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Antibacterial activities of *Bidens pilosa* L, *Hoslundia opposita* Vahl, and *Ageratum conyzoides* L against some common wound pathogens

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Bacterial infection cause delayed wound healing, and the rising cases of antibiotic resistance call for alternative solutions. This research aims to determine and compare the antibacterial activity of selected research plants when extracted by different methods using different solvents. The plants were extracted with ethanol, methanol, acetone, water, chloroform, and ethyl acetate using Soxhlet and maceration extraction. Agar well diffusion and resazurin dye reduction method were used to determine the antibacterial activity of the extracts against methicillin-resistant *Staphylococcus aureus* (MRSA), *S. aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. Agar well diffusion results showed that water, ethyl acetate (Soxhlet), and 100% ethanol (macerated) extracts for all 3 plants inhibited a broad spectrum of bacteria. MIC results showed that ethanol and 70% ethanol (macerated) extracts of *Hoslundia opposita* had the lowest MIC (1.95 mg/ml) against *S. aureus*. The ethanol extract of *H. opposita* Vahl showed the lowest MIC against MRSA. Water, ethanol, 100% ethyl acetate (macerated) and 70% ethanol (macerated) extracts *of Ageratum conyzoides* showed the lowest MIC against *E. faecalis*. Ethyl acetate extract of *A. conyzoides* showed the lowest MIC against *E. coli*. 100% methanol (macerated) extract of *H. opposita showed* the lowest MICs against *K. pneumoniae* and *P. aeruginosa,* respectively; All plants showed better antibacterial activity on gram positive than gram negative bacteria. The methanol extract of *H. opposita* showed better activity than the rest of the extracts.

Key words: *Ageratum conyzoides* L., antibacterial activity, *Bidens pilosa* L., *Hoslundia opposita* Vahl, wound healing.

INTRODUCTION

A wound is a disruption of cellular and anatomic continuity of tissue caused by physical, microbial,

thermal, chemical, or immunological tissue trauma (Maver et al., 2015). The wound healing process is meant to occur naturally but, bacterial infection can fatally disrupt the process (Andreu et al., 2015). A patient with an infected surgical wound is 60 times more likely to be hospitalised in intensive care unit (ICU). In developed countries, surgical site infections account for more than 50% of hospital admissions in intensive care units, and 5 to 15% in regular wards (Lubega et al., 2017). In Europe, out of 10,000 surgeries, 3 to 4% become infected, requiring treatment costing about 2 million euros/year (Andreu et al., 2015). The extent of the problem in developed countries is underrated (Lubega et al., 2017). In Uganda, 10% of the surgeries performed become infected leading to high morbidity and mortality (Seni et al., 2013).

Infection retards the inflammatory phase and synthesis of collagen, obstructs epidermal migration, and causes poor odour and tissue destruction (Andreu et al., 2015). Low levels of bacteria in chronic wounds facilitate the healing process. Nevertheless, when the number of bacteria exceeds a certain limit, infection sets in, and healing is disrupted (Dowsett, 2004; Sood et al., 2014). Infection occurs when there is multiplication and deposition of bacteria accompanied by a host reaction. Furthermore, bacteria in chronic wounds are known to create a biofilm (a protective polysaccharide coating). Biofilms are resistant to most systemic and topical antimicrobial agents *(*Andreu et al., 2015; Sood et al., 2014) and are rarely recognised by host defenses (Sood et al., 2014).

Some common bacterial pathogens present in infected wounds include *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* (Bowler et al., 2001), vancomycin-resistant *Enterococcus faecalis* (Tripathi et al., 2016), *Klebsiella pneumoniae* (Effah et al., 2020), and methicillin-resistant *S. aureus* (MRSA) (Manzuoerh et al., 2019), among others. *S. aureus* and *P. aeruginosa* are known to cause infections and form biofilms in chronic wounds. They are opportunistic and easily express destructive virulence factors (Gounani et al., 2020). *P. aeruginosa* is able to withstand different conditions in wounds and is resistant to various antibiotics. It has the ability to colonise and proliferate, resulting in problematic infection and eventually a high degree of mortality (Al-Azzawi and Abdullah, 2018). Methicillin-resistant *S. aureus* (MRSA) is the most life-threatening and has been of great concern in the medical field. MRSA is a major cause of nosocomial infections with a high rate of morbidity and mortality in surgical wound infections (Šiširak et al., 2010). *E. faecalis* is the third most frequently isolated pathogen across all types of wounds and has acquired intrinsic resistance to a variety of antibiotics, which makes treatment difficult (Chong et al., 2017). In Uganda, *K. pneumoniae* and *S. aureus* were reported as the leading cause of surgical site infections at Mbarara regional referral hospital (Lubega et al., 2017). While, extended spectrum beta lactamase (ESBL) producing Enterobacteriaceae and MRSA were reported in Mulago Referral Hospital (Seni et al., 2013).

Antibiotic resistance has recently become a great concern in the medical field. Bacteria have developed various defence mechanisms to modify or destroy antimicrobial agents, thus, there is an increasing number of pathogens that are resistant to drugs (Ugboko et al., 2020). *S. aureus* was reported to show 10 to 60% resistance rate to oxacillin, erythromycin and clindamycin, while *E. coli,*

P. aeruginosa and other Gram-negative bacteria showed less than 25% resistance to imepenem amikacin, and piperacillin-tazobactam. *S. aureus* and other Gram-positive bacteria showed a resistance rate of 0 to 25% to vancomycin (Seni et al., 2013). The raising resistance rate of bacterial calls for further research in finding potential solutions to wound infection.

Plants have long been used for the treatment of various diseases, especially in developing countries. Various plant extracts are utilised in wound treatment and management because their antimicrobial and healing properties arise from various mechanisms (Maver et al., 2015). They act as antimicrobial agents to prevent infection, and the rate of wound healing depends on the choice of material used (Andreu et al., 2015). Phytochemicals are chemical components found in plants. They occur naturally and possess antimicrobial, antioxidant and anti-inflammatory characteristics that are responsible for wound healing (Shah and Amini-Nik, 2017). These phytochemicals use different me chanisms to fight bacteria, thus, make it hard for bacteria to develop resistance. They are of low cost, and have minimal side effects (Onwa et al., 2016).

Bidens pilosa L. is utilised as a medicinal herb for several years in Africa, Asia, and America and is reported to treat over 40 diseases (Bartolome et al., 2013). It is an erect perennial herb that grows in tropical and temperate regions and has approximately 240 species (Yang, 2014) with potent wound healing properties (Hassan et al., 2011; Kakki et al., 2016). Several studies on this plant have also shown evidence that it possesses ant ulcerative activity against indomethacin-induced lesions (Kumadoh et al., 2021), possesses anti-hyperglycaemic activity (Hsu et al., 2009), anticancer (Shen et al., 2018), antimalarial activity (Laryea and Borquaye, 2019), antidiabetic (Chien et al., 2009), immunoresponsive and

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anti-inflammatory activity (Wahyuddin et al., 2020), antioxidant activity (Cortés-Rojas et al., 2013) and antileukemia properties (Maher et al., 2021).

Hoslundia opposita Vahl from Lamiaceae is mostly used in Uganda for cleansing the uterus after birth and treating vaginal laceration (Kiguba et al., 2016). It is also utilised in the treatment of herpes, skin diseases, sore throats, microbial infections, and wounds (Annan and Dickson, 2008). The plant has shown evidence as a potential treatment for wound healing (Annan and Dickson, 2008) and other properties, such as antimalarial activity (Tjitraresmi et al., 2020), antidiabetic, analgesic, antimicrobial, anti-inflammatory, acaricidal, and central nervous system activity (Kiguba et al., 2016).

Ageratum conyzoides L. belongs to Asteraceae and consists of approximately 30 species. The plant has been utilised in Africa, Asia and southern America to treat pregnancy disorders and menstrual issues (Ssegawa and Kasenene, 2007). It has been shown to possess wound healing properties (Oladejo et al., 2003; Lestari et al., 2018). Additionally, the plant has been utilized as a purgative, antimicrobial, anti-inflammatory, antidysenteric, and antiulcer and for wound treatment (Yadav et al., 2019).

The antibacterial activity of *H. opposita*, *A. conyzoides*, and *B. pilosa* has been studied elsewhere (Chah et al., 2006; Annan and Dickson, 2008; Mujovo et al., 2008; Ojo and Anibijuwon, 2010; Singh et al., 2013; Odeleye et al., 2014; Garg and Grewal, 2015; Lawal et al., 2015; İçöz et al., 2016; Agarwal et al., 2016; Njume et al., 2016; Voukeng et al., 2016; Said, 2017; Ogbole et al., 2018; Nakibuule et al., 2019). However, to the best of our knowledge, there is limited research that has investigated and compared the antibacterial activity of the three plants using different solvents and extraction methods against MRSA*, S. aureus, E. faecalis, P. aeruginosa, E. coli* and *K. pneumoniae*. The aim of the study was to determine and compare the antibacterial activity of these three plants extracted using different solvents against wound pathogens such as MRSA*, S. aureus, E. faecalis, P. aeruginosa*, and *E. coli* and *K. pneumoniae*. The results of this study will guide the further development of pharmaceuticals from these plants.

MATERIALS AND METHODS

Mueller Hinton Broth, Mueller Hinton Agar, ethanol, methanol, acetone, chloroform, ethyl acetate and distilled water were all obtained from Loba Chemicals (India). Dimethyl sulfoxide (DMSO) (99.7%) was obtained from Acros organics and was used as received. Resazurin dye was gotten from Glentham.

Collection of plants

The aerial (flowers, leaves and stalks) parts of mature plants (*B. pilosa*, *A. conyzoides* and *H. opposita*) were collected from Bbale, Kayunga district in Uganda, in a period between November 2019 and January 2020. Collection was performed during the rainy season between 9:00 and 12:00 EAT. The collected plant parts were placed in loosely woven open mesh bags to allow aeration.

Authentication

The three plants were identified and authenticated by a certified botanist at Makerere University Herbarium and given Voucher numbers: *H. opposita* 001, *B. pilosa* 002, *A. conyzoides* 003.

Plant preparation

Each plant was prepared separately. The aerial parts (leaves, flowers, stalks) of each plant were sorted to remove foreign matter as well as the damaged and sick parts. They were cleaned and air dried overnight. They were placed in an oven at 27 to 30°C until they were crispy dry. The dried plant parts were pulverised using an electric grinder into a fine powder and kept in airtight containers at room temperature for further processing.

Extraction by cold maceration

The solvents methanol, acetone and ethanol were used for extraction in absolute form and at 70% concentration. One hundred grams (100 g) of the plant powder was weighed and placed in a stoppered vessel, and 1000 ml of the desired solvent was added to the powder and shaken vigorously. The mixture was left to stand at room temperature for 7 days with frequent agitation. The mixture was strained, filtered through cotton wool, and through Whattman filter paper and pressed. The filtrate was concentrated in an oven at 50°C to remove excess solvent (Singh, 2008). The extract was weighed and the percentage extract yield was calculated as:

$$\frac{weight\ of\ extract\ obtained}{weight\ of\ dry\ powder} \times 100$$

All samples extracted by maceration as pure solvents were labeled 100% ETH, 100% MTH, 100% ACTN, 100% E.A and 100% CHF for ethanol, methanol, acetone, ethyl acetate and chloroform, respectively. Those extracted with 70% alcohol were labeled 70% ETH, 70% MTH, and 70% ACTN for ethanol, methanol and acetone, respectively. The water extract was labeled H_2O.

Soxhlet extraction

The extraction was done as described by Tandon and Rane (2008) but with modification. 10 g of plant powder was weighed and placed in a thimble in the extraction chamber of the assembled Soxhlet apparatus. 200 ml of solvent was placed in the distillation flask of the apparatus and heated to boiling until extraction was complete. The miscella was concentrated in an oven at 50°C. All extracts were stored in a refrigerator at 4°C for further processing. Soxhlet-extracted samples were labeled ETH SX, MTH SX, ACTN SX, E.A SX and CHF SX for ethanol, methanol, acetone, ethyl acetate and chloroform, respectively.

Phytochemical screening

Each extract was screened for phytochemicals such as flavonoids,

saponins (frothing test), terpenoids (Salkowski test), alkaloids (Wagner's test), and phenols (follin reagent) as described by (EL-Kamali and Elshikh, 2015).

Antibacterial susceptibility testing

Standard isolates were obtained from the Microbiology Laboratory at the School of Biomedical Sciences, College of Health Sciences, Makerere University. These included *S. aureus* (ATCC 25923), *K. pneumoniae* (ATCC 700603), *E. coli* (ATCC 25922), MRSA (ATCC 43300), *E. faecalis* (ATCC 29212), and *P. aeruginosa* (ATCC 278853).

Agar well diffusion method

Stock solutions of the extracts were prepared by dissolving 0.5 g of extract in 1 ml of DMSO (99.7%). Fresh bacterial cultures (24 h old) were prepared, and the suspension's turbidity was adjusted to that of a McFarland standard 0.5% by adding fresh colonies to 1 ml of normal saline until the turbidity matched. The standardised suspension had a concentration of approximately 1.5×10^8 colony-forming units (cfu) ml.

Standardised bacterial cultures were inoculated using a sterile rod on freshly prepared Mueller-Hinton agar plates to make a lawn. With the aid of a sterile cork borer, 6 mm wells were aseptically punched on the inoculated agar plates, allowing 30 mm between adjacent wells as well as between peripheral wells and the edge of the plate (Mbata et al., 2008).

Fifty microliters of the stock solution of the extract, ciprofloxacin 2 mg/ml (positive control) and DMSO (negative control) were each transferred to the wells made. The plates were incubated at 37°C for 24 h. The zones of inhibition were measured using a divider and a ruler and recorded in mm. The experiment was performed in triplicate for each sample, and the mean inhibition zone was calculated and recorded. Based on the results of agar well diffusion, 8 samples for each plant were chosen to run the MIC experiment.

Measuring MIC using the resazurin reduction method

Fresh cultures (24 h old) were prepared, and the suspension turbidity was adjusted to that of a 0.5% McFarland standard by emulsifying a colony in 1 ml of sterile normal saline. A 1:100 standardised bacterial suspension was made by adding 1 ml of the suspension matched with 0.5% McFarland standard to 99 ml of normal saline to make a 1:100 dilution. First, 0.5 g of extract was weighed and dissolved in 1 ml of DMSO to a concentration of 0.5 g/ml in sterile Bijou tubes.

Determination of MIC was performed according to Teh et al. (2017) but with modification. Fifty microliters of Mueller Hinton broth was dispensed into each well of a sterile 96-well microtiter plate. To the first row of the microtiter plate, 50 µl of each extract to be tested was placed into a separate well of a column leaving out two wells for the positive (ciprofloxacin) and negative (DMSO) controls. The mixture was mixed thoroughly and labelled. Fifty microliters of this mixture in the first row was transferred to the wells in the second row using a sterile multichannel pipette. The mixture in the second well was also mixed thoroughly, and 50 µl was transferred to the third well. This 2-fold dilution was repeated up to the eighth well. Finally, 50 µl of the mixture was removed from the eighth well and discarded. Fifty microliters of ciprofloxacin (0.5 mg/ml) and 50 µl of DMSO were placed in the 11 and 12th columns and serially diluted 2-fold. Twenty microliters of the standardised bacterial suspension was transferred to each of the wells and mixed thoroughly. The microtiter plates were covered and incubated at 37°C for 24 h.

After incubation, 20 µl of 0.015% *w/v* resazurin salt was added to each well, incubated at 37°C for 4 h and observed for colour change from purple to pink (Purple to pink indicates microbial reduction of the dye). To determine the MIC, the dilution of the last well showing no color change was multiplied by the original concentration of the extract, that is, lowest dilution with no color change × original concentration of the extract.

Statistical analysis

The experimental results of the inhibition zones were expressed as the means and standard deviations of 3 replicates. This analysis was done in Microsoft Excel 2013. Further analysis was done in Minitab-17 software to determine the difference in means using One-way analysis of variance at a 95% confidence interval. P values less than 0.05 were considered significantly different. A post hoc analysis where applicable was performed using Tukey pairwise comparisons.

RESULTS

Plant extraction yield

H. opposita, *A. conyzoides*, and *B. pilosa*, were extracted by Soxhlet and cold maceration using different solvents. The percentage yield for the Soxhlet and maceration extraction methods is shown in Table 1. Macerated samples showed better yield than Soxhlet samples. Water extracts had a higher yield than the rest of the solvents. Hydroalcoholic solvents (70% each of ethanol, methanol and acetone) had higher extract yields than the corresponding alcohols that were used at 100% concentration. For Soxhlet extraction, *A. conyzoides* had the highest yield (4%), followed by *H. opposita* (3.6%) and *B. pilosa* (3.4%). For maceration, *H. opposita* had the highest yield (10.4%), followed by *A. conyzoides* (9.5%) and *B. pilosa* (8.6%).

Qualitative phytochemical analysis

The results from the phytochemical analysis are shown in Table 2. Results showed the presence of most of the phytochemicals tested. All extracts showed the presence of alkaloids, flavonoids and phenols. Tannins and saponnins were mostly absent in ethyl acetate and chloroform extracts. Most Sohxlet extracts did not show the presence of saponnins. Terpenoids were more frequent in *H. opposita* and *A. conyzoides* extracts compared to *B. pilosa.*

Antibacterial activity by agar well diffusion

The plant extracts were screened for antibacterial activity against gram-positive and -negative bacteria using the agar well diffusion method, and the results are shown

Table 1. The extract yield (%) of *B. pilosa* (BP), *H. opposita* (HO) and *A. conyzoides* (AG) extracted by maceration and Soxhlet.

Solvent	HO yield (%)					
	Macerated	**Soxhlet**	**Macerated**	**Soxhlet**	**Macerated**	**Soxhlet**
ETH SX	n.a	5	n.a	5.2	n.a	5.4
ETH 70%	14	*n.a*	14.7	n.a	14	n.a
ETH 100%	6.6	n.a	5.1	n.a	6.9	n.a
MTHSX	n.a	4.2	n.a	6.3	*n.a*	5.2
MTH 70%	15	n.a	12.3	n.a	12.2	n.a
MTH 100%	7.7	n.a	7.6	n.a	9.3	n.a
ACTNSX	n.a	4.3	n.a	5	*n.a*	4
ACTN 70%	9.7	n.a	9.5	n.a	13.6	n.a
ACTN 100%	2.8	n.a	6	n.a	7.1	n.a
E.ASX	n.a	2.8	n.a	2	n.a	1.2
E.A 100%	2.2	n.a	3.4	n.a	7.4	n.a
CHF SX	n.a	1.8	n.a	1.5	n.a	1.2
CHF 100%	3	n.a	3	n.a	6.6	n.a
Distilled water	16.3	n.a	20.3	n.a	17	n.a
Average %yield	10.4	3.6	9.5	4	8.6	3.4

HO, AG and BP represent *Hoslundia opposita*, *Ageratum conyzoides*, and *Bidens pilosa* L, respectively. H2O, ETH, MTH, ACTN E. A and CHF represent water, ethanol, methanol, acetone, ethyl acetate and chloroform, respectively. 100 and 70% represent those extracted by maceration at 70 and 100% alcohol. SX represents those extracted by Soxhlet. n.ams\nz: Not applicable.

Table 2. Results of the qualitative phytochemical analysis of plant extracts.

Solvent extract	Alkaloids			Flavonoids			Phenols			Tannins			Saponnins			Terpenoids		
	HO	**AG**	**BP**	**HO**	**AG**	**BP**	**HO**	**AG**	**BP**	**HO**	**AG**	**BP**	**HO**	**AG**	**BP**	**HO**	**AG**	**BP**
ETH SX	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-
100%ETH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
70%ETH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
ACTN SX	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
100%ACT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
70%ACT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
MTH SX	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-
100%MT H	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
70%MTH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
H_2O	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E.A SX	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+
100%E.A	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+
CHF SX	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+
100%CHF	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+

HO, AG and BP represent *Hoslundia opposita*, *Ageratum conyzoides*, and *Bidens pilosa*, respectively. H_2O, ETH, MTH, ACTN, EA and CHF represent water, ethanol, methanol, acetone, ethyl acetate and chloroform, respectively. 100 and 70% represents those extracted by maceration at 100 and 70% alcohol. SX represents those extracted by Soxhlet.

in Tables 3 and 4 as well as Figure 1.

The negative control DMSO did not inhibit the growth of any of the organisms. The positive control ciprofloxacin showed significantly higher inhibition zones

Table 3. Mean inhibition zones (mm) of *H. opposita* (HO), *A. conyzoides*, (AG), and *B. pilosa* L (BP) on gram-positive bacteria.

Solvent	*Methicillin-resistant Staphylococcus aureus*			*Staphylococcus aureus*			*Enterococcus faecalis*		
	HO	**AG**	**BP**	**HO**	**AG**	**BP**	**HO**	**AG**	**BP**
ETH SX	15±0.58	13±1	15±2.3	13±2.08	16±1.4	11±0.58	11	12±0.58	11±0.58
100%ETH	19±1.15	17±1	15±2.3	19±1	13±2.52	17±0.58	15±0.58	0±0.00	12±0.71
70%ETH	15±2.65	13±1.15	12±1	13±1.73	10±0.58	11±0.58	10±1.53	12±0.58	0
ACTN SX	16±1	10±0.00	14±0.7	17±0.577	12±1	14±1.52	13±1.15	0	12±2.08
100%ACT	13±0.76	16±0.7	13±1	16±1.26	15±0.5	12±1.15	11±0.58	0	10±0.71
70%ACT	11±0.76	10±0.58	14±1.73	13±2.6	15±3	8±1.15	11±1.58	0	10
MTH SX	12±0.58	10±0.7	9±0.58	12±2.08	10±0.58	11±1.52	14±4.5	9±0.00	10±0.71
100%MTH	21±0.58	10±0.00	0	18±0.76	0	11±0.00	14±0.76	12±2	0
70%MTH	13±1	13±2	10±0.7	12±1.15	12±0.00	10±0.28	10±1.53	12±1.15	10±0.71
H_2O	12±2.08	13±1	9±0.00	10±1	12±0.58	14	11±2.5	11±1.15	8±2.1
E.A SX	17±1.15	11±1.53	15±1	15±1.15	13±0.58	15±1	13±1.15	10±1	13±1.73
100%E.A	16±1	13±2.08	19±0.58	14±0.29	13±1.15	18±0.76	11±0.5	11.±0.0	0
CHF SX	14±0.00	0	10±0.00	15±0.00	8±0.00	13±3	12±0.5	0	9±0.00
100%CHF	16±1.37	0	0	18±1.73	0	0	0	0	0
CIPRO		37±00			39±1			35±00	

HO, AG and BP represent *Hoslundia opposita* Vahl, *Ageratum conyzoides* L, and *Bidens pilosa* respectively. H_2O, ETH, MTH, ACTN E.A and CHF represent water, ethanol, methanol, acetone, ethyl acetate and chloroform, respectively. 100 and 70% represent those extracted by maceration at 70 and 100% alcohol. SX represents those extracted by Soxhlet.

Table 4. Mean inhibition zones (mm) of *H. opposita* (HO), *A. conyzoides* (AG), and *B. pilosa* (BP) on gram-positive bacteria.

Solvent	*Escherichia coli*			*Klebsiella pnuemoniae*			*Pseudomonas aeruginosa*		
	HO	**AG**	**BP**	**HO**	**AG**	**BP**	**HO**	**AG**	**BP**
ETH SX	12±0.7	12±2.8	10±1.73	0	0	0	11±0.00	10±0.58	12±0.00
100% ETH	0	0	0	10±0.00	0	0	0	0	0
70% ETH	0	0	0	9±0.00	0	0	9	0	0
ACTN SX	0	0	0	0	0	0	0	0	14±1
100% ACT	0	0	0	0	0	0	0	0	0
70% ACT	9±0.76	0	8±0.35	0	0		9±1.15	0	10±0.00
MTH SX	0	13±3.05	0	9±0.58		10	10	8±1.52	14±4.5
100%MTH	0	0	0	9	0	0	10±0.58	0	0
70%MTH	0	12±2.89	0	0	0	0	13±0.00	13±1.53	0
H2O	12±1	8±0.00	12±3.5	11±2.31	12±0.58	9±0.00	16±0.00	0	11±0.71
E.A SX	0	7±0.00	9±0.58	0	9±0.58	11±1	10±1	10	12±1
100%E.A	0	0	0	0	0	13±1.15	17±0.5	0	0
CHF SX	0	0	0	0	0		12±1.15	0	9±0.00
100%CHF	0	0	0	0	0	0	0	0	0
CIPRO		45±1			34±0.58			43±1.15	

HO, AG and BP represent *Hoslundia opposita* Vahl, *Ageratum conyzoides* L, and *Bidens pilosa* respectively. H_2O, ETH, MTH, ACTN, E. A and CHF represent water, ethanol, methanol, acetone, ethyl acetate and chloroform, respectively. 100 and 70% represent those extracted by maceration at 70 and 100% alcohol. SX represents those extracted by Soxhlet. CIPRO.

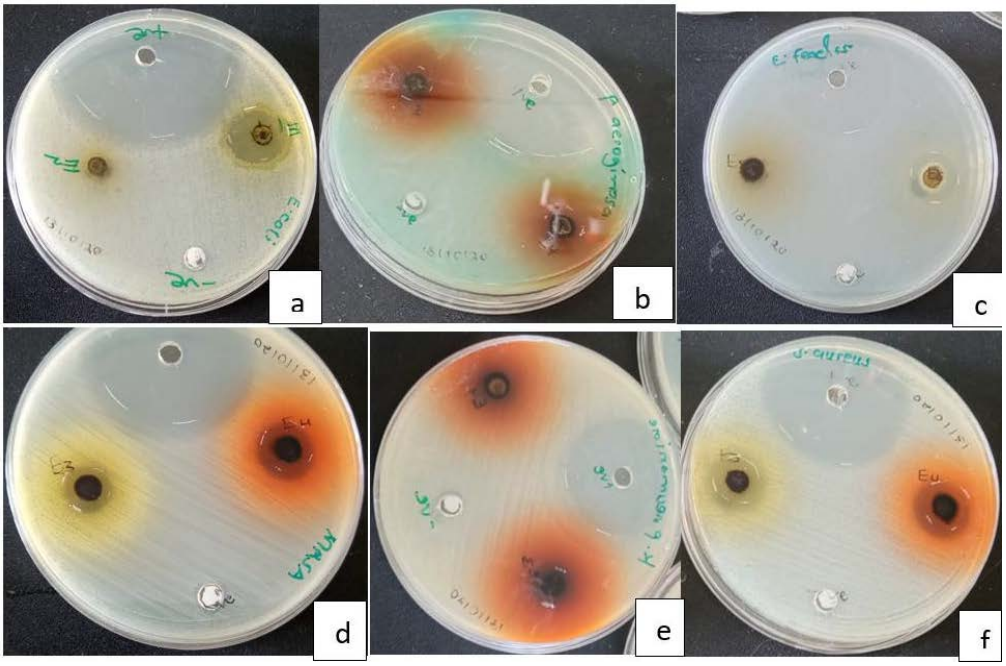

Figure 1. Growth inhibition of the bacterial strains *Escherichia coli* (a), *Pseudomonas aeruginosa* (b), *Enterococcus faecalis* (c), methicillin-resistant *Staphylococcus aureus* (d), *Klebsiella pneumoniae* (e), and *Staphylococcus aureus* (f).

above 30 mm for all organisms. The water extract of *H. opposita*, ethyl acetate (Soxhlet) extract of *A. conyzoides* as well as the water and ethyl acetate (Soxhlet) extracts of *B. pilosa* showed some clearance on all organisms.

MRSA was the most susceptible microorganism to most of the plant extracts. All extracts of *H. opposita* inhibited the growth of this organism. For *B. pilosa* and *A. conyzoides,* 12 out of 14 extracts showed clearance on *MRSA*. *H. opposita* extracts showed that the largest inhibition zone for *MRSA* was 21±0.58 mm by the 100% methanol extract (macerated), and the smallest was 13±1 mm by 70% acetone (macerated) and 70% methanol (macerated). For *B. pilosa* extracts, the largest inhibition zone was 19±0.58 mm by 100% ethyl acetate (macerated), and the smallest was 9±0.00 mm by distilled water extract. *A. conyzoides* showed the largest inhibition zone of 17±1 mm on *MRSA* by 100% ethanol (macerated) and the smallest 10±0.00 mm by 70% acetone (macerated) and 70% methanol (macerated) extracts.

The activity of the plant extracts against *S. aureus* was not significantly different from that against MRSA (P value=1.000). All *H. opposita* extracts inhibited this organism with the largest inhibition zone of 19±1.15 mm by 100% ethanol (macerated) and the smallest 10±1 mm by the distilled water extract. The largest inhibition zone for *B. pilosa* extracts was 18±0.76 mm by 100% ethyl acetate extract (macerated), and the smallest 8±1.15 mm by 70% acetone (macerated). For *A. conyzoides*, the largest inhibition zone was 16±1.4 mm by ethanol (Soxhlet), and the smallest, 8±0.00 mm by chloroform (Soxhlet).

E. faecalis was the least inhibited Gram-positive microorganism. All *Hoslundia* extracts except 100% chloroform (macerated) showed clearance of this bacterium, where the largest inhibition zone was 15±0.58 mm by 100% ethanol (macerated) and the smallest was 10±1.53 mm by 70% methanol (macerated). For *B. pilosa*, 10 out of 14 extracts showed clearance of the bacteria. The largest inhibition zone was 13±1.73 mm by ethyl acetate (Soxhlet), and the smallest was 8±2.1 mm by distilled water. Eight out of 14 extracts of *Ageratum* inhibited *E. faecalis,* where the largest inhibition zone was 12±0.71 mm by ethanol (Soxhlet), 70% ethanol (macerated), 100% methanol (macerated) and 70% methanol (macerated), while the smallest was 9±0.00 mm by methanol (Soxhlet).

P. aeruginosa was the most susceptible Gram-negative organism. Ten out of 15 extracts of *H. opposita* inhibited *P. aeruginosa*. The largest inhibition zone was 17±0.5 mm by ethyl acetate (macerated) extract, and the smallest was 9±1.15 mm by 70% ethanol (macerated) and 70% acetone (macerated). For *B. pilosa*, 7 out of 14 extracts showed activity on the organism. The largest inhibition zone was 14±4.5 mm by acetone (Soxhlet) and methanol (Soxhlet), and the smallest was 9±0.00 mm by the chloroform (Soxhlet) extract. *For A. conyzoides*,

4 out of 14 extracts inhibited the organism. The largest was 13±1.53 mm by 70% methanol (macerated), and the smallest was 8±1.52 mm by the ethyl acetate (Soxhlet) extract.

E. coli was quite resistant to most of the extracts, such that 5/14 extracts of *H. opposita* and *B. pilosa* showed some clearance of the organism, while for *A. conyzoides*, 4/14 extracts showed inhibition. The largest inhibition zone achieved for *H. opposita* was 12 mm by distilled water and ethanol (Soxhlet) extracts, and the lowest was 9±0.76 mm by 70% acetone (macerated). For *B. pilosa*, the largest was 12±3.5 mm by distilled water extract, and the lowest was 8±0.35 mm by 70% acetone (macerated). *A. conyzoides* extracts showed the largest inhibition zone of 13±0.35 mm by methanol (Soxhlet) and the smallest 7±0.00 mm by ethyl acetate (Soxhlet). *K. pneumoniae* was the most resistant microorganism, where 3 extracts of *H. opposita*, 4 extracts of *B. pilosa* L, and only 2 extracts of *A. conyzoides* showed inhibition. The largest inhibition zone obtained for *H. opposita* was 11±2.31 mm by distilled water extract, and the smallest was 9 mm by methanol (Soxhlet) and 70% acetone (macerated). For *B. pilosa*, the largest inhibition zone was 13±1.15 mm by 100% ethyl acetate (macerated), and the smallest was 9 mm by distilled water. For *A. conyzoides*, the largest was 12±0.58 mm by distilled water and 9±0.58 mm by ethyl acetate (Soxhlet).

Generally, the overall average inhibition zone of Gram-positive bacteria was significantly higher (P>0.001) than that of Gram-negative bacteria. The inhibition zones of *MRSA* and *S. aureus* were not significantly different (P=1.000) and these organism were the most susceptible to the plant extracts. *H. opposita* showed a significantly higher inhibition zone (p=0.003) than *A. conyzoides.* However, the overall inhibition zones for *B. pilosa* L and *A. conyzoides*, as well as *H. opposita* and *B. pilosa*, were not significantly different, with P values of 0.625 and 0.051, respectively.

The solvents that showed higher inhibition zones for all plant extracts were ethyl acetate (Soxhlet), water and ethanol (Soxhlet), with overall average inhibition zones (calculated across all three plants) of 10.8, 10.6 and 10.2 mm, respectively.

The Soxhlet-extracted samples generally showed a significantly higher average inhibition zone (p=0.03) than those extracted by maceration. However, the mean inhibition zones of extracts from the same type of solvent extracted by maceration or Soxhlet were not significantly different.

Determining the minimum inhibitory concentration (MIC)

From the first tests on antibacterial activity using agar well diffusion, 8 extracts of each plant were chosen according to their activity on a broad spectrum of bacteria by calculating the average inhibition zone obtained across the whole spectrum of bacteria tested. Their MIC was determined using the resazurin reduction method. The color change from purple to pink was observed. The change is an indication of the presence of active living bacterial cells that reduce resazurin (purple-blue) to resofurin (pink-colorless) (Elshikh et al., 2016). The MIC results are as shown in Figures 2 to 5.

Gram-positive bacteria were greatly affected by the plant extracts, with MICs ranging from 1.95 to 62.5 mg/ml (Figures 2 to 4). *S. aureus* was mostly susceptible to ethanol (Soxhlet) and 70% ethanol extracts of *H. opposita*, with the lowest MIC of 1.95 mg/ml. This was half as much as that of ciprofloxacin (3.9 mg/ml). Methanol (Soxhlet), 100% ethanol extracts of *H. opposita*, and 100% ethyl acetate and ethyl acetate (Soxhlet) extracts of *B. pilosa* showed MICs of 3.9 mg/ml for *S. aureu*s, similar to that of ciprofloxacin. *MRSA* was most affected by the ethanol (Soxhlet) extract of *H. opposita*, with the lowest MIC of 1.95 mg/ml, similar to that of ciprofloxacin.

E. faecalis was mostly susceptible to ethanol (Soxhlet), water, 100% ethyl acetate, and 70% ethanol extracts of *A. conyzoides*, with a MIC of 1.95 mg/ml, which was also similar to that of ciprofloxacin. Generally, as shown in Figure 5, *S. aureus* and *MRSA* were more susceptible than *E. faecalis;* however, their MICs were not significantly different (P=0.688). *S. aureus* and *MRSA* were mostly affected by *H. opposita*, with average MICs of 8.68 and 6.5 mg/ml, respectively, while *E. faecalis* was affected by *A. conyzoides*, with an average MIC of 11.81 mg/ml.

Gram-negative bacteria were less susceptible to the plant extracts, and their average MICs were not significantly different (0.648). *E. coli* was more susceptible to ethyl acetate (Soxhlet) extract of *A. conyzoides*, with 12.5 mg/ml MIC. *K. pneumoniae* and *P. aeruginosa* were more susceptible to the 100% methanol (macerated) extract of *H. opposita*, with MICs of 15.6 and 7.8 mg/ml, respectively. As shown in Figure 5, *A. conyzoides* extracts showed better activity on gram-negative bacteria, followed by *H. opposita* and *B. pilosa*. However, only the average MICs of *A. conyzoides L* and *B. pilosa* were significantly different (P<0.000). There was no significant difference between the average MICs of *A. conyzoides* and *H. opposita* (P=0.411) or *H. opposita* and *B. pilosa* (P=0.162). However, the average MICs of *A. conyzoides* were significantly lower than those of *B. pilosa* L. (P=0.006).

Generally*, H. opposita* extracts showed a higher antibacterial activity against Gram-positive bacteria, especially *S. aureus* and *MRSA,* compared to the rest of the plants, while *A. conyzoides* extracts showed a higher antibacterial activity against Gram-negative bacteria. Similar to the results of the inhibition zones, the Gram-positive bacteria were significantly more susceptible to all the plant extracts than the Gram-negative bacteria

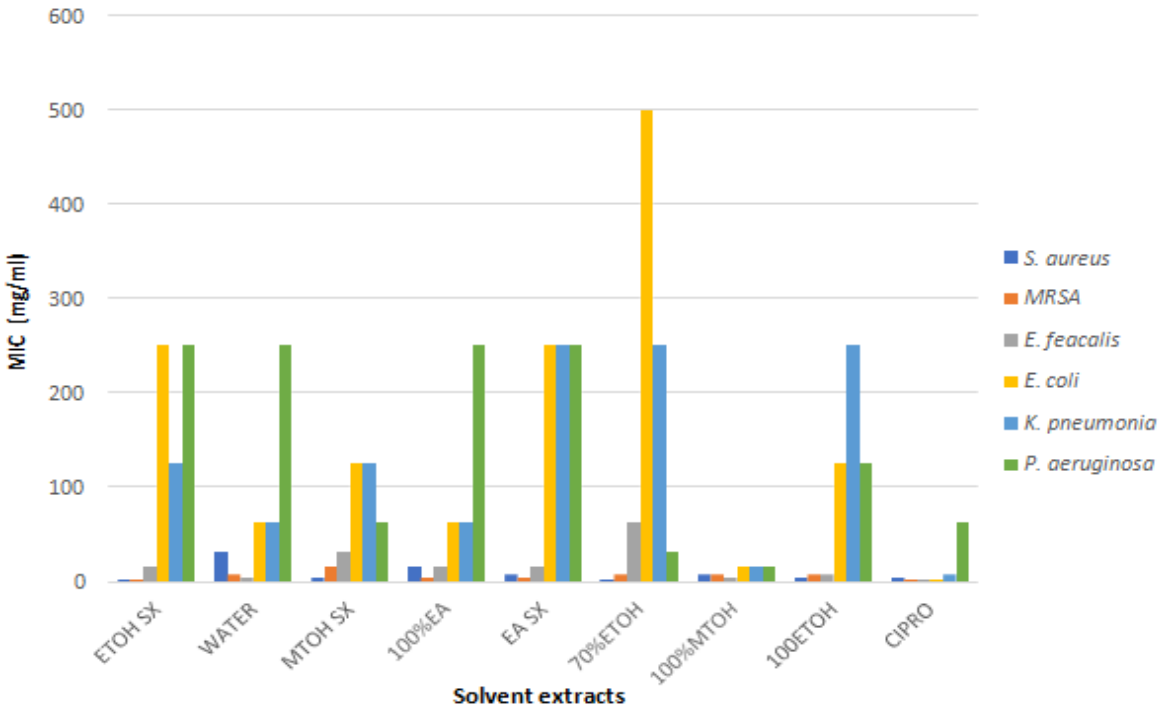

Figure 2. Minimum inhibitory concentrations for *H. opposita* extracts.
H_2O, ETH, MTH, E.A represent water, ethanol, methanol, ethyl acetate, respectively. 100 and 70% represents those extracted by maceration at 100 and 70% alcohol. SX represents those extracted by Soxhlet. CIPRO is for ciprofloxacin.

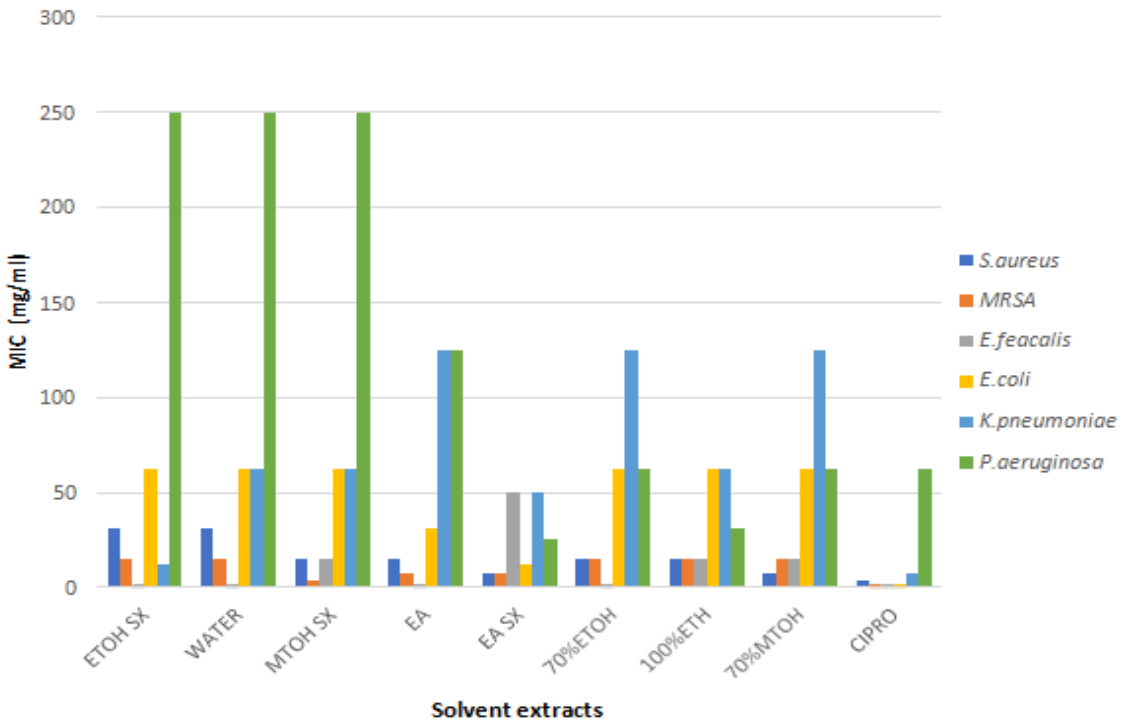

Figure 3. Minimum inhibitory concentration of *A. conyzoides* L extracts.
H_2O, ETH, MTH, E.A represent water, ethanol, methanol, ethyl acetate, respectively. 100 and 70% represent those extracted by maceration at 100 and 70% alcohol. SX represents those extracted by Soxhlet. CIPRO is for ciprofloxacin.

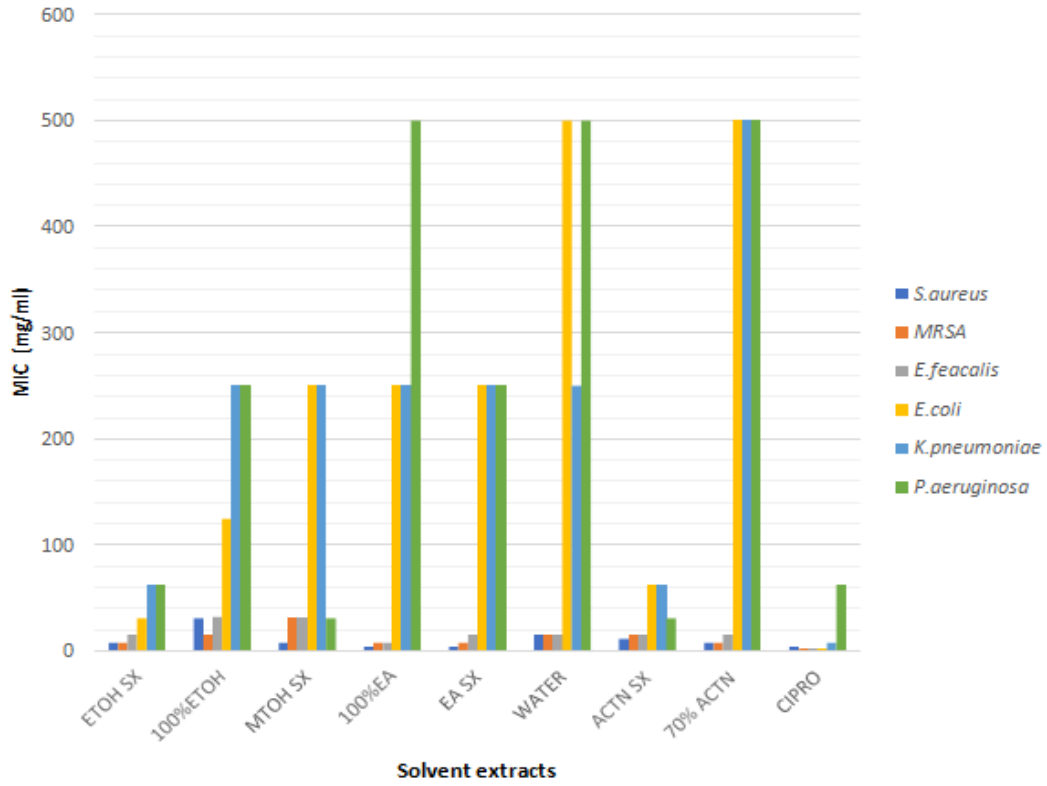

Figure 4. Minimum inhibitory concentration of *B. pilosa* extracts.
H_2O, ETH, MTH, E.A and ACTN represent water, ethanol, methanol, ethyl acetate and acetone, respectively. 100 and 70% represents those extracted by maceration at 100 and 70% alcohol. SX represents those extracted by Soxhlet. CIPRO is for ciprofloxacin.

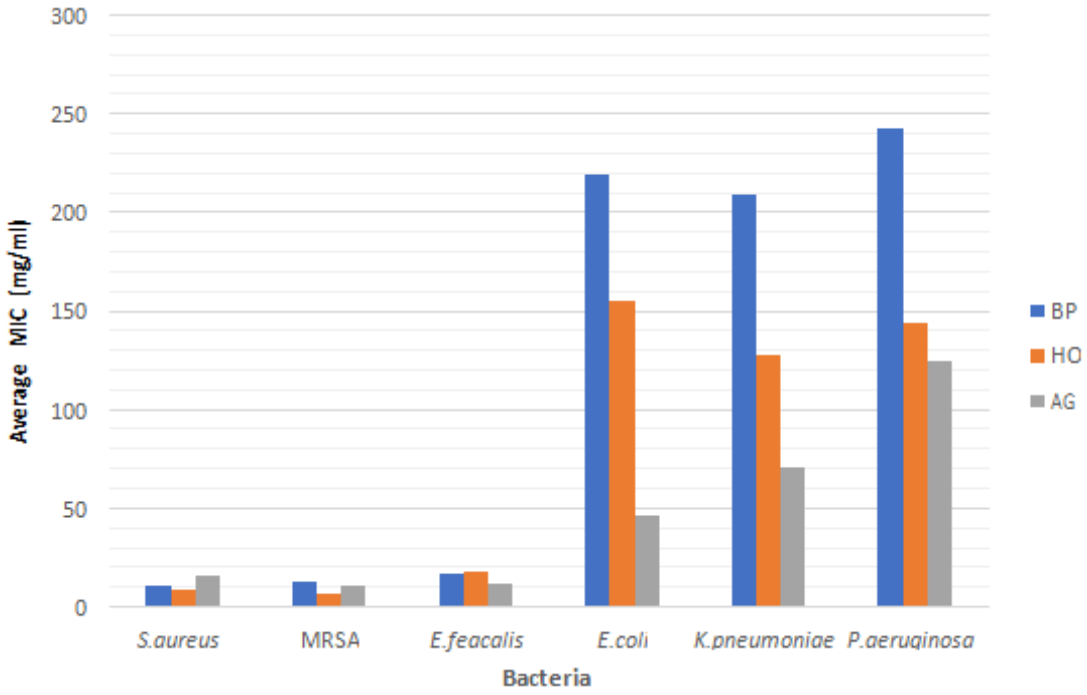

Figure 5. Average minimum inhibitory concentrations of extracts of *B. pilosa* (BP), *H.opposita* (HO) and *A. conyzoides* (AG).

(P<0.000). The lowest overall average MIC (calculated across all the bacteria) for *H. opposita* Vahl was 11.05 mg/ml by 100% methanol (macerated) extract. For *A. conyzoides* L., it was 25.5 mg/ml by ethyl acetate (Soxhlet) extract, and for *B. pilosa* L, it was 31.2 mg/ml by the ethanol (Soxhlet) extract. Therefore, the most effective extract was the 100% methanol (macerated) extract of *H. opposita*.

DISCUSSION

Extraction yield and phytochemical analysis

The difference in the yield obtained using different solvents can be attributed to the polarity of the solvent. Water is more polar than the rest of the solvents, which explains the higher yield obtained by the water extracts. The higher yield of hydroalcoholic solvents compared to pure alcoholic solvents could also be attributed to polarity because the addition of water increased the polarity of the solvent. Maceration gave a better yield than Soxhlet. This was expected since the former took a longer period of extraction (7 days). When the plant is in contact with the solvent for a longer period, it allows more diffusion of active compounds from the plant powder and thus a higher yield (Paz et al., 2018). The phytochemical analysis showed presence of flavonoids, tannins, and terpenoids in both Soxhlet and macerated. These compounds are responsible for the antibacterial activity of plants.

Antibacterial activity

From the results, it was observed that the water extracts of all plants inhibited at least 3 or more bacteria, and their inhibition zones and MICs were better than some of the solvent extracts. In traditional medicine, water is the main extractant for these plants during deconcoction (Ssegawa and Kasenene, 2007). The antibacterial activity of all the water extracts against a broad spectrum of bacteria justifies their use in traditional medicine to treat wounds and other bacterial infections.

H. opposita Vahl has been used in the treatment of several diseases, and its pharmacological properties have been explored (Said, 2017). In this study, the distilled water extract of this plant showed clearance of all organisms tested. A study by Ojo and Anibijuwon (2010) also found that aqueous extracts of *H. opposita* Vahl were able to inhibit all the organisms tested in this study (*MRSA* and *E. faecalis* were not tested). Furthermore, the results in this study also agree with other studies that have evaluated the antibacterial activity of this plant with other solvents. For example, methanol extracts of *H. opposita* showed clearance of *S. aureus* and *K. pnuemoniea* (Maregesi et al., 2008). Ethanol extracts of the same plant were found to inhibit the growth of *S. aureus* and *E. faecalis* (Atindehou et al., 2002). However, Ogbole et al. (2018) reported that methanol extracts (macerated) of *H. opposita*, from Nigeria did not show activity against *MRSA* and *P. aeruginosa*. Our results show that all methanol extracts (Soxhlet and macerated) of *H. opposita* Vahl had activity on both organisms, especially the 100% methanol (macerated). The difference in these results could be attributed to the difference in geographical location and environmental conditions, which may cause variation in secondary metabolites that are responsible for the antibacterial activity of the plant.

B. pilosa L. has been greatly explored in research for its pharmacological activities (Bartolome et al., 2013). The results obtained for extracts from this plant also correlate with other studies elsewhere. For example, in other similar studies, solvent extracts such as acetone (Adedapo et al., 2011; Shandukani et al., 2018), water (Khan et al., 2001; Adedapo et al., 2011; Silva et al., 2014; Lawal et al., 2015; İçöz et al., 2016), ethanol (İçöz et al., 2016), methanol (Ukwubile et al., 2014), and chloroform (Owoyemi and Oladunmoye, 2017) have been found to inhibit *S. aureus*. Ethanol (İçöz et al., 2016) and 70% ethanol (Silva et al., 2014) extracts were found to inhibit MRSA. Methanol (Njume et al., 2016), ethanol (Khan et al., 2001; İçöz et al., 2016) and water (Lawal et al., 2015) extracts were found to inhibit *E. faecalis.* Ethyl acetate, acetone and methanol extracts showed inhibition against *K. pneumoniae*. Ethanol (Khan et al., 2001), ethyl acetate, methanol, and acetone (Shandukani et al., 2018) extracts of *B. pilosa* L. have been found to have activity against *E. coli*. These results agree with those in our study. In contrast, İçöz et al. (2016) and Silva et al. (2014) reported that aqueous extracts of *B. pilosa L* showed no activity against *MRSA,* while in our study, aqueous extracts showed moderate activity against this organism.

A. conyzoids L has been used to treat wounds as well as other illnesses, and its pharmacological properties have also been researched (Okunade, 2002; Singh et al., 2013). Several studies have investigated the activity of this plant, and our results are in agreement with most of them. For example, 90% methanol (Soxhlet) (Neelabh et al., 2017), 80% ethanol (Agarwal et al., 2016) and water (Jagarlamudi and Kumar, 2017) showed activity against *S. aureus*. Elsewhere, 80% ethanol extracts (Voukeng et al., 2016), 40% ethanol and water (Akinyemi et al., 2005) extracts of *A. conyzoides* L showed inhibition against *MRSA*. Eighty percent ethanol has been shown to inhibit various VRE strains of *E. faecalis* (Agarwal et al., 2016). *E. coli* and *P. aeruginosa* were fairly affected by *A. conyzoides* L. extracts. Previous studies have found that methanol (Garg and Grewal, 2015; Jagarlamudi and Kumar, 2016; Neelabh et al., 2017), ethyl acetate, water and hydroethanolic (Jagarlamudi and Kumar, 2016), chloroform, acetone

(Garg and Grewal, 2015) and ethanol (Odeleye et al., 2014) extracts of *A. conyzoides* showed clearance of *E. coli* and *P. aeruginosa*. This was true for ethyl acetate, ethanol and methanol in our study but not acetone and chloroform. The contradictions in most of the studies could be attributed to the differences in extraction methods, geographical conditions, and environmental conditions. All these are reported to affect the quality and composition of the active components (Ncube et al., 2008) responsible for antibacterial activity.

Minimum inhibitory concentration

The MIC results were determined using the rezasurin reduction method. This method is recommended to quantify bacteria because it enables direct reading and the rezasurin indicator does not easily precipitate when reduction takes place (Cos et al., 2006). The color change from purple to pink that was observed is due to the presence of active living bacterial cells that reduce resazurin (purple-blue) to resofurin (pink-colorless) (Elshikh et al., 2016).

S. aureus has been reported to be a danger in surgical and burn wounds (Bowler et al., 2001). It has been globally perceived to be the number one cause of infections (Šiširak et al., 2010) and is fond of forming biofilms in chronic wounds (Fazli et al., 2009). In this study, 70% ethanol, methanol (Soxhlet), 100% methanol, and 100% ethanol extracts of *H. opposita*, as well as 100% ethyl acetate and ethyl acetate (Soxhlet) extract of *B. pilosa*, showed MICs between 1.95 and 3.9 mg/ml against this organism. This is an indication that these extracts contain compounds that are able to prevent or treat *S. aureus* infection in wounds. Moreover, *MRSA* is reported to be a resistant strain and a cause of mortality and morbidity in patients with infected surgical wounds (Šiširak et al., 2010). Our study found that *MRSA*, similar to *S. aureus,* was highly susceptible to all the plant extracts compared to other organisms. The organism was more susceptible to *H. opposita*, showing that compounds in these plants can be potential antibiotics against *MRSA.*

Vancomycin-resistant *E. faecalis* is the third most frequently isolated microorganism in infected surgical wounds and other types of infected wounds. It is resistant to a number of antibiotics, and is difficult to treat (Tripathi et al., 2016). In this study, *E. faecalis* was more susceptible to *A. conyzoides* extracts than other plants, where most of the extracts showed an MIC of 1.95 mg/ml. This is an indication that *A. conyzoides* has compounds that are likely to treat such a resistant strain.

P. aeruginosa is known worldwide for causing infection in chronic wounds and is involved in biofilm formation (Fazli et al., 2009). This organism was fairly resistant to a number of extracts. The 100% methanol (macerated) extract of *H. opposite* showed the most activity against this organism. Thus, compounds isolated from this extract could act as potential antibiotics against *P. aeruginosa*.

E. coli and *K. pneumoniae* were quite resistant to most of the extracts. *E. coli* isolates found in wounds have many virulence factors and have been reported to be resistant to drugs such as ampicillin, fluoroquinolones and tetracycline (Alharbi et al., 2019). *K. pneumoniae* isolates are responsible for one-third of all infections caused by Gram-negative bacteria, including wound infections (Effah et al., 2020). In this study, the 100% methanol extract (macerated) of *H. opposita* fairly inhibited the growth of these organisms with MIC of 7.8 mg/ml. This gives hope that further processing of compounds from this extract or making combinations with other antibiotics could lead to better results.

Generally, the activity of Gram-positive bacteria was significantly higher (P>0.001) than that of Gram-negative bacteria. This has also been reported in other similar studies (Owoyemi and Oladunmoye, 2017; Nakibuule et al., 2019). Gram-negative bacteria have a complex cell wall structure compared to that of Gram-positive bacteria. The structure consists of various proteins, polysaccharides and lipids. Additionally, the cell wall consists of an outer membrane hardly separated from it by a periplasmic space. The latter contains periplasm, which has been reported to contain bacterial enzymes responsible for destroying antibacterial agents before they can distort the cell membrane (Annan and Dickson, 2008).

The results from the agar well diffusion method did not necessarily correlate with the MIC results of some extracts, especially *A. conyzoides*. This difference could be attributed to the polarity of the bioactive compounds contained in the plants. It is stated that nonpolar compounds may not easily diffuse through agar (Eloff, 2019). Additionally, the difference in volatility, solubility and diffusion properties of the media could affect the results (Cos et al., 2006). This could explain the high activity *A. conyzoides* in regard to the MIC results, especially on *E. faecalis* and on all Gram-negative organisms, compared to the results of the inhibition zones.

From this study, it is not clear whether Soxhlet extraction gives better antibacterial activity compared to maceration because the extracts from the same solvent and plant but different extraction methods did not show significantly different antibacterial activity. In other studies, Paz et al. (2018) reported no significant difference in the antibacterial activity of 70% ethanol extracts of *Hamelia patens* extracted by maceration, percolation and Soxhlet extraction. However, Sankeshwari et al. (2018) reported that the antibacterial activity of cold macerated extracts of liquorice root were significantly different from those obtained by Soxhlet.
Therefore, it could be that the antibacterial activity may depend more on the type of solvent and type of plant rather than the extraction method. But, this may require further investigation.

It was also shown that the type of solvent greatly affected the antibacterial activity of the plant, as seen by the different values of the inhibition zone and MIC obtained by different solvent extracts. This could be because the quantity and composition of active com-pounds depend on the nature, polarity and concentration of the solvent (Ncube et al., 2008). In this study, ethanol, ethyl acetate, methanol and water were better solvents since most of their extracts had activity on a broad spectrum of bacteria for all three plants. Acetone seemed to be a better solvent for *B. pilosa* than for the other two plants. The best extract was the 100% methanol (macerated) extract of *H. opposita*.

The antibacterial activity of these plants can be attributed to the presence of active compounds such as phenols, flavonoids, terpenoids, sponnins, and tannins shown in Table 2. Their presence in these plants has already been investigated (Ajanaku et al., 2021; Jagarlamudi and Kumar, 2017; Odeleye et al., 2014; Okach et al., 2013; Owoyemi and Oladunmoye, 2017), and our results have confirmed the same. Their mode of action on microbes is linked to destruction of the microbial cell membrane, binding directly to the cell wall, interfering with the cell membrane and altering its integrity. They also synthesize microbial nucleic acids and inhibit multidrug resistant (MDR) pumps and microbial enzymes (Reichling, 2010). Flavonoids derive their antimicrobial activity by forming complexes with bacterial membranes and with soluble and extracellular proteins (Alihosseini, 2016). It is no wonder that they are synthesized by plants to counteract infection by microorganisms (Ncube et al., 2008). Their antimicrobial action is derived from their ability to form complexes with proteins via covalent bonding, hydrophobic effects and hydrogen bonding (Alihosseini, 2016). Most of the cholesterol-free gram-negative bacteria have their outer membrane covered by lipopolysaccharide (LPS); thus, it is claimed that saponins increase cell wall permeability by interacting with LPS (Arabski et al., 2012).

Therefore, the study confirms that these plants can be used in the further development of antibiotics for wound infections.

Conclusion

The objective of this study was to determine and compare the antibacterial activities of *H. opposita*, *B. pilosa*, and *A. conyzoides* against some common wound pathogens. We found that the type of solvent greatly affected the antibacterial activity of the plant. Ethanol, ethyl acetate, methanol and water were better solvents than acetone and chloroform. Gram-positive bacteria were more susceptible to the plant extracts than gram-negative bacteria. *MRSA* and *S. aureus* were the most affected organisms, while *K. pneumoniae* and *E. coli* were the least affected.

The antibacterial activity of these plants can be attributed to the presence of secondary metabolites shown in the phytochemical analysis. *H. opposita* showed better antibacterial activity against Gram-positive bacteria while *A. conyzoides* showed higher antibacterial activity against all Gram-negative bacteria than other plants. The best extracts from each plant were the 100% methanol (macerated) extract of *H. opposita*, followed by the ethyl acetate (Soxhlet) extract of *A. conyzoides* and the ethanol (Soxhlet) extract of *B. pilosa*. The overall most effective extract was the 100% methanol (macerated) extract of *H. opposite*. Since all the plant extracts had very good activity in one way or another, a combination of compounds isolated from these plants or with other antibiotics could produce syner-gistic action against a number of organisms. Antibiotic resistance is a great concern in the medical field. The research findings will guide the further development of pharmaceuticals from these plants as one step to combat the issue.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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