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Research Article

Employment of Gas Chromatography–Mass Spectrometry Analysis to Estimate Phytochemical Constituents and Antimicrobial Activity of *Bidens pilosa* Along Habitat Heterogeneity in Egypt

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Abstract

Background: Continuous exposure to a range of environmental conditions can induce the production of primary and secondary metabolites in plants. Thus, this study aimed to examine variations in phytochemical compounds and antimicrobial activity of crude extracts from *Bidens pilosa* plants across various Egyptian habitats. **Methods**: Plants were gathered from 10 habitats across five Southern and Middle Nile Delta Governorates. The phytochemical components of *B. pilosa* extracts were estimated both qualitatively and quantitatively. **Results and Conclusion**: The plant was found to contain terpenoids, flavonoids, phenols, tannins, alkaloids, sterols, saponins, carbohydrates, and amino acids, which are mainly concentrated in shoots. Citrus, guava, and mango orchards and wasteland habitats contained the highest contents of total flavonoids, phenols, tannins, and alkaloids. Fifty compounds were identified in the ethanol extract; the most dominant groups were aromatic and aliphatic compounds (22 and 17 compounds, respectively) with the highest peak area % recorded for nonadecane (9.58%), 14α-H-pregna (7.15%), pentane, 3-methyl (3.40%), and dodecane (4.20%). While 30 compounds were recorded in the chloroform:methanol extract with the dominance of organosilicons (30.00% of the total compounds), dicarboxylic acids (16.67%) and carboxylic acids (13.33%), in addition to the highest peak area % was recorded for cyclononasiloxane, octadecamethyl (10.98%), (Z)-5-ethylidene-3-hydroxy-4-(3'-methylbutanoyl)-2(5H)-furanone (9.55%), and silicone oil (7.95%). The raw extract of the *B. pilosa* shoots exhibited antimicrobial activity against many bacterial and fungal isolates. Most of the identified secondary metabolites exhibited physiological and ecological roles in plants and play roles in adverse environmental stressors. Many of the identified compounds possessed nutritional value and therapeutic effects. In addition, the methanol extract had a beneficial impact on these bioactive phyto-organic constituents, which can be harnessed and used in the food and pharmaceutical industries to produce drugs and raw materials for industrial purposes.

Keywords: *Bidens*; gas chromatography; flavonoids; pharmacology; habitats

1. Introduction

Plants are sessile organisms that are constantly subjected to a range of environmental biotic (pathogens) and abiotic (extreme temperature, drought, aridity, salinity, and pollution) stresses that seriously impair their growth, development, and productivity [1,2]. Plants have evolved strong, sophisticated immune systems over time in response to shifting environmental conditions [3]. Moreover, throughout their lives, plants biosynthesize primary (1^{ry}) and secondary (2^{ry}) metabolites; primary metabolites are necessary for plant growth and development, while 2^{ry} metabolites can be volatile or non-volatile and are produced as byproducts of 1^{ry} metabolic pathways in plants [4]. These 2^{ry} metabolites have broader uses because they influence plant physiological and ecological processes, helping the plants endure harsh environmental conditions [2,5]. More than 200,000 2^{ry} metabolites have been discovered, isolated, and described from plants as reported by Satish *et al.* [6] and Rabeh *et al.* [2]. In addition to the ecological functions of these metabolites in mitigating the effects of abiotic stressors, such as drought, salinity, UV radiation, and extreme temperatures [5,7], plant 2^{ry} metabolites can preserve different defense mechanisms against pathogens, insects, and predators through their toxicity and unpalatability [8].

Bidens pilosa (L. Asteraceae), also known as blackjack, is a weed that grows widely across heterogeneous habitats in Egypt [9]. *B. pilosa* has been used in folk medicine throughout America, Africa, Asia, and Oceania [10]. Compounds from this plant, including furan, phenols, flavonoids, and fatty acid esters, have antioxidant, antibacterial, antimicrobial, anti-inflammatory, antiproliferative, anticancer, antitumor, antidiabetic, antiarthritic, antimalarial, and autonomic nerve activities as reported in ethnobotanical databases [11,12]. Bartolome *et al.* [13] reported that *B. pilosa* is used to treat more than 41 diseases be-

cause it contains more than 200 phytochemical compounds. The plant and/or its polyenes have been shown to treat diabetes and hypertension [14–16]. According to Etukudo *et al.* [12], phytochemical screenings of *B. pilosa* revealed the presence of tannins, flavonoids, phytosterols, ascorbic acid, carotene, anthocyanins, saponins, steroids, and sugars. Additionally, Isakova *et al.* [17] recognized and isolated groups of chemical compounds, including polyacetylenes, flavonoids, and terpenes with biological activity [18]. *B. pilosa* can also be used to treat snake bites and wounds, while the aqueous solution from leaves is used to bathe infants and young children [19,20].

Field studies have explored the relationship between plant growth, productivity, and the concentration of 2^{ry} metabolites [21]. Specifically, few studies have estimated the number of 2^{ry} metabolites in *B. pilosa* and linked them to environmental conditions. Therefore, this study aimed to examine how habitat heterogeneity affects the phytochemical components and pharmacological activity of *B. pilosa* in the Egyptian Nile Delta. Such studies can increase public awareness about the therapeutic uses of the target species and may aid in plant conservation.

2. Materials and Methods

2.1 Plant Sampling

The *Bidens pilosa* plant was collected across five governorates (Al-Qalyubiya, Al-Dakahliya, Al-Sharkya, Cairo, and Giza) located in the Egyptian Nile Delta region (Fig. 1) during 2017 from 10 different habitats: crops, orchards (citrus, guava, apricot, pear, and mango), canal banks, plant nurseries, public gardens, and wastelands. The plant species was identified according to Boulos [9]. The average temperature in the study area varies between 13.7 °C and 17.4 °C during winter and 24.7 °C to 28.0 °C during summer; meanwhile, the mean relative humidity ranges from 46.35% in June to 70.18% in January, and the maximum amount of rainfall (1.04 mm) was recorded in December [22]. A composite sample of *B. pilosa* plants was gathered from each of the 48 sites, thereby representing the study habitats. Plant samples were transferred to the laboratory in plastic bags, then separated into roots and shoots. Plant samples were rinsed in tap water, then distilled water, and air-dried at room temperature in the shade before being homogenized in a planetary high-energy mill (RETSCH GmbH, Retsch-Allee 1-5, Haan, Germany), with a hardened chromium steel vial.

2.2 Qualitative Assessment of Phytochemical Constituents

A total of 50 g of ground plant roots and other shoots from the 10 habitats was soaked in distilled water at room temperature (25–30 °C) for 3 days, which served as the extraction solvent. The mixture was filtered with a Whatman No. 1 filter paper. The filtrates were used to test the following phytochemical constituents [23]:

Alkaloids: A total of 2 mL of plant extracts was dissolved in 2 N HCl. The presence of alkaloids was tested in the aqueous solution by:

a- Mayer's test: Mayer's reagent was added to the aqueous layer. A yellow, cream, or white precipitate or turbidity indicated the presence of alkaloids.

b- Wagner's test: Wagner's reagent was added to the aqueous layer. A brown or reddish-brown precipitate indicated the presence of alkaloids.

Phenols:

a- Ferric chloride test: A drop of neutral ferric chloride (5%) solution was added to 2 mL of the extract. A dark green/bluish-black color indicated the presence of phenols. b- Lead acetate test: A few drops of lead acetate solution were added to 2 mL of the extract. The formation of a yellow precipitate indicated the presence of phenols.

Terpenoids:

Salkowski's test: A total of 5 mL of plant extract was mixed with 2 mL of chloroform, then 3 mL of conc. H_2SO_4 was added carefully to the mixture to form a layer. The reddish-brown appearance on the inner face indicated the presence of terpenoids.

Flavonoids:

a- Ammonia test: A few drops of a 1% NH_3 solution were added to the plant extracts. A yellow color indicated the presence of flavonoids. b- Lead acetate test: A few drops of lead acetate solution were added to the plant extracts. The formation of a yellow precipitate indicated the presence of flavonoids. c- The H_2SO_4 test: A few drops of H_2SO_4 were added to the plant extracts. The formation of an orange color indicated the presence of flavonoids. d- Tannins: A total of 2 mL of 5% ferric chloride was added to 1 mL of the plant extracts. A greenish black color indicated the presence of tannins.

Protein and Amino acids:

Ninhydrin test: Two drops of freshly prepared 0.2% Ninhydrin reagent were added to 0.5 mL of plant extract, and the mixture was heated. The formation of a pink or purple color indicated the presence of proteins, peptides, or amino acids.

Sterols: A few drops of chloroform, 3–4 drops of acetic anhydride, and one drop of conc. H_2SO_4 was added to 2 mL of the plant extracts. A change in color from purple to green indicated the presence of steroids.

Saponins: A total of 2 mL of distilled water was added to 2 mL of plant extract, and the mixture was shaken in a cylinder for 15 minutes in a lengthwise motion. The presence of saponins is shown by the formation of a 1 cm layer of foam.

Carbohydrates: Molisch's reagent (1 mL) was added to 2 mL of the plant extracts. A few drops of concentrated sulfuric acid were added to the mixture. The formation of a purple ring confirmed the presence of carbohydrates.

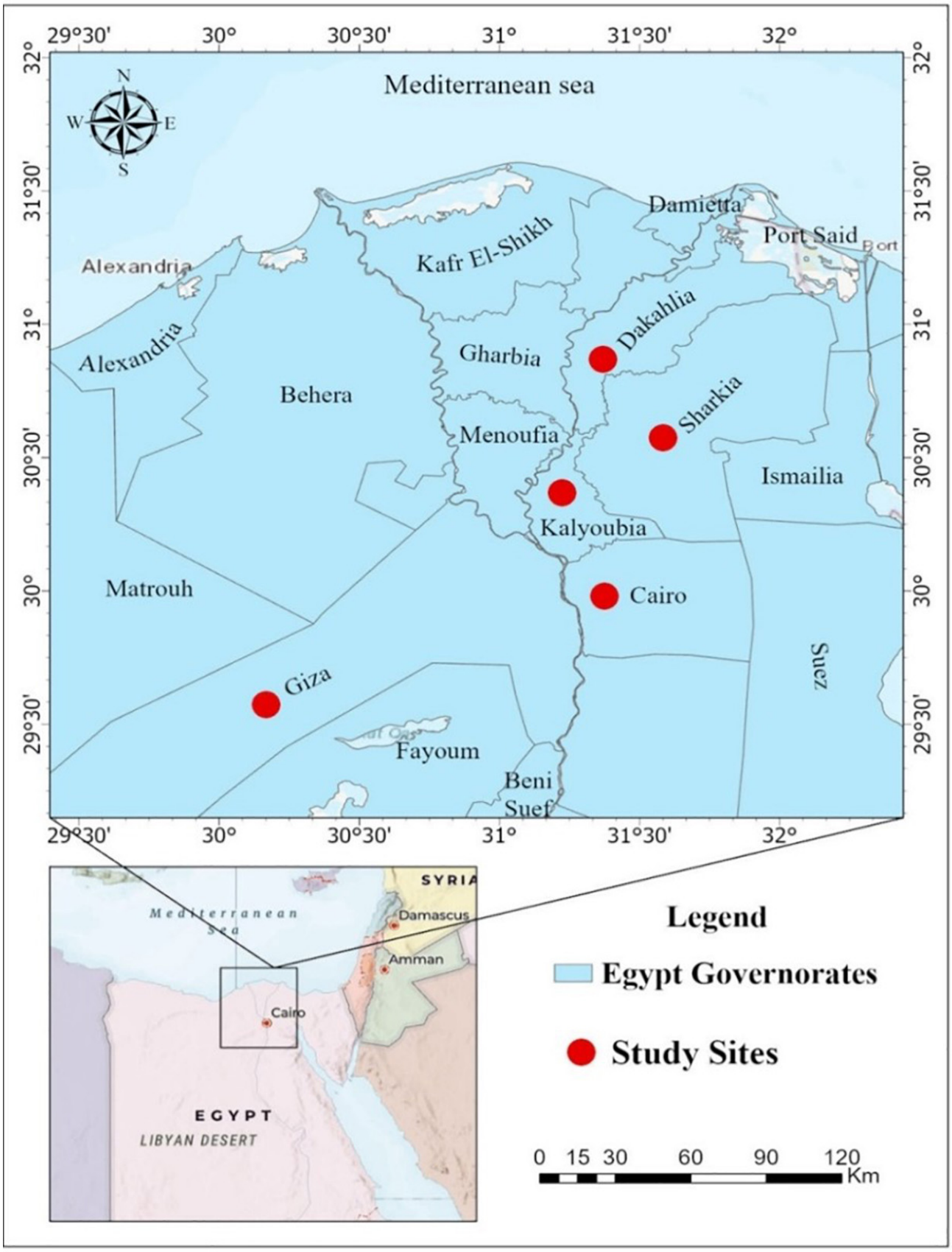

Fig. 1. Location map of the study locations in the Nile Delta Governorates.

3. Quantitative Estimation of Active Phytochemical Compounds

3.1 Total Phenolic Compounds (TPC)

The Folin–Ciocalteu reagent [24] was used to estimate the TPC content in the plant extract. Gallic acid (GA) was utilized as a standard, and the total phenolics were represented as mg GAE/g DW. GA concentrations of 2, 4, 6, 8, and 10 g/mL were made in methanol. A plant extract concentration of 1 mg/mL was likewise prepared in methanol; the test consisted of mixing 0.5 mL of each sample with 2.5 mL of a 10-fold diluted Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. After closing the tubes with parafilm and allowing them to sit at room temperature for 30 minutes, the absorbance was measured at 760 nm using a spectrophotometer (CECIL CE 1021, Cecil Instruments Limited, Corston, UK). All tests were performed in triplicate. Since reduced substances, such as polyphenols, are responsive to the Folin–Ciocalteu reagent, as a result of the reaction, the reduced substances turn blue. This blue color was spectrophotometrically measured. To estimate the unknown phenol content, a regression line based on GA was used. From the GA standard curve, the regression line was $y = 0.03 + 0.0913x$, $r^2 = 0.9976$. The absorbance is denoted by (y), while the mg GAE/g of the extract is denoted by (x). Hence, the goodness of fit of the chosen standard curve was determined to be excellent, after putting the absorbance (y = absorbance) of the test sample on the regression line of the previously mentioned (GA).

3.2 Total Saponins

According to Okwu and Ukanwa [25], 20 g of plant powder was dispersed in 200 mL of 20% ethanol. At around 55 °C, the hot water bath is used to heat the suspension for 240 minutes while the suspension is shaken constantly. After filtering the mixture, the residue was re-extracted with 200 mL of 20% ethanol. Then, the mixture volume was reduced to 40 mL using a 90 °C water bath. The concentration was combined with 20 mL of diethyl ether in a 250 mL separating funnel and vigorously agitated. The ether layer was discarded, whereas the aqueous layer was maintained. The purification procedure was repeated by adding 60 mL of n-butanol. The mixed n-butanol extract was washed twice with 10 mL of 5% aqueous sodium chloride. A water bath was used to heat the remaining solution. To obtain the desired weight, the samples were dried in an oven after evaporation. The saponin content was calculated as a proportion of the total saponin content.

3.3 Total Tannins

The gravimetric procedure (copper acetate method) entails quantitatively precipitating tannin from a copper acetate solution, burning copper tannate to CuO, and estimating the residual CuO [26]. A total of 2 g of *B. pilosa* shoots was extracted twice using 100 mL of acetone–water (1:1) for 60 minutes each and then filtered. The mixed extract was poured into a ¼-liter volumetric flask and brought to the required volume with distilled water. Then, the extraction was heated to boiling in a 500 mL beaker, and 30 mL of a 15% aqueous copper acetate solution was added while shaking. The ashless filter paper was used to separate the copper tannate product, which was subsequently burned in a crucible (crucibles had been previously burned at the same temperature and to a uniform weight) [26]. The weight was returned after the residue was treated with a few drops of nitric acid. The following correlation was used to evaluate the weight of CuO and the amount of tannin, where 1 g of CuO is equivalent to 1.305 g of tannin.

3.4 Total Alkaloids

The gravimetric method [27] was used to determine the total alkaloids, with 90% ethanol used to extract approximately 10 g of the plant powder until exhaustion (as determined by the Mayer reagent). At a temperature of 40 °C, the alcoholic plant extract was concentrated under reduced pressure until it became dry, then acidified with HCl (3%) and filtered. The acid–alkaloid component of the filtrate was extracted with chloroform until the alkaloid component was exhausted. Ammonia was used to convert the acidic aqueous layer to an alkaline medium (as determined by the Mayer and Dragendorff reagents). Anhydrous sodium sulphate was used to filter the chloroform extract, which was then evaporated under reduced pressure until completely dry, and the percentage was estimated by weighing it (w/w).

3.5 Total Flavonoid Content

Total flavonoid content (TFC) was calculated using the techniques described by [28,29].

The following Equation was applied to determine the TFC as quercetin in the plant material (mg/g; adjusted for moisture content), while the TFC represents the average of three determinations.

$$\mathrm{TFC}_{\text{plant material}} = \left(\mathrm{TFC}_{\text{tested solution}} \times 1.25 \times 50\right) / (w - ld)$$

where:

- $\mathrm{TFC}_{\text{test solution}}$: concentration of total flavonoids in the test solution (mg/mL),
- 1.25: dilution factor,
- 50: volume of the stock solution (mL),
- *w*: weight of plant material (g),
- *ld*: weight loss on drying of the plant material (g).

3.6 Identification of the Phytochemical Compounds Using Gas Chromatography–Mass Spectrometry (GC–MS)

A composite sample of 25 g of shoot powder (mixed from the 10 habitats) was added to 25 mL of ethanol (96%), and another sample was added to 25 mL of chloroform:methanol (2:1 v/v). The mixtures were shaken using an electronic shaker (KUKJE, Shaking Incubator, Ko-

rea model 36-SIN-125, Seoul, South Korea) at room temperature for 24 hours. Then, the extract was filtered using Whatman No. 1 filter paper. The residues were re-extracted twice, and the filtrates of each solvent were collected [30]. The solvent of each extract was evaporated under low pressure by an evaporator at a temperature not exceeding 50 ± 2 °C. The separation and identification of the pure active materials from plant extracts was performed using a GC–MS system (Shimadzu GC-MS-QP2050A at RCM, class 5000 software, Tokyo, Japan).

The chemical composition of the pure active materials from the plant extracts was performed using a Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 µm film thickness). The column oven temperature was initially held at 50 °C and increased by 5 °C/min to 230 °C, then held for 2 minutes, then increased to the final temperature of 290 °C by 30 °C/min and held again for 2 minutes. The injector and MS transfer line temperatures were kept at 250 and 260 °C, respectively; helium was used as the carrier gas at a constant flow rate of 1 mL/min. The solvent delay was 3 min, and 1 µL diluted samples were injected automatically using the Autosampler AS1300 (Foss Analytical, Hillerød, Denmark) coupled with the GC in split mode. EI mass spectra were collected in full-scan mode at an ionization voltage of 70 eV over the m/z range 40–1000. The ion source temperature was set at 200 °C. The components were identified by comparing their retention times and mass spectra with those in the WILEY 09 and NIST 11 mass spectral databases.

4. Antimicrobial Bioassay

4.1 Extraction

Benzene, methanol, and ethanol (70%) were used as solvents for plant extraction. The extractions were prepared as follows: three weights, each of 50 g of air-dried plant shoot powder, were packed separately into each solvent [31,32]. After 5 days, the solvent from each extract was removed under low pressure using a rotary evaporator at 60 ± 2 °C. The dried extracts were kept at –20 °C in sterile vials until further use [33].

4.2 Microorganisms Used

The examined microbes (*Bacillus subtilis* (ATCC6633), *Staphylococcus aureus* (MTCC96), *Pseudomonas aeruginosa* (MTCC424), *Escherichia coli*, and the fungal strains of *Aspergillus flavus*, *Penicillium chrysogenum*, *Syncephalastrum racemosum* and *Candida albicans*) were obtained from the culture collection unit in the Regional Centre for Mycology and Biotechnology (RCMB), Al-Azhar University. The bacteria and fungi were cultured on nutrient agar and malt extract agar, respectively.

4.3 Assessment of the Antimicrobial Activity In Vitro

Antimicrobial activity of the dried plant shoot extracts, dissolved in dimethyl sulfoxide (DMSO) to obtain a 100 mg/mL final extract, was tested against the microbes. The inhibition zone diameter was used to estimate the antimicrobial potential of the extracts. Inoculum suspensions of all bacterial and fungal strains were spread on the medium surface. The antimicrobial activity of the microbes was investigated using the agar plate well diffusion method. A 6 mm corkborer was used to make wells (6 mm in diameter) into the media. The wells in each plate were filled with 100 µL extract. The inoculated plates for bacterial strains were incubated at 37 °C for 24 hours, and those for fungal strains at 25 °C for 48 hours. The diameters of the inhibition zones were measured after 24 hours and 48 hours of incubation for bacteria and fungi, respectively. The experiment was conducted in triplicate, and the diameters of inhibition zones were measured by a standard scale [34]. DMSO was found to have no antimicrobial effect and was therefore used as a negative control.

4.4 Statistical Analysis

After testing the data for normality, the differences in the phytochemical constituents of the *B. pilosa* shoots and in antimicrobial activity across different habitats were analyzed using one-way analysis of variance (ANOVA) in the SPSS software (SPSS Inc., Chicago, IL, USA) [35]. When differences were significant, a post hoc test was applied using Tukey's test.

5. Results

5.1 Phytochemical Screening

The phytochemical screening of the aqueous extract of *B. pilosa* shoots and roots from the 10 habitats detected the presence of flavonoids, phenols, terpenoids, tannins, alkaloids, sterols, saponins, and amino acids in the plant shoots (Table 1). However, alkaloids and sterols were not recorded in the roots. Carbohydrates, as primary metabolites, showed intense reactions in the two parts of the *B. pilosa* plant from the 10 habitats. The aqueous extracts of the *B. pilosa* shoots collected from citrus, mango, and guava orchards, and wasteland habitats showed more intense reactions with all tested phytochemical reagents than those of other habitats.

5.2 Active Phytochemical Compounds

Statistically highly significant variation was detected in the investigated phytochemical compounds of *B. pilosa* shoots across different habitats ($p < 0.001$; Table 2). The significantly highest content of total flavonoids, total tannins, alkaloids and total saponins (295.80 ± 24.95 mg/g, 1.95 ± 0.13%, 2.04 ± 0.21%, and 1.92 ± 0.17%, respectively) were recorded in *B. pilosa* shoots from wastelands, while the highest total phenols (392.55 ± 25.00 mg/g) were

Table 1. Screening of the phytochemical compounds in aqueous extracts of *B. pilosa* plant shoots (Sh) and roots (R) collected from different habitats.

Habitats	Terpenoid		Flavonoids		Phenols		Tannins		Alkaloids		Sterols		Saponins		Amino acids		Carbohydrates	
	Sh	R	Sh	R	Sh	R	Sh	R	Sh	R	Sh	R	Sh	R	Sh	R	Sh	R
Crops	++	+	++	++	++	–	++	–	–	–	++	–	++	–	++	++	+++	+++
Citrus	+++	++	+++	+++	+++	–	+++	–	+++	–	–	–	–	–	+++	++	+++	+++
Guava	+++	++	+++	+++	+++	++	+++	++	+++	–	–	–	+++	++	+++	++	+++	+++
Apricot Orchards	++	–	++	++	++	++	++	–	++	–	–	–	++	++	++	++	+++	+++
Mango	+++	++	+++	+++	+++	++	+++	++	+++	–	–	–	+++	++	+++	++	+++	+++
Pear	++	+	++	++	++	++	++	–	++	–	–	–	++	++	++	++	+++	+++
Public garden	++	–	++	++	++	–	++	–	++	–	–	–	++	++	++	++	+++	+++
Plant nurseries	++	–	++	++	++	–	++	–	++	–	–	–	++	++	++	–	+++	+++
Canal banks	++	+	++	++	++	++	++	–	++	–	–	–	++	–	++	–	+++	+++
Wastelands	+++	+	+++	+++	+++	++	+++	++	+++	–	–	–	+++	++	+++	++	+++	+++

Based on the color intensity of the reaction: +: trace, ++: moderate, +++: intense, –: not detected.

Table 2. Phytochemical compounds content in *Bidens pilosa* shoots from different habitats.

Habitats	Phytochemical compounds				
	Flavonoids (mg GA/g DW)	Phenols (mg GA/g DW)	Tannins (%)	Alkaloids (%)	Saponins (%)
Crops	244.65 ± 24.16^{de}	320.22 ± 23.00^{d}	1.25 ± 0.12^{ef}	1.09 ± 0.09^{e}	$\underline{1.06 \pm 0.11^{e}}$
Citrus	285.16 ± 26.01^{bc}	386.61 ± 24.00^{ab}	1.73 ± 0.08^{b}	1.83 ± 0.15^{b}	1.79 ± 0.14^{b}
Guava	276.32 ± 26.00^{c}	379.83 ± 24.51^{b}	1.71 ± 0.15^{b}	1.98 ± 0.17^{a}	1.88 ± 0.16^{ab}
Apricot Orchards	251.51 ± 23.06^{d}	318.11 ± 23.00^{d}	1.33 ± 0.12^{cd}	1.28 ± 0.14^{c}	1.56 ± 0.16^{cd}
Mango	291.06 ± 27.00^{ab}	$\underline{392.55 \pm 25.00^{a}}$	1.71 ± 0.13^{b}	1.75 ± 0.15^{b}	1.85 ± 0.15^{ab}
Pear	235.62 ± 12.00^{ef}	354.71 ± 23.50^{c}	1.21 ± 0.13^{f}	1.22 ± 0.12^{cd}	1.62 ± 0.15^{c}
Public garden	241.61 ± 22.00^{ef}	344.82 ± 23.00^{c}	1.34 ± 0.14^{c}	1.14 ± 0.11^{de}	1.49 ± 0.15^{d}
Plant nurseries	$\underline{233.54 \pm 23.00^{f}}$	$\underline{285.21 \pm 25.00^{e}}$	1.25 ± 0.13^{ef}	$\underline{1.05 \pm 0.10^{e}}$	1.44 ± 0.14^{d}
Canal banks	241.27 ± 23.00^{ef}	320.53 ± 22.66^{d}	$\underline{1.18 \pm 0.12^{ef}}$	1.20 ± 0.13^{cd}	1.53 ± 0.15^{cd}
Wastelands	$\underline{295.80 \pm 24.95^{a}}$	388.77 ± 23.51^{ab}	$\underline{1.95 \pm 0.13^{a}}$	$\underline{2.04 \pm 0.21^{a}}$	$\underline{1.92 \pm 0.17^{a}}$
F-value	73.89***	95.25***	46.04***	31.28***	13.20***

The means with the same letters in the same column are not significantly different. ***Significant difference at $p < 0.01$. Maximum and minimum values are underlined.

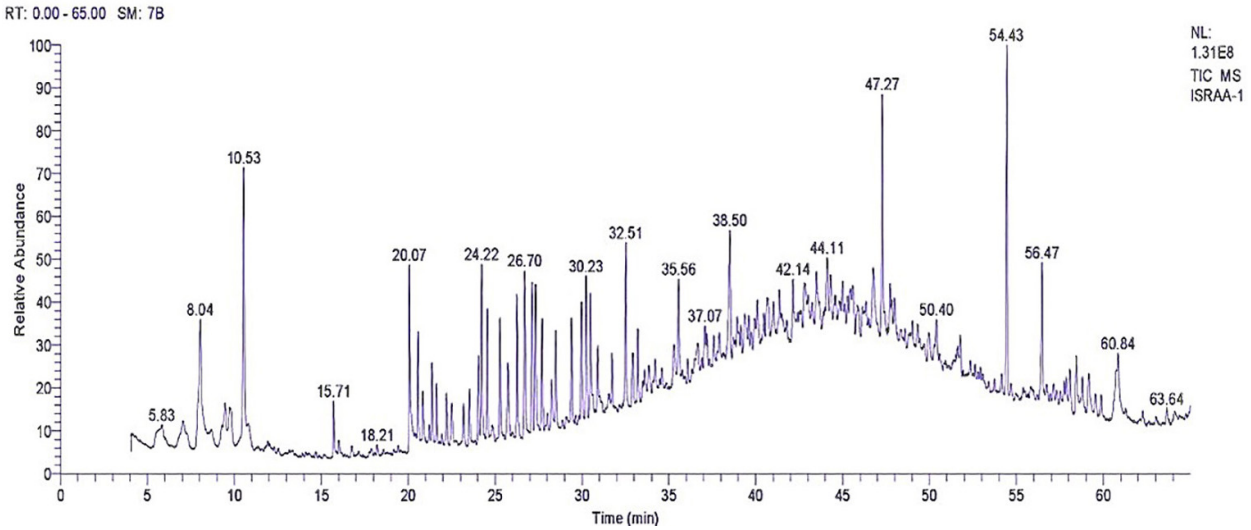

Fig. 2. Typical GC–MS total ion chromatograms (TICs) of phytochemical compounds in the alcohol extract of *Bidens pilosa* shoots.

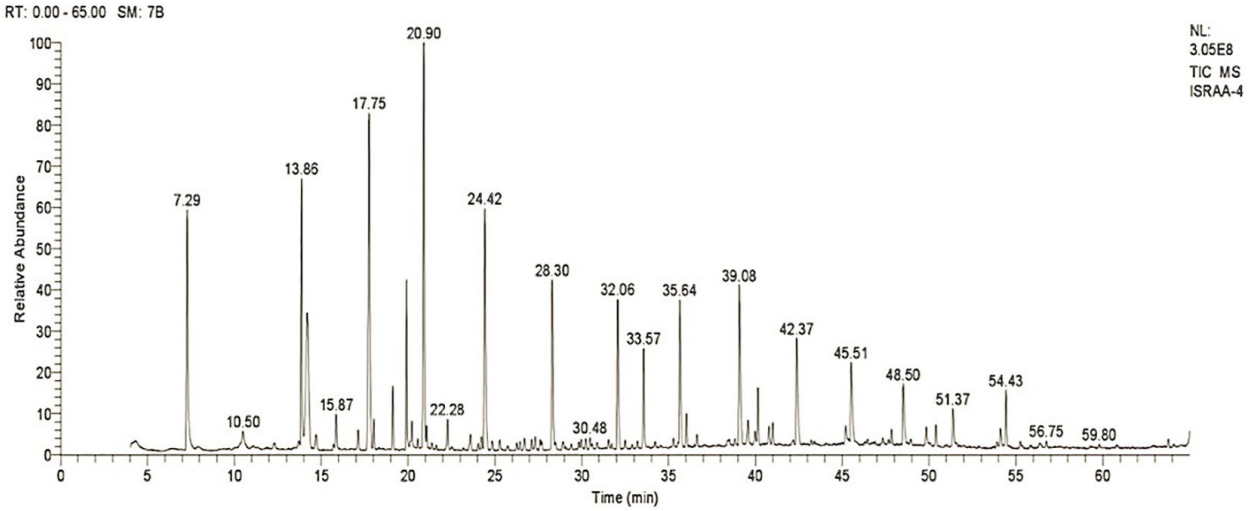

Fig. 3. Typical GC–MS total ion chromatograms (TICs) of phytochemical compounds in chloroform: methanol extract of *Bidens pilosa* shoots.

recorded in plant tissues from mango orchards. Conversely, the significantly lowest contents of total flavonoids, phenolics, and alkaloids (233.54 $\pm$ 23.00 mg/g, 285.21 $\pm$ 25.00 mg/g, and 1.05 $\pm$ 0.10%, respectively) were recorded in the nursery habitat. Moreover, the lowest content of total tannins and saponins (1.18 $\pm$ 0.12% and 1.06 $\pm$ 0.11%) was recorded in canal banks and crops, respectively. Notably, *B. pilosa* plants from citrus, guava, and mango orchards, as well as wasteland habitats, contained the highest concentrations of all investigated phytochemicals.

5.3 GC–MS Analysis of the Phytochemical Compounds

The phytochemical groups and compounds identified in ethanol and chloroform:methanol extracts by GC–MS analysis, along with their molecular formulas, molecular weights, and peak area percentages, are presented in Tables 3,4, and Figs. 2,3. Fifty compounds belonging to nine phytochemical groups (fatty alcohols, thiols (mercaptan), alkaloids, aromatic compounds, aliphatic natural products, steroids, terpenoids, flavonoids, heterocycloalkanes) were identified in the ethanol extract by GC–MS (Table 3 and Fig. 2). The most dominant phytochemical groups in the ethanol extract were aromatic compounds (22 compounds with 44% of the 50 compounds) and aliphatic compounds (17 compounds with 34%). The peak area % of the 50 compounds identified in the ethanol extract had its lowest value (0.68%) for the steroid hahnfett and the aliphatic compound docosane, while its highest (12.07%) was for the thiol tert-hexadecanethiol. Other compounds that recorded high peak areas were: nonadecane (9.58%), 14α-H-pregna (7.15%), dodecane (4.20%), and pentane, 3-methyl (3.40%).

Table 3. The chemical compounds identified in the ethanol extract of *B. pilosa* shoots using GC–MS.

No.	RT	Compound name	Molecular formula	Molecular weight (g/mol)	Peak area %
			Fatty alcohols		
1	7.04	1-octanol,2-butyl	$C_{12}H_{26}O$	186	1.55
			Thiols (mercaptan)		
2	46.76	Tert-hexadecanethiol	$C_{16}H_{34}S$	258	12.07
			Alkaloids		
3	42.79	2-piperidinone,N-[4-bromo-n-butyl]	$C_9H_{16}BrNO$	233	1.10
			Amino acids		
4	36.65	Glycine, N-[N-(2-hydroxybenzoyl)-a'alanyl]-methyl ester (aspartyl-phenylalanine or dimethyl aspartame)	$C_{13}H_{16}N_2O_5$	280	0.75
			Aromatic compounds		
5	20.07	Thiophene, tetrahydro-,1,1-dioxide (sulfolane)	$C_4H_8O_2S$	120	2.31
6	21.37	Benzene, (1-butylhexyl)	$C_{16}H_{26}$	218	0.97
7	21.62	Benzene, (1-propylheptyl)	$C_{16}H_{27}$	218	0.72
8	30.90	Benzene, (1-propylheptadecyl)	$C_{26}H_{46}$	358	1.20
9	22.20	Benzene, (1-ethyloctyl)	$C1_6H_{26}$	218	0.76
10	28.49	Benzene, (1-ethyldecyl)	$C_{18}H_{30}$	246	1.42
11	23.51	Benzene, (1-methylnonyl)	$C_{16}H_{26}$	218	0.86
12	24.04	Benzene, (1-pentylhexyl)	$C_{17}H_{28}$	232	1.23
13	30.23	Benzene, (1-pentyloctyl)	$C19H_{32}$	260	2.02
14	27.12	Benzene, (1-pentylheptyl)	$C_{18}H_3O$	246	1.98
15	24.22	Benzene, (1-butylheptyl)	$C_{17}H_{28}$	232	2.54
16	24.54	Benzene, (1-propyloctyl)	$C_{17}H_{28}$	232	1.70
17	27.70	Benzene, (1-propylnonyl)	$C_{18}H_{30}$	246	1.57
18	25.28	Benzene, (1-ethylnonyl)	$C_{17}H_{28}$	232	1.68
19	26.70	Benzene, (1-methyldecyl)	$C_{17}H_{28}$	232	2.68
20	27.32	Benzene, (1-butyloctyl)	$C_{18}H_{30}$	246	2.15
21	29.97	Benzene, (1-methylundecyl)	$C_{18}H_{30}$	246	1.72
22	30.48	Benzene, (1-butylnonyl)	$C_{19}H_{32}$	260	2.04
23	30.90	Benzene, (1-propyldecyl)	$C_{19}H_{32}$	260	1.20
24	31.73	Benzene, (1-ethylundecyl)	$C_{19}H_{32}$	260	0.74
25	30.48	Benzene, (1-butylhexadecyl)	$C_{26}H_{46}$	358	2.05
26	33.20	Benzene, (1-methyldodecyl)	$C_{19}H_{32}$	260	1.47
			Aliphatic natural products		
27	23.18	1-chlorooctadecane	$C_{18}H_{37}Cl$	288	1.46
28	8.04	Tetradecane	$C_{14}H_{30}$	198	3.43
29	9.46	1-decanol,2-hexyl	$C_{16}H_{34}O$	242	0.72
30	10.53	Dodecane	$C_{12}H_{26}$	170	4.20
31	38.50	Nonadecane	$C_{19}H_{40}$	268	9.58
32	20.84	5-octadecene, (E)	$C_{18}H_{36}$	252	0.72
33	56.47	Tricosane	$C_{23}H_{48}$	324	2.34
34	60.85	Dotriacontane	$C_{32}H_{66}$	450	1.38
35	35.55	Heneicosane	$C_{21}H_{44}$	296	1.60
36	32.91	10-heneicosene (c,t)	$C_{21}H_{42}$	294	0.90
37	47.72	Dodecane, 1-cyclopentyl-4-(3-cyclopentylpropyl)	$C_{25}H_{48}$	348	1.37
38	20.56	Hexadecane cetane	$C_{16}H_{34}$	226	0.93
39	26.26	Heptadecane, 2,6-dimethyl	$C_{19}H_{40}$	268	0.97
40	19.92	Decane, 2,2,3-trimethyl	$C_{13}H_{28}$	184	1.24
41	20.56	Eicosane	$C_{20}H_{42}$	282	0.84

Table 3. Continued.

No.	RT	Compound name	Molecular formula	Molecular weight (g/mol)	Peak area %
42	38.48	Docosane	$C_{22}H_{46}$	310	0.68
43	19.91	Pentane, 3-methyl	C_6H_{14}	86	3.40
		Steroids			
44	43.49	14α-H-pregna	$C_{21}H_{36}$	288	7.15
45	49.97	Hahnfett	N/A	0	0.68
		Terpenoids			
46	28.25	Phytol 2-hexadecen-1-ol,3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]-(T-phytol)	$C_{20}H_{40}O$	296	0.95
47	25.74	Heptadecane, 2,6,10,15-tetramethyl	$C_{21}H_{44}$	296	1.44
		Flavonoids			
48	45.86	2-dodecen-1-yl(-) succinic anhydride 2,5-furandione,3-(dodecenyl)dihydro	$C_{16}H_{26}O_3$	266	0.86
		Heterocycloalkanes			
49	56.78	1-methyl-1-undec-10-enyloxy-1-silacyclohexane	$C_{17}H_{34}OSi$	282	1.78
50	36.02	4H-1,3-benzodioxin-4-one, 2-(1,1-dimethylethyl) hexahydro-, [2S-(2a',4aa',8aa')]	$C_{12}H_{20}O_3$	212	0.90

Moreover, 30 compounds were found to belong to 11 phytochemical groups in the chloroform:methanol extract: fatty alcohols, cyclic ether, anhydrides, diesters, fatty aldehyde, alkaloids, carboxylic acids, dicarboxylic acids, tricarboxylic acids, flavonoids, and organosilicons (polymers or siloxanes) (Table 4 and Fig. 3). The most dominant phytochemical groups were the organosilicons, which included nine compounds (30.00%), dicarboxylic acids (five compounds (16.67%)), and carboxylic acids (four compounds (13.33%)). Among the 30 compounds identified in the chloroform: methanol extract, the following compounds recorded the highest peak area; cyclononasiloxane, octadecamethyl (10.98%), (Z)-5-ethylidene-3-hydroxy-4-(3'-methylbutanoyl)-2(5H)-furanone (9.55%), silicone oil (7.95%), 1,2-cyclohexanedicarboxylic acid, didecyl ester (5.47%), cyclooctasiloxane, hexadecamethyl (5.91%). Notably, Six of the total identified compounds in the two *B. pilosa* shoot extracts had peak areas below 1%.

5.4 Antimicrobial Activity

The antimicrobial activity of *B. pilosa* shoot extracts from different habitats, obtained using benzene, ethanol, and methanol solvents, was evaluated against a spectrum of bacterial and fungal strains (Table 5). Benzene extracts of *B. pilosa* shoots collected from crops, mango orchards, canal banks, plant nurseries, and public gardens did not affect the tested bacteria and fungi. However, all tested bacterial strains were sensitive to the benzene extract of *B. pilosa* shoots from wastelands, with inhibition zones ranging from 15.5 $\pm$ 0.75 mm (for *P. aeruginosa*) to 25.8 $\pm$ 2.07 mm (for *S. aureus*). Conversely, the tested fungal strains showed low sensitivity to the benzene extract of *B. pilosa* shoots, and their sensitivity varied with habitat. The highly significant inhibition zone for *A. flavus*, *S. racemosum*, *P. chrysogenu*, and *C. albicans* were 15.2 $\pm$ 1.05, 16.6 $\pm$ 1.04, 8.3 $\pm$ 0.42, and 10.0 $\pm$ 0.94 mm, respectively, resulting from benzene extracts of *B. pilosa* from mango orchards, wastelands and, guava orchards (Table 5).

Moreover, *Candida albicans* showed resistance to the *B. pilosa* shoot ethanolic extract. The highest significant inhibition zones resulted from treating bacteria and fungi with ethanolic extract of *B. pilosa* from habitats in wastelands, where the highest inhibition zones were 20.05 $\pm$ 1.75, 20.0 $\pm$ 1.89, 13.6 $\pm$ 1.24, and 19.6 $\pm$ 0.08 mm for *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*, respectively. However, the highest significant inhibition zones for *S. racemosum* and *P. chrysogenum* (12.5 $\pm$ 1.13 and 20.8 $\pm$ 1.62 mm) were recorded by applying plant ethanol extracts from guava orchards. Moreover, the ethanol extract of the wasteland plants showed efficient activity (17.0 $\pm$ 1.15 mm) against *A. flavus*.

Furthermore, the methanol extracts of *B. pilosa* showed no activity against the tested bacterial and fungal strains, which exhibited high resistance in canal banks, plant nurseries, and public gardens habitats. The methanolic extracts from apricot and pear orchards had slight antimicrobial activity against *E. coli* and *P. aeruginosa*. Conversely, the extracts from the citrus, guava, and mango or-

Table 4. The chemical compounds identified in the chloroform:methanol extract of *B. pilosa* shoots using GC–MS.

No.	RT	Compound name	Molecular formula	Molecular weight (g/mol)	Peak area %
		Fatty alcohols			
1	10.49	1-Decanol,2-ethyl	$C_{12}H_{26}O$	186	0.58
		Cyclic ether			
2	13.86	Oxirane, 2-methyl-3-(1-methylethyl)	$C_6H_{12}O$	100	2.95
		Anhydrides			
3	57.89	Decanoic anhydride	$C_{20}H_{38}O_3$	326	1.15
		Diester			
4	7.29	Sulfuric acid, dimethyl ester	$C_2H_6O_4S$	126	3.26
		Fatty aldehydes			
5	50.39	2,2-dideutero octadecanal	$C_{18}H_{36}O$	270	0.62
6	51.62	Octadecanal, 2-bromo	$C_{18}H_{35}BrO$	346	0.82
		Alkaloids			
7	33.21	2-oxabicyclo[3.3.1]nonane, benzenemethane sulfona	$C_{16}H_{21}NO_6S$	355	0.64
8	51.37	1H-purin-6-amine, [(2-fluorophenyl)methyl]	$C_{12}H_{10}FN_5$	243	1.04
		Carboxylic acids			
9	47.27	Tributyl acetylcitrate 1,2,3-propanetricarboxylic acid, 2-(acetyloxy)-,tributyl ester	$C_{20}H_{34}O_8$	402	2.29
10	53.59	Isopropyl 8-(3-octyl-2-oxiranyl) octa noate	$C_{21}H_{40}O_3$	340	1.05
11	47.85	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	376	1.14
12	53.57	Octadecanoic acid, 10-oxo-, methyl ester (stearic acid), and stigmasterol compounds)	$C_{19}H_{36}O_3$	312	1.30
		Dicarboxylic acids			
13	54.45	1,2-benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	5.09
14	47.23	1,2-cyclohexanedicarboxylic acid, bis(2-ethylhexyl)ester	$C_{24}H_{44}O_4$	396	3.03
15	47.34	1,2-cyclohexanedicarboxylic acid, didecyl ester didecyl-1,2-cyclohexanedicarboxylate	$C_{28}H_{52}O_4$	452	5.47
16	54.43	1,2-benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	1.05
17	40.63	Nonanedioic acid, dibutyl ester azelaic acid, dibutyl ester	$C_{17}H_{32}O_4$	300	0.76
		Tricarboxylic acid			
18	45.19	Butyl citrate	$C_{18}H_{32}O_7$	360	1.92
		Flavonoids			
19	48.62	2H-pyran-2-carboxylic acid, 6-butoxy-3,6-dihydro-, ethyl ester	$C_{12}H_{20}O_4$	228	3.13
20	36.03	(Z)-5-ethylidene-3-hydroxy-4-(3'-methylbutanoyl)-2(5H)-furanone	$C_{11}H_{14}O_4$	210	9.55
21	46.30	a',a'-dimethyl-5-(methylsulfinyl)-2-furfuryl propargyl ether	$C_{11}H_{14}O_3S$	226	10.01

Table 4. Continued.

No.	RT	Compound name	Molecular formula	Molecular weight (g/mol)	Peak area %
		(Organosilicons) polymers or siloxanes			
22	10.49	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	444	0.62
23	14.20	Cycloheptasiloxane, tetradecamethyl	$C_{14}H_{42}O_7Si_7$	518	4.06
24	15.87	Hexasiloxane, tetradecamethyl	$C_{19}H_{42}O_5Si_6$	458	1.06
25	19.12	Heptasiloxane, hexadecamethyl	$C_{16}H_{48}O_6Si_6$	532	4.30
26	28.30	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	$C_{16}H_{50}O_7Si_8$	578	5.17
27	17.75	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	592	5.91
28	35.65	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	666	10.98
29	28.20	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16, 18,18, 20, 20-Icosamethylcyclodecasiloxane	$C_{20}H_{60}O_{10}Si_{10}$	740	3.10
30	39.08	Silicone oil silikonfett SE30 (grevels)	$C_8H_{24}O_2Si_3$	424	7.95

Table 5. Antimicrobial responses of pathogenic bacterial and fungal strains to benzene extracts of *B. pilosa* collected from different habitats. Data are expressed as the diameter of the inhibition zone (mm).

Habitat	Inhibitory zones diameter (mm)							
	Bacteria				Fungi			
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*A. flavus*	*S. racemosum*	*P. chrysogenum*	*C. albicans*
	Benzene extract							
Crops	-	-	-	-	-	-	-	-
Citrus	1.8 ± 0.13^{c}	7.1 ± 0.62^{d}	-	-	7.2 ± 0.73^{b}	-	-	8.6 ± 0.20^{b}
Guava	15.1 ± 1.06^{a}	20.3 ± 1.07^{b}	10.4 ± 0.24^{b}	-	15.2 ± 1.05^{a}	-	7.6 ± 0.52^{a}	10.0 ± 0.94^{a}
Apricot Orchards	8.4 ± 0.31^{c}	7.3 ± 0.52^{d}	10.6 ± 0.94^{b}	7.4 ± 0.68^{b}	8.8 ± 0.63^{b}	-	-	-
Pear	7.2 ± 0.51^{c}	10.4 ± 0.8^{c}	7.4 ± 0.72^{cd}	-	7.2 ± 0.03^{b}	-	-	-
Mango	-	-	8.2 ± 0.71^{c}	-	-	16.6 ± 1.04^{a}	-	-
Canals banks	6.2 ± 0.02^{d}	7.3 ± 0.42^{d}	6.3 ± 0.31^{d}	-	-	-	-	-
Plant nurseries	-	-	7.5 ± 0.53^{e}	-	-	-	-	-
Public gardens	-	-	-	-	-	-	-	-
Wastelands	25.8 ± 2.07^{a}	24.4 ± 2.13^{a}	20.5 ± 2.26^{a}	15.5 ± 0.75^{a}	-	-	8.3 ± 0.42^{a}	10.0 ± 0.83^{a}
F-value	15.6**	10.34**	12.5***	8.4***	6.6***	11.8***	3.4***	5.8***
	Ethanol extract							
Crops	6.2 ± 0.42^{c}	-	7.6 ± 0.61^{c}	-	-	7.8 ± 0.62^{b}	6.5 ± 0.42^{c}	-
Citrus	-	-	10.5 ± 1.02^{b}	-	-	12.2 ± 1.14^{a}	-	-
Guava	12.1 ± 1.14^{b}	15.5 ± 1.14^{b}	10.0 ± 1.53^{b}	-	-	12.5 ± 1.13^{a}	20.8 ± 1.62^{a}	-
Apricot Orchards	6.4 ± 0.31^{c}	6.4 ± 0.52^{c}	8.6 ± 0.75^{bc}	7.6 ± 0.52^{b}	-	7.5 ± 0.44^{b}	7.4 ± 0.62^{c}	-
Pear	-	7.1 ± 0.03^{c}	-	-	-	-	-	-
Mango	-	7.3 ± 0.52^{c}	9.2 ± 0.80	9.5 ± 0.03^{b}	7.5 ± 0.62^{b}	-	8.7 ± 0.64^{c}	-
Canals banks	-	6.6 ± 1.02^{c}	-	-	-	-	-	-
Plant nurseries	-	-	-	-	-	-	-	-
Public gardens	-	-	-	-	-	-	-	-
Wastelands	20.05 ± 1.75^{a}	20.0 ± 1.89^{a}	13.6 ± 1.24^{a}	19.6 ± 0.08^{a}	17.0 ± 1.15^{a}	-	15.5 ± 1.06^{b}	-
F-value	13.6***	8.5***	7.4***	5.8***	4.2***	6.6***	5.2***	0.00
	Methanol extract							
Crops	-	-	-	-	-	-	-	-
Citrus	10.0 ± 0.82^{c}	13.5 ± 1.24^{b}	15.5 ± 1.04^{b}	9.6 ± 0.83^{c}	-	-	10.0 ± 0.84^{bc}	12.4 ± 1.14^{bc}
Guava	18.8 ± 1.52^{b}	-	9.6 ± 0.73^{c}	12.4 ± 1.03^{b}	20.0 ± 2.01^{b}	10.0 ± 0.94^{b}	10.0 ± 0.93^{bc}	18.8 ± 1.65^{a}
Apricot Orchards	-	-	7.7 ± 0.73^{cd}	8.7 ± 0.82^{c}	-	-	-	-
Pear	-	-	6.4 ± 0.51^{d}	7.3 ± 0.72^{c}	-	-	-	-
Mango	12.6 ± 1.03^{c}	8.7 ± 0.73^{c}	10.0 ± 0.94^{b}	14.4 ± 1.25^{b}	10.0 ± 1.06^{c}	-	8.8 ± 0.74^{c}	-
Canals banks	-	-	-	-	-	-	-	-
Plant nurseries	-	-	-	-	-	-	-	-
Public gardens	-	-	-	-	-	-	-	-
Wastelands	25.5 ± 2.16^{a}	22.2 ± 2.14^{a}	19.9 ± 1.87^{a}	15.5 ± 1.23^{a}	27.7 ± 2.51^{a}	27.7 ± 2.04^{a}	15.6 ± 1.33^{a}	-
F-value	35.25***	24.86***	3.91***	8.24***	6.28***	3.86***	4.52***	2.89***

** and ***: significant differences at $p < 0.01$ and 0.001, respectively. The means with the same letters in the same column are not significantly different according to Tukey's test.

chards exhibited a broad spectrum and the strongest antimicrobial activity. For the bacterial strains, the highest inhibition zones (25.5 ± 2.16, 22.2 ± 2.14, 19.9 ± 1.87, and 15.5 ± 1.23 mm) were observed against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*, respectively, when methanolic extracts from wasteland plants were applied. Likewise, the methanol extract from the wasteland plant against the tested fungal strains indicated that the highest inhibition zones (27.7 ± 2.51, 27.7 ± 2.04, and 15.6 ± 1.33 mm) were recorded for *A. flavus*, *S. racemosum*, and *P. chrysogenum*, respectively.

6. Discussion

Traditionally, *Bidens pilosa* is used to cure a wide range of disorders, with various formulations of the whole plant and/or its parts reported to treat over 40 ailments [12,18]. The phytochemical screening of the aqueous extract of *B. pilosa* roots found that terpenoids, phenols, saponins, and amino acids exhibited moderate, low, or no reaction with their reagents, depending on habitat type. In addition, alkaloids and sterols were not found in the root extract, which runs counter to the study by Ajanaku *et al.* [36], who observed these compounds in the aqueous root extract of *B. pilosa*. Conversely, all estimated phytochemical compounds in the shoot aqueous extract were intensively or moderately reacted based on the kind of habitat. Similar findings were reported by Ajanaku *et al.* [36] and Son *et al.* [37], who also noted that the presence or absence of phytochemical compounds depended on habitat type, solvent, solubility of the chemical categories, or their fractions. According to Pfoze *et al.* [38], the leaves and flowers of *B. pilosa* store high levels of antioxidants, polyphenolic compounds, and total flavonoids. Moreover, the concentrations of the 2^{ry} metabolites in the plant parts varied across collection sites. These results aligned with those of Olawale *et al.* [18], who found that *B. pilosa* shoots are the main source of phytochemical compounds. This finding is economically valuable for using the shoots of *B. pilosa* to extract considerable amounts of the estimated phytochemical components.

Plants are continuously subjected to adverse environmental conditions throughout their lives, such as strong light, ultraviolet (UV) radiation, dehydration, salt stress, and microbial diseases [39]. Plants adapt and flourish across diverse environmental conditions, in large part due to the production and fluctuation of secondary metabolites such as phenolics, flavonoids, terpenoids, and tannins [4]. Notably, the *B. pilosa* shoots from wastelands under extreme conditions, such as high salinity and drought, had the greatest level of total flavonoids, total tannins, alkaloids, and total saponins. Indeed, these metabolic processes enable plants to optimize stomatal control, osmotic adjustment, and pathogen defense under stressors such as drought and herbivory [40]. Simpson *et al.* [41] claimed that terpenoids and flavonoids are essential for the control of both abiotic and biotic stress responses. The main functions of flavonoids in plants are to scavenge free radicals and protect plants from sun radiation [42]. Moreover, tannins play a critical role in regulating plant life during plant–environment interactions [39] and in preventing various diseases by minimizing oxidative stress and restricting herbivore feeding as anti-digestive compounds, in addition to their antimicrobial properties [43]. Furthermore, tannins are synthesized in plants in response to environmental stresses, such as pollution, drought, and UV radiation, in habitats such as wastelands [3].

About 200 compounds, including flavonoids, terpenoids, aliphatics, aromatics, porphyrins, phenylpropanoids, and other compounds, have been isolated from *B. pilosa* [13,44]. In the present study, 80 phytochemical compounds were identified by GC–MS analysis in ethanol (50 compounds) and chloroform:methanol (30 compounds) extracts of *B. pilosa* shoots. Several compounds, such as tricosane, dotriacontane, docosane, octacosanol, docosanoic acid, octadecanoic acid, eicosanoic acid, hexadecanoic acid, (Z)-9-octadecenoic acid, (E)-5-octadecenoic acid, and phytol, identified in the present study, were also recognized by Bartolome *et al.* [13] in the same species. Fatty alcohols separated from both ethanol and chloroform:methanol extracts are used in medicines, cosmetics, skin care products, plasticizers, detergents, and fuels [45]. Certain siloxanes, such as octadecamethyl, cyclononasiloxane, and cyclooctasiloxane, hexadecamethyl, with high peak areas, may enable the plant to withstand herbicides [46].

A total of 22 compounds were aromatic, with benzene (1-methyldecyl) having the largest fraction. Moreover, 17 compounds were aliphatic, with the highest contribution of nonadecane. Additionally, two steroid compounds, 14α-H-pregna and hahnfett, were also recognized. These compounds exhibited high antimicrobial activity and were also found in many plants, such as *Duranta erecta*, *Allium rotundum*, and *Petrea volubilis* [47,48]. The enzyme activator tert-hexadecanethiol is a thiol (mercaptan) compound found in high concentrations in *B. pilosa* shoots and sugar cane [49]. A total of 3 alkaloid compounds (1H-purin-6-amine, (2-fluorophenyl) methyl > 2-piperidinone, N-(4-bromo-n-butyl) > 2-oxabicyclo (3.3.1) nonane, benzenemethane sulfona) were found in *B. pilosa* shoot extracts. These alkaloids with antimicrobial activity were found in higher concentrations than those of *Psidium guajava* (0.04%) and *G. latifolium* (0.12%) [50].

The amino acid glycine (aspartyl-phenylalanine) was detected in *B. pilosa* shoots in the ethanol extract. Glycine is a xenobiotic human metabolite that aids in the formation of bile acids, transforms benzoic acid to hippuric acid in the liver, combines with cholic acid to form glycocholate, and is used in the biosynthesis of heme, creatine, and purines [51]. Additionally, several studies indicate that glycine enhances the plant resistance to salinity,

drought, and cold temperatures through increasing the reactive oxygen species (ROS) scavenging system [52]. Furthermore, glycine regulates carbohydrate accumulation (sucrose, glucose, fructose), which can supply carbon rings for polyphenol production and serves as a source of energy [53]. Importantly, glycine promoted sugar accumulation, which may favorably enhance flavanol glycoside production by increasing glycoside availability and carbon availability [53]. Glycine applied topically may promote the accumulation of health-promoting chemicals and improve the antioxidative activity of lettuce, which may be advantageous for human nutrition [54].

Two terpenoids (heptadecane, 2,6,10,15-tetramethyl > phytol), and four flavonoid compounds {(Z)-5-ethylidene-3-hydroxy-4-(3'-methylbutanoyl)-2(5H)-furanone > a',a'-dimethyl-5-(methylsulfinyl)-2-furfuryl propargyl ether > 2H-pyran-2-carboxylic acid, 6-butoxy-3, 6-dihydro-, ethyl ester > 2-dodecen-1-yl(-) succinic anhydride} were found in the *B. pilosa* shoots. According to Shohaib *et al*. [55], flavonoids have several pharmacological activities against ageing, ulcers, fungi, bacteria, inflammation, hepatotoxicity, diabetes, tumors, and cancer. Moreover, flavonoids cure burns, scars, wounds, and ulcers, and act against leukemia and herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) infections [38,56]. In addition, nine compounds of organosilicons or siloxanes were detected in *B. pilosa*. Interestingly, silicone oil is the most common compound in this group, found at high percentage. The powerful antioxidant octadecamethyl cyclononasiloxane, found in *B. pilosa* shoots, was also reported in the essential oil of *Thaumatococcus daniellii* and is used in the food and pharmaceutical industries, as well as in traditional medicine in West African countries [57].

The antibacterial activity of medicinal plant extracts varied with the extraction solvent and the tested bacterium [37]. Methanol extracts showed the broadest spectrum and strongest antimicrobial activity, while ethanol extracts displayed low to moderate inhibition, with considerable activity against *S. aureus*, *B. subtilis*, and *P. aeruginosa*. Moreover, the non-polar benzene extracts exhibited the lowest activity, with limited inhibition, mainly against fungal strains. Similar findings for *B. pilosa* have also reported strong antimicrobial properties of the methanol extract and moderate activity of the ethanol extract [58,59]. Xuan and Khanh [60] recommend the *B. pilosa* plant as a viable source for identifying antimicrobial agents because the leaf extract exhibited strong antimicrobial activity against *S. aureus*, *P. aeruginosa*, and *E. coli* [61]. Shandukani *et al*. [62] stated that the lower antibacterial activities of *B. pilosa* are typically oriented towards the non-polar solvents. This is consistent with the dominance of antioxidant activities and phenolic content, which are separated more in the non-polar solvents. However, the benzene extract of the *B. pilosa* shoots from wastelands was effective against all evaluated bacterial strains. The identified 2^{ry} metabolites, including terpenoids, steroids, and flavonoids, may be responsible for the antibacterial activity of *B. pilosa* extracts [37]. Moreover, the ethanolic extract had high efficacy against *S. aureus* in accordance with da Silva *et al*. [63]. Certain extracts from *B. pilosa*, such as methanolic, aqueous, and ethanolic extracts, have been demonstrated to have potent free radical-scavenging properties, indicating their ability to block ROS and prevent oxidative damage [12,64].

7. Conclusion

The phytochemical screening indicated that terpenoids, flavonoids, phenols, tannins, alkaloids, sterols, saponins, carbohydrates, and amino acids were more abundant in the aqueous extract of *Bidens pilosa* shoots than in the roots. It was found that the *B. pilosa* shoots are a storehouse of phytochemicals. The GC–MS analysis of the ethanol extract of the plant shoots comprised 50 phytochemical compounds; however, 30 compounds were identified in the chloroform:methanol extract. Most of these compounds benefit human health, both in nutrition and in medical use. Two steroid compounds (14α-H-pregna and hahnfett), which are toxic and carcinogenic at high concentrations, were recorded at low concentrations, thereby demonstrating benefits for human health. Therefore, to use *B. pilosa* shoots in human nutrition and medicine, they must be tested for safety due to the presence of toxic and carcinogenic compounds, and the safe dose must be calculated to prevent cumulative effects. These findings have strengthened the scientific foundation and therapeutic value of *B. pilosa*, as well as the potential of the plant for use in creating functional foods with antioxidant properties or the use of the plant extract as a substitute for antibiotics in the treatment and prevention of microbial illnesses.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

AK and RM designed the research study. AK, EE, DB and RM performed the research. AK, EE and RM conducted experiments. AK and TG analyzed the data and write the draft. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The plant materials of *Bidens pilosa* were gathered from different Egyptian habitats with personal permission. According to Egyptian regulations and policies, this study

does not involve human or animal subjects; therefore, ethical committee approval is not necessary.

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Conflict of Interest

The authors declare no conflict of interest.

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