



Investigation of the Phyto-constituents and the Antibacterial Activity of *Burkea africana* against *P. aeruginosa*, *E. coli*, *S. aureus* and *E. faecalis*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Bacterial skin infections are common in the tropics and the emergence of resistant bacterial strains has complicated treatment outcomes especially in immune compromised patients. This study focused on determining the antibacterial activity and preliminary phytochemical screening of the plant, *Burkea Africana*, which has been used for decades to treat various ailments in Southern Africa. Phyto-constituent determination and antibacterial assay was carried out on the hydro-ethanolic and aqueous leaf extracts of *Burkea Africana*. The Agar well diffusion method was used to investigate the antibacterial efficacy of both extracts. Activity was measured against *P. aeruginosa*, *E. coli*, *S. aureus* and *E. faecalis* using ciprofloxacin as the standard. For both extracts, phytochemical analysis revealed the presence of steroids, tannins, saponins and carbohydrates. The 70% hydro-ethanolic extract however revealed more phyto-constituents including flavonoids and alkaloids. For both extracts antibacterial activity was comparable to the standard. The highest zone of inhibition recorded for the water extract was 22 ± 0.4 mm at 500 mg/ml for *E. coli* while the standard, Ciprofloxacin had a zone of inhibition of 25 ± 0.33 mm. The ethanolic extract showed higher antibacterial efficacy when compared to ciprofloxacin against *S. aureus*. The zone of inhibition for the 70% ethanolic extract was 25.4 ± 0.50 mm and that of the standard was 27 ± 0.48 mm. Antibacterial activity was generally higher in the ethanolic extract than the water extract. It is therefore concluded that *B. africana* exhibits antibacterial activity.

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1. INTRODUCTION

The human skin can be classically defined as the largest organ of the body. It performs several functions which include protection against microbes such as fungi and bacteria [1]. Bacterial skin infections are fairly common and normally occur after there has been a break in the skin's integrity [2]. They can be classified as either primary skin infections or secondary skin infection. Primary skin infections have a distinctive clinical features and disease course. These primary infections are consequent of one specific incidental pathogen, typically affecting normal skin. Common types of such, include forms of Impetigo as well as folliculitis and certain boils. On the other hand, secondary skin infections usually occur on diseased skin and typical examples include toe web infections³. Studies have shown that when the skin has been compromised or under moist occlusive conditions, it can support the growth of both commensal and pathogenic bacteria. The best studied cutaneous pathogen is *Staphylococcus aureus*, however little is known regarding the pathogenic mechanisms employed [2,3].

Staphylococcus aureus is one of the most common bacteria that results in primary skin infections. Recent studies have also shown an expression of *S. aureus* and *E. coli* in squamous cell carcinoma lesions. An expression of *S. aureus* DNA has also been observed in actinic keratosis and seborrheic keratosis biopsies. It is unclear whether the bacteria is a result of the lesions or possibly contributing to tumour formation. A possible mechanism however, where bacteria might contribute to tumour formation is by the production of chronic inflammation which is a classic mechanism of carcinogenesis [4]. Enterococci and *P. aeruginosa* infections are also on the rise, although the reason for the increasing incidence is unknown it is believed that the selection pressure from increasing consumption of cephalosporins is an important factor. Skin and soft tissue infections caused by enterococci are on the rise particularly in developing countries [5,6].

The increasing incidence of bacterial skin infections is believed to be a result of antibacterial resistance. Antimicrobial resistance is considered to be a very serious public health

threat. The source of antimicrobial resistance is believed to be from two groups that is, from people and in animals for prophylaxis and growth promotion [7]. The development of antibacterial resistance in dermatology is even more troubling in immune compromised patients, for example organ transplant recipients because they are more likely to develop bacterial infections. Infections developed by such patients tend to be more aggressive and hard to treat [8].

Antibacterial resistance has resulted in researchers trying to develop new antimicrobials. Plant sourced antimicrobials have become very important because antimicrobials derived from plants are believed to be able to target resistant bacterial strains [9,10]. Medicinal plants are playing an increasing role in the discovery of new therapeutic agents and developing new drugs. In 2008, out of the top 150 prescription drugs prescribed in the United States of America, at least 118 (74%) were based on natural sources [11]. Roughly 7000 different pharmaceutical compounds found in the western pharmacopeia as well as top selling drugs such as Taxol, quinine and camptothecin were sourced from plants [9].

Burkea africana also known as *Wild syringe* is a common tree found throughout tropical Africa particularly in sandy soil, in woodland and wooded grassland. The medium sized deciduous tree belongs to *caesalpinioideae* family [12]. The fruits of *B. africana* resemble *Terminalia* at first glance however, fruits of *B. africana* are regularly separated by its compounded leaves [13]. The tree is used in Zimbabwe to treat a wide array of conditions including bacterial infections.

Ethno-medically the bark and leaves are used to treat a variety of ailments including inflammation, heavy menstruation, pneumonia, abdominal pain and as a wound dressing for topical ulcers [14]. In Zimbabwe the plant has numerous medicinal uses including treating topical infections [15,16]. In central Zimbabwe the powdered bark and crushed leaves are often used to treat scabies and infected topical ulcers. Herbalists in northern Zimbabwe treat boils using the powdered leaves of the tree. The powdered leaves work by drawing out the salve from inside the boil. Herbalists in eastern Zimbabwe use *B. africana* leaves to treat infected topical wounds, ear aches and tooth ache.

This study focused on determining the antibacterial activity and preliminary screening of one plant that has been used to treat various ailments in Zimbabwe and most of the African continent.

2. MATERIALS AND METHODS

2.1 Research Facilities

The research was carried out at the University of Zimbabwe, School of Pharmacy laboratories in Harare, Zimbabwe in 2019.

2.2 Plant Collection

Burkea africana leaves were collected in August 2018, 40kms south-east outside Harare, Zimbabwe (18°04'56.7"S 31°20'41.1"E) and identified by a taxonomist at the national herbarium. The leaves were cleaned and air dried to constant weight. Size reduction of the plant leaves was done using a laboratory grinder. The powder material was stored in polythene bags at room temperature until they were required for extraction.

2.3 Preparation of Plants Extracts

2.3.1 Ethanolic extracts

B. africana leaves (200 g) were macerated in 800 ml, 70% ethanol for 120 hours. The sample was sonicated before the bulk material was filtered of using a mutton cloth. Removal of bulky material was followed by vacuum filtration using Whatman number 1 filter paper. Ethanol was removed from extracts using a rotary vapor (Buchi Rota-vapor R-114) at 55°C. This was followed by freeze drying the sample using a freeze dryer (Bio-base freeze-dryer). To reconstitute, the extracts were dissolved, at 0.2 g/ml, in 12% (v/v) dimethyl sulphoxide (DMSO) in water, to give a stock solution. The stock solution was kept at 4°C in the dark until used [17].

2.3.2 Preparation of aqueous extracts

The dried powdered leaves of *B. africana* were gently heated and stirred in distilled water for 4 hours. 200g of plant material was extracted in 2000ml of distilled water. The prepared sample was allowed to cool to room temperature. After which it was initially filtered with a clean mutton cloth followed by vacuum filtration using Whatman number 1 filter paper. The filtrate was

then frozen in an ice cube container and then concentrated via freeze drying [18].

Determination of plant extract yield (% w/w)

The yield (% w/w) from all dried extracts was calculated by the formula given below.

$$\text{Yield (\%)} = \frac{w_2 - w_1}{w_0} \times 100$$

Where:

w_2 : is the weight of the extract and the container.

w_1 : Weight of the container alone

w_0 : The weight of the plant powder [19]

2.3.3 Preliminary phytochemical studies

Both extracts were subjected to qualitative analysis for the identification of various primary and secondary metabolites.

Test for alkaloids: 1 ml of Mayer's reagent was added to 1 ml of the extract and observed after shaking the test tube for the formation of white precipitate which indicates the presence of alkaloids [10].

Test for flavonoids: 1 ml of neutral ferric chloride was added to 1 ml of the extract and observed for a brown colour change which indicates the presence of flavonoids [20].

Test for terpenoids: 1 ml of extract was treated with 1 ml of chloroform and 1 ml of concentrated sulphuric acid was added. Observations were made for the formation of a reddish brown colour which indicates the presence of terpenoids [10].

Test for steroids: 1 ml of chloroform was mixed with 1 ml of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed together. Observations were made for the formation of a dark red colour or dark pink colour indicates the presence of steroids [21].

Test for tannins: 5 ml of extract was added to 2 drops of 1% lead acetate and observed for a yellow precipitate indicated the presence of tannins [21].

Test for saponins: 2 ml of the extract with 20 ml of distilled water was agitated in a graduated cylinder for 15 min. The formation of a layer of foam indicated the presence of saponins [22].

Test for phenols: About 1 ml of lead acetate solution was added to 1 ml of the extract. The solution was observed for the formation of a brown colour precipitate indicated the presence of phenolic compounds [10].

Test for carbohydrates: Four drops of Molisch reagent was added to 1 ml of the extract followed by 1 mL of concentrated sulphuric acid which was added carefully by sliding it down the test tube walls. The resultant concoction was left standing for a couple of minutes and then further diluted with a 5 ml addition of distilled water. The mixture was observed for the development of red or dull violet ring at the junction of the liquids, which showed the presence of carbohydrates [21].

Test for proteins: 1 ml of ninhydrin was dissolved in 1 ml of acetone and added to 1 ml of extract. The solution was heated for 5 minutes in a water bath. The solution was observed for the formation of blue or purple colour which revealed the presence of protein [10].

2.3.4 Antimicrobial testing

2.3.4.1 Bacteria strains

In order to determine the antibacterial activity of all extracts a total of four bacterial strains obtained from Parirenyatwa group of Hospital Department of Medical Microbiology were used. Two gram negative strains (*P. aeruginosa atcc27853* and *E. coli atcc25922*) two gram positive (*S. aureus atcc25923* and *E. faecalis atcc 29212*). All the test strains were maintained on nutrient agar slants at 4°C and sub-cultured on to nutrient broth for 24 hours prior to testing. These bacteria strains served as test pathogens for antibacterial activity assay.

2.3.4.2 Antibacterial tests

The Agar well diffusion method was used to determine the antibacterial activity of all the extracts following methods described by the National Committee for Clinical Laboratory Standards (NCCLS) [23]. The bacteria inoculum was equally spread. The inoculum which was matched to the turbidity equivalent to 0.5 McFarland solution ($1-2 \times 10^8$ CFU/mL) with the addition of sterile saline, was spread on nutrient agar plates with a sterile swab moistened with the bacterial suspension. Subsequently, wells of 8 mm diameter were punched into the agar

medium and filled with 100 μ l (50,100, 250 and 500 mg/ml) of plant extract and allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 37° for 24 h. Wells containing the same volume of DMSO (10%) served as negative controls while standard antibiotic discs of Ciprofloxacin (100 μ g) was used as the positive control. After incubation, the diameters of the growth inhibition zones were measured in mm. The experiments were carried out in triplicates for each extract against each of the test organism. Data were expressed as mean \pm standard deviation [24,25].

3. RESULTS

3.1 Preliminary Phytochemical Analysis

The percentage yield of *B. africana* extracts were recorded in Table 1. The ethanolic extract had a better yield than the aqueous extract.

Both extracts tested positive for tannins, saponins, carbohydrates and steroids (Table 2). Confirmation for phyto-constituents was confirmed by colour changes. No colour changes were observed in the water extract for alkaloids, flavonoids, terpenoids, phenols and proteins meaning the phyto constituents were not detected. The ethanolic extract tested positive for every phyto-constituent that was tested except for proteins.

3.2 Antibacterial Assay

The zone of inhibition increased with increasing concentration of the aqueous extract (Table 3). Better inhibition was observed at 500 mg/mL in *E. coli*, gram positive bacteria, with the aqueous extract achieving a zone of inhibition of 22 ± 0.45 mm while the lowest zone of inhibition was recorded in *P. aeruginosa* (3.2 ± 0.17 mm), gram negative bacteria, at 50 mg/mL. Although antibacterial activity was recorded the standard had higher zones of inhibitions.

The zones of inhibition recorded against the four strains of bacteria using ethanolic extract (Table 4) were generally higher than that of the aqueous extract. Increase in antibacterial activity was noted with an increase in concentration of the extract. The highest zone of inhibition was $25.4 \text{ mm} \pm 0.55$ (*S. aureus*) recorded at 500 mg/mL while the lowest zone of inhibition was recorded at 50 mg/mL (*P. aeruginosa*).

Table 1. Percentage yield of extracts and characteristics

	<i>B. africana</i> water extract	<i>B. africana</i> hydro-ethanolic extract
% yield	12.75	26.43
Texture	Powder	Powder
Colour	Light green	Dark green-brown

Table 2. Results for preliminary phytochemical screening of *B. africana* leaves

Phytoconstituent	Ethanolic extract	Water extract
Alkaloids	++	-
Flavonoids	+++	-
Terpenoids	+	-
Steroids	++	+
Tannins	++	++
Saponins	++	+++
Phenols	++	-
Carbohydrates	+	+
Proteins	-	-

Key+++ = strongly present (positive within 5 minutes) ++ = moderately present (positive after 5min but within 10 min), + weakly present (positive after 10 min but within 15 min), - = absent

Table 3. Antibacterial activity (zone of inhibition) of aqueous extract of *B. africana*

Type of strain	Zone diameters (mm) with respect to Conc. of the Aqueous extract					
	50 mg/ml	100 mg/ml	250 mg/ml	500 mg/ml	DMSO	Standard
<i>P. aeruginosa</i>	3.2±0.17	3.8±0.23	4.5±0.15	5.9±0.22	NIL	15.2±0.29
<i>E. coli</i>	5.1±0.12	8.8±0.52	16±0.22	22±0.4	NIL	25.7±0.33
<i>E. faecalis</i>	6.9±0.82	11.2±0.32	17.6±0.32	20.3±0.11	NIL	22.3±0.45
<i>S. aureus</i>	7.3 ±0.28	9.4±0.11	11.6±0.29	18.4±0.50	NIL	27.8±0.48

Table 4. Antibacterial activity (zone of inhibition) of ethanolic extract of *B. africana*

Type of strain	Zone diameters (mm) with respect to Conc. of the Hydro-ethanolic extract					
	50 mg/ml	100 mg/ml	250 mg/ml	500 mg/ml	DMSO	Standard
<i>P. aeruginosa</i>	5.0±0.55	11.7± 0.13	17.3±0.68	23.1±0.16	NIL	25±0.29
<i>E. coli</i>	6.2±0.32	9.8±0.43	15.3±0.2	21.9±0.72	NIL	20±0.52
<i>E. faecalis</i>	7.8±0.44	12.6±0.82	16.3±0.35	22±0.40	NIL	23±0.19
<i>S. aureus</i>	8.3 ±0.28	10.4±0.11	18.6±0.29	25.4±0.50	NIL	27±0.48

4. DISCUSSION

Antibiotics are used to provide the main form of treatment for infections. However, the high genetic variability of bacteria results in them developing antibiotic resistance. The development of antibiotic resistance has resulted in plants being investigated for antibacterial activity. The search of antimicrobials from natural sources has received a lot of attention mainly because phytochemicals obtained from plant products act as a prototype for the development of less or even non-toxic antimicrobial agents that may potentially target antimicrobial resistant strains as well as non-resistant strains [20,26].

Preliminary phyto-chemical studies and antibacterial studies were carried out on the extract of *B. africana* leaves. The hydro-ethanolic extract had more phyto-constituents than the aqueous extract. Both extracts had steroids, tannins, saponins and carbohydrates in common. The percentage yield of both extracts of *B. africana* were recorded in Table 1. The ethanolic extract had a higher percentage yield of 26.43% compared to that of the aqueous extract (12.75%). Both extracts were in powder form after freeze drying. The higher yield obtained by the hydro-ethanolic might be explained by the polarity of the solvent. The 70% ethanolic solvent is more polar than water this will result in a

higher solubility of polar phyto-constituents hence a bigger yield [27].

In addition to that the hydro-ethanolic had alkaloids, flavonoids, terpenoids and phenols. Studies have shown that flavonoids exhibit antimicrobial activity and their mode of action by complexing with the cell wall and by binding to adhesins. Tannins have also shown to have antimicrobial activity and their mechanism of action is believed to be binding to adhesins, enzyme inhibition and metal ion complexation. The mechanism of action for terpenoids however is believed to be via membrane disruption while that of alkaloids is believed to be by intercalating into the cell wall and DNA of parasites [28,21].

Antibacterial activity was assayed on both ethanolic and hydraulic extracts of *B. africana*. In all cases Gram-positive bacteria was more susceptible to plant extracts. Difference in sensitivity might be caused by the structural differences between Gram-positive and Gram-negative bacteria. Gram-negative bacteria is not as susceptible due to the lipid based outside membrane which acts as a semi-permeable barrier to the uptake of antibiotics and substrate molecule [29,30]. This lipid based membrane is not present in Gram-positive bacteria. The cell wall of Gram-negative bacteria provides an extra layer of protection to the organism without hindering the exchange of material required for sustaining life²⁶. The combination of a highly hydrophobic lipid bilayer with pore-forming proteins of specific size-exclusion properties allows the cell wall to act as a selective barrier. The permeability properties of this barrier, therefore, have a major impact on the susceptibility of the microorganism to antibiotics, which, to date, are essentially targeted at intracellular processes [26,28].

Ethanolic extracts of *B. africana* presented higher antibacterial activity compared to its aqueous extract. This might be explained by one or two reasons. Namely the nature of the biological active components (alkaloids, flavonoids) may be enhanced by the presence of ethanol [20]. The differing inhibition zones presented may be caused by the different diffusion capability of the active phyto-constituents of the extract in the agar medium. Furthermore the growth and metabolic activity of micro-organisms as well as antimicrobial activity of diffused active may play a role [31,32,30].

The ethanolic extract of this plant revealed the presence of alkaloids, which have several functions including, antimicrobial, anti-cancerous and antioxidant activity [33]. Flavonoids have also shown to possess antimicrobial, antioxidant as well as anti-allergic activity. They have the ability to alter how the body responds to carcinogens, bacteria and various allergens. Tannins are known to have antibacterial as well as antioxidant behavior. Recent reports suggest that tannins might have antineoplastic behavior [34].

Studies have also shown that Saponins are bioactive antibacterial agents, they also have anti-inflammatory and hypercholesterolemia activity [33]. Steroids on the other hand are believed to have cardio tonic activity and antimicrobial activity. They are generally used in cosmetics and herbal medicines [21]. Terpenoids are the most numerous and widespread group of natural products found in most plants. Their variety also accounts for their numerous functions these include anti-malarial, anti-inflammatory and anti-cancer activity. It should be noted that some terpenoids are toxic [35].

5. CONCLUSION

Ethanol proved to be a better extraction solvent for the extraction of bio-active compounds of *B. africana* leaves. This conclusion was supported by the higher yield that was obtained as well as the presence of more phyto-constituents. Both extracts exhibited antibacterial activity against the selected bacterial strains. Higher activity was however, observed in the 70% ethanolic extract. The presence of phyto-constituents and antibacterial activity therefore validates its use by traditional healers to treat bacterial infection.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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