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Evaluation of Secondary Metabolites, Antibacterial, Antiplasmodial and Acute Toxicity Potentials of Chloroform Crude Extract of *Boswellia dalzielii* Stem Bark

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ABSTRACT

Medicinal plants contain bioactive compounds that have the potentials to cure many human ailments without unnecessary side effects like most of the chemotherapeutic drugs used today. Therefore, the need for phytochemicals in medicinal plants for potentials application in the treatments of these human ailments as alternatives. Drug resistance parasite has rendered most of the drugs used in treating many human diseases ineffective. There is an urgent need and continuous search for new drugs from natural sources because most of the drugs used are either derived from plant or end-product of the natural source. Antibacterial and antiplasmodial activities of *Boswellia dalzielii* stem bark chloroform extract against some pathogens and *P. bergei* was investigated using the serial dilution method**.** Phytochemical studies (GC-MS RT profiling) revealed the presence of some secondary metabolites. The extract was tested against thirteen bacterial strains (*Styphylococcus epidermidis, Mycobacterium smegmatis, Enterococcus faecalis, Styplococcus aureus, Bacillus subtilis)* and Gram-negative strains *Klebsiella aerugninosa, Proteus vulgaris, K. pneumonia, Klebsiella oxytoca,Entrobacter cloacae, Peptostreptococcus asaccharolyticus, Escherichia coli, Proteus mirabilis).* Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the extract showed activities against *Mycobacterium smegmatis,Escherichia coli, Klebsiella oxytoca, Klebsiella aerugninosa and Proteus vulgaris.* The extract demonstrated high safety with LD50 value greater than 5000 mg/kg body weight. The extract shows a high potent of antiplasmodial activities with *P. bargie inhibition of 66.95%*. The results demonstrated that *Boswelliadalzielii* stem bark extract can be used as a source of cheaper, less toxic novel antibiotic and antimalarial substances for drug development.

Key words: Acute toxicity, Antibacterial, Antiplasmodial, *Boswellia dalzielii*, GC-MS RT, Medicinal plants.

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INTRODUCTION

Medicinal plants contain bioactive compounds that have the potentials to cure many human ailments without unnecessary side effects like most of the chemotherapeutic drugs used today. Therefore, the need for phytochemicals in medicinal plants for potentials application in the treatments of these human ailments as alternatives.[1] Drug resistance parasite has rendered most of the drugs used in treating many human diseases ineffective.[2] Therefore, the urgent need and continuous search for new drugs from natural sources because most of the drugs used are either derived from plant or end-product of the natural source.[2-4] *Boswellia dalzielii* from *Burseraceae* family, it is known as frankincense tree. The plant which is called "*Hannu*" in the Hausa language is a plant that is used medically by the West Africa people especially Northern part of Nigeria, Ivory Coast and Cameroon where the plant is found in abundance.[5-8] The decoction of the plants part is used in treating many ailments such as treating malaria, fever, gastrointestinal diseases, septic sores, rheumatism, venereal diseases.[9-11] The stembark secretes white gum that is burnt to fumigate cloth and to drive out flies, mosquitoes etc., from room.[7-8] Medicinal plants are parts of some African diets and are very important to their health. Traditional medicine began when man started searching for food in the bush by ploughing and eating all types of leaves and fruits.[12] Natural product compounds are also of great interest in the process of drug discovery and design. Mainly plant-derived natural product constituents have long been sources of drugs. A large proportion (30–40%) of the pharmaceuticals available in modern medicine is directly or indirectly derived from natural sources.[13-14] Due to their large diversity in nature, natural products serve as a source for the designating of lead molecules of interest for the development of new drugs. They support biochemical and molecular instrument needed to clarify complex cellular and molecular mechanisms of action involved in most pathological and physiological processes.[2,15]Plants are used as the primary source of medicinal agents due to its availability and cheapness by 80% of the world's population.[16] Some of the diseases treated using drugs from the plant include bacterial infection and malaria. Some of these drugs include Artemisinin derived from the Qinghao plant (*Artemisia annua L, China 4th century)* and quinine from the cinchona tree (South American, 17th century), penicillin

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found in 1947, methicillin found in 1959, tetracycline found in 1948, erythromycin found in 1952 and streptomycin found in 1943.[17-20]

The *Boswellia dalzielii* stem bark decoction is used to treat septile sore, venereal diseases, rheumatism, and gastrointestinal infections.[10-21]Phytochemical studies revealed that alkaloid is absence, [9-10,] while tannins, saponins, flavonoids, steroids, terpenes cardiac glycosides were found to be present.[22-23]It was reported by [23-24] that the methanolic and aqueous crude extracts of the *Boswellia dalzielii* stem bark has shown a good result on antibacterial and anti-fungal activities. This research was carried out because the previous investigation was on stem bark methanolic, ethanolic, aqueous and hexane extract. This was the first-time chloroform crude extract was been investigated on antibacterial, antimalarial and toxicity of *Boswellia dalzielii.* stem bark.

MATERIALS AND METHODS

Collection and preparation of plant materials

The stem bark of *Boswellia dalzielii* was collected from Kontagora LGA, Niger state, Nigeria in March 2017. Identification was undertaken where the voucher specimen [*Boswellia dalzielii* (Musa /KNT/FHI: 1481)].

Extraction and Isolation of the plant's extract

The stem bark (1 kg) were air-dried at 37°C and grounded to powder. Extraction was carried out following the method described by [26] with slight modification, the solvents used were hexane, ethyl acetate, chloroform and methanol. A 250 g of powdered plant materials were macerated separately in 600 mL of hexane for 72 hours, filtered using Whatman filter paper number 1 and concentrated using a rotary vacuum evaporator (South Africa). The plant's residue obtained after hexane extraction was macerated separately in 600 mL of ethyl acetate for another 72 hours, filtered and concentrated using a rotary vacuum evaporator. The residue from ethyl acetate extraction was then macerated in 600mL of chloroform for 72 hours, filtered and concentrated using a rotary vacuum evaporator.[25] The extracts were dried and stored in differently labelled vials. The percentage yield calculated as follows:

Percentage yield = mass of extract /Mass sample taken x 100 (1)

GC- high-resolution TOF-MS profile screening of the extracts

Mass calibration of the instrument was initially performed and passed before analyses to ensure that accurate mass data were collected on the LECO Pegasus GC-HRTOF-MS system (LECO Corporation, St Joseph, MI, USA). Perfluorotributylamine (PFTBA) was used as the mass calibration compound and 11 masses were used for pre-analysis calibration: CF3 (m/z 68.9952), C2F4 (m/z 99.9936), C2F4N (113.9967), C2F5 (m/z 130.9920) C3F6 (m/z 149.9904), C4F9 (m/z 218.9856), C5F10N (m/z 263.9871), C8F16N (m/z 413.9775), C9F18N (m/z 463.9743) and C9F20N (m/z 501.9711). The observed intensity and resolution were 41.392 and 40.200, respectively, with a mass accuracy root mean square (RMS) of < 1 ppm. Samples were subsequently analyzed on the GC-HRTOF-MS system equipped with an Agilent 7890A gas chromatograph (Agilent Technologies, Inc., Wilmington, DE, USA) operating in high-resolution, equipped with a Gerstel MPS multipurpose autosampler (Gerstel Inc. Germany) and a Rxi®-5 ms column (30 m × 0.25 mm ID × 0.25 µm) (Restek, Bellefonte, USA). One microliter of each sample was injected in a spitless mode using helium as a carrier gas pumped at a constant flow rate of 1 mL/min. The inlet and transfer line temperatures were 250 and 225 °C, respectively, the initial oven temperature was set at 70 °C, held for 0.5 min, ramped at 10 °C/min to 150 °C, held for 2 min, ramped at 10 °C/min to 330 °C and held for 3 min for the column to bake out. The MS data acquisition rate was a recommended rate of 13 spectra/s, m/z range of 30–1000, electron ionization at 70 eV, ion source temperature at 250 °C and a system recommended extraction frequency of 1.25 kHz. Sample extracts of three biological replicates were analyzed twice, yielding a total of six analytical injections for each sample.[26]

Qualitative examination of phytochemicals

Test for flavonoids

Shinoda Test: About three pieces of magnesium chips were dropped into the plant's chloroform crude filtrate, few drops of concentrated hydrochloric acid were added. A red to purple or pink, orange coloration indicated that flavonoids are presence.[27,28]

Ferric chloride: 2 ml of the plant's chloroform crude filtrate, the addition of a few drops of 10% ferric chloride solution was made. Aviolet coloration or green-blue confirmed the presence of a phenolic hydroxyl group. [27-28]

Terpenoids test: (Salkowski test): 0.5 g of the chloroform crude extracts was added to 2 ml of chloroform. 3 ml of concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer. A reddish-brown coloration of the interface indicated the presence of terpenoids.[31]

Tannins:3 drops of 0.1% ferric chloride were added to 5 ml of chloroform crude extract. A blue-black or brownish-green coloration indicated the presence of tannins.[28-29]

Saponins test: 5ml of water was added to 10ml of chloroform crude extract and agitated vigorously for a stable persistent froth. 3 drops of Olive oil lead to the formation of emulsion which confirmed the presence of saponins.[28,29]

Steroids test:2 ml acetic anhydride was added to about 5 ml of plant's chloroform crude extract. Some drops of concentrated sulphuric acid were added to the mixture. A violet to blue or green coloration indicated steroid presence. [28-29]

Test for Alkaloid: 0.5 ml of Bismuth potassium iodide solution known as "Dragendorff's reagent" was mixed with 2 ml of plant's extract. Orange precipitate indicated alkaloids presence. [28-29]

Crude extracts acute toxicity tests

Acute toxicity of the plant's extract was tested using oral administration method. The oral administration of the plant's extract at a single high dose of 5,000 mg/kg body weight was carried out according to OECD methods.[30]

Anti-plasmodial screening of the extracts

Inoculation of parasite

Highly parasitized (20-30% parasitemia) blood was obtained by cardiac puncture from *Plasmodium berghei* infected mice. The blood was diluted with phosphate buffer saline and 0.2ml of the diluted blood was intraperitoneally inoculated into the mouse.[31]

Treating of inoculated mice with plant extracts

Four days (4) suppressive test were carried out to evaluate the antimalarial properties of the extracts according to the method described by.[32] A single dose of 300 mg/kg body weight (bw) of each extract was tested with each group consisting of 3 mice. Two groups of mice (3 each) were set up as negative and positive controls and were treated with normal saline (2 ml/100 g) and chloroquine (5 mg/kg body weight) respectively. All the treatments were done orally for four consecutive days. Parasitemia count was carried out by preparing a Giemsa stained-thin film and viewed under the microscope as described by.[32] Changes in weight and Packed Cell Volume (PCV) were monitored throughout the study period. The PCV was determined using the microhaematocrit method as described by.[33]

Collection of Blood and Preparation of Serum

The collection of blood samples for biochemical analyses was done as described by.[34] At the end of the four days treatment, the animals were fasted but still had water *ad libitum* for 24 h before they were sacrificed under ether anaesthesia. The blood was collected in a clean, dry centrifuge tube. The blood sample was allowed to stand for 10 minutes at room temperature and then centrifuged at 1000 rpm for 15 minutes to get the serum.

Biochemical parameters

Aspartate aminotransferase activity (AST)

Principle: An Aspartate aminotransferase (AST) test kit (Randox Laboratories Ltd, Crumlin, UK) was used. Aspartate aminotransferase was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4 – dinitrophenylhydrazine.

GOT

α-oxoglutarate + L-aspartate glutamate +oxaloacetate (2)

Procedure: Solution A contained phosphate buffer, L – aspartate, α – oxoglutarate while solution B contained 2, 4 – dinitrophenylhydrazine. 0.05 ml of the sample was added to 0.25 ml of solution A. It was mixed and incubated for exactly 30 minutes at 37˚C. Afterwards, 0.25 ml of solution B was added, mixed and allowed to stand for 20 minutes at 25˚C. Thereafter 0.25 ml of 0.1 M NaOH was added to the mixture and thoroughly mixed. 0.05 ml of distilled water was added to the blank test tube and the same procedure as done for the sample was repeated. The absorbance was taken at 546 nm after 5 minutes. The activity of aspartate aminotransferase (AST) in the sample was read off the standard curve. This was carried out based on the procedure.[35]

Alanine aminotransferase activity (ALT)

Principle: An Alanine aminotransferases test kit (Randox Laboratories Ltd, Crumlin, UK) was used. Alanine transaminase (ALT) or Glutamate pyruvate transaminase (GPT) catalyzes the formation of pyruvate and glutamate from alanine and α – oxoglutarate.

GPT

α-oxoglutarate + alanine
L-glutamate + pyruvate (3)

Procedure: Solution A contained buffer (phosphate buffer), L – alanine, α – oxoglutarate while solution B contained 2, 4 – dinitrophenylhydrazine. 0.05 ml of sample was pipetted into the sample test tube, 0.25 ml of solution A was added to it. It was mixed and incubated for exactly 30 minutes at 37˚C. Afterwards, 0.25 ml of solution B was added, mixed again and allowed to stand for 20 minutes at 25˚C. 0.25 ml of 0.1 M NaOH was then added to the mixture. It was mixed, and the same procedure was repeated for blank using 0.05 ml of distilled water in place of the sample. The absorbance was taken at 540 nm after 5 minutes. The activity of ALT in the sample was read off the standard curve. This was carried out based on the procedure.[36]

Alkaline phosphatase (ALP)

An alkaline phosphatase test kit (Randox Laboratories Ltd, Crumlin, UK) was used for the estimation of alkaline phosphatase (ALP). The principle of this method is based on the reaction involving serum alkaline phosphatase and colourless substrate of phenolphthalein monophosphate. The ALP hydrolyses phenolphthalein monophosphate to phosphoric acid and phenolphthalein. The mixture turned pink at pH 9.8 and ALP was measured spectrophotometrically at 540 nm.

ALP

(P-nitrophenylphosphate)+ H_2O PO_4 + (P-nitrophenol) (pink, pH= 9.8) (4)

The 0.05 ml of sample and 0.05 ml of distilled water were dispensed into their respective labelled test tubes. Three milliliters (3.0 ml) of the substrate was added to each test tube and then mixed. The initial absorbance (A1) was taken at 1 min and subsequent absorbances were taken every minute for an additional 3 minutes at 405 nm and 37˚C. The difference between absorbances (ΔA) and the average absorbance differences per minute (ΔA/min) was calculated. It was carried out as described by.[37]

ALP activity (U/L) = (ΔA/min) x 2750 (5)

Where: ΔA is changed in absorbance per minute

Determination of serum total protein concentration

Serum total protein concentration was calculated using Randox kit from Randox Laboratories Limited, U. K.

Principle: Cupric ions in an alkaline medium interact with protein peptide bonds resulting in the formation of a coloured complex.

Procedure: Serum (0.02 ml), 0.02 ml of standard regent and 0.02 ml of distilled water were respectively pipetted into test tubes labelled serum, standard and blank. One millilitre (1 ml) of R1 (Biuret reagent) was added to all test tubes, mixed gently and incubated for 30 minutes at 25˚C. The absorbances of sample and standard were measured at 546 nm against the blank.[41] The concentration was calculated using Equation 4:

Total protein concentration (mg/dL) = A_{sample} x 190 (6)

R1 reagent consists of 100mmol/l of sodium hydroxide, 16mmol/l of Na-K-tartrate, 15mmol/l of Potassium iodide and 6mmol/l of cupric sulphate.

Antibacterial assay

The crude extracts was evaluated by the micro-dilution.[42,43]Thirteen both Gram-positive bacteria (*Styphylococcus epidermidis (ATCC12228TM), Mycobacterium smegmatis (ATCC14468TM),Enterococcus faecalis (ATCC14506TM), Styplococcus aureus (ATCC25923TM), Bacillus subtilis (ATCC19659TM))* and Gram-negative strains *Klebsiella aerugninosa (ATCC13048TM), Proteus vulgaris (ATCC33420TM), Klebsiella oxytoca (ATCC8724TM), Entrobacter cloacae(ATCC13047TM), Peptostreptococcus asaccharolyticus (ATCC14963TM), Escherichia coli (ATCC25922TM), Proteus mirabilis (ATCC7002TM).* Stock solution (1mg/mL) of every single extract were prepared in DMSO out of which 100 µL of each of them was serially diluted (1:1) to give 500, 250, 62.5 and 31.2 µL/mL and were seeded in 96-well plate. An overnight fresh culture (50 µL) containing 1.5x10^8cfu/Ml of each strain in the muller-Hilton broth was selectively arranged in 96-well plates which contained 50 µL different concentrations in duplicate to make a final volume of 100µLand allowed to grow overnight with 5% CO_2 flow at 37°C. Streptomycin and Nalidixic acid were used as control. Cells viability was confirmed using Resazurin dye after 5 hours incubation and the Minimum Inhibition Concentration was recorded for each extract. The Minimum Bactericidal Concentration value was determined by plating directly the content of the wells with concentrations higher than the MIC value. The MBC value was determined when there was no bacterial growth from the directly plated contents of the wells. In addition, the contents of the well showing indication of growth inhibition were serially diluted to quantify an endpoint neutralizing the bacteria.[38-39]

RESULTS AND DISCUSSION

Table 1 shows the total protein contents of the mice.

Infected, untreated mice have lower serum Alkaline phosphatase, total proteins and albumin concentrations when compared with the normal control, standard and extract-treated groups. However, mice treated with BC02 recorded higher protein concentrations compared with the negative control group. Alanine transaminase (ALT) activities, on the other hand, were much higher in the untreated control. Treatments with the compound significantly lower activities of this enzyme towards normalization. The total protein content of the mice after treatment with the extract of BC02 is 51.07±6.76 mg/dL. The biochemical parameters monitored in the liver and serum are useful 'markers' for assessing tissue damage.[39]The liver which plays a vital role in the intermediary metabolism of biomolecules and drugs could also be affected by the toxic side effects of these drugs and diseases. AST and ALTare markers of liver damage and can be used to assess liver cytolysis during parasitic infection.[40] In the present study, infected untreated mice have lower serum Alkaline phosphatase, total proteins and albumin concentrations when compared with the normal control, standard and extract-treated groups.

The increase in ALT activities in the infected untreated mice may be related to liver inflammation and is an indication of abnormal function of the liver.[41] Alkaline phosphatases are often used to assess the integrity of the plasma membrane and endoplasmic reticulum.[48] The alteration in serum ALP activities in *P. bergei* infected untreated rats suggested that the integrity and functionality of endoplasmic reticulum and plasma membrane have been comprised by the malaria infection.[42] Mice treated with the compound shows satisfactory activities of biomarker enzyme which were an indication of preserved organ integrity. The total proteins and albumin play major roles in assessing the integrity of kidney and liver.[43]*The malaria parasite* has a varying effect on total protein as hypoproteinemia as well as hyperproteinemia.[44]This study, however, showed significant hypoproteinemia in the serum of *P. berghei* infected untreated mice. This significant decrease may be due to the mobilization of defensive enzymes (which are known proteins) to counter the effect of parasite-induced oxidative stress which were consequently ameliorated by some of the plant's extracts. The decrease in albumins and total proteins reported in this study could lead to overhydration which is injurious to cellular homeostasis. This will harmfully compromise the normal metabolic activities of the liver and consequently the health of the animals.[44] The improvement in the concentrations of albumin and total proteins in rats that are cured with the plant's extract is an indication of the reduced pathological effect of the parasite.

Infected mice body weight changes

The body weight change in *P. berghei infected mice following treatment with extracts Boswellia dazillie, is* shown in Table 3. There was a significant decrease in body weight of all the experimental animals after induction. Treatment of the infected mice with 5mg/kg bw chloroquine (standard drug) increases the bodyweight of the animals at the end of the experiment however, infected untreated mice show a significant decrease in body weight of the mice. BC02extractsdecrease the weight of animals after treatment.

Packed cell volume

The packed cell volume (PCV) in *P. berghei*infected mice following treatment with). 5000 mg/kg, *Boswellia dalzielii* chloroform extracts (BCO2) which indicated acute toxicity of LD_{50}>5000 mg/kg. There was a significant decrease in packed cell volume (PCV) of all the experimental animals after induction. The infected micewere treated with 5mg/kg body weight (bw) chloroquine (standard drug) and the resulted animal showed an increase in the packed cell volume (PCV), however, infected untreated mice show a decrease in packed cell volume (PCV) of the animals. However, the plant's chloroform extract decreases the PCV of treated mice from 33.50±0.65% to 31.21±1.53%.

Acute toxicity

The extract shows a varying degree of behavioral changes including; restlessness, hyperactivity, weakness, erythraemia and Rubbing of mouth on the wall of the cage upon acute (5000 mg/kg bw). Exposure of the plant extracts in mice demonstrates high safety with LD_{50} value higher than 5000mg/kg bw(Table 4). *Boswellia dalzielii* chloroform crude extract (BC02) has shown less toxicity >5000 in vivo, it was reported by, [45] that *Boswellia dalzielii* aqueous extract gave >1500 IC_{50} *in vitro*.

Parasitaemia

Table 5 presented the parasitaemia counts of *P. berghei* infected mice treated with chloroform crude extracts from *Boswellia dazielii,* plants. Treatment of the infected mice with 5mg/kg bw chloroquine (standard drug) produce significant antiplasmodial activities with 97.39% inhibitions of the parasite. Similarly, treatment with medicinal plant produces a varying degree of antiplasmodial effect with percentage parasite inhibition of 66.95%. It was reported by [46-47] that the antiplasmodial activities in vitro against *P. falciparum* of chloroform, methanol and water extracts of *Boswellia dalzielii leaves* to be 41.01±11.30 µg/ml, 18.85±1.93 µg/ml and >100 (IC50. 3D7±SD µg/ml) respectively.

Table 1: Mice total protein.

Extracts	Albumin (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Proteins (mg/dL)
BCO_2	5.53 ± 0.5714	13.16 ± 0.34	21.6 ± 0.27	168.34 ± 2.67	51.07 ± 6.76
Negative	2.32 ± 0.32	25.04 ± 0.50	49.6 ± 0.36	134.05 ± 3.90	21.34 ± 0.91
Normal control	5.20 ± 0.3452	29.68 ± 0.78	38.9 ± 0.42	178.43 ± 0.97	49.78 ± 1.89
Standard control	5.17 ± 0.8571	37.6 ± 0.79	68.4 ± 0.67	189.05 ± 1.37	52.83 ± 3.57

Data are Mean ± SEM of duplicate determination, BC02= Chloroform extract of *Boswellia dalzillie.*

Table 2: Effect of plant extracts body weight changes in (*P. berghei)* infected mice.

Extracts	Before Inoculation	After inoculation	After treatment
BCO2	22.50 ± 0.50	19.00 ± 0.00	22.00 ± 0.00
Control	19.50 ± 2.50	21.50 ± 1.50	24.00 ± 1.00
Negative	24.00 ± 1.00	21.50 ± 0.50	18.00 ± 3.00
Standard control	23.50 ± 1.50	20.00 ± 1.00	23.00 ± 1.00

Data are Mean ± SEM of duplicate determination.

The phytochemicals screening of the chloroform extracts shows that there were mild contents of polyphenone, medium contents of alkaloids, saponins, steroids and triterpenes and anthraquinone. The extract gave a high intensity of flavonoids and tannins.

Table 6. The extract showed high sensitivity on *M. segmentis* which has the MIC of 62.5µg/mL. MBC of 125µg/mL and MBC/MIC ratio of 2 as the smallest value. When the ratio of the MBC/MIC value was lower than 2, the extract exhibits a bactericidal effect.[48] some of these MIC values obtained over a wide range pathogenic strains are lower than some of the values exhibited by the standard drugs used in this study (Streptomycin (STM) and Nalidixic acid (NLD) as shown in the Table 6. MIC value of *E. cloacae* bacterial strain 250µg/ml is lower than one of the drug used Streptomycine with MIC value >512µg/ml, *P. vulgaris* MIC value 125 µg/mL is lower than the other control drug Nalidixic acid 500µg/mL, *E. coli* 125µg/mL lower than Nalidixic acid 512 µg/mL, *M. segmatis*62.5µg/mL lower than Nalidixic acid value >512µg/mL while*E. faecalis* 250 µg/mL is lower than Nalidixic acid 512µg/mL. The MIC and MBC tested showed that the *Boswellia dalzielii* (BC02) chloroform crude extract has activity against some gram-positive and gram-negative bacteria; it is therefore termed broad spectrum as reported by.[46-50] *M. segmatis* was the most sensitive while *B. subtilis* was the least for gram-positive while *E. coli, P. vulgaris, K. aerogenes, K. oxytoca* was the most sensitive and *P. asaccharolyticus, Kneumonia, E. cloacae, P. mirabilis* was the least for gram-negative. The sensitivity of these bacterial representatives to the studied extract is concentration-dependent. The extract MIC values as shown in Table 6 was able to inhibit some gastrointestinal pathogens (GI) like *E.faecalis, E. coli and K. aerogenes.* The extract inhibits some respiratory pathogens such as *K. pneumonia, P. vulgaris and K. oxytoca.* It also inhibited some skin pathogens like *S. epidermidis, M. smegmatis* and *K. oxytoca.* Pathogens of urinary were also inhibited by the extract *P. mirabilis* and *P. vulgaris.*

Table 3: Effect of plant extracts on packed cell volume (PCV) in *P. berghei* infected mice.

	Packed Cell Volume (%)		
Extracts	Before Inoculation	After inoculation	After treatment
BCO2	46.29 ± 7.89	33.50 ± 0.65	31.21 ± 1.53
Negative	41.26 ± 1.90	21.50 ± 3.23	21.78 ± 1.45
standard control	45.56 ± 0.73	30.50 ± 2.50	39.50 ± 2.45

Data are Mean ± SEM of duplicate determination.

Table 4: parasitaemia count in *P. berghei* infected mice treated with plant Boswellia dalzielii chloroform crude extract BC02.

	Parasitaemia			
Plant's extract	One	Three	Five	% Parasite Inhibition
BCO2	5.00 ± 2.00	24.00 ± 2.00	19.00 ± 2.00	66.95[b]
Standard control	5.00 ± 3.00	11.50 ± 0.50	1.50 ± 0.03	97.39[a]

Data are Mean ± SEM of triplicate determination. The mean parasite inhibition with different superscript alphabet are significantly ($p<0.05$) difference.

Table 5: Phytochemical screening of *Boswellia dalzielii* stem bark chloroform extracts.

Plant extract	Alkaloid	Saponnin	Steroids & Triterpenes	Phenolic; Anthraquinone	Flavonoids	Polyphenone	Tannin
BCO2	++	++	++	++	+++	+	+++

+ = mild, ++ = medium, +++ = high intensity%yield of BCO2 extracts was 3.4%.

Table 6: Antibacterial activity result of *Boswellia dalzielii*stem bark chloroform crude extracts.

Gram positive (G+) Bacteria strain	Streptomycin	Nalidixic Acid	Mic(µg/ml)	mbc (µg/ml)	mbc/mic ratio
S. epidermidis (ATCC NO.12228TM	8	64	250	500	2
B. subtilis (ATCC NO.19659TM)	16	16	500	>500	2
S. aureus (ATCC NO.25923TM)	256	64	250	500	2
M. smegmatis (ATCC NO.14468TM)	<4	>512	62.5	125	2
E. faecalis (ATCC NO.14506TM)	128	>512	250	500	2
Gram-negative (G-) Bacteria strain					
P. asaccharolyticus (ATCC NO.14963TM)	128	128	250	500	2
K. pneumonia (ATCC NO.12228TM)	64	64	250	500	2
E. cloacae (ATCC NO.13047TM)	>512	16	250	500	2
K. oxytoca (ATCC NO.8724TM)	16	8	125	250	2
P. mirabilis (ATCC NO.7002TM)	16	256	250	500	2
K. aerogenes (ATCC NO.13048TM)	64	64	125	250	2
P. vulgaris (ATCC NO.33420TM)	30	8	125	250	2
E. coli (ATCC NO.25922TM)	64	512	125	250	2

MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration

CONCLUSIONS

The plant under study is traditionally used for the treatment of various bacterial infections which includes gastrointestinal (*E. faecalis, E. coli and K. aerogenes,* respiratory (*K. pneumonia, P. vulgaris and K. oxytoca),* skin (*S. epidermidis, M. smegmatis* and *K. oxytoca) and* urinary (*P. mirabilis* and *P. vulgaris)* pathogens contributes to its validity as traditional treatments for such ailments. The chloroform extract showed activity of 66.95% against plasmodium bergei with little or none toxicity and also exhibit excellent activity against some of the pathogens may be due to the presence of certain terpenes, flavonoids, saponnins and other metabolites identified in the stem bark. A positive result may be anticipated for further studies involving isolation of active compounds, future phytochemical studies and toxicity assays will lead to the development of other effective alternatives for the treatment of malaria and bacterial infections. The results demonstrated that *Boswellia dalzielii* stem bark chloroform crude extract can be used as a source of cheaper and less toxicity novel antibiotic and antimalarial substances for drug development.

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ETHICAL CLEARANCE

The ethical clearance for this study was obtained from the Federal University of Technology Minna, Niger state of Nigeria where the antimalarial assay was conducted.

REFERENCES

1. Tijjani A, Ya S, Fi A, Iz K, Gm T, Li C. Isolation and Structural Elucidation of 20 Hydroxyecdysterone from Vitex doniana Sweet Stem bark (Black plum) Mustapha. Medicinal Chemistry. 2017;7(3):828-31.
2. Gathirwa JW, Nyang'acha RM. Why medicinal plants could play a role in treating malaria. Kenyan Medical Research Institute, Kemri. April 12, 2017.
3. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: efficacy models for compound screening. Nature reviews Drug discovery. 2004;3(6):509-20.
4. Organization for Economic Cooperation and Development O.E.C.D. Guidelines for the testing of chemicals/ no. 407: Repeated dose oral toxicity test method. 2008, Paris, France.
5. Hilou A, Nacoulma OG, Guiguemde TR. *In vivo* antimalarial activities of extracts from Amaranthus spinosus L. and Boerhaaviaerecta L. in mice. Journal of ethnopharmacology. 2006;103(2):236-40.
6. Tukur F. Pharmacological Evaluation of Three Locally Used Medicinal Plants Against Carbon Tetrachloride-Induced Hepatotoxicity pn Rats, 2012.
7. Dalziel JM. The useful plants of west tropical Africa. The useful plants of West Tropical Africa. 1937.
8. Younoussa L, Nukenine EN, Esimone CO. Toxicity of Boswellia dalzielii (Burseraceae) leaf fractions against immature stages of Anopheles gambiae (Giles) and Culex quinquefasciatus (Say)(Diptera: Culicidae). International Journal of Insect Science. 2016;8:IJIS-S37188.
9. Olukemi MA, Kandakai-Olukemi YT, Mawak JD. Antibacterial activity of the stem bark of Boswellia dalzielii. J Pharm & Bioresourses. 2005;2:131-6.
10. Burkill HM. The useful plants of west tropical Africa; Vol.1 UK: white Friars Press. 1965:300.
11. Trease GE, Evans WC. Pharmacognosy (13th edn). Bailliere Tindall, London. 1989:176-80.
12. Lewinsohn R. Três Epidemias: lições do passed, 200; 81-85, Editora unicamp, ISBN 8526805827, Campinas, Brazil.
13. de Araújo-Júnior JX, de Oliveira MSG, Aquino PGV, Alexandre-Moreira MS, Sant'Ana AEG. A phytochemical and ethnopharmacological review of the genus *Erythrina*. In phytochemicals — A global perspective of their role in nutrition and health, Rao V, (Ed.). InTech. Available from: http://www.intechopen.com/books/phytochemicals-a-global-perspective-of-their-role-in-nutrition-and-health/a-phytochemical-and-ethnopharmacological-review-of-the-genus-erythrina.2012 Mar 21.
14. Nmorsi OP, Egwunyenga AO. Antioxidant status of Nigerian children with Plasmodium falciparum malaria. African Journal of Microbiology Research. 2007;1(5):61-4.
15. Oliveira AB, Dolabela MF, Braga FC, Jácome RL, Varotti FP, Póvoa MM. Plant-derived antimalarial agents: new leads and efficient phythomedicines. Part I. Alkaloids. Anais da Academia Brasileira de Ciencias. 2009;81(4):715-40.
16. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: efficacy models for compound screening. Nature reviews Drug discovery. 2004;3(6):509-20.
17. Krettli AU, Andrade-Neto VF, Brandão MD, Ferrari W. The search for new antimalarial drugs from plants used to treat fever and malaria or plants randomly selected: a review. Memórias do Instituto Oswaldo Cruz. 2001;96(8):1033-42.
18. Additional material adapted from the centers foWr Disease control (CDC) Malaria website, 2005.
19. Greenwood D. Antimicrobial drugs: chronicle of a twentieth century medical triumph. Oxford University Press; 2008.
20. Bhattacharjee M. Chemistry of antibiotics and related drugs. Cham: Springer Science + Business media; New York, NY; 2016.
21. Trease GE, Evans WC. Pharmacognosy (13th edn). Bailliere Tindall, London. 1989:176-80.
22. Baoua M, Fayn J, Bassiere J. Preliminary phytochemical testing of some medicinal plants of Niger. Plant Med Phytother. 1976;10:251-66.
23. Alemika TO, Oluwole FS. An investigation of the potentials of Boswellia dalzielii and Commiphorakerstingii in the treatment of peptic ulcer. W Afr J Pharmacol Drug Res. 1991;9(10):91-4.
24. Adelakun EA, Finbar EA, Agina SE, Makinde AA. Antimicrobial activity of Boswellia dalziellii stem bark. Fitoterapia. 2001;72(7):822-4.
25. Ntiejumokwu S, Alemika TO. Antimicrobial and phytochemical investigation of the stem bark of Boswellia dalzielii. West African Journal of pharmacology. Drug Rese. 1991;10:100-4.
26. Ocheng F, Bwanga F, Joloba M, Borg-Karlson AK, Gustafsson A, Obua C. Antibacterial activities of extracts from Ugandan medicinal plants used for oral care. Journal of ethnopharmacology. 2014;155(1):852-5.
27. Adebo, OA, Kayitesi, E, Tugizimana, F, Njobeh, PB, Differential metabolic signatures in naturally and lactic acid bacteria (LAB) fermented *ting* (a Southern African food) with different tannin content, as revealed by gas chromatography-mass spectrometry (GC–MS)-based metabolomics. Food Res Int. 2019;121:326-35.
28. Ocheng F, Bwanga F, Joloba M, Borg-Karlson AK, Gustafsson A, Obua C. Antibacterial activities of extracts from Ugandan medicinal plants used for oral care. Journal of ethnopharmacology. 2014;155(1):852-5.
29. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005;4(7):685-8.
30. Organization for Economic Cooperation and Development O.E.C.D. Guidelines for the testing of chemicals/ No. 407: Repeated dose oral toxicity test method. 2008 Paris, France.
31. Hilou A, Nacoulma OG, Guiguemde TR. *In vivo* antimalarial activities of extracts from Amaranthus spinosus L. and Boerhaaviaerecta L. in mice. Journal of ethnopharmacology. 2006;103(2):236-40.
32. Jigam AA, Abdulrazaq UT, Egbuta MN. *In-vivo* antimalarial and toxicological evaluation of Chrozophora senegalensis A. Juss (euphorbiaceae) extracts. Journal of Applied Pharmaceutical Science. 2011;1(10):9.

33. Dacie JV, Lewis SM. Investigation of Coagulation Factors In: Practical Haematology. Edinburgh: Churchill Livingstone. 2000.
34. Shittu OK, Lawal B, Adeniyi AK, Kilani LT, Saka RB. Effect of Methanol extract of Musca domestica larva on some Enzymes and Haematological parameters in Trypanosoma brucei brucei-infected rats. Nigerian Journal of Basic and Applied Sciences. 2017;25(2):66-74.
35. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology. 1957;28(1):56-63.
36. Wright P. J., & PlummerD. T. The use of urinary enzyme measurement of direct renal damage caused by nephrotoxic compounds. Biochem Pharmacy. 1972;23:65-73.
37. Weichselbaum CT. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. American Journal of Clinical Pathology. 1946;16(3_ts):40-9.
38. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta medica. 1998;64(08):711-3.
39. Andrews JM. Determination of minimal inhibitory concentration. Journal Antimicrob Chemother. 2001;48:5-6.
40. Elshikh M, Ahmed S, Funston S, Dunlop P, McGaw M, Marchant R, *et al*. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnology Letters. 2016;38(6):1015-9.
41. Yusuf AA, Mohammed BA, Abafi MJ, Garba R, Usman OM, Ibrahim J, *et al*. Alterations in Serum Urea, Creatinine and Electrolytes Concentrations in Wister Rats Following Repeated Administration of Methanol Extracts Ofazanzagarckeana Pulp.
42. Akanji MA, Adeyemi OS, Oguntoye SO, Sulyman F. Psidium guajava extract reduces trypanosomosis associated lipid peroxidation and raises glutathione concentrations in infected animals. EXCLI J. 2009;8:148-54.
43. Umar SI, Ndako M, Jigam AA, Adefolalu SF, Ibikunle GF, Lawal B. Anti-plasmodial, Anti-inflammatory, anti-nociceptive and safety profile of Maytenus senegalensis root bark extract on hepato-renal integrity in experimental animals. Comparative Clinical Pathology. 2019;28(6):1571-9.
44. Lawal B, Shittu OK, Oibiokpa FI, Mohammed H, Umar SI, Haruna GM. Antimicrobial evaluation, acute and sub-acute toxicity studies of Allium sativum. Journal of Acute Disease. 2016;5(4):296-301.
45. Eghianruwa KI. The effect of supplemental antioxidants vitamin C and dimethyl sulfoxide on weight gain and survival in T. brucei infected and diminazene treated rats. Veterinarskiarhiv. 2012;82(5):519-29.
46. Bhonsle HS, Korwar AM, Kote SS, Golegaonkar SB, Chougale AD, Shaik ML, *et al*. Low plasma albumin levels are associated with increased plasma protein glycation and HbA1c in diabetes. Journal of Proteome Research. 2012;11(2):1391-6.
47. Jansen O, Angenot L, Tits M, Nicolas JP, De Mol P, Nikiéma JB, *et al*. Evaluation of 13 selected medicinal plants from Burkina Faso for their antiplasmodial properties. Journal of Ethnopharmacology. 2010;130(1):143-50.
48. Michel-Briand Y. Molecular mechanisms of antibiotic action. MASSON, PARIS (FRANCE).1986.
49. Ntiejumokwu S, Alemika TO. Antimicrobial and phytochemical investigation of the stem bark of Boswellia dalzielii. West African Journal of Pharmacology. Drug Rese. 1991;10:100-4.
50. Rezghi M, Hajimehdipoor H, Zakerin S, Fahimi S. Preparation of a Traditional Poly Herbal Gel for Abnormal Vaginal Discharge. Research Journal of Pharmacognosy. 2019;6(4):27-35.

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