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Evaluation of the antimicrobial activity of *vangueria volkensii* bark, fruit, leaf, and stem extracts

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Abstract
Introduction: Vangueria volkensii used in Sub-Saharan African countries to treat venereal diseases has no published data on its antimicrobial activities.
Materials and Methods: Vangueria volkensii bark, fruits, leaves, and stems were sequentially extracted via Soxhlet extraction using petroleum ether (PE), acetone (ACE), and ethanol (ETOH). Using disc diffusion assay, antimicrobial activities were evaluated against six bacteria (Gram-negative [Escherichia coli B strain, Salmonella enterica Subsp. enteritidis, Shigella flexneri] and gram-positive [Enterococcus faecalis, Staphylococcus aureus, methicillin-resistant S. aureus]) using 50 mg/ml extract concentrations.
Results: Salmonella enterica Subsp. enteritidis, S. flexneri, E. faecalis and methicillin-resistant S. aureus were sensitive to all extracts prepared. E. coli B strain was resistant to bark ETOH and stem ACE extracts. Fruit extracts prepared from ACE had the most inhibitory activity for all microorganisms, except E. faecalis.
Conclusions: This study shows that V. volkensii contains antimicrobial properties as all extracts exhibited broad spectrum activity against both gram-positive and gram-negative bacteria.

Keywords: Vangueria volkensii, antimicrobial, disc diffusion, Vangueria fruits, medicinal plants

Introduction
Natural products from plants have traditionally been excellent sources of small molecule antibacterial agents which are known to be effective antimicrobial substances against a wide range of microorganisms [1, 2]. Plant-derived antibacterials have always been sources of novel drugs and may continue to serve as a critical source of novel drugs to combat antimicrobial resistance [3, 4]. Medicinal plants have been used by humans for thousands of years and continues to play a critical role in the healthcare system in many areas in the world [5]. The widespread use of herbal medicine in many African countries is not limited to just rural areas, but also in urban areas [6-9]. The World Health Organization (WHO) estimates that about 80% of the global population, primarily those in developing countries, still rely on traditional medicine [7, 8, 10-14]. This is due to poverty, cultural acceptability, the lack of access to orthodox medicine and medical facilities, and the low ratio of trained medical doctors to patients. In some areas, there is integration of plant-based medicine with orthodox medicine [15, 16]. Plant or herbal based medicines is not limited to developing countries. Developed countries like the United States and the United Kingdom have seen growth in this area and there is a blossoming industry of herbal medication labeled as nutritional supplements [17, 18].
The family Rubiaceae, commonly called the coffee family, is one of the most species-rich angiosperm families in terms of number of genera and species, with about 611 genera and more than 13,000 species [10, 19, 20]. It is a predominantly tropical and subtropical family, but representatives occur on all continents, except Antarctica [20]. Shrubs, trees, lianas or herbs are the growth forms of Rubiaceae, with shrubs being the most common. Species are mainly woody, and less than 20% of the genera are herbaceous [10]. Members of the Rubiaceae family exhibit great medicinal potential and value. Numerous studies have documented the widespread use of Rubiaceae members throughout the world as treatment for a wide range of symptoms and diseases such as diarrhea, headaches, diabetes, malaria, and many more ailments [21-23].

According to Karou *et al*. (2011), at least 34 different genera of Rubiaceae native to Sub-Sahara Africa have been documented for treating or managing over 70 different diseases [10]. The genus *Vangueria*, a major angiosperm in the Rubiaceae family is made up of about 50 small trees and shrubs [24]. *Vangueria volkensii*, locally referred to as "Kimuluet," is a species of Rubiaceae native to Kenya. *V. volkensii* (bark, fruit, leaves, roots, and stems) is known to treat and manage venereal diseases and malaria [9, 13, 14, 25].

Rubiaceae is considered a major component of sub-Saharan folk medicine. However, due to the vast number of species, scientists have not been able to screen all Rubiaceae for their ethnopharmacological uses and the identification of their medicinal properties is still ongoing. Therefore, the aim of this study is to investigate the antibacterial activity of *V. volkensii* with the intent of establishing the antimicrobial potentials of *V. volkensii* extracts on bacterial pathogens. Species in the genus *Vangueria* have been shown to have high antibacterial activity against several microorganisms; thus, *V. volkensii* is predicted to have moderate to high antibacterial activity against several different microorganisms [26, 27]. The antibacterial activity of *V. volkensii* against both gram-negative and gram-positive bacteria will be conducted via the disc diffusion method by measuring the zone of inhibitions (ZOI in mm).

Impact of study: This study will give information on the antimicrobial activities of various parts of a traditional medicinal plant. Results from this study will serve as a preliminary data for further phytochemical studies on this plant to discover novel antimicrobial compounds in the plant.

Methods

2.1 Plant Material

Collection of *V. volkensii* bark, fruit, leaves, and stems was contracted out to Caleb Rugut and Haron Koech, local natives, who excavated the plant material from naturally growing scrubs located near Kapkoros Village, Nandi North District, Rift Valley Province, Kenya. The plant parts were collected in May 2016 and shipped to Texas A&M International University (TAMIU) and authenticated by Amede Rubio, an ecologist associated with TAMIU. Plant components were thoroughly washed with water and chopped into pieces. They were sun-dried for 10 days, grounded to coarse powder with a High-speed Universal Grinder, kept in Ziploc bags and stored at room temperature in the dark.

2.2 Preparation of Plant Extracts

The extraction method from Addo-Mensah *et al*. (2015) was adopted with a few modifications [28]. Briefly, sequential Soxhlet extractions were performed for each of the dried powdered plant components. The bark, fruits, leaves, and stems were extracted in 3 days successively with 400 mL of petroleum ether (PE), acetone (ACE), and 9:1 ethanol/deionized water (ETOH). The solvents were removed from each extract via evaporation using a rotary evaporator. The extracts were then dissolved in water and lyophilized in a freeze-dryer. Finally, the crude extracts were weighed and stored in the dark at 10ºC to prevent photo-degradation. Extracts were then dissolved in dimethyl sulfoxide (DMSO) to produce 100 mg/mL stock solutions. Using DMSO as the solvent, concentrations of 5, 15, 25, 50 mg/mL was prepared from the stock solutions. The prepared extract concentrations and the stock solutions were stored in the dark at 10ºC until further use.

2.3 Microorganisms

Antimicrobial studies were carried out using gram-negative and gram-positive bacteria. All bacteria strains used in this study were obtained from Presque Isle Cultures (PIC). The gram-negative bacteria included: *Escherichia coli* strain B (EC; PIC 337), *Salmonella enterica* Subsp. *enteritidis* (SE; PIC +371) and *Shigella flexneri* (SF; PIC +387). Whereas the gram-positive bacteria included: *Enterococcus faecalis* (EF; PIC +522), *Staphylococcus aureus* (SA; PIC 4651) and methicillin-resistant *Staphylococcus aureus* (MRSA; PIC +4656). Common aetiologic agents of venereal diseases such as *Chlamydia trachomatis*, *Neisseria gonorrhea* and *Treponema pallidum*, were unavailable, therefore *E. coli*, *S. flexneri*, *E. faecalis*, and *S. aureus* were used because they are representative of pathogenic organisms associated with venereal diseases [29].

2.4 Preparation of Microorganisms

Sterile Mueller-Hinton Agar (MHA) was prepared according to the manufacturer's instructions (Addo-Mensah *et al*. 2015) to prepare agar plates which were stored in a walk-in refrigerator until use [28]. Stock bacterial strains (EC, SE, SF, EF, SA, and MRSA) was sub-cultured on Muller-Hinton agar plates, incubated at 30ºC for 20 h and stored at 10ºC until further use.

2.5 Inoculum Preparation

Sterile nutrient broth was prepared, and 2 mL of the broth was added to sterile tubes. Using sterile toothpicks, a single colony of a microorganism was selected from its respective agar plate culture and the toothpick containing the colony was transferred into one of the nutrient broth tubes. The broth culture and a tube with only 2 mL of the sterile nutrient broth (negative control) was placed in a water bath shaker and incubated at 37ºC for 18 h. After incubation, the sterile nutrient broth was used as a blank for adjusting the turbidity of the actively grown broth culture using a spectrophotometer. The absorbance of the suspension was adjusted to a level of A = 0.132 ± 0.005 at 625 nm and the suspension was used later for inoculation onto Mueller-Hinton agar plates. This procedure was repeated for all microorganisms.

2.6 Determination of Antimicrobial Activity

The antimicrobial activity of extracts was determined via disc diffusion method (Addo-Mensah *et al*. 2015) using MHA plates [28]. Briefly, 100 µL of the respective inoculum was added to moisture-free agar plates utilizing sterile L-shaped rods to evenly distribute the inoculum. Individual 6 mm disc filter paper discs were impregnated with 20 µL of the respective previously prepared extract concentrations (5, 15, 25, 50 mg/mL) and applied onto the plates. A single disc was impregnated with 20 µL of the DMSO, which served as the negative control, and applied to the plates. Antibacterial positive controls (APC) made up of vancomycin 30 µg (gram-positive bacteria) antibiotic disc and tetracycline 30 µg (gram-negative bacteria) antibiotic disc (BD BBL™ Sensi-Disc) were purchased from BD Life Sciences After all the discs had been applied to the plates, the plates were incubated at 38ºC for 18-20 h. Following incubation, the zone of inhibition (ZOI) was observed. Using a Vernier caliper, the inhibition zone diameter was measured in millimeters for all plates. The mean ZOI in mm ± the standard error of the means (SEM) was recorded. All tests were carried out by sextet and repeated four times to minimize experimental error.

2.7 Experimental Design and Statistical Analysis

Statistical analyses were performed on the response variable, size of the zone of inhibition (ZOI in mm) for each plant extract material (i.e. bark, fruit, leaf, and stem). The first was a two-way Analysis of Variance (ANOVA) associated with a 5x4 factorial experiment for each of the six microorganisms (i.e., EC, SE, SF, EF, SA, and MRSA). The first factor was the type of extract and the other factor was the level of concentration. The second set was a three-way ANOVA, which combined the data across microorganisms, with the addition microorganism as a factor, resulted in a 6x5x4 factorial experiment. The factors were microorganism (EC, SE, SF, EF, SA, MRSA), type of extract (ACE, ETOH, PE, APC, DMSO) and concentration (5, 15, 25, 50), which translates to 120 treatment combinations in each complete block [30]. The blocks were comprised of the time that each of the four replications was performed (i.e., week 1, week 2, week 3, and week 4). A Duncan's Multiple Range Test (DMRT) was employed for each plant material to identify which of the extract means was or was not significantly different [30]. To compare the 18 interaction means for each plant material, a 95% confidence interval was implemented for each of the microorganism-extract, where overlapping intervals indicated no significant difference. All analysis was carried out using the general linear model facility of the software Statistical Packages for the Social Sciences version 24.0 (SPSS, Chicago, Illinois).

Results & Discussions

3.1 Plant Extract Yield

Extraction yields for all plant parts were generally low. The percent yield of the ACE crude extracts as seen in Table 1 ranged from 0.52% to 2.71% with the leaf extract having the highest percentage yield of 2.71% followed by the fruit and bark extracts (1.12% and 0.97%), respectively. The lowest ACE yield obtained was from the stem extract (0.52%). The percentage yield of the ETOH extracts ranged from 0.84% to 3.88% with the leaf and bark extracts having the highest percentage yield of 3.88% and 3.30%, respectively. The lowest ETOH yield obtained was from the stem and fruit extracts (0.84% and 1.12%), respectively. The percentage yield of the PE extracts ranged from 0.038% to 1.67% with the fruit extract having the highest percentage yield of 1.67% followed by the bark and leaf extracts (0.34% and 0.33%), respectively. The lowest yield obtained was from the stem PE extract (0.038%).

The extraction yield was determined for each extraction using the percent yield equation:

$$\text{Extract yield} = \frac{(\text{Dry Extract weight})}{\text{Dry starting material weight})} \text{ x } 100$$

Table 1: Extraction yields of *V. volkensii* plant components by PE, ACE and ETOH

Plant part	Solvent	Wt. of plant material (g)	Wt. of extract (g)	Plant material extracted (%)
Bark	ACE	15.0058	0.1451	0.97±0.012
	ETOH	15.0058	0.4796	3.20±0.012
	PE	15.0058	0.0512	0.34±0.011
Fruit	ACE	50.0279	0.5595	1.12±0.011
	ETOH	50.0279	0.5610	1.12±0.012
	PE	50.0279	0.8355	1.67±0.013
Leaf	ACE	10.0003	0.2706	2.71±0.014
	ETOH	10.0003	0.3885	3.88±0.013
	PE	10.0013	0.3664	0.33±0.004
Stem	ACE	50.0000	0.2595	0.52±0.005
	ETOH	50.0000	0.4198	0.84±0.005
	PE	130.0096	0.0498	0.038±0.006

±: Standard error.

3.2 Antimicrobial Activity of Extracts

Disc diffusion assays were conducted to determine if antimicrobial activity was present in the extracts of *V. volkensii*, therefore extracts concentration with the highest measured zones of inhibition for each extract, which was the 50 mg/ml concentrations was used for analysis. The susceptibility of bacteria to an extract is indicated by an inhibition zone diameter of 9 mm or more around the disc [31-33]. The sensitivity status of the bacteria is determined by measuring the inhibition zone of diameter to the nearest whole millimeter, which defines the bacteria as resistant (≤9 mm), moderately sensitive (10-11 mm), or sensitive (≥12 mm) to the antibiotic [32]. The negative control (DMSO) in all assays performed did not exhibit any inhibition, thus indicating that the inhibition observed in the assays was attributed to the positive control (APC) or the antibacterial properties exhibited by active components of the extract.

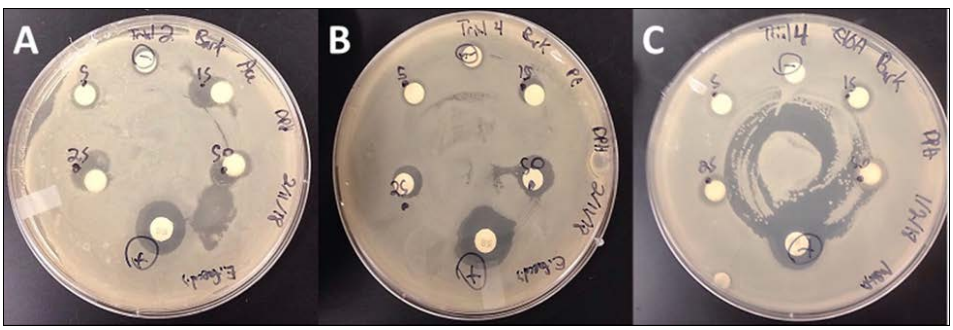

Fig 1: Antimicrobial disc diffusion assay results of *V. volkensii* bark extract.

Note: (a) ACE extract against *E. faecalis* (EF) (b) PE extract against *E. faecalis* (EF) (c) ETOH extract against methicillin-resistant *Staphylococcus aureus* (MRSA). On plate, 5, 15, 25, 50: extract concentrations (5, 15, 25, 50 mg/ml, respectively), +: APC (Vancomycin), -: negative control (DMSO).

3.2.1 Antimicrobial Activity of Bark Extracts

All bacteria were susceptible to *V. volkensii* bark extracts, except *S. aureus* for ACE and PE extracts and *E. coli* B strain for ETOH extracts (Table 2). The zones of inhibition (mm) for the 50 mg/ml concentration ranged from 8.27 to 11.50 mm for ACE extract, 8.89 to 10.94 mm for ETOH extract, and 8.21 to 11.20 mm for PE extract. The highest zone of inhibitions for the ACE and PE extracts (11.50 and 11.20 mm, respectively) was against *E. faecalis.* Whereas the highest zone of inhibition for ETOH (10.94 mm) was against methicillin-resistant *S. aureus*. *Staphylococcus aureus* was resistant and *E. faecalis* was sensitive to ACE extract (Figure 1). The other four bacteria (*E. coli* B strain, *S. enterica* Subsp. *enterica*, *S. flexneri* and methicillin-resistant *S. aureus*) were moderately sensitive to ACE extract. All bacteria were moderately sensitive against ETOH and PE extracts, except for *E. coli* B strain and *S. aureus*, which exhibited resistance to ETOH and PE extracts, respectively. These findings corroborated by previous antimicrobial studies conducted on *Vangueria edulis* and *Vangueria infausta*, which showed bark ETOH and PE extracts against *E. coli* and *S. aureus* of having low antimicrobial activity [34, 35].

Table 2: Antimicrobial activity of *V. volkensii* bark extracts (50 mg/ml)

Extract	Mean Zone of Inhibition in mm ± SEM					
	Gram Negative Bacteria			Gram Positive Bacteria		
	EC	SE	SF	EF	SA	MRSA
ACE	9.77 ± 0.24	9.84 ± 0.35	9.72 ± 0.46	11.50 ± 0.37	8.27 ± 0.29	10.17 ± 0.41
ETOH	8.89 ± 0.26	10.48 ± 0.25	9.54 ± 0.40	10.73 ± 0.09	10.63 ± 0.70	10.94 ± 0.45
PE	9.53 ± 0.41	9.84 ± 0.25	10.78 ± 0.09	11.20 ± 0.37	8.21 ± 0.31	10.85 ± 0.34
APC	16.86 ± 0.47	20.64± 0.31	15.46 ± 0.48	16.53 ± 0.39	17.70 ± 0.42	16.65 ± 0.58
DMSO	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00

±: standard error of the means (SEM)

3.2.2 Antimicrobial Activity of Fruit Extracts

All bacteria were susceptible to *V. volkensii* fruit extracts, except *S. aureus* for ETOH extract (Table 3). The zones of inhibition (mm) for the 50 mg/ml concentration ranged from 11.07 to 14.01 mm for ACE extract, 8.30 to 12.88 mm for ETOH extract, and 9.65 to 11.61 mm for PE extract. The highest zone of inhibitions for the ETOH and PE extracts (12.88 and 11.61 mm, respectively) were against *S. enterica* Subsp. *enteritidis*. Whereas the highest zone of inhibition for ACE (14.01 mm) was against methicillin-resistant *S. aureus*. All bacteria were either sensitive or moderately sensitive to the extracts, except for *S. aureus*, which was resistant to ETOH extract. All bacteria, except for *E. coli* B strain, were sensitive to ACE extract. *E. coli* B strain and *S. flexneri* were moderately sensitive against ETOH extracts, whereas *S. enterica* Subsp. *enteritidis*, *E. faecalis* and methicillin-resistant *S. aureus* were sensitive to ETOH extract. *S. enterica* Subsp. *enteritidis* was sensitive to PE and the other five bacteria were moderately sensitive to PE. The antibacterial activity of the fruits extracts is similar to that obtained by Ali and Elshiekh (2020) [36]. In their study, extracts from *V. madagascariensis* fruits exhibited either high or partial antibacterial activities against four bacteria strains including EC and SA at 50mg/ml concentration level. This observation underscores the medicinal uses of the fruits of *Vengueria* species.

Table 3: Antimicrobial activity of *V. volkensii* fruit extracts (50 mg/ml)

Extract	Mean Zone of Inhibition in mm ± SEM					
	Gram Negative Bacteria			Gram Positive Bacteria		
	EC	SE	SF	EF	SA	MRSA
ACE	11.07 ± 0.54	13.61 ± 0.67	12.67 ± 1.07	12.00 ± 0.46	12.47 ± 0.38	14.01 ± 1.50
ETOH	10.82 ± 0.32	12.88 ± 0.55	10.51 ± 0.18	11.68 ± 0.23	8.30 ± 0.34	12.23 ± 0.46
PE	10.28 ± 0.17	11.61 ± 0.44	9.87 ± 0.31	11.09 ± 0.26	9.65 ± 0.09	10.85 ± 0.58
APC	14.39 ± 0.24	23.64± 0.46	23.50 ± 3.30	19.36 ± 0.54	23.53 ±2.41	25.51 ± 1.18
DMSO	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00

±: standard error of the means (SEM)

3.2.3 Antimicrobial Activity of Leaf Extracts

All bacteria were susceptible to *V. volkensii* leaf extracts, except *S. aureus* for ACE and PE extracts (Table 4). The zones of inhibition (mm) of the 50 mg/ml concentration ranged from 8.48 to 11.56 mm for ACE extract, 10.68 to 12.34 mm for ETOH extract, and 7.55 to 12.50 mm for PE extract. The highest zone of inhibitions for the ACE and PE extracts (11.56 and 12.50 mm, respectively) was against methicillin-resistant *S. aureus*, whereas the highest zone of inhibition for ETOH (12.34 mm) was against *S. aureus*. All bacteria were moderately sensitive to ACE extract, except for *S. aureus* and methicillin-resistant *S. aureus*, which were resistant and sensitive, respectively, to ACE extract. The gram-negative bacteria (*E. coli* B strain, *S. enterica* Subsp. *enteritidis*, *S. flexneri*) were moderately sensitive to ETOH extract, while the gram-positive bacteria (*E. faecalis*, *S. aureus*, methicillin-resistant *S. aureus*) were sensitive to ETOH extract. *S. aureus* was the only bacteria deemed resistant to PE extract, the others were deemed either moderately sensitive (*E. coli* B strain, *S. flexneri*) or sensitive (*S. enterica* Subsp. *enteritidis*, *E. faecalis*, methicillin-resistant *S. aureus*) to PE extract. Shia *et al*. (2013) studied the antibacterial activity of sixteen plant species from South Africa, including *V. infausta*. The *V. infausta* leaf ETOH

extract, exhibited the highest antibacterial activity compared to the other fifteen plant species. In the present study, the zones of inhibition for leaf ETOH extract against *E. faecalis* (11.48 mm), *S. aureus* (12.34 mm), *E. coli* (10.97mm) and *S. enterica* Subsp. *enteritidis* (10.68 mm) was relatively high, indicating good antimicrobial activity. Thus, corroborating reported results of the study conducted by Shia *et al*. (2013) [27].

Table 4: Antimicrobial activity of *V. volkensii* leaf extracts (50 mg/ml)

Extract	Mean Zone of Inhibition in mm SEM					
	Gram Negative Bacteria			Gram Positive Bacteria		
	EC	SE	SF	EF	SA	MRSA
ACE	10.17 ± 0.13	10.48 ± 0.28	10.18 ± 0.18	11.24 ± 0.49	8.48 ± 0.20	11.56 ± 0.56
ETOH	10.97 ± 0.14	10.68 ± 0.80	11.48 ± 0.72	12.21 ± 0.11	12.34 ± 1.94	11.55 ± 0.36
PE	11.25 ± 0.55	11.60 ± 0.44	10.37 ± 0.16	11.70 ± 0.35	7.55 ± 0.38	12.50 ± 1.62
APC	15.21 ± 3.48	21.45± 1.77	20.21 ± 7.30	18.53± 0.64	18.14 ±0.69	20.36 ± 4.60
DMSO	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00

±: standard error of the means (SEM)

3.2.4 Antimicrobial Activity of Stem Extracts

All bacteria were susceptible to *V. volkensii* stem extracts (ACE and ETOH), except *S. aureus* for ACE (Table 5). The zones of inhibition (mm) for the 50 mg/ml concentration ranged from 8.02 to 11.38 mm for ACE extract and 10.00 to 11.36 mm for ETOH extract. The highest zone of inhibition for ACE extract (11.38 mm) was against *E. faecalis* and for ETOH extract (11.36 mm) was against methicillin-resistant *S. aureus*. Antimicrobial activity of *V. volkensii* stem PE extract was only conducted on *S. aureus* due to insufficient crude PE extract. Therefore, *S. aureus* was not susceptible (8.19 mm) to PE. Stem ETOH extract against all microorganisms exhibited relatively high zones of inhibition. *Escherichia coli* strain B and *S. aureus* were resistant to ACE extract while the other four bacteria were moderately resistant to ACE extract. All bacteria were moderately sensitive to ETOH extracts. *Staphylococcus aureus*, which was the only bacteria tested against PE extract, was resistant to PE extract.

Table 5: Antimicrobial activity of *V. volkensii* stem extracts (50 mg/ml)

Extract	Mean Zone of Inhibition in mm ± SEM					
	Gram Negative Bacteria			Gram Positive Bacteria		
	EC	SE	SF	EF	SA	MRSA
ACE	9.28 ± 0.55	10.46 ± 0.31	10.66 ± 0.40	11.38 ± 0.05	8.02 ± 0.30	10.84 ± 0.30
ETOH	10.21 ± 0.41	10.52 ± 0.56	10.00 ± 0.38	10.66 ± 0.30	11.15 ± 0.82	11.36 ± 0.59
PE	*	*	*	*	8.19 ± 0.36	*
APC	15.21 ± 3.48	21.45± 1.77	20.21 ± 7.30	18.53± 0.64	18.14 ±0.69	20.36 ± 4.60
DMSO	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00

* Not observed, due to lack of production of extract

±: standard error of the means (SEM)

Overall, microorganisms that were sensitive to the extracts (ACE, ETOH, PE) was from the fruit and leaf extracts. The general trend that gram positive bacteria are more sensitive to plant extracts than gram negative bacteria has been well documented [10, 26, 27, 33]. The cell wall biochemical composition of gram-positive bacteria (outer peptidoglycan layer) and gram-negative bacteria (outer phospholipidic membrane) may result in susceptibility of gram-positive bacteria, compared to gram negative bacteria [10]. Nearly all extracts (bark, fruit, leaf, and stem) exhibited broad spectrum activity against both gram-positive and gram-negative bacteria. However, *E. coli* B strain (gram negative) and *S. aureus* (gram positive) bacteria were resistant to some extracts. Those extracts against *E. coli* B strain were the bark ETOH and stem ACE and those extracts against *S. aureus* were the bark, leaf and stem ACE and PE extracts and the fruit ETOH extract.

3.3 Inhibitory Activity of Extracts for Each Microorganism

Members of the Rubiaceae family contain inhibitory properties [19, 22, 31, 37]. The inhibitory activity of *V. volkensii* extracts were examined via Analysis of Variance (ANOVA) and Duncan's Multiple Range Tests (DMRT). All analyses showed significant ($p<0.05$) interactions of mean ZOIs between microorganisms and extracts. In addition, all analyses indicated a significant difference ($p<0.05$) of mean ZOIs among microorganisms and among concentrations. Analysis of variance was repeated for all extracts (bark, fruit, leaf, stem) by removing the mean ZOIs of DMSO and APC to determine if any significant difference ($p<0.05$) initially observed, was due to these controls, which did not change the significance ($p<0.05$).

Overall, microorganisms that were sensitive to the extracts (ACE, ETOH, PE) was from the fruit and leaf extracts. Nearly all extracts exhibited broad spectrum activity against both gram-positive and gram-negative bacteria. However, *E. coli* B strain (gram negative) and *S. aureus* (gram positive) bacteria were resistant to some extracts. *V. volkensii* fruit extracts prepared from ACE showed the most antimicrobial activity, measured by mean zones of inhibition, against five of the six bacteria tested. Methicillin-resistant *S. aureus* had the highest zone of inhibition (12.0263 mm) among all extracts and microorganisms tested. These results support the notion that acetone or alcohol are the solvents of choice for extracting antibacterial compounds from Rubiaceae [10, 28, 38]. This is primarily because acetone, and alcohol to an extent, has the capability of extracting both polar and nonpolar components, thus, a greater number of components will be extracted [38, 39]. This study confirmed that *V. volkensii* bark, fruit, leaf, and stems, do in fact possess antimicrobial properties, however, the extent as to which are present, and quantity is unknown.

Conclusion

In conclusion, the disc diffusion assay revealed potential antimicrobial capabilities by *V. volkensii*. In general, all extracts exhibited broad spectrum activity against both gram-

positive and gram-negative bacteria. Most microorganisms were sensitive to all extracts, except for *E. coli* B strain and *S. aureus*, which were resistant to some extracts. More specifically, *E. coli* B strain was resistant to bark ETOH and stem ACE extracts, while *S. aureus* was resistant to bark, leaf and stem ACE and PE extracts, and the fruit ETOH extract. It is recommended that phytochemical analysis be conducted on *V. volkensii*. Additionally, since antimicrobial compounds are known to act in synergy with one another, future studies should be carried out to isolate and characterize active compounds and determine the synergy relationship between the antimicrobial compounds.

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