

# Studies on bioactivity and secondary metabolites of crude extracts of *Bidens pilosa* L. (Asteraceae): A medicinal plant used in the Transkei region of South Africa

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**Abstract:** Whole plant-parts of *Bidens pilosa* were powdered and extracted in concentrated hexane, acetone, ethanol, methanol and water. The extracts were tested for antimicrobial activity against *Escherichia coli* (25922), *Bacillus subtilis* (ATCC 6051), *Enterococcus faecalis* (51299) *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC127853), using standard microbiological techniques. Active crude extracts were macerated in concentrated methanol and tested for secondary metabolites including tannins, saponins, alkaloids, cardiac glycosides, anthraquinones, steroids and flavonoids using standard phytochemical procedures. Hexane and methanol extracts demonstrated similar activity producing 8-17 mm and 11-18 mm inhibition zone-diameter ranges respectively. Further analysis for minimum inhibitory concentrations (MIC<sub>50</sub>) recorded 1.25-20mg/mL and 2.5-20mg/mL for hexane and methanol extracts respectively. The highest zones of inhibition diameters (22-36mm) and lowest MIC<sub>50</sub> values (0.0002-0.0006mg/mL) were recorded for Gentamicin, the positive control. Minimum bactericidal concentration (MBC) ranges were between 10-80mg/mL and 0.001-0.005mg/mL for extracts and control antibiotic respectively. With the exception of anthraquinones, the plant crude extracts tested positive for all secondary metabolites analyzed. These results provide scientific basis for the use of *B. pilosa* in South African traditional medicine. The antibacterial activity reported herein may be attributed to one or more of the 6 secondary metabolites detected in the plant crude extracts.

**Keywords:** Antibacterial resistance; susceptibility tests; plant extracts; transkei; South Africa.

## INTRODUCTION

*Bidens pilosa* L. (Asteraceae) has a wide distribution, mainly in tropical and subtropical regions of the world (Deba *et al.*, 2008; Bartolome *et al.*, 2013). It is an erect perennial herb that may grow to a height of 0.3-1.5m (Shivakumar *et al.*, 2014; Silva *et al.*, 2014). The plant has a remarkable method of seed dispersal characterized by the production of barbed awns, which stick on animal fur or clothing and can cause injury if directly exposed to the skin (Bairwa *et al.*, 2010; Njume *et al.*, 2014a). It is called Black Jack or Spanish needles in English and *Uhlabangulo* in isiXhosa, a language spoken by the people of the Transkei region of South Africa. In Brazil, it is popularly known as "picão-preto" and decoctions, infusions and tincture of whole plant parts are used in the treatment of gastrointestinal discomfort, diabetes/malaria and hepatitis C respectively (Borges *et al.*, 2013). In some parts of the Transkei region of South Africa, the leaves and young shoots are used in combination with other indigenous plants as leafy vegetable especially in times of food scarcity (Adedapo *et al.*, 2011). Decoctions of roots and leaves are used in the neighbouring villages of Mthatha in the Eastern Cape Province of South Africa to treat arthritis, headache and abdominal discomfort. Its uses are embedded in the culture and tradition of the

indigenous population of Oliver Reginald (O.R) Tambo District Municipality, the people of Transkei, reported to be more rural and traditional in their way of life than other regions of South Africa (Dold and Cocks, 2002). However, inaccessibility to western drugs and inadequate healthcare facilities in the Transkei region of South Africa have also encourage reliance on the use of this plant and other traditional herbs as useful alternatives to allopathic healthcare system (Njume *et al.*, 2014b). *B. pilosa* is readily available, accessible and virtually free to everybody who desires its use. In the Kwa-Zulu Natal Province of South Africa, the Zulus crush the leaves in water and take them orally for the treatment of dysentery, diarrhoea and colic (Arthur *et al.*, 2012).

Almost every part of *B. pilosa* including roots, stems and flowers are ethnically used for treatment of inflammatory diseases or bacterial infections in Africa, Asia and America (Chang *et al.*, 2007). Despite its folkloric uses in the alleviation of gastrointestinal discomfort in South Africa and many parts of the developing world, only few studies have concentrated on its antimicrobial activity against bacteria etiologic agents of diarrhoea and dysentery. However, literature survey reveals a large amount of potentially useful bioactive compounds isolated from this plant, most of them with anti-inflammatory, anti-malarial, anti-diabetic, hypotensive and immunomodulatory effects (Silva *et al.*, 2011; Yang

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*et al.*, 2014). Not many studies have concentrated on its antibacterial compounds, especially not with the South African ecotype. Some of the compounds structures and bioactivities including flavonoids, terpenoids, phenylpropanoids, aromatics, porphyrins, aliphatics and polyenes as reported by Silva *et al.* (2011); Bartolome *et al.* (2013) and Yang *et al.* (2014) are presented on table 1.

Apart from scarcity of adequate healthcare resources, bacterial drug resistance against many therapeutic agents is a persistent problem in many parts of South Africa. Resistance rates of about 30% have been reported for ceftriaxone and cefotaxime, two very important drugs used in the treatment of enterobacteria infections in the country (Brink *et al.*, 2012). *Klebsiella pneumoniae*, a stubborn nosocomial pathogen has been reported to exhibit a resistance of up to 46.5% against ciprofloxacin (Perovic *et al.*, 2012). Other studies have documented the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in South Africa with resistance to erythromycin, tetracycline, trimethoprim/sulfamethoxazole, gentamicin and ciprofloxacin (Marais *et al.*, 2009; Heysell *et al.*, 2011). This is a particularly distressing problem especially in South Africa where about 6.4 million people may have their immune systems compromised by ~~due to~~ HIV/AIDS (Hanson *et al.*, 2015). The search for new drugs therefore becomes even more important considering this ever disturbing phenomenon of antimicrobial resistance, which seems to render previously existing drugs obsolete. Different drug combinations and longer duration of therapies have been helpful in alleviating this problem. However, the high pill burdens and adverse effects associated with some therapies have been quite unfriendly to some patients. Equally important is the fact that not many new drugs are being developed on a yearly basis. Medicinal plants such as *B. pilosa*, which are already in use by the local community in South Africa and other parts of the developing world may have the potential to serve as alternative reservoirs of chemical ingredients for the pharmaceutical industry. This study was therefore designed to evaluate the medicinal properties of *B. pilosa*; to test the plant solvent-extracts against some medically important bacteria for the determination of Minimum inhibitory and Minimum bactericidal concentrations; and to screen the most active extracts for secondary metabolites in a bid to determine the phyto-components likely responsible for its pharmacological properties.

## Methodology

### Selected micro-organisms

All the micro-organisms selected for this study were reference bacteria strains (MD, USA) obtained from the stock culture of the National Health Laboratory Services (NHLS), Nelson Mandela Academic Hospital, Mthatha South Africa. They included *Pseudomonas aeruginosa* (127853), *Escherichia coli* (25922), *Staphylococcus*

*aureus* (29213), *Enterococcus faecalis* (51299) and *Bacillus subtilis* (6051). Approval for the study was obtained from the Department of Health and the Ethics Committee of Walter Sisulu University (WSU), South Africa. Nutrient agar was used as our primary isolation medium for all the bacteria under investigation.

### Preparation of plant material

Whole plants including roots, stems, leaves and flower-parts were harvested from the vicinity of WSU, Nelson Mandela Drive (NMD) campus in late September 2014. Identification of the specimen was conducted in the university's herbarium under the supervision of the curator. Vouchers (CN03) were kept in the herbarium. Plant samples were cleaned with running tap water, chopped into smaller pieces and sun dried for 1 ~~one~~ week. The plant material was ground to fine powder (ATO Mix, Cambridge), placed in brown bottles and kept in a dark cupboard until needed.

### Preparation of plant crude extracts

Crude extracts were prepared by soaking 400 g of ground samples in 700mL concentrated hexane, acetone, ethanol, and methanol in 2L volumetric flasks (Schott, Durban). Another 400g of sample material was soaked in water. The flasks were labeled and placed in a shaker incubator (Labcon, Maraisburg) for 2 days. The test material was centrifuged (1006.2 x g) for 5-10 minutes and separated from the solvent by filtration through a filter funnel with a pore size of 60 Å. Centrifugation and filtration was performed thrice, the plant debris was discarded and the solvent removed by evaporation in a rota vapour (Büchi, Switzerland). In order to remove residual solvents, the extracts were collected in open-mouth evaporating dishes (Haldenwanger, Berlin) and left at room temperature for ~~one~~ 1 week. The filtrate of extracts prepared in water was rapidly frozen in 99% acetone and the water removed by sublimation in a process known as freeze-drying (Castillo-Juárez *et al.*, 2009). Approximately 2g of each extract was used in the analysis and the rest wrapped in aluminium foil and stored at 4°C.

### Determination of inhibition zone diameters

The plant crude extracts were screened following previously established procedures (Njume *et al.*, 2014b). Saline (0.9%) was used to prepare an emulsion of the test bacteria (McFarland turbidity standard 0.5). Mueller Hinton agar plates were inoculated with the bacteria emulsion by spread plate technique and left to dry for 20 minutes. A sterile stainless steel cork borer was used to punch holes measuring 6mm in diameter in the agar. Concentrations for extracts, Gentamicin (positive control) and acetone (negative control) were prepared at 100 mg/mL, 0.0005mg/mL and 80%v/v respectively. Approximately 75µL of various samples and controls were carefully placed in the holes using a micropipette (Tokyo, Japan). The plates were placed in the incubator

set at 37°C and the inhibition zone diameters (mm) were measured 24 hours later. These tests were repeated once and the means for inhibition zone diameters were computed.

#### **Test for minimum inhibitory concentrations (MIC<sub>50</sub>)**

Methanol and hexane extracts were considered for MIC<sub>50</sub> determination. The test was conducted in accordance with previously established procedures (Bonacorsi *et al.*, 2009). Briefly, test extracts and controls were diluted double fold in micro titre plates using Mueller Hinton broth. Concentrations ranges of 0.31-40mg/mL and 0.0001-0.0008mg/mL were prepared and analyzed for extracts and control antibiotic respectively. Bacteria strains harvested from an 18-hour old culture and broth only were used as negative control. At McFarland turbidity standard 0.5, twenty microliters of bacteria was emulsified in 180µL of broth-containing extract wells. The plates were placed in an automatic enzyme-linked immunosorbent assay (ELIZA) plate reader (Winooski, VT) and the absorbance read at 600 nm. The plates were incubated at 37°C and the absorbances read again, 24 hours later. Both sets of absorbances were compared to determine differences in bacteria growth patterns. Test concentrations that caused a decrease in 50% of bacterial growth (MIC<sub>50</sub>) were determined after plotting a graph of absorbance values against concentration.

#### **Test for minimum bactericidal concentrations (MBC)**

Minimum bactericidal concentrations were carried out in accordance with the method of Nethathe and Ndip (2011). Wells with concentrations that inhibited 50% of bacteria growth were emptied in sterile test tubes containing saline (0.9%). The volume collected from each well was approximately 200µL. A tenfold serial dilution was performed and approximately 10µL was withdrawn from the tubes and spread on Mueller Hinton agar plates followed by incubation at 37°C. The plates were observed for absence of bacteria growth after 24 hours. The lowest concentrations of extract or antibiotic that did not produce visible growth of bacteria at the later cultivation were recorded as MBC.

#### **Phytochemical screening**

Methanol crude extracts were analyzed for secondary metabolites. Qualitative analysis for tannins, saponins, alkaloids, cardiac glycosides, steroids and flavonoids were carried out according to standard procedures (Harbourne, 1983; Akinpelu *et al.*, 2008; Ben *et al.*, 2013).

#### **Analysis for Alkaloids**

A small amount of the plant crude extract weighed to 0.5 g was dissolved in 5mL of 1% aqueous hydrochloric acid placed in a steam bath. The mixture was filtered and a few drops of Dragendorff's reagent (potassium bismuth iodide solution) were added. Cloudiness was recorded as positive test for alkaloids.

#### **Analysis for tannins**

A small amount of the extract weighed to 1.0g was dissolved in 20mL of distilled water. The mixture was filtered and 2-3 drops of 10% FeCl<sub>3</sub> was added to 2mL of the filtrate. A blackish-blue or blackish-green colouration was recorded as positive test for tannins. Another 2mL of the filtrate was added to 1mL of bromine water and the presence of precipitate was again recorded as positive test for tannins.

#### **Analysis for flavonoids**

The crude extract was weighed to 0.2g, dissolved in 2mL of methanol and heated. Magnesium metal chip was added to the mixture followed by a few drops of concentrated HCL. Intense cherry red (flavonones) or orange (flavonols) colouration was recorded as positive test for flavonoids (Ben *et al.*, 2013).

#### **Analysis for saponins**

A sterile stainless steel cork borer was used to bore holes on a freshly prepared 7% blood agar plate. Approximately 0.2g of the extract dissolved in methanol was placed in one of the holes. An equivalent amount of commercial saponin (BDH) solution was included in the experiment as positive control while distilled water and methanol were placed in the negative control wells. The plates were incubated at 35°C for 6 hours. Complete haemolysis of the blood around the extract was recorded as positive test for saponins.

#### **Analysis for cardiac glycosides**

The extract was weighed to 0.5g and dissolved in 2mL of glacial acetic acid containing 100µL of 1% FeCl<sub>3</sub>. This was underlain with concentrated H<sub>2</sub>SO<sub>4</sub>. The presence of a brown ring at the interphase was indicative of presence of a deoxy sugar, characteristic of cardiac glycosides. A violet ring sometimes appears below the ring while in the acetic acid layer; a greenish ring may form just above and gradually spread throughout this layer.

#### **Analysis for steroids**

Steroids were tested in accordance with the method of Akinpelu *et al.* (2008). Approximately 0.5g of the extract was dissolved in 3mL of CHCl<sub>3</sub> and filtered. About 3ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added to the filtrate to form a lower layer. Appearance of a reddish brown ring was recorded as positive test for steroids.

#### **Test for anthraquinones**

Anthraquinones were tested by placing 0.5g of the extract in a dry test tube and adding 5mL of concentrated chloroform (Maobe *et al.*, 2013). The mixture was shaken vigorously for 5 minutes, filtered and the filtrate shaken with an equal volume of concentrated ammonia solution for 2-3 minutes. The ammonia or lower layer in the tube was observed for red/pink or violet colouration (Maobe *et al.*, 2013).

**Table 1:** Some Bioactive Compounds isolated from *B. pilosa* as reported by Silva *et al.* (2011); Bartolome *et al.* (2013) and Yang *et al.* (2014)

Compound	Structure	Bioactivity
Flavonoids		
Centaurein		Causes cytotoxicity, possesses antiviral and anti-listerial properties
Butein		Produces cytotoxic effects on colon adenocarcinoma cell proliferation.
Jacein		Compound possesses free radical scavenging properties and classified as antioxidant.
Quercetin 3-O-β-D-galactopyranoside		Compound possesses anti-inflammatory properties
Luteolin		Possesses multiple bioactivities; effective against tumour cell adhesion and proliferation. Activation of apoptosis.
Centaureidin		Anti-mitotic by inhibition of tubulin polymerization <i>in vitro</i> and induces mitotic figure formation in CA46 Burkitt lymphoma cells.

Continued...

Compound	Structure	Bioactivity
Polyynes		
1,2-Dihydroxytrideca-5,7,9,11-tetrayne		Compound with anti-cell proliferative activity (anti-angiogenic)
(R)-1,2-Dihydroxytrideca-3,5,7,9,11-pentayne		Compound with anti-T-cell differentiation activity
Cytopiloyne, 2-β-D-glucopyranosyloxy-1-hydroxytrideca-5,7,9,11-tetrayne		Compound possesses anti-differentiation activity of helper T (Th0) to Th1 cells
3-β-D-glucopyranosyl-1-hydroxy-6(E)-tetradecene-8,10,12-triayne		Compound possesses antidiabetic and antiinflammatory properties

**Table2:** Zones of inhibition diameters (mm) of plant crude extracts and control antibiotic (mean ± SD)

Bacteria sp	Crude Extracts					
	Hexane	Acetone	Ethanol	Methanol	Water	Gentamicin
<i>B. subtilis</i>	17.0±0.5	0.0±0.0	13.0±1.1	18±1.0	11.0±0.0	29.0±1.5
<i>S. aureus</i>	10.0±1.1	0.0±0.0	9.0±0.0	18.0±1.5	9.0±1.1	36.0±0.5
<i>E. faecalis</i>	8.0±0.0	8.0±0.5	12.0±1.1	12.0±1.1	11.0±0.5	22.0±1.0
<i>E. coli</i>	11.0±0.5	10.0±0.5	11±0.0	11±0.0	0.0±0.0	22.0±0.5
<i>P. aeruginosa</i>	10.0±0.5	0.0±0.0	13.0±0.5	12.0±0.5	0.0±0.0	22.0±1.1

Results shown are representative of mean ± SD of triplicate determinations for each bacteria/extract tested. SD, standard deviation

**Table 3:** Minimum inhibitory and bactericidal concentration (mg/mL) values of plant crude extracts and control antibiotic

Bacteria sp	MIC <sub>50</sub>			MBC		
	Hexane	Methanol	Gentamicin	Hexane	Methanol	Gentamicin
<i>B. subtilis</i>	1.25	2.5	0.0002	10	10	0.001
<i>S. aureus</i>	5	10	0.0004	40	20	0.003
<i>E. faecalis</i>	5	10	0.0004	40	20	0.003
<i>E. coli</i>	10	15	0.0004	40	40	0.003
<i>P. aeruginosa</i>	20	20	0.0006	80	40	0.005

MIC<sub>50</sub>, minimum inhibitory concentration for 50% susceptibility; MBC, minimum bactericidal concentration

## STATISTICAL ANALYSIS

Without taking the wells into consideration, the zones of inhibition diameters (mm) of *B. pilosa* extracts and control antibiotic were presented as mean ± standard

deviation. The antimicrobial activity (zone diameters, MICs and MBCs) of extracts and control antibiotic were statistically compared to determine significant differences (95% confidence interval) using One-way analysis of variance (ANOVA) as employed in SPSS (v19.0 Chicago, USA).

## RESULTS

The largest zone of inhibition diameter of 36 mm was recorded for the control antibiotic, followed by methanol (18mm) and hexane (17mm) plant extracts (table 2). Acetone, ethanol and aqueous extracts were also active producing zones that ranged from 0-10mm, 9-13mm and 0-11mm respectively. Based on these results, hexane and methanol extracts were considered for further antimicrobial determinations. In the MIC<sub>50</sub>-determinations, both extracts produced values ranging from 1.25-20mg/mL and 2.5-20mg/mL for hexane and methanol extracts respectively. Both extracts were also bactericidal at higher concentrations of 10-80mg/mL for the hexane extract and 10-40mg/mL for the methanol extract (table 3). The control antibiotic recorded 0.0002-0.0006mg/mL and 0.001-0.005mg/mL for MIC and MBC tests respectively. Overall, activities demonstrated by crude extracts of *B. pilosa* and Gentamicin against the reference bacterial strains were different from each other (P<0.05).

**Table 4:** Phytochemical composition of crude methanol extracts of *B. pilosa*

Secondary Metabolite	Strength of Reaction
Tannins	+
Saponins	++
Alkaloids	++
Flavonoids	++++
Steroids	++
Cardiac glycosides	+
Anthraquinones	-

-, no reaction; +, weak reaction; ++, moderate reaction; +++++, strong reaction

Phytochemical analysis of methanol extracts revealed that secondary metabolites including tannins, saponins, alkaloids, cardiac glycosides, steroids and flavonoids were present (table 4). A very strong positive reaction was recorded for flavonoids. Anthraquinones were not detected.

## DISCUSSION

Antimicrobial resistance is a threat to the efficacy of modern medicine (Shallcross *et al.*, 2015). In order to mitigate the effects of this threat, new therapies have to be developed from medicinal plants used in different parts of the world. These plants are known to possess a chemical diversity and phytochemical complexity that may help in maintaining stability/activity in complex gut environments and therefore interfere with the ability of infectious organisms to become resistant (Dahiya and Purkayastha, 2012). In our study of *Bidens pilosa*, screening results indicate varying activity against some bacteria of medical importance. *Bacillus subtilis*, a Gram

positive facultative aerobe demonstrated high susceptibility to test extracts and control antibiotic. Largest inhibition zone-diameters of 17mm and 18mm for hexane and methanol extracts respectively were recorded against this organism (table 2). Its susceptibility relative to the other bacteria was further confirmed in the MIC and MBC determinations both for the crude extracts and control antibiotic (table 3). Other studies have reported the high susceptibility of *B. subtilis* to plant extracts and antimicrobials (Rabe and Van Staden, 1997; Maskovic *et al.*, 2011; Gull *et al.*, 2012; Njume *et al.*, 2014b). In fact, in the study by Rabe and van Staden (1997), 12 of the 21 plant species tested were active against *B. subtilis*.

The ethanol, acetone and water extracts did not produce very large zones of inhibition diameters in the screening (table 2). In fact, all the zones of inhibition diameters produced by these three extracts were lower than 14 mm, the CLSI breakpoint susceptibility guidelines for gentamicin, which we used also for extracts (Hombach *et al.*, 2011). For this reason, the activity of these extracts was considered weak and they were not further evaluated for MIC and MBC determinations. However, such extracts can still be useful after fractionation and purification of the active ingredients as previously established (Akinpelu *et al.*, 2008). Our classification of these extracts as weak is equally in contradiction to the findings of Rojas *et al.* (2006) and Lawal *et al.* (2015) who reported the aqueous extracts of this plant to be highly active against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* and other bacteria species. In the Rojas *et al.* (2006) study, the ethanol extract was also active against *Staphylococcus aureus*. However, it is important to note that the methods used in the evaluation of the extracts in the above mentioned studies were different from ours. In addition, the activity of the same plants may differ with location, age, nature/type of soil and sometimes climate (Patra *et al.*, 2013; Raja and Sreenivasulu, 2015). The results of this study are consistent with the works of Rabe and van Staden (1997); Okoli *et al.* (2009); Ashafa and Afolayan (2009) and Balangcod *et al.* (2012) who also reported the methanol extract of *B. pilosa* to be highly active against bacterial pathogens.

Generally, antimicrobial susceptibility tests results indicated that all the bacteria tested were susceptible to the extracts and Gentamicin. However, the overall activity of Gentamicin was significantly different from extracts (P<0.05). The potent nature of this antibiotic can be noticed by looking at its performance as displayed on tables 1 and 3. This activity was not surprising considering that the control antibiotic is an isolated chemically purified agent with a known mechanism of action against bacteria while the crude extracts contain a mixture of complex substances some of which may possess antagonistic characteristics to one another.

The Asteraceae are rich in sesquiterpene lactones, which could be useful in the maintenance of a healthy system, nutritionally and chemotherapeutically (Chadwick *et al.*, 2013). In many rural areas of Nigeria, Benin and South Africa, *B. pilosa* is used as food and medicine (Voster and Van Rensberg, 2005; Arthur *et al.*, 2012; Sanoussi *et al.*, 2015). As a vegetable, the plant is a source of fibre, vitamins and minerals (Odhav *et al.*, 2007). However, concerns have been raised about consumption of the plant leaves and its possible association with oesophageal cancer in South Africa (Arthur *et al.*, 2012). Plans to evaluate the mutagenicity of various plant parts of *B. pilosa* in the Transkei region of South Africa have already received attention in our research laboratory. Nonetheless *B. pilosa* has demonstrated anti-mutagenic potentials in the bacterial reverse mutation assay reported by Hong *et al.* (2011), suggesting that it is a potentially safe plant to use in traditional medicine.

The medicinal activities of *B. pilosa* have been attributed to the abundance of flavonoids and polyacetylenes whether glycosylated or non-glycosylated (Ubillas *et al.*, 2000; Wang *et al.*, 2010; Cortés-Rojas *et al.*, 2013; Lawal *et al.*, 2015). It is interesting to note that flavonoids were the most abundant secondary metabolites reported in this study (table 4). Other secondary metabolites included saponins, alkaloids and steroids. Tannins and cardiac glycosides gave a weak positive reaction while anthraquinones were completely absent. The absence of anthraquinones corroborates the findings of Silva *et al.* (2014) who also recently reported their absence after analyzing leaf, flower, stem and roots extracts of *B. pilosa*. All the plant secondary metabolites detected in this study have been reported by other investigators to possess antimicrobial, anti-fungal, anti-spasmodic, anti-inflammatory, anti-diabetic and anti-parasitic properties (Cowan, 1999; Aiyelaagbe and Osamudiamen, 2009; Teke *et al.*, 2011). Their detection herein is therefore consistent with the reports of the aforementioned investigators. These findings further justify the antibacterial activity reported against the tested bacterial pathogens and scientifically rationalize the use of *B. pilosa* in South African medicinal practices.

## CONCLUSION

This study provides fundamental information on the secondary metabolites and antibacterial potential of *B. pilosa*. In the light of the results obtained herein, it can be concluded that *Bidens pilosa* has a diverse group of phytochemical components which include tannins, saponins, alkaloids, flavonoids, steroids and cardiac glycosides. These components are likely responsible for its antimicrobial activity exhibited against medically important bacteria; *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus subtilis*, thus scientifically validating

its use in the treatment of abdominal discomfort in the Transkei region of South Africa. Our next objective is to isolate the specific plant principles responsible for its antimicrobial activity, determine their mechanism of action and assess their *in vivo* potencies.

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