two-way ANOVA for the antipyretic test; subjected to Dunnet's *post hoc* test for multiple comparisons between the untreated and treated groups.

3. Results

3.1. Antiplasmodial activity

Results of antiplasmodial screening of the ethanol extract and fractions are shown in Fig. 1a and b. The ethanol extract displayed moderate antiplasmodial activity against the two parasite strains, with IC_{50} values of 28.69 µg/ml and 25.19 µg/ml against 3D7 and W2mef strains respectively (Fig. 1a and b). Of the three fractions tested, only the dichloromethane (DCM) fraction showed high antiplasmodial activity against CQ-sensitive 3D7 (Fig. 1a, IC_{50} = 6.44 µg/ml) and drug-resistant W2mef (Fig. 1b, IC_{50} = 6.30 µg/ml). The ethyl acetate and residual fractions were inactive ($IC_{50} > 100$ µg/ml) against both parasite strains.

3.2. In vitro cytotoxicity and hemolysis

The results of the *in vitro* cytotoxicity test (Table 1) show that the extract did not cause cytotoxicity or significant hemolysis at all concentrations compared to the positive controls. Hemolysis was considered as significant for values above 10%, and the percentage (%) hemolysis displayed by *Burkea africana* at the concentration of 1 mg/ml was 9.28%.

3.3. Acute toxicity

At a limit dose of 2000 mg/kg, the extract caused a reduction in physical activity and sedation within 30 min–1 h in extract-treated mice. No gross behavioral changes such as tremors, convulsions, or other signs of toxicity were observed. There were no delayed signs of toxicity or mortality recorded up to 14 days after the initial administration of the extract.

3.4. Effect on acetic acid-induced abdominal writhing

As shown in Fig. 2, the extract produced a significant ($p < 0.05$), decrease in the writhing response at 300 and 900 mg/kg doses in extract-treated groups compared to the negative control. At 900 mg/kg, the highest effect was seen which was higher than that produced by the standard drug, aspirin at a dose of 150 mg/kg.

3.5. Antipyretic activity

Lipopolysaccharide injection produced a time-dependent initial increase in rectal temperature from baseline (Table 2). The increase from baseline temperature was highest within 1–2 h, with a subsequent decrease at 3–5 h after LPS injection. The antipyretic effect
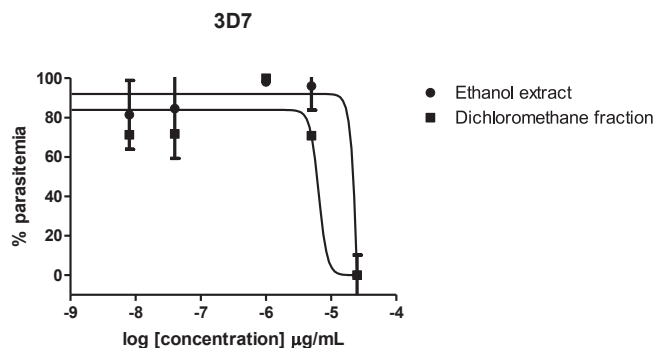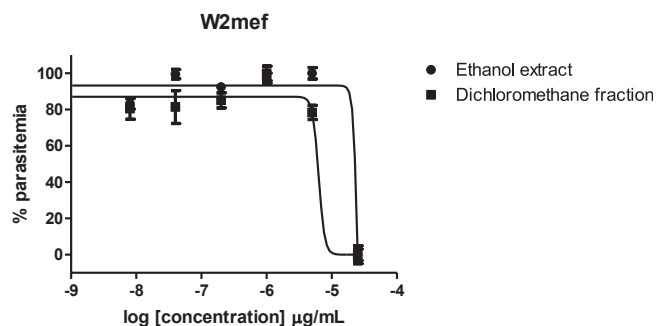

Fig. 1b. Retain caption as "Concentration-response curves of *B. africana* extract and dichloromethane fraction against *P. falciparum* W2mef. Each data point represents the mean ± SEM (n=3). IC_{50} values: Extract = 25.19 µg/ml, DCM fraction = 6.30 µg/ml, Artesunate = 16.79 nM, Chloroquine = 147.5 nM.

Table 1
In *vitro* cytotoxicity and hemolysis assay.

Treatment	Concentration	Inhibition (%)	Red Blood Cells Lysis (%)
		HEK 293	
BA	0.125 mg/ml	NI	NT
	0.250 mg/ml	NI	NT
	0.500 mg/ml	NI	NT
	1.000 mg/ml	NI	9.28
Etoposide	100 nm	33.00	NT
Triton X	0.1%	NT	100

PBS – Phosphate buffered saline, BA – *Burkea africana,* NT – Not tested, NI - No Inhibition.
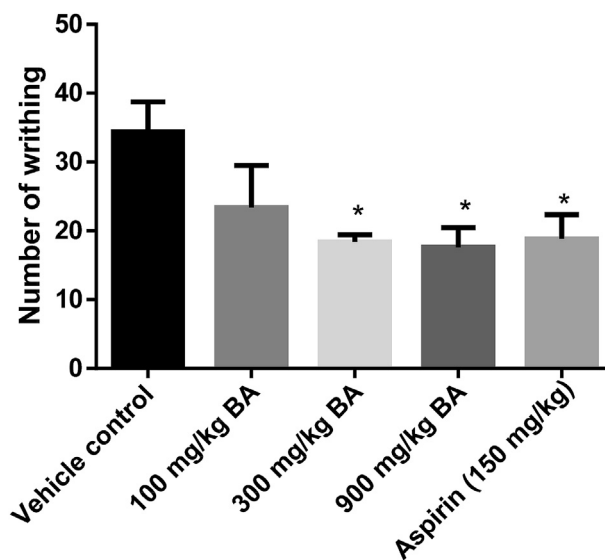

Fig. 2. Effect of ethanol extract of *Burkea africana* stem bark on acetic acid-induced abdominal writhing. Values are expressed as mean ± SEM; n = 5. Significant at *$p < 0.05$ compared to vehicle control (negative control).

seen in the 300 mg/kg extract-treated group was observed to be significant ($p < 0.05$) at 2 and 3 h. A similar significant ($p < 0.01$, 0.05) antipyretic effect was produced by paracetamol (PCM) when compared with the hyperthermic control group.

3.6. HPLC fingerprints

The HPLC fingerprints of the ethanol extract of *Burkea africana* show major peaks at the retention times of 3.745, 3.938, 4.182,

Fig. 1a. Concentration-response curves of *B. africana* extract and dichloromethane fraction against *P. falciparum* 3D7. Each data point represents the mean ± SEM (n=3). IC_{50} values: Extract =28.69 µg/ml, DCM fraction = 6.44 µg/ml, Artesunate = 10.80nM, Chloroquine = 31.65 nM.

Table 2
Effect of ethanol extract of *B. africana* stem bark on LPS-induced fever.

Group/treatment	Difference in Temperature from Baseline (°C)				
	1 h	2 h	3 h	4 h	5 h
Normothermic/vehicle	−0.15 ± 0.40	0.30 ± 0.21	0.07 ± 0.29	0.25 ± 0.10	0.43 ± 0.30
Hyperthermic/vehicle	1.07 ± 0.25	1.73 ± 0.33	1.57 ± 0.30	1.30 ± 0.38	1.38 ± 0.19
100 mg/kg BA	1.03 ± 0.27	1.08 ± 0.33	0.98 ± 0.17	1.27 ± 0.26	1.22 ± 0.22
300 mg/kg BA	0.67 ± 0.36	0.60 ± 0.23*	0.48 ± 0.35*	0.90 ± 0.19	0.62 ± 0.36
900 mg/kg BA	0.46 ± 0.22	1.08 ± 0.22	1.12 ± 0.17	0.52 ± 0.21	0.58 ± 0.23
PCM 300 mg/kg	0.23 ± 0.21	0.23 ± 0.42**	0.42 ± 0.55*	0.95 ± 0.31	1.05 ± 0.17

Values are expressed as mean ± SEM; n = 6. Significant at *$p < 0.05$, **$p < 0.01$ compared to hyperthermic vehicle control (negative control).

5.333 and 6.405 min (Fig. 3a). In the DCM fraction fingerprint (Fig. 3b), peaks with retention times of 4.405 and 6.490 min corresponded to caffeic acid and rutin (Fig. 3c and d). The peak with a retention time of 6.405 min in the ethanol extract corresponded to rutin (Fig. 3c).

4. Discussion

In this study, the ethanol extract of *Burkea africana* stem bark was evaluated for its antiplasmodial potential. The moderate activity produced by the extract appeared enhanced in the dichloromethane (DCM) fraction, which exhibited high antiplasmodial activity while other fractions were inactive. It was observed that IC_{50} values of the DCM fraction against both drug-sensitive and resistant strains were almost equivalent. This may imply that the fraction mediates antiplasmodial effects in mechanism(s) that are distinct from the antiplasmodial mechanisms of the standard drugs, which the W2mef strain displays resistance to. Chloroquine mediates lethality in parasites by inhibiting heme detoxification in the acidic food vacuole and resistance to this drug is by overexpression of a calcium ion-dependent efflux transporter which prevents the accumulation of chloroquine.[26] Artesunate elicits its action by the cleaving of the endoperoxide bond through reaction with heme. This leads to the production of free radicals which alkylate *P.falciparum* proteins resulting in DNA damage and cytotoxicity.[27] Resistance to artesunate is caused by the mutation of the *P. falciparum*'s K13 gene which allows the parasite to withstand the sudden oxidative damage usually caused by artemisinins resulting in slow parasite clearance and greater survival of early ring-stage parasites *in vitro*.[28] Therefore the activity of the extract and DCM fraction suggests that they may act through mechanisms that are different from chloroquine and artesunate. The concentration of the antiplasmodial activity in the DCM fraction may indicate that the antiplasmodial-active principles are mainly non-polar, compared with the other fractions of increasing polarity. Other authors have also reported antiplasmodial activity in non-polar fractions of plant extracts.[6,16,27] Thus, the nature of the extracting solvent is an important determinant of antiplasmodial activity.[29] The absence of significant cytotoxic or hemolytic effects in the extract suggests that the extract selectively targets the parasites within the red blood cells. In a different study, the authors reported that a hydromethanol extract of *B. africana* stem bark preserved erythrocyte integrity and displayed hematopoietic effects, and this may support the absence of significant hemolytic effect of *B. africana* extract in this study.[30] Besides, an ethanol extract of the plant showed a non-cytotoxic effect on normal cells and a non-cancerous cell line but was preferentially cytotoxic to a cancerous cervical cell line.[31] This renders the extract as a potential antiplasmodial agent that will not exert deleterious effects on host erythrocytes and other body cells.

Acute toxicity testing of the plant extract showed that the extract was acutely safe as no overt toxicity manifestations or mortality was observed. The extract may, therefore, be described as being non-toxic following acute oral ingestion of an equivalent dose in humans. Further studies on the effect of the extract on body organs may be useful to ascertain safety following repeat-dose administration.

The acetic acid writhing test is normally used to study the peripheral effects of drugs on pain and inflammation.[22] Acetic acid is an irritant that produces pain when injected into the peritoneum. The pain which is usually steady and prolonged is associated with tissue damage and also involves increased movement and postural adjustment of abdominal muscles. The constriction of the abdominal muscles is due to the sensitization of nociceptive receptors to prostaglandin.[32] This effect is attenuated by analgesics and anti-inflammatory agents and is useful for screening substances with peripheral antinociceptive and anti-inflammatory activity. Aspirin is a potent non-steroidal anti-inflammatory agent that works via inhibition of cyclooxygenase that catalyzes the production of prostaglandins through the arachidonic pathway.[33] The extract attenuated acetic acid writhing response in mice. It is probable, therefore, that the plant exerts its effect peripherally via peripheral inhibition of nociception. A recent study reported the central and peripheral antinociceptive activity of *B. africana* stem bark, which did not show an anti-inflammatory effect.[34] This may partly explain the higher efficacy of the extract seen in this study compared to the anti-inflammatory agent, aspirin. Further studies would be required to definitively account for the possible mechanism of action of the extract in peripheral and central pain.

Fever is a pathophysiological response produced by toxins and certain endogenous substances which include tumor necrosis factor-α (TNF-α) and prostaglandins.[35] Lipopolysaccharide produces fever by stimulating the production of endogenous TNF-α.[35,36] Paracetamol has been shown to suppress fever by inhibiting prostaglandin synthetase resulting in the blockade of synthesis of prostaglandin in the brain.[37] From our findings, it may be suggested that the extract inhibits the production of tumor necrosis factor-α and prostaglandins stimulated by the presence of the LPS. The short duration of the effect of the extract may be associated with low concentrations of antifebrile principles in the extract, which is a complex mixture of different phytochemicals.

The HPLC chromatogram revealed peaks that may correspond with rutin and caffeic acid, which may be employed as the chromatographic marker compounds of the active DCM fraction. The chromatogram also showed a higher concentration of these corresponding peaks, and this may be related to the higher antiplasmodial activity of the fraction compared with the parent ethanol extract. The presence of rutin and other phenolic acids suggests an antioxidant effect, although this may not completely account for the antiplasmodial activity of the extract and fraction.[16]

In conclusion, the findings of this study suggest that *B. africana* possesses antiplasmodial, anti-inflammatory and antipyretic effects thereby justifying its use in traditional medicine in the management of malaria. However, further *in vivo* studies are necessary to ascertain its antimalarial effect, fully elucidate its mechanism of action and to identify active antiplasmodial components resident in
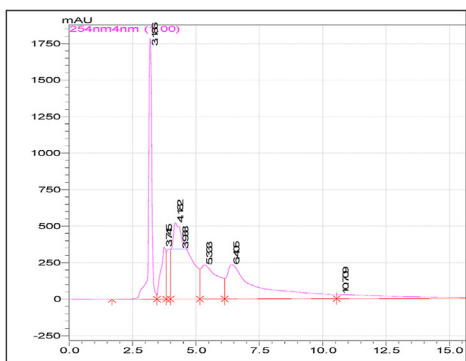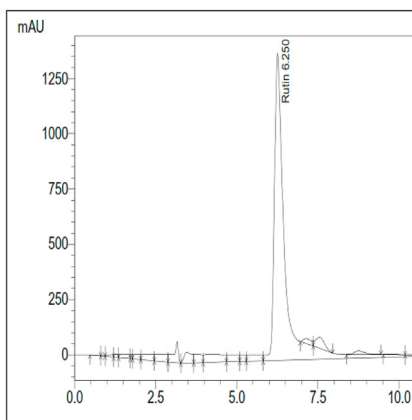
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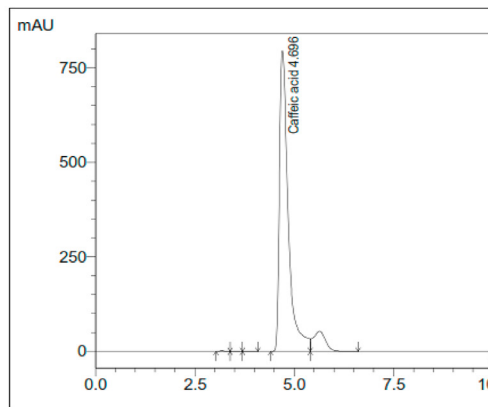
d. HPLC profile of caffeic acid
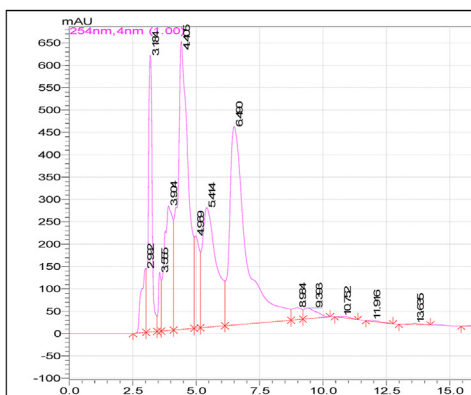
b. HPLC fingerprint of dichloromethane fraction of ethanol extract of *Burkea africana* stem bark

c. HPLC profile of rutin

Fig. 3. a. HPLC fingerprint of ethanol extract of *B. africana* stem bark, b. HPLC fingerprint of dichloromethane fraction of ethanol extract of *Burkea africana* stem bark., c. HPLC profile of rutin, d. HPLC profile of caffeic acid.

its dichloromethane fraction. To the best of our knowledge, this is the first time the antiplasmodial activity of *B.africana* is being reported.

Ethical approval

All authors hereby declare that "principles of laboratory animal care" (NIH publication no. 8523, revised 1985) were followed, as well as specific national laws where applicable. All experiments were examined and approved by Research and Ethics Committee of the Department of Pharmacology and Toxicology, NIPRD (NIPRD/ 05:03:05–6).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

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