RESEARCH ARTICLE

Phytochemical analysis and anti-mycobacterium activity of *Bidens pilosa* crude extracts

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Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis*. The increase of drug-resistant strains of the bacterium necessitates a continuous search for alternative antimicrobial drugs from other sources such as medicinal plants like *Bidens pilosa*. *Bidens pilosa* has been used extensively in South Africa as a traditional medicine to treat diseases such as TB, inflammation, immunological disorders, digestive disorders, infectious diseases, cancers, metabolic syndrome, and wounds. Several studies have shown the extracts and compounds of *B. pilosa* to possess anti-mycobacterial, antitumor, anti-inflammatory, antidiabetic, antimalarial, and anticancer activities. Therefore, this study aimed to conduct a phytochemical analysis and determine the anti-mycobacterial activity of crude extracts of *Bidens pilosa*. Phytochemical analysis was done by using standard methods. Total phenols, flavonoids, and tannins were evaluated by using colorimetric techniques. The antioxidant activity was assessed by using Thin Layer Chromatography (TLC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2, 2-azino-bis (3-ethyl-benzthioziline-6-sulfonic acid) (ABTS) assays. The anti-mycobacterial activity was determined by using well diffusion and micro-dilution assays against selected *Mycobacterium* strains. The High-Performance Liquid Chromatography (HPLC) analysis was done to profile phenolic compounds in the extracts. The phytochemical screening showed that *B. pilosa* contained tannins, alkaloids, steroids, cardiac glycosides, phenols, and terpenoids in the fresh plant material (FPM) and dried plant material (DPM). The quantitative analysis showed that the DPM extracts had higher phenol, tannin, and flavonoid content than that of the FPM extracts. The highest phenolic and tannin contents were found in methanolic extracts of the DPM with 130 ± 0.01 GAE mg/g and 588.84 ± 0.30 mg TAE/g, respectively. The highest total flavonoid content (TFC) was found in the DPM of the ethanol extracts at 51.23 ± 0.40 QE mg/g. The IC_{50} values for FPM and DPM ranged from 0.511 mg/mL to 0.5113 mg/mL by using DPPH and ABTS, respectively. The DPM of ethanol and acetone extracts exhibited anti-mycobacterial activity against *M. avium*, *M. smegmatis*, *M. terrae*, and *M. tuberculosis* with a MIC value of 6.25. The HPLC analysis revealed the presence of phenolic compounds. In conclusion, the DPM extracts of *B. pilosa* had more phytochemicals present in the extract and exhibited higher antioxidant activity and anti-mycobacterial activity than that of the FPM extracts, which could be explored for potential drugs related to mycobacterium diseases.

Keywords: *Bidens pilosa*; phytochemicals; antioxidant activity; anti-mycobacterial activity.

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Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* [1, 2]. It is a severe health issue, especially in rural areas of developing countries [3, 4]. The top 8 countries with the most infections are India, China, Indonesia, Philippines, Pakistan, Nigeria, Bangladesh, and South Africa [5]. In 2021, the World Health Organization (WHO) reported that

about 304,000 people in South Africa had been diagnosed with TB, and approximately 56,000 had died due to this illness [6, 7]. In 2021, 450,000 cases of Multidrug-resistant tuberculosis (MDR-TB)/rifampicin-resistant TB (RR-TB) worldwide were caused by strains resistant to isoniazid (INH) and rifampin (RIF) [5]. COVID-19 has also become a global threat [6, 7]. Individuals infected by TB, human immunodeficiency virus (HIV), and acquired immunodeficiency syndrome (AIDS) are at a higher risk of infection by coronavirus as it shows symptoms similar to TB [8, 9]. Consequently, the incidence of TB observed a surge of approximately 3% compared to the 437,000 cases documented in 2020. This increase was associated with a substantial mortality rate of approximately 191,000 deaths, predominantly caused by MDR/RR-TB [6]. It is predicted that the increasing number of COVID-19 infections may result in an additional 6.3 million cases of TB in 2020-2025 with 1.4 million deaths [10]. In 2019, approximately half a million people had RR-TB, resulting in about 78% of MDR-TB cases globally [7]. In South Africa, MDR-TB is a serious threat with about 76% of MDR-TB patients co-infected with HIV resulting in a mortality rate of about 71% in KwaZulu-Natal Province [11, 12].

Irrespective of the new antibiotics produced against diseases, TB remains a global health threat [13, 14], and new drugs are required to counteract the disease [15]. Medicinal plants have been used for decades to treat, manage, and control various diseases [16-18]. The determination of antibacterial activities of different medicinal plants has become of particular interest due to the current global issue of increasing antibiotic resistance of microorganisms. Resistance continues to intimidate the prevention and treatment of infections caused by bacteria, parasites, viruses, and fungi. *Bidens pilosa* L. from the family *Asteraceae* [19, 20] commonly known as Black Jack [21] is a medicinal plant from tropical and Central America and is distributed worldwide [22]. The plant has been used as a traditional medicine to treat diseases such as TB, fever, influenza, angina, diabetes, oedema (water retention), fungal and bacterial infections, inflammation, and gastroenteritis [23, 24]. *Bidens pilosa* has about 201 compounds comprising 70 aliphatics, 60 flavonoids, 25 terpenoids, 19 phenylpropanoids, 13 aromatics, 8 porphyrins, and 6 other compounds [25]. Phytochemicals play a significant role in identifying crude drugs [26], which implies that compounds or a mixture of compounds that have positive activity in mammalian cells are potential therapeutic agents and can be used as leads toward the development of new drugs [27]. The study of numerous medicinal plants has improved rapidly due to their antibacterial and antioxidant activities, low toxicity, and the potential to be a cheaper alternative to costly synthetic drugs. Van Puyvelde *et al*. showed the leaves of *B. pilosa* sampled from Rwanda having antimicrobial activity against *M. tuberculosis* [28]. However, there is limited scientific information on the anti-mycobacterial activity of *B. pilosa* extracts [29]. The phytochemical analysis and anti-mycobacterium activity research of *Bidens pilosa* crude extracts hold substantial significance in medicinal applications and drug development. Understanding the phytochemical makeup of the plant can offer insights into its therapeutic properties and potential uses, while evaluating its anti-mycobacterium activity can contribute to finding new treatments for diseases like tuberculosis, especially given the rise of drug-resistant strains. This research validated the traditional usage of the plant in medicine. Moreover, developing effective, affordable medications from *Bidens pilosa* could have profound economic implications, particularly in low- and middle-income countries facing a high burden of diseases like tuberculosis.

Materials and Methods

Collection of plant

Bidens pilosa plants were collected from the Vaal University of Technology, Vanderbijlpark, South Africa. A botanist authenticated the sample. The

voucher specimen (PUC0015461) was deposited in the AP Goossens Herbarium, North-West University, Potchefstroom, South Africa.

Preparation of plant extracts

The whole plant of *B. pilosa* was washed and divided into two equal parts. One part was used as fresh material (FPM), and the other was air-dried (DPM) for 2 weeks. The FPM was extracted by using five solvents including water (crude extract was boiled at 100°C for 10 minutes and cooled), acetone, methanol, hexane, and ethanol (Sigma-Aldrich, Darmstadt, Germany) by maceration 500 g plant materials in 1,000 mL of solvent for 24 h for each solvent with constant shaking. Five solvents were also used to extract the DPM with 500 g plant materials into 1,000 mL of solvent by maceration for 24 h for each solvent with constant shaking. The homogenates were then filtered through 0.45 µm pore size Whatman® filter paper (Cytiva, Malborough, MA, USA), and the solvents were dried by using fume hood evaporation (Labex, Edenvale, South Africa). At the same time, the aqueous extracts were lyophilized by using Scanvac Coolsafe freeze dryer (Apex Scientific, Durban, South Africa). The plant extracts using water, acetone, methanol, hexane, and ethanol were annotated as below:

FPM and DPM using methanol: FME & DME
FPM and DPM using ethanol: FEE & DEE
FPM and DPM using acetone: FAE & DAE
FPM and DPM using hexane: FHE & DHE
FPM and DPM using water: FWE & DWE

The percentage yield of each solvent extract was calculated by using the following equation:

$$\text{Percentage yield (\%)} = \frac{\text{Actual yield (g)}}{\text{Theoretical yield (g)}} \text{ X } 100$$

where the actual yield (g) was the amount of extracted sample, and the theoretical yield (g) was the amount of raw plant material. Extracts were prepared to a concentration of 100 mg/mL for subsequent assays.

Qualitative phytochemical screening

Bidens pilosa extracts (100 mg/mL) were subjected to preliminary phytochemical screening according to standard methods reported by Harbone [30] for the detection of the following constituents.

1. Flavonoid alkaline reagent test for flavonoids

Three (3) milliliters of plant extract were treated with 1 mL of 10% NaOH solution. The formation of an intense yellow color indicated the presence of flavonoids.

2. Ferric chloride test for tannins and phenols

Two milliliters of 5% solution of $FeCl_3$ was added to 1 mL of crude extract. A black or blue green color indicated the presence of tannins and phenols.

3. Keller-Killani test for cardiac glycosides

Two milliliters of plant extracts were treated with 2 mL of glacial acetic acid containing a drop of $FeCl_3$. A brown-colored ring or brown violet under a brown-greenish layer indicated the presence of cardiac glycosides.

4. Mayer's test and Wagner's test for alkaloids

Approximately 3 mL of extracts were added to 3 mL of 1% HCl and heated for 20 minutes. The mixtures were then cooled, and 1 mL of Mayer's reagent or 1 mL of Wagner's reagent (Sigma-Aldrich, Darmstadt, Germany) was added dropwise. The formation of a greenish-colored or cream precipitate (Mayer's test) or a reddish-brown precipitate (Wagner's test) indicated the presence of alkaloids, respectively.

5. Terpenoids

Approximately 2 mL of chloroform and 3 mL of H_2SO_4 were added to 5 mL of plant extracts. A reddish-brown coloration was taken as a positive test for terpenoids.

6. Saponins

About 3 mL of plant extracts were added to 3 mL of distilled water and shaken vigorously. A stable, persistent froth was formed as a positive test for saponins.

7. Phlobatannins

Two milliliters of 1% HCl were added to 3 mL of plant extracts and boiled. The deposition of a red precipitate was taken as evidence of the presence of phlobatannins.

8. Steroids

Five milliliters of chloroform and 5 mL of H_2SO_4 were added to 500 µL of the prepared plant extracts. The presence of steroids was indicated by a color change from violet to blue or green or a ring of blue/green or if the upper layer turned red and the sulfuric layer was yellow with green fluorescence.

Quantitative Phytochemical Analysis

1. Determination of total phenolic content (TPC)

The concentration of TPC in all the plant extracts was measured according to the oxidation/reduction reaction reported by Škerget *et al*. [31] by using the Folin-Ciocalteu reagent (Sigma-Aldrich, Darmstadt, Germany). A volume of 0.5 mL of diluted extracts (100 mg/mL), 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with distilled water), and 2 mL of Na_2CO_3 (7.5%) were mixed and incubated in a 50°C oven (Labcon, Krugersdorp, South Africa) for 5 mins and then cooled. Distilled water (0.5 mL) was used as a negative control for the experiment. The absorbance of the standard Gallic acid solution (0.5 mg/mL) was measured by using T60 UV-Visible Spectrophotometer (PG Instruments, Leicestershire, UK) at 760 nm with 500 µL of 50, 100, 150, 200, and 250 µg/mL methanolic Gallic acid solutions. All tests were performed in triplicate, and a standard curve was established. The total phenol value was obtained from the equation:

$$Y = 0.0106X + 0.1246$$

and was expressed as mg/g Gallic acid equivalent (mg GAE/g) by using the formula below.

$$C = cV/M$$

where C was the total content of phenolic compounds (mg GAE/g). c was the concentration of Gallic acid (µg/mL) established from the calibration curve. V was the volume of extract (0.5 mL). m was the weight of pure plant extract (0.05 g).

2. Determination of total tannin content (TTC)

The total tannin content was determined by using Folin Ciocalteu method [32]. Approximately 0.5 mL of Folin Ciocalteu reagent and 1 mL of 35% Na_2CO_3 solutions were added to 0.1 mL of plant extracts (100 mg/mL) diluted with distilled water up to 10 mL. The absorbance was measured at 725 nm after 45 minutes of incubation at ambient temperature. The standard absorbance curve of Tannic acid solution (0.1 mg/mL) was established by using 500 µL of 50, 100, 150, 200, and 250 µg/mL tannic acid solutions. All tests were performed in triplicate. The total tannic acid values were obtained from the equation:

$$Y = 0.0046X + 0.0098 \qquad R^2 = 0.9994$$

and expressed as tannic acid equivalent (TAE) using the formula below.

$$C = cV/M$$

where C was the total content of tannins compounds in mg TAE/g of dry weight. c was the concentration of tannic acid (µg/mL) established from the calibration curve. V was the volume of extract (0.5 mL). m was the weight of pure plant extract (0.05 g). All the measurements were carried out in triplicate.

3. Determination of total flavonoid content (TFC)

Total flavonoid content was measured by using Aluminium chloride colorimetric method [33] with some modifications. Approximately 0.5 mL aliquot of $AlCl_3$ (1.2%) and potassium acetate (120 mM) were mixed with 0.5 mL of the plant extracts (100 mg/mL) and diluted with acetone to 2 mL. The reaction was allowed to stand at ambient temperature for precisely 30 minutes before the absorbance was measured at 415 nm. Quercetin was used as a standard with 20, 40, 60, 80, and 100 µg/mL methanol quercetin solutions

to establish a calibration curve. The total flavonoid content expressed as mg quercetin equivalent (QE)/g of dried plant material was calculated based on the calibration curve by using the following equation:

$$Y = 0.0175X - 0.0061$$

where X was the absorbance and Y was the concentration (mg QE) of the methanol quercetin solutions. All the experiments were carried out in triplicate.

Antioxidant activity of *B. pilosa* crude extracts

1. Thin Layer Chromatography (TLC)

The chemical constituents and antioxidant activity of *B. pilosa* extracts were detected by using Silica gel 60, F_{254} TLC plates (Merck KGaA, Darmstadt, Germany). 4 µL of each extract (100 mg/mL) was loaded on the TLC plates and saturated in two different eluent solvent systems with different polarities including benzene/ethyl acetate/ammonia hydroxide (BEA) (9:1:0.1) and chloroform/ethyl acetate/formic acid (CEF) (5:4:1). The TLC plates were dried in a fume hood. The compounds on the TLC plates were examined and sprayed with vanillin-sulphuric acid reagent (0.1 g vanillin:28 mL methanol:1 mL concentrated sulfuric acid) and heated at 110°C for color development. A second TLC chromatogram of the same extracts was sprayed with DPPH reagent (1,1-diphenyl-2-picrylhydrazyl) (Sigma-Aldrich, Darmstadt, Germany) and allowed to stand for 5 minutes. Antioxidants were detected by a color change from deep purple to yellow white.

2. DPPH radical scavenging assay

1 mL of the extracts at different concentrations of 0.01, 0.1, 0.5, and 1.0 mg/mL were mixed with 1 mL of 0.12 mM DPPH solution. After shaking, the mixture was incubated at ambient temperature in the dark for 30 mins, and then the absorbance was measured at 517 nm. Acetone was used as a negative control while L-ascorbic acid was used as a positive control. The radical scavenging activity was determined as the percentage of inhibition using the following equation:

$$\text{Scavenging activity (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

where $A_{control}$ was the absorbance of DPPH solution without any sample. A_{test} was the absorbance of DPPH solution with sample. The inhibitory concentration (IC_{50}) value was the sample concentration required to scavenge 50% DPPH free radicals. All the tests were carried out in triplicate.

3. ABTS radical scavenging assay

A 7 mM stock solution of 2, 2-azino-bis (3-ethyl-benzthioziline-6-sulfonic acid) (ABTS) (Sigma-Aldrich, Darmstadt, Germany) was prepared in double-distilled water. The ABTS radical cation was prepared by adding 88 µL of 140 mM potassium persulfate to 5 mL of ABTS solution and stored in the dark for 12-16 h. The ABTS solution was then diluted with cold ethanol to a final absorbance of 0.70 ± 0.02 at 734 nm and 37°C before the test. Plant extracts at different concentrations of 0.01, 0.1, 0.5, and 1.0 mg/mL were prepared. L-ascorbic acid was used as the positive control, while acetone was used as the negative control. The extracts' total scavenging capacities were quantified by adding 1,000 µL of ABTS to 50 µL of each plant extract. The reactions were incubated at 37°C for 4 mins before the absorbance at 734 nm was recorded. All the experiments were carried out in triplicate.

Anti-mycobacterial activity

1. Preparation of Mycobacterium media and culture

The Gram-positive *Mycobacterium smegmatis ATCC 19420™, Mycobacterium avium ATCC15769/2529™, Mycobacterium terrae ATCC 15755™,* and *Mycobacterium tuberculosis ATCC 25177™* (Microbiologics, Johannesburg, South Africa) were used for the current study. *Mycobacterium* cultures were inoculated in Middlebrook 7H9 broth (Sigma-Aldrich, Darmstadt, Germany) for ± 2-3 days at 37°C with

constant shaking. The same broth was also used with the enrichment albumin dextrose catalase supplement (ADC) (Sigma-Aldrich, Darmstadt, Germany) for *Mycobacterium* sp. Culture. Following the initial incubation, the organisms were subcultured on Middlebrook 7H11 agar (Sigma-Aldrich, Darmstadt, Germany) and incubated at 37°C for 24 hours to detect contamination. The oleic albumin dextrose catalase (OADC) enrichment (Sigma-Aldrich, Darmstadt, Germany) with glycerol was used to culture *Mycobacterium* sp. and maintained in Middlebrook broth at 4°C. Grown *mycobacterium* cultures were sub-cultured on freshly prepared broth for 24 h at 37°C before the anti-mycobacterial test. The Middlebrook 7H11 agar was used for the well diffusion assay, and the Middlebrook broth was used to perform the minimum inhibition concentration (MIC) assay.

2. Well diffusion test

A modified well diffusion test was used as a preliminary screening method for the antimicrobial potential of the plant extracts [34]. The agar plates were uniformly inoculated with the appropriate test organism to obtain confluent growth. Wells (made from sterile bottom parts of 200 µL pipette tips, 5 mm in diameter) were created in the Middlebrook extract. Approximately 20 µL of the plant extract (100 µg/mL) was added to the wells. Sterile distilled water (20 µL) was used as a negative control with Rifampicin as a positive control. The Petri dishes were incubated at 37°C for 24 hours. The level of mycobacterial susceptibility was determined according to the size of the zone of inhibition, which was measured in mm using a ruler.

3. Microtiter minimum inhibitory assay

The minimum inhibitory concentration (MIC) for the anti-mycobacterial activity of *B. pilosa* crude extracts was determined by the method of Elloff with some modifications [35]. Briefly, *Mycobacterium* cultures were grown at 37°C overnight with constant shaking. 100 µL of each extract (100 mg/mL) were diluted two-fold with sterile broth in the 96-well microtiter plate for each of the four *Mycobacterium* strains. 100 µL of the *Mycobacterium* culture were then added to a 96-well plate with Rifampicin at 50 mg/mL as the positive control and sterile broth as the blank. The plates were covered and incubated at 37°C for 24 hours before 50 µL of 0.2 mg/mL p-iodonitrotetrazolium chloride (INT) (Sigma-Aldrich, Darmstadt, Germany) was added to all wells and incubated at 37°C for additional 8 h. A reddish pink color indicated bacterial growth in the wells. MIC was determined as the lowest concentration inhibiting the respective microorganisms' growth. All the assays were performed in triplicate.

Identification of phenolic compounds in *B. pilosa* crude extracts

1. Preparation of standard solutions and crude extracts

Ascorbic acid, Gallic acid, Resorcinol, Vanillin, Tannic acid, Acetyl Salicylic acid, and Benzoic acid (Sigma-Aldrich, Darmstadt, Germany) were used as standard solutions for the identification of phenolics present in *B. pilosa* extracts according to the method of Mradu *et al*. with slight modifications [36]. 1 g of each standard compound was dissolved in 10 mL of high-performance liquid chromatography (HPLC) grade methanol (Sigma-Aldrich, Darmstadt, Germany). The compounds (10 mg/mL) were sonicated and filtered by using a 0.45 µm, 47 mm diameter nylon membrane filter (Labchem, Darmstadt, Germany) before being analyzed in Agilent 1200 Infinity Series HPLC (Thermo scientific, Waltham, MA, USA). 2 g of fresh and air-dried *B. pilosa* extracts prepared using different solvents was dissolved in 4 mL of acetone, ethanol, and methanol, resulting in 500 mg/mL concentration. Before injection into the column, the samples were sonicated and subsequently filtered through a nylon membrane filter with a 47 mm diameter and 0.45 µm pore size (Labotec, Midrand, South Africa). All the standard samples were analyzed by using 515 HPLC pumps and 2489 UV/VIS with Symmetry C18 (5 µm, 4.6 × 250 mm) reverse-phase water guard column and Hamilton microliter syringe (Thermo Scientific, Waltham, MA, USA) at the

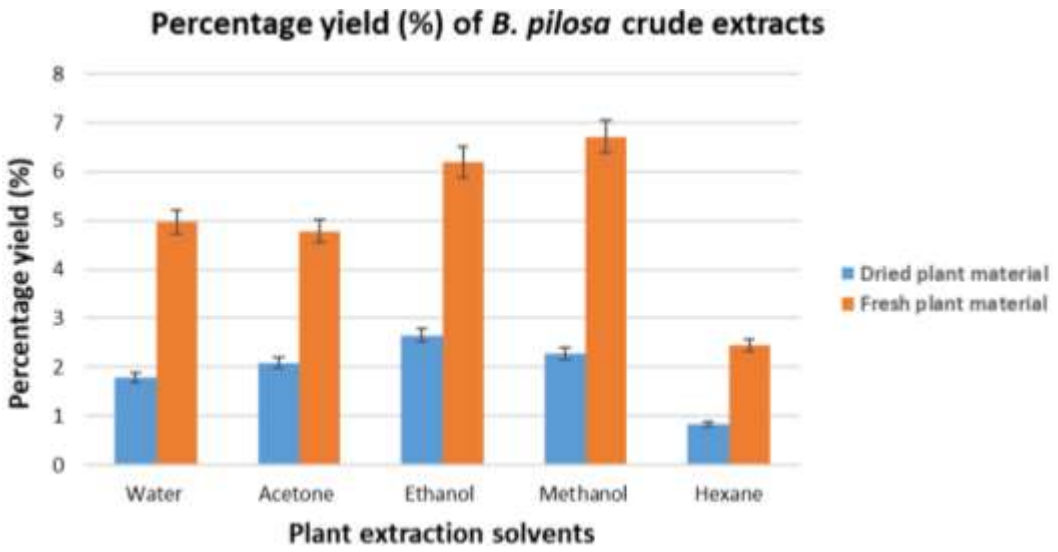


Figure 1. Percentage yield (%) of extracts from fresh and dried *B. pilosa* using different solvents.

injection volume of 20 µL. The data analysis was done by using the Agilent software. The gradient elution of Solvent A (Acetonitrile) and Solvent B (0.1% Phosphoric acid in water) was used. The gradient program started with 8% of Solvent A for the first 35 minutes and then increased to 22% for the next 10 minutes before bringing it down again to 8%. The UV wavelength was set at 280 nm at 25°C with a flow rate of 1.0 mL/min for 45 minutes.

Statistical analysis

Each experiment was conducted in triplicate for replicability. Subsequent data analysis was performed through Microsoft Excel, and the results were represented as mean values with standard deviations (n=3). IC_{50} values were determined by using the software Graphpad Prism 8.0.2 (GraphPad Software, Boston, MA, USA). A *P* value of less than 0.05 was set as the threshold for statistical significance.

Results

The percentage yield of *B. pilosa* (%) using different extraction solvents

The percentage yields of crude extracts ranged from 2.28 - 6.72% in methanol, 2.65 - 6.20% in ethanol, 2.09 - 4.78% in acetone, 1.79 - 4.97% in water, and 0.83 - 2.45% in hexane (Figure 1). The highest percentage yield of the extracts from DPM was obtained in ethanol, while hexane giving the lowest yield. Methanol produced the highest potential yield for FPM, while hexane gave the lowest yield.

Qualitative preliminary phytochemical analysis

The preliminary phytochemical screening was conducted on all ten crude extracts of *B. pilosa*. Tannins, phenols, terpenoids, and saponins were presented in both FPM and DPM extracts (Table 1). Cardiac glycosides were presented in all the extracts except for FAE, DAE, and FEE. Alkaloids were presented in FWE, DWE, FAE, DAE, FEE, DEE, and DME and absented in FME, FHE, and DHE. Steroids were presented in FWE, FAE, DEE, FME, DME, and FHE and absented in DWE, DAE, FEE, and DHE. Phlobatannins were absent in all crude extracts.

Quantitative phytochemical analysis

The Folin-Ciocalteu and Aluminium chloride methods were used to determine the total phenolic, tannin, and flavonoid contents of the crude extracts of *B. pilosa*. DPM extracts had higher phenols, tannins, and flavonoid contents than that of FPM (Table 2). In FPM extracts, the

Table 1. Phytochemical screening results for *B. pilosa* crude extracts obtained from five different solvents for FPM and DPM.

No.	Compounds	FWE	DWE	FAE	DAE	FEE	DEE	FME	DME	FHE	DHE
1.	**Flavonoids**	++	++	++	++	+	++	+	++	+	++
2	**Tannins**	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
3	**Phenols**	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
4	**Cardiac glycoside**	+	+	-	-	-	++	-	++	+++	+++
5	**Alkaloids**										
	Mayer's test	+	++	+	++	+	++	-	++	-	-
	Wagner's test	++	++	+	++	+	++	-	-	-	-
6	**Tepernoids**	+	++	+++	+	+++	+++	+++	+++	+++	++
7	**Saponins**	++	++	+	++	++	++	+	++	++	+
8	**Phlobatannins**	-	-	-	-	-	-	-	-	-	-
9	**Steroids**	+	-	++			+	+	++	++	-

Notes: +++: intense positive; ++: strong positive; +: weak positive; -: negative.

Table 2. Total phenolic, tannin, and flavonoid contents of FPM and DPM of *B. pilosa* plant crude extracts.

Plant extract	Phenolic content (mg GAE/g)		Tannin content (mg TAE/g)		Flavonoid content (mg QE/g)	
	FPM	DPM	FPM	DPM	FPM	DPM
Acetone	106.97±0.01a	111.29±0.30 a	187.39±0.01a	328.70±0.20a	49.43±0.01a	38.16±0.40a
Methanol	113.76±0.1 a	130.43±0.01 a	204.05±0.01a	588.84±0.30b	50.11±0.92b	50.96±0.60b
Ethanol	108.35±0.01 a	124.29±0.30a	214.92±0.10a	383.04±0.30c	50.79±1.00c	51.23±0.40c
Aqueous	102.65±0.01 a	87.83±0.01 *a*	170.72±0.10a	311.30±0.01d	48.09±0.97d	36.03±0.10a
Hexane	83.51±0.03 *a*	86.60±0.03*b*	162.75±0.01a	217.83±0.02d	37.22±0.40*a*	37.15±0.10*a*

Notes: Data represented the mean ± SD of FPM and DPM extracts (n = 3). Means within a column showing the same small letter were not significantly different ($P > 0.05$) according to the t-test.

highest phenolic content was found in ethanol at 113.76 ± 0.1 mg GAE/g, whereas for DPM, the highest TPC was found in methanol at 130.43 ± 0.01 mg GAE/g. No statistically significant difference in the phenolic contents of FPM and DPM extracts was observed (P > 0.05) except for the hexane extracts. There was a higher concentration of tannins (214.92 ± 0.10 mg TAE/g) in the ethanol extracts of FPM and methanol (588.84 ± 0.30 mg TAE/g) by using DPM. The total tannin content of DPM extracts showed significant differences among all the extracts ($P < 0.05$) except in water and hexane. No significant differences were observed among FPM extracts ($P > 0.05$). The TFC of DPM extracts was found to be 51.23 ± 0.40 mg QE/g in DEE, while TFC of FPM extracts was 50.11 ± 0.92 mg QE/g of dry/fresh weight in FEE. The total tannin contents of DPM and FPM extracts showed significant differences between methanol, acetone, and ethanol ($P < 0.05$), while there was no difference in water and hexane ($P > 0.05$).

Antioxidant activity of *B. pilosa* using thin layer chromatograph, DPPH, and ABTS assays

Thin layer chromatograph (TLC) was used as a qualitative indicator for detecting antioxidant activity in FPM and DPM extracts of *B. pilosa* by using DPPH as an indicator. The results showed various bands with more bands in DEE and DAE. An intense color resolution displaying different compounds in *B. pilosa* extracts was better resolved in BEA (benzene : ethyl acetate : ammonia hydroxide) than in CEF (chloroform : ethyl acetate : formic acid) solvent system. Antioxidant activity indicated by yellow-whitish bands after spraying with DPPH was intense in both FPM and DPM extracts, especially in CEF solvent system (Figure 2). The DPPH scavenging activity of DPM extracts using different solvents

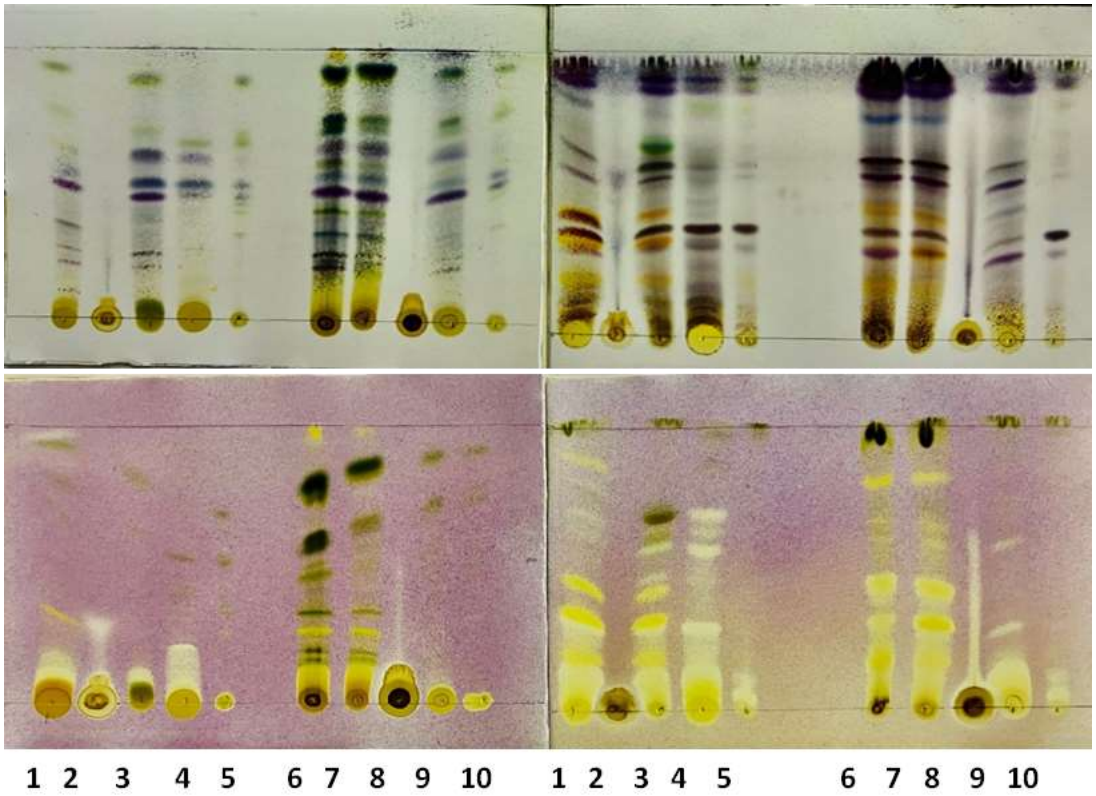


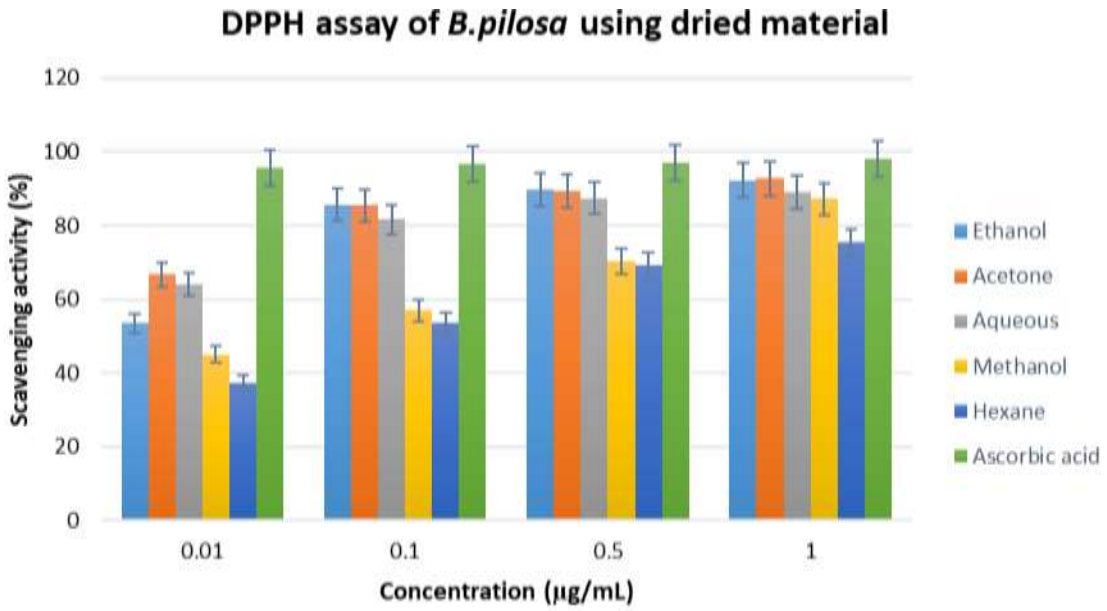
Figure 2. Thin layer chromatogram fingerprint profile of FPM and DPM extracts of *B. pilosa* (100 mg/mL) were separated in BEA (upper) and CEF (below) solvent system and sprayed with vanillin sulfuric acid and DPPH reagents for visualization. Lanes 1: FEE, 2: FWE, 3: FAE, 4: FME, 5: FHE, 6: DEE, 7: DWE, 8: DAE, 9: DME, 10: DHE.

Table 3. IC_{50} values of *B. pilosa* for DPPH and ABTS assays using FPM and DPM.

Plant Extract	DPPH IC_{50} (mg/mL)		ABTS IC_{50} (mg/mL)	
	DPM	FPM	DPM	FPM
Acetone	0.061	0.223	0.076	0.239
Ethanol	0.055	0.078	0.057	0.155
Methanol	0.061	0.209	0.173	0.349
Water	0.067	0.256	0.167	0.504
Hexane	0.127	0.132	0.058	0.188
Ascorbic acid	0.00243	-	0.00141	-

ranged between 37.39 ± 0.01 to 92.75 ± 0.01% was shown in Figure 3A. The highest scavenging activity was observed in DAE (66.67 ± 0.02 - 92.75 ± 0.01%) and the lowest in DHE (37.39 - 75.33 ± 0.01%). Ascorbic acid was used as a positive control and displayed higher antioxidant activity in all concentrations. Contrary to DPM, FPM showed a slightly lower DPPH scavenging activity ranging from 31.28 to 89.97% (Figure 3B). The highest antioxidant scavenging activity was observed in FEE (45.03 ± 0.03 - 89.97 ± 0.00%) and the lowest in FHE (31.28 ± 0.03 - 65.92 ± 0.05%). The DPM of *B. pilosa* showed higher scavenging activity than that of FPM when using ABTS at different concentrations (0.01 to 1.0 µg/mL) (Figure 4A). The highest scavenging activity was observed in DEE ranged from 71.52 ± 0.01 to 90.62 ± 0.02%. In FPM, the highest scavenging activity was observed in FEE ranging from 46.29 ± 0.01 to 87.00 ± 0.00% (Figure 4B).

A.



B.

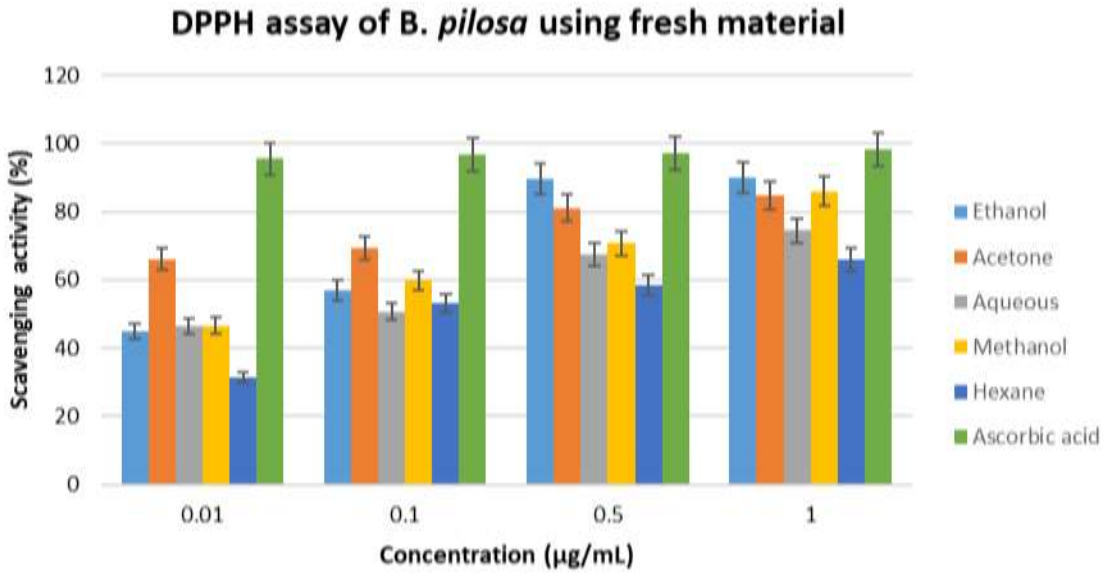


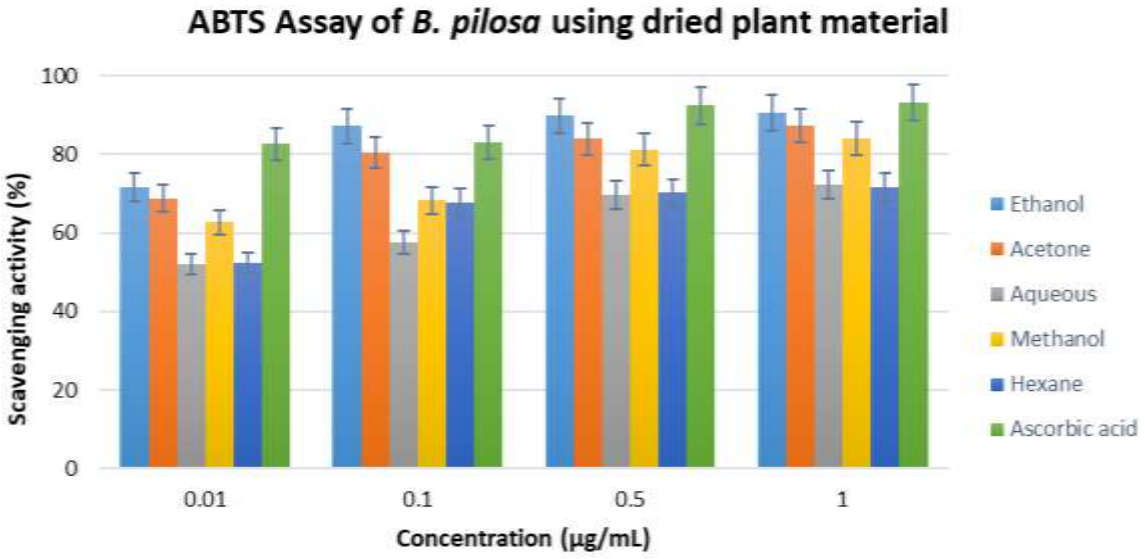
Figure 3. DPPH radical scavenging activity (%) of DPM (A) and FPM (B) of *B. pilosa* extracts.

The half-maximal inhibitory concentration (IC_{50}) is a quantitative measure that shows the amount of a specific inhibitory substance required at 50% inhibition [37]. The crude extracts of *B. pilosa* had low IC_{50} values in DPPH and ABTS assays (Table 3). The DPM extracts showed lower IC_{50} values than that of FPM extracts. The lowest IC_{50} was observed in ethanol extract at the concentrations of 0.055 and 0.078 mg/mL in DPPH assay and 0.057 and 0.155 mg/mL in ABTS assay for DPM and FPM, respectively.

The anti-*Mycobacterium* activity of FPM and DPM of *B. pilosa*

The well-diffusion assay initially determined the antimicrobial activity of the crude extracts. The

A.



B.

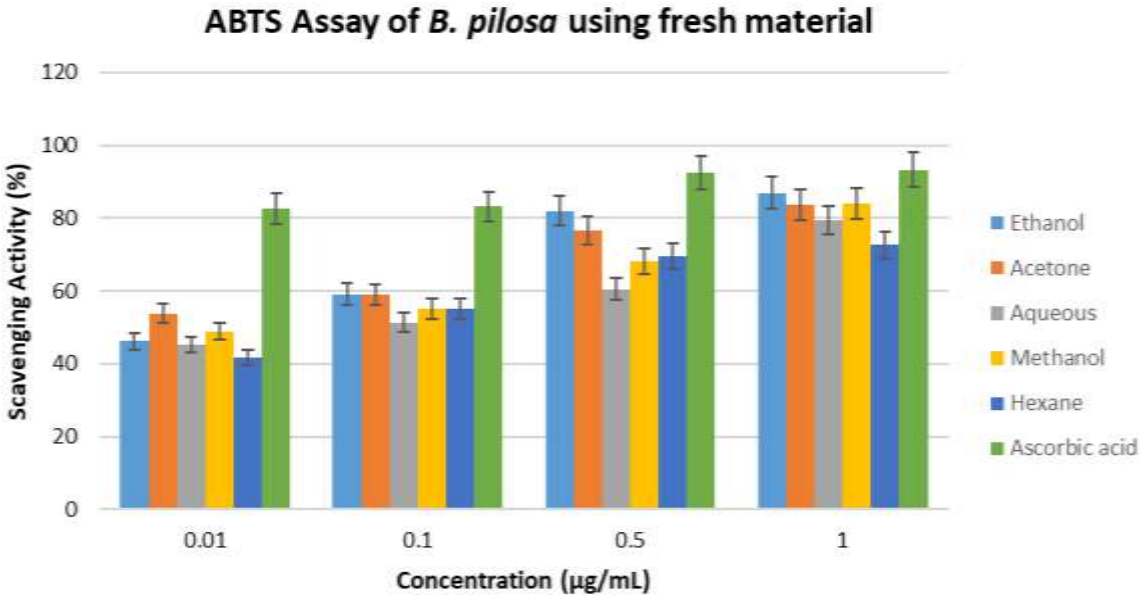


Figure 4. ABTS radical scavenging activity (%) of DPM (A) and FPM (B) of *B. pilosa* extracts.

inhibition zones ranged from 1.75 to 13.00 mm for FPM and DPM extracts. DAE observed the highest zone of inhibition against *M. avium* at 14.00 ± 2.65 mm (Table 4). For *M. smegmatis*, the highest zone of inhibition was observed in DEE at 13.00 ± 1.41 mm. *Mycobacterium terrae* showed the highest zone of inhibition with DAE extracts, which was 10.00 ± 1.41 mm. The highest zone of inhibition against *Mycobacterium tuberculosis* was 12.33 ± 3.79 mm and also in DAE. The crude hexane and water extracts displayed no anti-*Mycobacterium* activity against all the tested strains. Rifampicin was used as a positive control. Minimum inhibitory concentration (MIC) of the crude extracts of *B. pilosa* ranged from 6.25 to 50 mg/mL (Table 5). The lowest MIC value of 6.25 mg/mL was with the acetone and ethanol extracts against all the test strains. The other

Table 4. Diameter of inhibition zones of plant extracts against mycobacterial strains at 100 mg/mL.

Plant extracts	Zones of inhibition (mm)							
	M. avium		*M. smegmatis*		*M. terrae*		*M. tuberculosis*	
	FPM	DPM	FPM	DPM	FPM	DPM	FPM	DPM
Acetone	12.00±2.65	14.00±2.65	-	10.00±2.65	8.00±1.41	10.00±1.41	7.67±0.58	12.33±3.79
Ethanol	9.33±1.53	10.00±2.00	2.75±1.10	13.00±1.41	8.50±0.71	9.5±0.71	11.33±1.53	11.00±2.65
Methanol	11.00±1.73	12.00±1.73	-	5.50±2.21	7.00±2.83	9.50±0.71	4.33±1.15	7.67±2.50
Water	-	-	-	2.25±1.06	1.75±0.35	-	-	-
Hexane	-	-	-	-	-	-	-	-
Rifampicin	31.3332.00±1.53		44.00±5.66		38.00±4.24		32.00±2.65	

Notes: Data represented the mean ± SD.

Table 5. Minimum inhibitory concentration (mg/mL) of DPM and FPM of *B. pilosa* crude extracts against selected mycobacterial strains.

Plant extracts	Minimum inhibitory concentration (mg/mL)							
	M. avium		*M. smegmatis*		*M. terrae*		*M. tuberculosis*	
	FPM	DPM	FPM	DPM	FPM	DPM	FPM	DPM
Acetone	12.5	6.25	12.5	6.25	6.25	6.25	12.5	6.25
Ethanol	12.5	6.25	6.25	6.25	6.25	6.25	12.5	6.25
Methanol	25	12.5	25	12.5	12.5	12.5	25	25
Aqueous	25	25	50	25	12.5	12.5	25	50
Hexane	50	50	50	50	50	50	50	50
Rifampicin	1.562		3.125		1.562		6.25	

extracts, methanol, aqueous, and hexane, presented MIC values ≥ 12.5 mg/mL. It was also noted that DPM extracts showed lower MIC values than that of FPM extracts.

HPLC profiles of *B. pilosa* extracts for the identification of phenolic compounds

The HPLC profiles of FPM and DPM using methanol, acetone, and ethanol extracts were analyzed for 5 phenolic compounds with modifications [38]. The HPLC profiles were separated as (i) ascorbic acid, (ii) gallic acid, (iii) resorcinol, (iv) vanillin, and (v) benzoic acid with retention times (RT) of 2.521, 4.849, 10.880, 18.186, and 39.823 minutes, respectively. Phenolic compounds in each chromatogram were shown in Figure 5 from AI to C2 with peaks showing the RT. The results of FME at 280 nm showed various constituents with different retention times (Figure 5: A1). The phenolic compounds were separated on FME at RTs of 2.525, 3.792, 10.663, 17.592, and 40.109 minutes, indicating the presence of ascorbic acid, gallic acid, resorcinol, vanillin, and benzoic acid (Figure 5: A1). The methanol extract from DME had higher amounts of phenolic compounds than that of FME with ascorbic acid, gallic acid, resorcinol, vanillin, and benzoic acid at 2.326, 3.303, 10.384, 21.649, and 40.112 minutes, respectively (Figure 5: B1). The FEE of *B. pilosa* exhibited different constituents at various retention times. The prominent peaks were observed at 2.762, 10.642, 22.285, and 40.371 minutes, which represented ascorbic acid, resorcinol, vanillin, and benzoic acid, respectively (Figure 5: B1). Similarly, DEE showed various constituents with different retention times. The highest peak in DEE was observed as ascorbic acid with an RT of 2.559 minutes (Figure 5: B2). Dried acetone extracts showed more phenolic compounds than that of FAE in peak areas. Chromatograms of 5 out of 5 phenolic compounds were observed in FAE and DAE at different RTs (Figure 5: C1 and C2). In FAE, the highest peak was observed at 3.401 min as gallic acid, whereas in DAE, the highest peak was

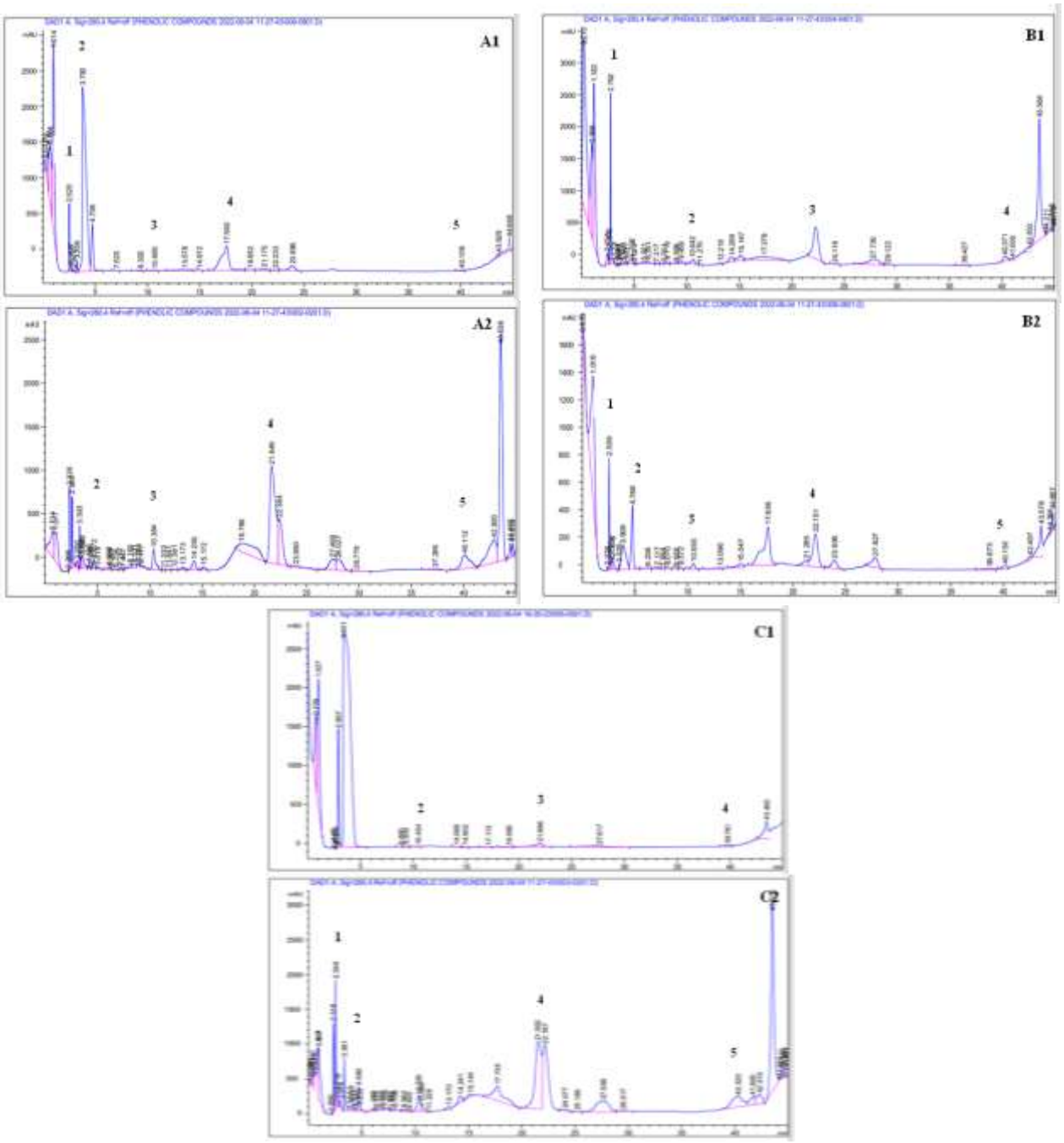


Figure 5. HPLC of FME (A1), DME (A2), FEE (B1), DDE (B2), FAE (C1), and DAE (C2) showed the presence of different phenolic compounds. 1: ascorbic acid, 2: gallic acid, 3: resorcinol, 4: vanillin, 5: benzoic acid.

observed at 2.546 mins as ascorbic acid.

Discussion

The percentage yield of *B. pilosa* crude extracts

Different solvents produced different extraction yields from *Bidens pilosa* plant samples (Figure 1), which was associated with the differences in the solvent polarities [39]. Methanol yielded 6.7%, followed by water, ethanol, acetone, and hexane. Ethanol had the highest DPM yield,

followed by methanol, acetone, water, and hexane, demonstrating that solvent polarity affected extraction efficiency. The kind, biological activity, and yield of isolated chemicals depended on medicinal plant preparation for experimental research [40]. More yield means more material for downstream studies and fewer extractions, especially if the plant material is uncommon or endangered.

Phytochemical screening of *Bidens pilosa* crude extracts

1. Qualitative phytochemical analysis

The crude extracts of *B. pilosa* contained flavonoids, phenols, tannins, alkaloids, terpenoids, saponins, glycosides, and steroids (Table 2), which were known to exhibit therapeutic and physiological activities. Extracts obtained from DPM showed more phytocompounds than that from FPM. Flavonoids were presented in moderation in all crude extracts (Table 2). Flavonoids have protective effects against cardiovascular diseases and have antimicrobial, anticancer, antivirus, anti-inflammatory, and anti-aging activities [41-43]. Lechner *et al*. looked at the modulation of isoniazid (INH) susceptibility by flavonoids in *Mycobacterium* and found that the flavonoids decreased the MIC of INH [44]. They also suggested that the flavonoids' activity was related to antioxidant activity and inhibition of the mycobacterial efflux pumps. Tannins and phenols were presented in all crude extracts (Table 2). Tannins are phenolic compounds with a high molecular weight, soluble in water and alcohol, and act as primary antioxidants or free radical scavengers [45, 46]. Tannins showed antibacterial, antiviral, and antifungal activities [47]. The high tannin content reported in this work supported the traditional use of *B. pilosa* to treat various ailments. Biological properties such as anti-carcinogen, anti-inflammation, anti-atherosclerosis, anti-aging, cardiovascular protection, endothelial function improvement, apoptosis, angiogenesis inhibition, and cell proliferation activities had been associated with phenolic compounds [48]. The DPM's water, acetone, and ethanol extracts showed the presence of cardiac glycosides, whereas, in FPM, they were presented only in methanol extracts (Table 2). Glycosides are utilized in treating congestive heart failure and cardiac arrhythmia and have been reported to lower blood pressure [49, 50]. Alkaloids were presented in the aqueous, acetone, and ethanol extracts and absented in the methanol and hexane crude extracts (Table 2). Alkaloids are effective for pain relief and have antispasmodic and antibacterial activities [51]. Saponins in the extracts (Table 3) possess anti-inflammatory activity, antidiabetic, anti-HIV, and anti-atherosclerotic properties [52, 53]. Terpenoids have various biological activities such as antiparasitic, antiallergenic, anti-inflammatory, antimalarial, and antibacterial properties [54-56], and play a vital role in the prevention and therapy of several diseases such as cancer [57]. All crude extracts lacked phlobatannins. These results agreed with the study by Owoyemi and Oladunmoye who found no phlobatannins in dried *B. pilosa* leaves extracted with ethanol and water [58]. Ezeonwumelu *et al*. found phlobatannins in *B. pilosa* aqueous leaf extracts [59]. Environmental factors, plant physiological and biosynthetic processes, and extraction solvent could explain the presence or lack of phytochemicals in different studies [60]. Plant extracts had antibacterial steroids [61]. Table 1 showed that *B. pilosa* crude extracts contained secondary metabolites like tannins, flavonoids, phenols, and alkaloids.

2. Quantitative phytochemical analysis

The DME and FME extracts had the most phenolics of 130.43 ± 0.01 and 113.76 ± 0.1 mg GAE/g, respectively (Table 3). Muchuweti *et al*. observed 1,102.797 ± 2.239 mg GAE/g TPC in *B. pilosa* methanol crude extracts [64]. Lee *et al*. found 538.1 mg GAE/g dry weight of phenolics in *B. pilosa* flowers using methanol [65]. In previous research, Singh *et al*. found 72 µg of GAE/mg of dry weight in fresh *B. pilosa* leaves [66], whereas Falowo *et al*. observed 75.9 ± 0.08 mg GAE/g dry weight in ethanol:water (70:30) leaves [67]. This study found significant phenolic content which could be attributed to plant material that was

gathered in summer. Jorgensen noted that light and radiation were necessary for phenol biosynthesis [68], which explained solar radiation's high phenolic compound production [69]. Light stimulates phenolic biosynthesis enzymes. Phenylalanine ammonia-lyase (PAL), a key enzyme in phenolic chemical production, rises under light [70, 71]. Compared to methanol, acetone extracted fewer phenolic compounds in *B. pilosa*, which might be due to the low solubility of polyphenols in acetone because of the hydrogen interactions between polyphenols and proteins [72]. Other researchers had alluded that because phenolic compounds included a hydroxyl group, they were more soluble in polar organic solvents. Hence various studies showed that methanol and acetone could extract high amounts of phenolic compounds, which influenced the biological activity of the extracts [73-76]. The extracts had more tannin than phenols or flavonoids. FPM extracts had the highest tannin concentration in FEE at 214.92 ± 0.10 mg TAE/g, whereas DPM extracts had the highest in DEE at 588.84 ± 0.30 mg TAE/g. Mbokazi showed that *B. pilosa* methanolic seed and radicle extracts had tannin contents of 416.36 ± 1.14 mg GAE/g and 69.05 ± 0.05 mg GAE/g dry weight, respectively [77]. In water extracts, *B. pilosa*'s vegetative and reproductive sections had tannin contents of 1,030 ± 0.9436 mg GAE/100 g and 827 ± 0.9428 mg GAE/100 g, respectively. Tannic acid concentrations in *B. pilosa* crude extracts have been shown to be high in several solvents. The plant uses tannins to defend against parasites and harsh climatic conditions. These tannins have immune-modulating, cardio-protective, anticancer, antibacterial, antiviral, and anti-inflammatory activities [79, 80]. Thus, *B. pilosa* can be used in traditional medicine to cure microbial infections due to its tannic concentration. Ethanol has been identified as one of the most commonly used solvents for flavonoid extraction because of its capacity to solubilise moderately polar flavonoids with no environmental impact [81]. Flavonoids are generally extracted from plant sources using organic solvents, water, and combinations of various solvents [82]. The highest flavonoid content for the ethanol extracts from FPM was 50.79 ± 1.00 mg QE/g and 51.23 ± 0.40 mg QE/g from DPM. The flavonoid content in ethanol extracts was almost similar to that of the methanol extracts. The TFC of the ethanolic extracts in this study was higher than that reported by Falowo *et al*. using ethanol:water (70:30) and equivalent to 14.9 ± 0.03 mg Ru/g [67], while Singh *et al*. used methanol on *B. pilosa* leaves and recorded 123.3 µg Quercetin per mg dry weight [66]. Flavonoids, the bioactive secondary metabolites of plants, provide flavour, color, and pharmacological and antioxidant activities [83, 84]. Flavonoids protect plants from UV radiation and scavenge free radicals [85]. Flavonoids have potent antioxidant and anti-inflammatory in humans [86, 87]. Compared to other research, this study's variation in TPC, TTC, and TFC might be related to sugars, solvent, genetic variation [88], the process, and the length of the extraction [89].

Antioxidant activity of FPM and DPM of *B. pilosa* using TLC, DPPH, and ABTS

Thin layer chromatography (TLC) is one of the techniques that has been applied for qualitative analysis of antioxidants in plant extracts [90, 91]. Different compounds of *B. pilosa* in FPM and DPM were indicated by the different colours (Figure 2) on the TLC plate sprayed with vanillin. The free radical scavenging activity of *B. pilosa* extracts was evaluated by spraying the TLC plate with a DPPH reagent. Figure 2 displayed the presence of compounds with antioxidant activity, indicated by yellow bands on the TLC plates against a purple background. The presence of antioxidants inhibited the production of free radicals, and the appearance of a yellow-white colour was often based on suppressing the accumulation of oxidized products [92]. DPPH and ABTS tests were employed to quantify the free radical scavenging activities of *B. pilosa* extracts [93]. Electron and hydrogen atom transport underlies the ABTS and DPPH reaction processes [94]. *Bidens pilosa* crude extracts had stronger antioxidant activity than that of ascorbic acid. Ethanol extracts scavenged best at 1 mg/mL. Ethanol extracts' significant DPPH

scavenging activity corresponded with the intensity of antioxidant compounds (Figure 2). This study showed radical scavenging activities similar to those reported by Adedapo *et al*. using methanol, acetone, and water extracts of *B. pilosa* leaves [95] and Falowo *et al*. using ethanol-water solution (7:3) extracts [67]. *B. pilosa*'s significant scavenging activity demonstrated its proton-donating ability, which might make it a primary antioxidant. The lowest DPPH IC_{50} value was 0.055 mg/mL in the ethanol extract from DPM, which was lower than that reported by Singh *et al*. who reported a DPPH IC_{50} value of 80.45 µg/mL in the methanolic extract of *B. pilosa* [66]. Adedapo *et al*. found a DPPH IC_{50} value of 94.2 mg/mL in the leaves of *B. pilosa* [95]. The antioxidant activity of the essential oils from the leaves and flowers of *B. pilosa* had a DDPH IC_{50} of 47 and 50 µg/mL, respectively [96]. This value was also slightly lower than that obtained in this study. In ABTS assay, the lowest IC_{50} was observed in the ethanol extract from DPM at 0.057 mg/mL, which was lower than that reported by Singh *et al*. (171.6 µg/mL) [66] and Adedapo *et al*. (0.75 mg/mL) [95]. Strong antioxidant activity in ABTS and DPPH assays requires physiological action and oxidation of hydroxyl/superoxide radicals [65]. ABTS outperformed DPPH in radical scavenging. ABTS and DPPH tests react differently [97]. Lee *et al*. found ABTS to be more sensitive to antioxidants due to its quicker reaction kinetics and responsiveness [65]. ABTS is more soluble in both aqueous and organic solvent media, making it adaptable for measuring physiological fluids' hydrophilic and lipophilic antioxidant capabilities [98]. Stereo selectivity of radicals and extract solubility in different testing methods also affect extracts' ability to react and quench diverse radicals [99]. The tannins, phenols, and flavonoid levels in this study matched *B. pilosa*'s high antioxidant levels as measured by DPPH and ABTS assays (Table 2). Phenolic substances such as simple phenolics, polyphenols, flavonoids, tannins, and phenolic terpenes, which have redox characteristics, are substantially related to medicinal plant antioxidant activity [100]. Phenols are aromatic rings with one or more hydroxyl substructures that allow extracts to scavenge free radicals [101]. Flavonoids are phenolic compounds having a wide range of antioxidant effects. Flavonoids with hydroxyl substituents on the nucleus prevent lipid peroxidation, but methoxy groups or glycogens decrease antioxidant action [102]. Antioxidative, anticarcinogenic, antibacterial, and anti-inflammatory properties make plant phenolic substances valuable pharmacologically [103, 104].

Anti-mycobacterial activity of FPM and DPM of *B. pilosa* using well diffusion and MIC assays

The *B. pilosa* FPM and DPM extracts' anti-mycobacterial activities were assessed by using well diffusion and microtiter assays. The DAE and DEE inhibited the bacteria best in the well diffusion tests (Table 4), but hexane and water extracts did not. Secondary metabolites in *B. pilosa* were responsible for the inhibitory effect on *Mycobacterium* strains (Table 1). These secondary metabolites were responsible for inhibiting and repressing the growth of human bacterial pathogens through mechanisms that differed from existing antibiotics [105, 106]. For example, alkaloids target DNA topoisomerases and disrupt bacterial morphology and growth. Phenols, terpenoids, and saponins damage bacterial membranes [107, 108]. Flavonoids change membrane permeability and pathogenicity [109]. This emphasizes the usage of secondary metabolites of plant constituents as resources for exploring new antibiotics [110, 111]. The specific molecular targets or mechanisms of action of *B. pilosa* on mycobacteria are still unknown. Further investigations are required to detect and quantify the active constituents of *B. pilosa* responsible for the inhibition of *Mycobacterium* strains. The minimum inhibitory concentration (MIC) of *B. pilosa* FPM and DPM ranged between 6.25 and 50 mg/mL for the different solvents against *M. avium*, *M. smegmatis*, *M. terrae*, and *M. tuberculosis*. The DPM extract showed higher activity against these species, with the lowest MIC value of 6.25 mg/mL in DAE and DEE. Van Vuuren classified the anti-mycobacterial

activities of crude plant extracts using three different categories based on their MIC values, where 1 mg/mL or below was regarded as good, greater than 1 mg/mL and less than 10 mg/mL as moderate, and large than 10 mg/mL as poor activity [112]. Based on the above interpretation, the inhibitory activity of *B. pilosa* on mycobacterial growth was average (Table 6). This moderate anti-*Mycobacterium* activity observed in this study might be attributed to lipids such as mycolic acids and the bacteria's thicker, hydrophobic cell walls [113]. On the other hand, the higher sensitivity of *Mycobacterium* strains to DAE, DEE, and DME could be attributed to the fact that these polar solvents could dissolve various hydrophilic and lipophilic compounds leading to higher permeability of the lipid cell membranes and inhibited growth [114]. Hexane, which extracted non-polar chemicals, had no effect on the strains (Table 6). Non-polar chemicals are inactive against infections like *Mycobacterium* [115]. Although moderate activity was observed in this study, these extracts might still be of value because they could offer compounds with different binding sites to those of current drugs and possibly overcame the problem of antibiotic resistance. In general, medicinal plants are more active against Gram-positive bacteria than Gram-negative bacteria, and the difference in susceptibility is related to structural differences in the cell walls [116]. This study's anti-mycobacterial activity matched that of Ajanaku *et al*. who tested the stem and root parts of *B. pilosa* using the proportion method on an L-J medium [117]. The study found that the hexane fraction of *B. pilosa*'s root had weak activity against drug-susceptible *M. tuberculosis* (DS-MTB) and drug-resistant *M. tuberculosis* (DR-MTB). The stem showed no activity against DS-MTB and DR-MTB. Ajanaku *et al*. later examined the anti-mycobacterial properties of *B. pilosa* leaf extracts [118]. The hexane/methanol fraction showed good anti-tubercular activity against DS-MTB, DR-MTB, and *M. tuberculosis* strain (H37Rv) with a MIC value of 6.25 mg/mL, while the other fractions were not active. Several plants with promising anti-tubercular activity have been described in previous investigations [119-122]. The asteraceae, lamiaceae, fabaceae, and apiaceae form part of the few plant families that have been studied [123]. *Bidens pilosa* belongs to asteraceae and showed anti-mycobacterial activity consistent with those of previously reported plant families.

HPLC for identifying phenolic compounds present in *B. pilosa*

HPLC is widely used to separate and quantify chemicals in complex materials such as plant extracts [124]. The phenolic compounds from fresh and dried *B. pilosa* were identified by using HPLC. The phenolic compounds were indicated by peaks (Figure 5) that showed the presence of ascorbic acid, gallic acid, resorcinol, vanillin, and benzoic acid. The DPM extracts of *B. pilosa* showed greater separation of compounds than that of FPM extracts. The DPM extracts of *B. pilosa* were more effective than FPM extracts based on the high amount of phytochemicals screened, high phenolic, flavonoid, and tannins content, high antioxidant activities, and lastly, low MIC values. This study showed that *B. pilosa* contained phytocompounds with antioxidant and anti-mycobacterial activity, which could be explored for new potential and effective drugs related to *mycobacterium* diseases.

Conclusion

This study demonstrated that the extraction process of *Bidens pilosa* greatly influenced the yield of bioactive compounds and their respective activities. DPM extracts of *B. pilosa* were more effective than FPM extracts, indicated by the higher amount of screened phytochemicals, elevated phenolic, flavonoid and tannins content, more substantial antioxidant activities, and lower MIC values. Therefore, the results of this study underscored the therapeutic potential of *B. pilosa* and the importance of extraction methods in harnessing this potential, leading the way for future research to enhance drug development, particularly for diseases related to mycobacterial infections.

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