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Research Article

Antimicrobial Efficacy, Cytotoxicity, Acute Oral Toxicity, and Phytochemical Investigation of the Aqueous and Methanolic Stem Bark Extracts of *Bridellia micrantha* (Hochst.) Baill

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ABSTRACT

Introduction: Microbial infections cause high morbidity and mortality in humans globally. Antimicrobial resistance, emergence of new strains, high costs of antibiotics, inaccessibility-especially in remote areas, and adverse effects, impede successful eradications of pathogens, hence the need for novel strategies. *Bridellia micrantha* is used in traditional medicine to treat microbial infections; however, it has not been empirically validated. **Methods:** Antimicrobial activity of the aqueous and methanolic stem bark extracts of *Bridellia micrantha* was investigated using the disk diffusion and broth microdilution techniques, described by the Clinical Laboratory Standards Institute (CLSI) guidelines. The brine shrimp lethality assay technique was used to investigate the cytotoxic effects of the studied plant extracts in exposed nauplii. Acute oral toxicity effects of the studied plant extracts in Winstar rats were investigated following the up-and-down procedure described by the Organisation for Economic Development and Co-operation (OECD). Qualitative phytochemical screening was performed following standard procedures. **Results**: The aqueous and methanolic extract of *B. micrantha* indicated varied antimicrobial activities against *E. coli, S. typhimurium, S. aureus,* and *C. albicans*, with inhibition zones ranging from 6.00mm to 19.00mm. Furthermore, the studied plant extracts exhibited low MIC values (≤100 µg/ml) on selected microbes. Since the MIC values were much lower than 1000µg/ml (the cutoff for antimicrobial efficacy appraisal), it is anticipated that, the studied plant extracts can be strong antibiotics. The aqueous and methanolic stem bark extracts of *B. micrantha* were cytotoxic to brine shrimp nauplii, with LC_{50} values of 486.67±3.15 µg/ml and 458.33±2.87 µg/ml, respectively; however, these extracts did not elicit any observable signs of toxicity in rat models. Pharmacologically active phytochemicals, including flavonoids, alkaloids, saponins, tannins, phenols, and anthraquinones were detected in the two studied extracts. **Conclusions:** The aqueous and methanolic stem bark extracts of *B. micrantha* have appreciable antimicrobial activity against *E. coli, S. typhimurium, S. aureus* and *C. albicans*. Besides, the studied plant extracts are cytotoxic to brine shrimp nauplii; but they do not cause acute oral toxicity effects in rat models. Additionally, the studied plant extracts contain bioactive phytochemicals, with antimicrobial activity.

Key words: Brine shrimp lethality Assay, Minimum inhibitory concentration, Median lethal concentration (LC_{50}), median lethal dose (LD_{50}), Zone of inhibition.

INTRODUCTION

Microbial infections are among the major causes of morbidity and mortality in humans globally, especially in vulnerable groups [1–3]. Furthermore, the increased rate of antimicrobial resistance to the available medications has caused major healthcare challenges[4,5]. The persistence of microbial infections during and after the treatment cycles has precipitated an overuse of antibiotics leading to other unprecedented outcomes [6].

Owing to the drawbacks and inefficiencies of conventional antimicrobial chemotherapy, alternative stratagems are required to curb infectious diseases at relatively cheaper costs than those involved in orthodox medicine, and with fewer or no toxic effects[7]. Medicinal plants on the other hand are a valuable source of antimicrobials owing to their long-term applications. Further, these plants are considered to easily accessible, affordable and with fewer side effects compared to western medicine [8,9].

Bridellia micrantha (Hochst.) Baill is a medium to large deciduous tree which grows up to 20 m above the ground with spreading crown[10]. It belongs to Euphorbiaceae family of herbs and trees which are characterized by succulent leafless branches; milky or watery latex; glands at the leaf base; and 3-lobed fruits. It is locally known as '*mukuigo*' by the Kikuyu of Murang'a County, Kenya [11,12].

The stem bark tinctures and decoctions of *Bridellia micrantha* are used to cure burns, soft tissue injuries, sexually transmitted infections, protozoa infections, gastrointestinal conditions, typhoid, pneumonia and dental diseases [12,13]. Leaf preparation is used to manage eye problems [13].

Previous studies have indicated that *B. micrantha* has anti-ulcer activity against *H. pylori*-induced ulcers and antimicrobial activities against *S. typhi, S. enteritidis, S. flexneri, E. coli* and *M. tuberculosis* bacterial strains [13]. Furthermore, antidiabetic, hypolipidemic and antioxidant effects of extracts derived from *B. micrantha* have been reported [10,14]. Phytochemical investigations have revealed presence oftaraxerone, Friedelin, Taraxerol, Epifriedelinol, gallic acid, ellagic acid, anthocyanidin, delphiniridin and Benzene 1,3-bis(3-phenoxyphenoxy),2-pinen-4-one [13].

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In spite of the extensive usage of medicinal plants to curb microbial infections, there are few focused empirical studies formulated to validate the claimed potencies [15]. Moreover, due to the lack of clear dosage regimens, formulation guidelines, marketing and practice regulations in traditional medicine practice, toxicity and safety profiles of most medicinal plants have remained unknown [16].

Therefore, this study was designed to investigate the antimicrobial, cytotoxicity and acute oral toxicity effects of the aqueous and methanolic stem bark extracts *B. micrantha* to lay a framework towards their validation and as potential sources of safe and potent antimicrobials.

MATERIALS AND METHODS

Plant collection, identification, and processing

The stem barks of the studied plants were selected for this study based on their ethnomedical usage among the Agikuyu community of Murang'a County [12]. The plant specimen was submitted to the East African herbarium hosted at the National Museum of Kenya in Nairobi and botanically identified and authenticated by a taxonomist at National Museums of Kenya and assigned voucher specimen numbers as follows; *Bridellia micrantha* (NMK/01/2019), where the voucher specimens were prepared and deposited. The plant materials were then collected and transported to the Department of Public Health, Pharmacology and Toxicology laboratories, at the College of Agriculture and Veterinary Sciences, Kabete Campus, University of Nairobi. The collected materials were then sorted and evenly spread to dry at room temperature for 2 weeks. They were then ground into a powder by an electric mill and stored in plastic containers awaiting extraction.

Extraction of the collected plant materials

The crude extracts of the studied plant materials were prepared according to the procedures described by Harborne [17]. The methanolic extracts of the studied plant materials were obtained by cold maceration method. Briefly, 250 g of respective plant powders were soaked in 1 litre of analytical-grade methanol in 2-litre conical flasks. The respective flasks containing the merc-menstruum mixtures were gently shaken and covered with aluminum foil papers at their mouths. They were shaken once daily for two days, thereafter, the mixtures were decanted and filtered through Whatman filter papers (No.1). They were then concentrated under vacuum by the help of a rotary evaporator. The resultant extracts were transferred into glass bottles and further dried in a hot-air oven at 35 °C for 5 days. They were weighed and their respective percentage yields determined.

For the aqueous extracts, about 50 g of respective plant powders were macerated in 500 ml of distilled water and heated for minutes at 58 °C. The mixtures could cool to room temperature and then filtered through the Whatman filter papers. The filtrates were transferred into freeze-drying flasks and fitted into a freeze-dryer, where they were lyophilized for 48 hrs. The dried extracts were weighed, and their respective percentage yields determined. All the extracts were stored in a refrigerator (4 °C) and only retrieved when required.

Investigation of the antimicrobial activities of the aqueous and methanolic bark extracts of *B. micrantha* on selected microbes

In this study, *Escherichia coli* (ATCC 25925), *Salmonella typhimurium*, (ATCC 14028), *Staphylococcus aureus* (ATCC 25923) and *C. albicans* (ATCC 10231) were obtained from the Department of Public Health, Pharmacology and Toxicology of the College of Agriculture and Veterinary Sciences, University of Nairobi, Kabete Campus. These strains were selected based on their clinical significance and availability. To investigate the effects of the studied plant extracts on the selected microbial strains, the disc diffusion and broth microdilution techniques described by the Clinical and Laboratory Standards Institute were followed [18].

Preparation and standardization of microbial inoculum for experimentation

The fungal strain (*C. albicans*) was grown in Sabouraud dextrose agar (SDA; Oxoid) for 24 hrs according to the directions of the M100-S23 document of the CLSI [18]. Thereafter, sterile normal saline was used to standardize the inoculum to achieve a 0.5 McFarland standard at 530 nm using a UV-vis spectrophotometer. Ranges of between 0.11 and 0.14 at OD_{530} were obtained. This was considered to be $1\text{-}5\times10^6$ cfu/ml.

On the other hand, the bacterial strains (*E. coli, S. typhimurium* and *S. aureus*) were grown in Mueller-Hinton agar as per the CLSI guidelines for 24 hrs. Thereafter, the inocula were standardized to a turbidity equivalent to 0.5 McFarland scale of approximately $1\text{-}2\times10^8$ cfu/ml[18].

The disc diffusion assay for antimicrobial susceptibility

In this assay, 1 g of each of the studied extracts were dissolved in 10 ml of 1 % DMSO (in sterile water) in a 15 ml centrifuge tube and thoroughly vortexed to make stock solutions of containing 100 µg/ml. The stocks were then serially diluted two-fold to give 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml and 3.125 µg/ml respectively.

Afterwards, 20 µl were aspirated and carefully impregnated on sterile Whatman discs of 6 mm diameter. The discs were gently pressed on the media containing 1 ml of the bacterial or fungal inocula to allow for proper drug-microbe contact. The assays were performed in triplicate with DMSO as negative control and streptomycin or ciprofloxacin or amphotericin B as positive controls. All the plates were incubated for 24 hrs at 37°C, then diameters of zones of inhibition of microbial growth measured in millimeters.

The Broth microdilution technique for minimum inhibitory concentration (MIC) determination

In this determination, the modified CLSI method described by Golus et al.[19] was adopted. Briefly, cultures were prepared and adjusted in Mueller-Hinton Broth media to 0.5 McFarland equivalent turbidity. Carefully, 10 µl of the previously prepared test extracts at a 10-fold concentration were transferred into Eppendorf tubes containing 90 µl of molten Mueller-Hinton agar in triplicate and gently vortexed. Microdilution was done in volumes of 100 µl in sterile 96-U-shaped multiwell plates in two-fold. In each of the micro-titre plates, the growth, sterility control and negative (1 % DMSO) controls were included for each of the tested microbial strains.

All the multiwell plates could settle at room temperature for the agar to solidify. Then, 2 µl of freshly prepared inoculate at concentration of 10^4 cfu/spot were dispensed into the wells using a multichannel micropipette and allowed to interact at room temperature. The wells at the sides were added sterile water, and the plates were covered in zip-lock plastic bags and incubated at 35 °C for 18 hrs. The MIC was determined as the lowest concentration of the studied extracts which could completely inhibit microbial growth as per the CLSI recommendations [18].

Evaluation of the effects of the studied plants extracts on brine shrimp nauplii

In this study, the brine shrimp lethality assay method described by Meyer et al.[20] was used. Briefly, approximately 0.5 g of *Artemia salina* cysts (Sanders Great SaltLake, Brine Shrimp Company L.C., U.S.A.) were placed in an artificial sea containing 500 ml of brine water. They were incubated for two days to hatch into nauplii under continuous normal bulb illumination at 25 – 29 °C temperature and enough aeration.

Thereafter, ten nauplii were transferred using Pasteur pipettes into three sets of sample vials containing the either the studied plant extracts at concentrations of 0, 10, 100 and 1000 µg/ml or podophyllotoxin in 5 ml brine solutions in triplicate. The nauplii were then incubated for 24 hours and the number of survivors in each test vial were counted and documented. The percentage lethality was determined as a ratio of surviving nauplii in the test groups to those in the control (vehicle treated) group. LC_{50} values were derived from the line of best from a plot of percentage survival against concentration.

Evaluation of the Acute Oral Toxicity effects of the aqueous and methanolic bark extracts of the studied plant extracts

In this study, the guidelines posited by the Organization for Economic Co-operation and Development (OECD) in protocol document number 425 were adopted [21]. Experimental female Winstar Rats weighing 150± 20g were sourced from the Department of Public Health, Pharmacology and Toxicology animal breeding unit, acclimatized for 72 hrs before dosing. The studied plant extracts were reconstituted in normal saline solution to achieve the appropriate dose for administration.

On the experimentation day, the animals were fasted for 4 hrs and randomly assorted into groups of three rats. The experiment was initiated by administering a single dose of 175 mg/Kg bw orally to the first group and normal saline (10 ml/Kg bw) to the control group.

Observations of wellness parameters (skin fur, eye colour, mucus membrane, salivation, lethargy, sleep, coma, convulsions, tremors and diarrhoea) were recorded at intervals of 30 min., 4 h., 24 h., 48 h., 1 week and 2 weeks for each individual rat. In the absence of observable signs of toxicity or mortality during the 14-day experimentation period, the next subsequent higher doses of 550 mg/Kg bw and 2000 mg/kg bw respectively were administered into new groups of rats. All the experiments were done in triplicate [21]. At the end of the experiments, the experimental rats were euthanized and disposed of according to the set protocols.

Qualitative phytochemical composition of the aqueous and methanolic bark extracts of *B. micrantha*

In this study, the standard protocols for qualitative phytochemical screening described by Harborne[20] were followed. The phytochemicals that were evaluated include alkaloids, flavonoids, tannins, Saponins, Anthraquinones and phenols.

TEST FOR ALKALOIDS

Dragendorff test

About 0.1 g of theaqueous and methanolic extracts of *B. micrantha* were extracted by boiling with 10 ml of 1% hydrochloric acid in independent test tubes. The mixtures were filtered and to about 2 ml of the filtrate, a few drops of the Dragendorff reagent were added. The formation of the red precipitates in the respective tubes indicate presence of alkaloids.

Mayer's test

To the 2ml remaining portion of the filtrate of the respective extracts in the Dragendorff test, a few drops of the Mayer's reagent were added along the sides of the respective tubes. The formation of the white creamy precipitates in the respective tubes indicated presence of alkaloids.

Test for flavonoids

To approximately 5 ml of ethanolic filtrates of the respective extracts, 2ml of 2% sodium hydroxide were added. The formation of an intense colour that decolorize on addition of a few drops of diluted hydrochloric acid indicate presence of flavonoids.

Test for tannins (ferric chloride test)

About 0.1 g of the aqueous and methanolic bark extracts of the studied plants were extracted by boiling with 20 ml of the distilled water. The mixtures were filtered through Whatman filter paper, and into 2 ml of the filtrate, a few drops of 5 % ferric chloride were added. The appearance of the dark green colour indicates a positive test for tannins.

Test for phenols

About 0.1 g each of the studied extracts were boiled with 10 ml of 70 % of ethanol for 5 minutes in water bath and then filtered while hot. The filtrates were cooled to room temperature and 2 ml of it be transferred into a clean test-tube then followed by dropwise addition of 5% ferric chloride solution. The appearance of green precipitates will indicate the presence of phenols.

Test for saponins

About 0.5 g of the aqueous and methanolic extracts of the studied plants were dissolved in 5 ml of warm distilled water and vigorously shaken. The appearance of persistent frothing indicated presence of saponins.

Test for anthraquinones

Approximately 0.1 g of each of the studied extract were warmed 1 ml of chloroform in a water bath for 5 minutes. Afterwards, they were filtered through Whatman filter paper and allowed to cool to room temperature before adding equivalent volumes of 10 % ammonia. The mixtures were then shaken and the presence of pink coloration on the upper layer indicates presence of anthraquinones.

Data management and analysis

Quantitative data from antimicrobial and brine shrimp lethality experiments were tabulated on Excel spreadsheet (Microsoft 365) and exported to Minitab version 19.1 statistical software. Descriptive statistics were performed, and values were expressed as $\bar{x} \pm SEM$ One-Way ANOVA was used to determine differences among means followed by Tukey's *post hoc* test for pairwise comparisons and separations of means. Means that showed p values <0.05 were considered statistically significant. Acute oral toxicity results were treated according to the OECD[21] guidelines. Qualitative data on wellness parameters in the acute oral toxicity and qualitative phytochemical screening studies were only tabulated. The obtained findings were presented in tables.

RESULTS

Antimicrobial effects of the aqueous bark extract of *Bridellia micrantha* on selected microbial strains

The antimicrobial effects of the aqueous bark extracts of *Bridellia micrantha* were also investigated in this study. The results showed that at the lowest three concentrations tested, the mean zones of inhibition in *E. coli* bacterial strain were not significantly different (p>0.05; Table 1). Likewise, at the two upper concentrations (50 µg/ml and 100 µg/ml), the obtained zones of inhibition of *E. coli* were significantly similar (p>0.05). However, the positive control antibiotic gave the largest zone of inhibition (26.67±0.33 mm) compared with the zones of all the other treatments in *E. coli* (p<0.05; Table 1).

The effects of the aqueous bark extract of *B. micrantha* on *S. typhimurium* were also investigated in this study, the results revealed no significant difference in zones of inhibition at extract concentrations of 6.25 µg/ml and 12.5 µg/ml and at concentrations of 50 µg/ml and 12.5 µg/ml (p>0.05; Table 1). Similarly, at a concentration of 3.125 µg/ml, the recorded zone of inhibition was not significantly from that of the negative control (p>0.05). However, the positive control drug showed a significantly larger zone of inhibition than the zones produced in all the other treatments (p<0.05; Table 1).

The effects of the aqueous bark extract of *B. micrantha* on *S. aureus* bacterial strain were determined. In this study, at all the extracts concentrations, the observed zones of inhibition were not significantly different ($p>0.05$; Table 1). However, the reference drug produced a significantly larger zone of inhibition compared with the zones produced by the studied extract at all concentrations and the negative control ($p<0.05$; Table 1).

The susceptibility of *C. albicans* fungal strain to the aqueous bark extract of *B. micrantha* was investigated in this study. The results showed no significant differences in zones of inhibition recorded at all the extract concentrations were observed ($p>0.05$; Table 1). Notably, *C. albicans* was not susceptible to the reference drug, hence, the zone of inhibition was like that of the negative control ($p>0.05$; Table 1).

The effects of the methanolic bark extract of *B. micrantha* on selected microorganisms were also investigated in this study. The results showed that, upon application of 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, and 50 µg/ml of the methanolic bark extract of *B. micrantha*, the exhibited zones of inhibition were not significantly different ($p>0.05$; Table 2). However, at 100 µg/ml, the observed zone of inhibition was significantly larger compared to zones of inhibition in other extract concentrations and negative control ($p<0.05$; Table 2). In this setup, the positive control showed the highest zone of inhibition compared with all the other zones of inhibition ($p<0.05$; Table 2).

For *S. typhimurium*, the exhibited zones of inhibition by the methanolic extract of *B. micrantha*, ranged from 7.33±0.33 mm at 3.125 µg/ml to 11.33±0.67 mm at 100 µg/ml with significant differences ($p<0.05$; Table 2). No significant differences in zones of inhibition of *S. typhimurium* were observed in extract concentrations of 12.5 µg/ml, 25 µg/ml, and 50 µg/ml($p>0.05$; Table 2). Generally, a dose-dependent increase in zone of inhibition sizes was observed with the increasing concentration of the extract with 100 µg/ml showing a significantly larger zone than those in lower concentrations ($p<0.05$; Table 2). In this setup, the positive control drug showed the largest zone of inhibition compared with all the other zones ($p<0.05$; Table 2).

Minimum inhibitory concentrations (MICs)

The lowest concentrations of the studied plant extracts and reference antibiotics, which completely inhibited microbial growth in the 48-hr incubation period at 37 °C, were considered as the minimum inhibitory concentrations (MICs).

The MICs of the aqueous bark extract of *B. micrantha* were 50 µg/ml and 100 µg/ml for *E. coli* and *S. typhimurium* bacterial strains respectively and 12.5 µg/ml for both *S. aureus* and *C. albicans* (Table 3). Similarly, the MICs of the methanolic bark extract of *B. micrantha* were 50 µg/ml for *E. coli*, 100 µg/ml for *S. typhimurium* and 12.5 µg/ml for both *S. aureus* and *C. albicans* microbes (Table 3).

Table 1: Antimicrobial effects of the aqueous bark extract of *Bridellia micrantha* on selected microbial strains.

Concentration (µg/ml)	Zone of inhibition (mm)			
	E. coli	*S. typhimurium*	*S. aureus*	*C. albicans*
3.125	6.17 ± 0.17^{d}	6.67 ± 0.17^{d}	10.33 ± 1.20^{bc}	11.00 ± 0.58^{a}
6.25	6.83 ± 0.60^{d}	7.00 ± 0.00^{cd}	10.67 ± 1.45^{bc}	11.67 ± 0.33^{a}
12.5	8.50 ± 1.76^{cd}	7.00 ± 0.00^{cd}	11.33 ± 1.33^{bc}	12.17 ± 0.17^{a}
25	10.50 ± 1.04^{c}	7.83 ± 0.60^{c}	13.33 ± 1.67^{b}	12.67 ± 1.33^{a}
50	14.33 ± 0.88^{b}	10.00 ± 0.58^{b}	13.33 ± 1.67^{b}	12.67 ± 1.33^{a}
100	15.33 ± 1.67^{b}	10.00 ± 0.58^{b}	13.67 ± 1.20^{b}	13.00 ± 0.58^{a}
-ve	6.00 ± 0.00^{d}	6.00 ± 00^{d}	6.00 ± 00^{d}	6.00 ± 0.00^{b}
+ve	26.67 ± 0.33^{a}	27.00 ± 0.00^{a}	24.33 ± 0.33^{a}	6.00 ± 0.00^{b}

Values are expressed as $\tilde{x}\pm$SEM; means which do not share a lowercase alphabet superscript within the same column are significantly different (One-Way ANOVA followed by Tukey's test ($p<0.05$); Positive control: For *E. coli* and *S. typhimurium* it was Ciprofloxacin (10 µg); For *S. aureus* it was Streptomycin (µg) and for *C. albicans* it was Amphotericin B (µg); Negative control: DMSO (1.4 %).

Table 2: Antimicrobial effects of the methanolic bark extract of *Bridellia micrantha* on selected microbial strains.

Concentration (µg/ml)	Zone of inhibition (mm)			
	E. coli	*S. typhimurium*	*S. aureus*	*C. albicans*
3.125	10.00 ± 1.53^{c}	7.33 ± 0.33^{e}	8.33 ± 0.88^{de}	6.67 ± 2.85^{d}
6.25	12.67 ± 2.85^{bc}	9.00 ± 0.58^{d}	9.00 ± 1.00^{d}	11.00 ± 0.00^{c}
12.5	12.67 ± 2.96^{bc}	9.33 ± 0.67^{cd}	10.67 ± 1.86^{cd}	12.00 ± 0.00^{b}
25	13.00 ± 2.00^{bc}	10.00 ± 0.58^{bcd}	12.67 ± 0.88^{bc}	12.33 ± 0.33^{b}
50	14.67 ± 0.33^{bc}	10.67 ± 0.88^{bc}	14.33 ± 1.20^{b}	13.17 ± 0.44^{b}
100	16.00 ± 1.53^{b}	11.33 ± 0.67^{b}	15.33 ± 0.33^{b}	19.00 ± 3.06^{a}
-ve	6.00 ± 0.00^{d}	6.00 ± 0.00^{e}	6.00 ± 0.00^{e}	6.00 ± 0.00^{d}
+ve	27.33 ± 1.45^{a}	27.00 ± 0.00^{a}	23.33 ± 0.67^{a}	6.00 ± 0.00^{d}

Values are expressed as $\tilde{x}\pm$SEM; means which do not share a lowercase alphabet superscript within the same column are significantly different (One-Way ANOVA followed by Tukey's test ($p<0.05$); Positive control: For *E. coli* and *S. typhimurium* it was Ciprofloxacin (10 µg); For *S. aureus* it was Streptomycin (µg) and for *C. albicans* it was Amphotericin B (µg); Negative control: DMSO (1.4 %).

Table 3: Minimum inhibitory concentrations of the aqueous and methanolic extracts of *Bridellia micrantha* on selected microbial strains.

Plant extract	Minimum inhibitory concentration(µg/ml)			
	E. coli	*S. typhimurium*	*S. aureus*	*C. albicans*
B. micrantha (aq)	50	100	12.5	12.5
B. micrantha (met)	50	100	25	25
+Ve control	0.30	0.30	0.62	>100

Positive control: For *E. coli* and *S. typhimurium* it was Ciprofloxacin (10 µg); For *S. aureus* it was Streptomycin (µg) and for *C. albicans* it was Amphotericin B (µg); aq: aqueous extract; met: Methanolic extract.

Notably, the lowest MICs were recorded for aqueous bark extract of *B. micrantha* on *S. aureus* and *C. albicans* microbes (Table 3). Additionally, Ciprofloxacin (reference antibiotic) exhibited the lowest MICs on of 0.30 µg/ml on *E. coli* and *S. typhimurium* bacterial cultures while Streptomycin had an MIC of 0.62 µg/ml on *S. aureus* bacterial strain. Amphotericin B showed an MIC of > 100 µg/ml on *C. albicans* fungus. The MICs of the methanolic bark extract of *B. micrantha* were 50 µg/ml for *E. coli*, 100 µg/ml for *S. typhimurium* and 12.5 µg/ml for both *S. aureus* and *C. albicans* microbes (Table 3).

Cytotoxic effects of the aqueous and methanolic extracts of the studied plants of *Bridellia micrantha* on brine shrimp nauplii

The effects of the studied plant extracts on brine shrimp nauplii were also investigated in this study. The concentrations of the aqueous and methanolic extracts of *B. micrantha* that could kill 50 % of the exposed brine shrimp nauplii were determined and considered as mean lethal concentration (LC_{50}). Generally, the LC_{50} values ranged from 10 µg/ml for the positive control (cyclophosphamide) to 486.67±3.15 µg/ml for the aqueous bark extract of *B. micrantha* (Table 4). The LC_{50} value obtained for the aqueous extract of *B. micrantha* was significantly higher than those of the methanolic extract of *B. micrantha* and the positive control drug ($p<0.05$; Table 4).

Acute oral toxicity effects of the aqueous and methanolic extracts of *Bridellia micrantha* in rat models

In this study, the acute oral toxicity effects of the aqueous and methanolic stem bark extracts of the studied plants in laboratory rats were evaluated. Various wellness parameters were monitored throughout the 14-day experiment period and the findings recorded.

The results showed that, at all the orally administered doses (175 mg/Kg bw, 550 mg/Kg bw and 2000 mg/Kg bw) of the studied extracts, there were no observable signs of toxicity recorded in all the experimental rat models. Since the wellness parameters were normal to the limit dose level of 2000 mg/Kg bw, the LD_{50} values of all the studied plant extracts were considered to be >2000 mg/Kg bw according to the OECD/OCDE (2008) guidelines.

Qualitative phytochemical composition of aqueous and methanolic bark extracts of *Bridellia micrantha*

The results showed presence of alkaloids, anthraquinones, saponins, tannins, glycosides, flavonoids and phenols in the aqueous and methanolic extracts of B. micrantha (Table 5).

DISCUSSION

The resurgences of multi-drug resistant microbial strains have rendered the management and treatment of associated infections a challenging endeavor, leading to increased morbidity and mortality[22]. It is estimated that annually, over 2 million persons are diagnosed with mortal infections which are exacerbated by resistance, and, of the diagnosed cases, over twenty thousand patients succumb as a result of therapeutic failure [4,23]. Globally, research has shown that antimicrobial resistance could cause over 10 million deaths by the year 2050 if not arrested early enough [23].

Due to the inadequacy of therapeutic tools to thwart microbial infections, there is an urgent need for the search of alternative and complementary strategies to curb these infections [22,24]. As a result, medicinal plants have proved to be a viable alternative with a high propensity for potent antimicrobials [25,26]. As a result, the current study was designed to investigate the antimicrobial, cytotoxicity, acute oral toxicity effects, and phytochemical composition of the aqueous and methanolic stem bark extracts of *B. micrantha*. Since this plant is used traditionally to fight microbial infections, its scientific exploration serves as a guide towards the discovery of lead compounds for antimicrobial chemotherapy [12,27].

We adopted the most recommended antimicrobial susceptibility methodology described by the NCCLS[18], to determine the antimicrobial efficacy of the aqueous and methanolic stem bark extracts of *B. micrantha*. In this study, the standard disc diffusion and broth microdilution techniques were followed do determine the effects of the studied plant extracts on microbial growth. The zones of inhibition and the minimum inhibitory concentrations were considered indicators of antimicrobial activity.

Previous studies have shown that plant extracts exhibiting a zone of inhibition of above 6 mm on selected microbial strains have antimicrobial activity [28–31]. Plant extracts which show a zone of inhibition of between 6 mm and 9 mm are deemed to possess slight antimicrobial activity, those

Table 4: Effects of the aqueous and methanolic extracts of *Bridellia micrantha* brine shrimp nauplii.

Plant extract	LC_{50}(µg/ml)
C. megalocarpus (aq)	486.67±3.15^{b}
C. megalocarpus (met)	458.33±2.87^{c}
+ control	10.00±1.31^{f}

Values are presented as $\tilde{x}$±SEM; means with different lowercase alphabet superscript within the same column are significantly different by One-Way ANOVA followed by Tukey's test ($p<0.05$); aq: aqueous extract; met: Methanolic extract; Positive control; cyclophosphamide.

Table 5: Presence of alkaloids, anthraquinones, saponins, tannins, glycosides, flavonoids and phenols in the aqueous and methanolic extracts of B. *micrantha*.

Phytochemical	*B. micrantha*	
	Aqueous extract	Methanolic extract
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Glycosides	+	+
Flavonoids	+	+
Anthraquinones	+	+
Phenols	+	+

+: Present; -: Absent

showing zones of between 9 mm and 12 mm have moderate activity while those exhibiting inhibition zones of 13-16 mm are considered to have high antimicrobial activity. Additionally, plant extracts which have inhibition zones ranging from 16-19 mm have very high antimicrobial activity while those exhibiting zones of inhibition with diameters of 20 mm or above on selected strains are considered to have remarkable antibiotic potency [29,31,32].

In the present study, the aqueous extract of *B. micrantha* indicated slight to high antimicrobial activities against *E. coli* strain based on the produced zones of inhibition sizes. Slight to moderate effects were observed against *S. typhimurium* strain while moderate to high effects were exhibited on *S. aureus* bacterial strain and *C. albicans* fungal strain [31].

For the methanolic extract of *B. micrantha,* moderate to high antimicrobial activities were recorded against *E. coli* while slight to moderate effects were observed in *S. typhimurium* strain. Slight to high and slight to very high antimicrobial effects were noted in *S. aureus* and *C. albicans* respectively. Remarkably, at a concentration of 100 µg/ml of the methanolic extract of *B. micrantha* a zone of 19.00±3.06mm was recorded on *C. albicans,* indicating very high antifungal effects [31]. These findings corroborate those of Douglas and Gitonga[33].

Moreover, research has shown that plant extracts which have MIC values that are less than 1 mg/ml (1000 µg/ml) have antimicrobial activity with a potential of offering potent antibiotics [14,34]. In this study, the studied plant extracts exhibited low MIC values on selected microbes. Since the MIC values were much lower, it is anticipated that, the studied plant extracts can be strong antibiotics.

Medicinal plants are a host of various bioactive compounds with a broad spectrum of pharmacologic efficacies [17]. Research has shown that tannins, phenols, flavonoids, terpenoids among other phytocompounds are responsible for the antimicrobial activity of plants [35–37]. Therefore, bioactivities reported in this study are attributable to these phytochemicals which work either solely or in synergy with others to cause the pharmacologic effects.

Despite the long-standing utilization of herbals and their products for the management of various health conditions, serious concerns regarding their safety have been raised [16]. Various factors that affect the therapeutic potency of herbal medicines are generally not adhered to. There is lack of standard procedures and regulations governing the preparation, labelling, marketing and dispensing of herbal medicines [15]. This has led to an emergence of unscrupulous practioners of herbal medicine thereby raising safety concerns. There are no dosage guidelines, clearly outlines contraindications, conventional drug-herbal drug interactions, and toxicity profiles of herbal preparations [38,39]. As a result, improper use of these medicines could cause life-threatening effects considering the insufficiency of scientific and clinical data. Accordingly, we evaluated the cytotoxicity and acute oral toxicity effects of the aqueous and methanolic stem bark extracts of *B. micrantha* to appraise their safety.

We adopted the brine shrimp lethality assay technique described by Meyer *et al.* [20] to assess the cytotoxic/safety effects of the aqueous and methanolic stem bark extracts of *B. micrantha* in exposed shrimp nauplii. In this method, the concentration of the plant extracts that can kill 50 % of nauplii following exposure is considered as the LC_{50}. Research has shown that plant extracts with LC_{50} values that are < 30 µg/ml are very cytotoxic. Furthermore, plant extracts exhibiting LC_{50} values ranging from 30-100 µg/ml are toxic, while those having LC_{50} values that are over 100 µg/ml are considered to be of low toxicity or safe [40,41]. Based on this criteria [40,41], both the aqueous and methanolic extracts of *B. micrantha* were toxic as their LC_{50} values were between 30 µg/ml and 100 µg/ml. Thus, caution should be exercised whenever extracts from *B. micrantha* are administered at cellular level to avert adverse events.

Since most of herbal medicines are administered orally, the acute oral toxicity effects of the studied plant extracts were investigated in rat models. In this study, the acute oral toxicity study top-down procedure described the OECD document number 425 was adopted [21]. The results showed that all the studied plant extracts were non-toxic at oral doses and therefore safe. Considering these results, the studied plant extracts are safe for use in traditional medicine.

It is however notable that both the aqueous and methanolic extracts of *B. micrantha* were toxic to brine shrimp but non-toxic to experimental mice. This could imply that multicellular organisms have efficient machineries and mechanisms of handling drug agents as opposed to unicellular and lower organisms [42]. Additionally, the toxicity exerted by these extracts at the cellular level could be negligible so as to cause no observable signs of toxicity in experimental rat models [42,43]. The safety of the studied plant extracts reported in this study could be attributed to low concentration or absence of toxicity associated phytochemical compounds[44]. Furthermore, the antimicrobial bioactive compounds anticipated to be present in the studied plant extracts in varied degrees do not cause observable signs of toxicity [45]. These findings indicate that the studied plant extracts can be good alternative sources of safe antimicrobial compounds. Therefore, this study supports the traditional use of the studied plant extracts for the management of the claimed conditions.

CONCLUSIONS AND RECOMMENDATIONS

Based on this study's findings, the aqueous and methanolic bark extracts of *B. micrantha* have appreciable antimicrobial effects on *E. coli, S. typhimurium, S. aureus* and *C. albicans* microbial strains. Also, the aqueous studied plant extracts of *B. micrantha* are toxic, to brine shrimp nauplii, but do not cause any observable signs of toxicity in experimental rats. Additionally, the aqueous and methanolic bark extracts of *B. micrantha* possess antimicrobial associated phytochemicals. Further investigations aimed at establishing the specific modes of action of the studied plant extracts are recommended. Moreover, drug interaction studies involving the studied plant extracts are encouraged. Further antimicrobial activity studies using other microbial strains, and toxicological investigations of the studied plant extracts in other experimental models should be done. Also, isolation and characterization of specific antimicrobial phytocompounds from the studied plant extracts should be done.

DATA AVAILABILITY

All data in this study are included within the manuscript; however, any additional information is available from authors upon request.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest whatsoever regarding this study.

AUTHOR CONTRIBUTIONS

Joseph Kathare conceived the research idea and performed the experiments under the close supervision of James Mbaria and Joseph Nguta. Gervason Moriasi designed, guided the experiments, and assisted with data analysis and interpretation. All authors reviewed and approved the final manuscript for publication.

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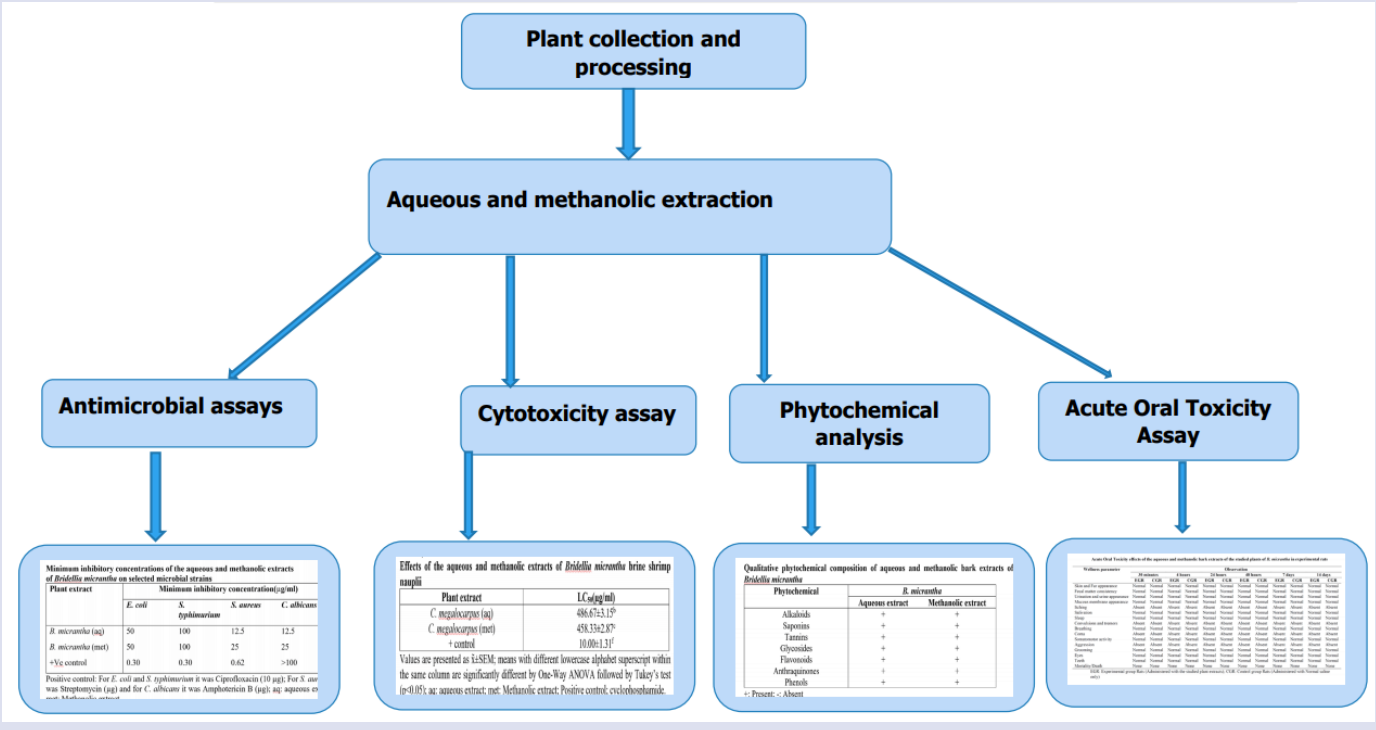
GRAPHICAL ABSTRACT



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