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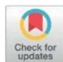

Original Article

Phytochemical Analysis of Essential Oil, Total Phenol and Flavonoid Content, Antibacterial, Antimalarial, and Antioxidant Activities of *Chenopodium album* and *Bassia prostrata* Methanol Extracts

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Abstract

Introduction: *Chenopodium album L.* and *Bassia prostrata L. Beck* from the Chenopodiaceae family are widespread species in Asia. This study aimed to assay the total phenol and flavonoid contents, the activities of the antioxidant, antibacterial, and antimalarial methanol crude extracts, and to determine the chemical composition of the essential oil of *C. album* and *B. prostrata*.

Materials and Methods: Total phenol and flavonoid contents were assayed via the Folin-Ciocalteu colorimetric and Modified Woisky and Salatino $AlCl_3$ colorimetric methods. Appropriate total phenolic and flavonoid content was confirmed to perform antibacterial and antimalarial tests. Antibacterial susceptibility test, determination of minimum inhibitory concentration (MIC), and determination of minimum bactericidal concentration (MBC) were performed on 5 Gram-positive strains and 5 Gram-negative strains of bacteria. The antimalarial potential was assessed using the heme detoxification inhibition (ITHD) test, and antioxidant potential was measured by the DPPH assay.

Results: The results represented the inhibitory effects of *C. album* and *B. prostrata* methanolic extracts on *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, two important pathogenic bacteria in humans. Although they did not present antimalarial activity through ITHD, both extracts exhibited weak antioxidant activity. *B. prostrata* showed much better antioxidant potential (IC_{50} = 134.4 μg/ml). The analysis of essential oil of *C. album* and *B. prostrata* by Gas chromatography/mass spectrometry revealed 14 and 26 compounds, mostly diterpenes.

Conclusions: The findings of our study suggest that these plants are good sources of natural compounds with antioxidant and antibacterial properties, specifically antipseudomonal, that could be considered as therapeutic agents.

Keywords: Antibacterial Activity, Antimalarial Activity, Antioxidant, *Bassia prostrata*, *Chenopodium album*, Essential Oil

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Introduction

Herbal medicine is becoming more prevalent in developing nations as a supplemental therapy alongside conventional therapies. There is an ongoing endeavor to investigate alternative natural and holistic choices, prompted by the harmful adverse effects of conventional chemical medications and pharmaceuticals. Plant-based natural products are organic substances that originate from various natural sources, either as intricate combinations obtained from unprocessed components or as individual compounds.[1,2] The utilization of medicinal plants by humans can be traced back to ancient times as a means to treat various ailments. Subsequently, these invaluable resources have successfully preserved countless lives by combating severe ailments including infections and cancer. It is still considered highly relevant in the current era as an essential means of healthcare for almost 80% of the global population, as stated by the World Health Organization (WHO).[3-6] Additionally, it serves as a valuable source for generating novel pharmaceuticals, with 80% of all synthetic drugs originating from this source. In recent centuries, there has been a significant increase in investigation, advancement, and refinement of the analysis of herbal ingredients.[7] Recently, there has been an increasing

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demand to study natural products to discover active substances with antibacterial, antioxidant, and anticancer effects derived from plants.[8,9] Phytochemicals, which are natural molecules derived from medicinal plants, offer a viable alternative to a range of pharmaceutical medications. These chemicals have intriguing biological properties and have the potential for use in medicine.

Antibacterial Drug Resistance (ADR) is a pivotal issue that has emerged in hospitals and occurred in community settings. Increasing mortality due to ADR, high costs of hospitalization, and severe adverse effects of some anti-bacterial agents are the major causes that require discovering new, more potent, and safe antibacterial agents. Among the medicinal plants' chemical compounds with antioxidant capacity, flavonoids and other phenolic compounds have an antibacterial potential.[10-13] Essential oils, complex mixtures of volatile compounds from plants, have been used for centuries in various applications, including medicine, agriculture, and industry.[14] Recent studies have proven their usage in traditional medicines as antibacterial agents and demonstrated their effects on major diseases such as cardiovascular disease, rheumatoid arthritis, Non-Alcoholic Fatty Liver Disease (NAFLD), Alzheimer's, cancers diabetes, and skin disorders.[15-23]

The *Chenopodiaceae* family consists of flowering herbs distributed in temperate steppes and deserts worldwide.[24] This family includes about 100 genera and 1500 species.[25] *Chenopodium album* L. (*C. album*) from Chenopodiaceae is an annual shrub that grows in Asia, Africa, Europe, and North America.[26] This herb is used as a hepatoprotective, diuretic, and anthelmintic agent in traditional medicine.[27] A study revealed that aqueous and methanol extracts of *C. album* had significant effects against bacterial strains.[28] According to Külcü *et al*., various extracts of *C. album* leaves were potent antimicrobials against 10 bacterial species.[29] *Bassia prostrata* (M. Bieb.) Fisch. (*B. prostrata*) is an annual perennial shrub of Chenopodiaceae. This herb germinates in deserts from March to April; moreover, it is an Eurasian herb, which its main use is as animal feed.[30,31] In this study, we aimed to quantify the total phenol and flavonoid contents of crude methanol extracts of *C. album* and *B. prostrata*, assaying their antimalarial, antibacterial, and antioxidant activities. We also extracted essential oils of *C. album* and *B. prostrata* and identified their chemical components to find common compounds.

Materials and Methods

Methanol and ethanol of HPLC grade were purchased from Chem-Lab, Belgium. DPPH, hemin, chloroquine diphosphate, rutin, and Tween 20 and 80 were obtained from Sigma-Aldrich Company, USA. Other materials such as *n*-hexane, dimethylsulfoxide (DMSO), Folin-Ciocalteu reagent, gallic acid, $AlCl_3$, MHA medium (Mueller-Hinton Agar), and MHB (Muller-Hinton Broth) were provided by Merck, Germany.

Plant Materials

All parts of *C. album* (Voucher Specimen No. 48404), and all parts of *B. prostrata* (No. 48405) were harvested from Karaj city (Alborz state, Iran), and voucher specimens were deposited. The herbs were cleaned of debris, then stored in a dry and dark place for about two weeks to dry, and finally milled to a fine powder.

Extraction

Essential Oil

C. album and *B. prostrata* dried powder (200 g) were separately soaked in 1000 ml water in a flask overnight. They were then subjected to the Clevenger-type apparatus as recommended by the British Pharmacopoeia[32] and hydro-distillated for 5 hours with *n*-Hexane (2 ml) following the method described by Guan *et al.*[33] for better extraction. The obtained essential oils were dried over anhydrous sodium sulfate and immediately injected into a GC/MS system using a Hewlett-Packard 6890/5972 system, split-less, with helium as the carrier gas at a flow rate of 0.8 ml/min and a DB-5 fused silica column (30 m × 0.5 µm, film thickness 0.33 mm). The column temperature was programmed to rise at a rate of 5 °C/min from 60 to 260 °C. The quadrupole mass spectrometer was set to m/z 35 –360 amu with an ionizing voltage of 70 eV and an ionization current of 150 mA. The essential oil components were identified by comparing their mass spectra fragmentation pattern with the Wiley 275. L library.

Crude Extract

100 g of finely powdered parts of each plant were immersed in 300 ml of 80% methanol and mixed for 24 hours on a shaker (GFL, Germany) at room temperature (maceration), and this process was repeated two more times. They were filtered (Whatman, UK), and each extraction's solvents were concentrated under reduced pressure using a rotary evaporator (Heidolph, Germany). The concentrated extracts were dried in a dry oven at 42 °C to remove the solvent. The resulting extracts were kept in sterile and light-protected containers at 4 °C for further tests.

Antibacterial Assay

Bacterial Strains and Culture Media

Five Gram-positive strains of lyophilized bacteria, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Kocuria rhizophila* ATCC 9341, *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* ATCC 1247, as well as five strains of Gram-negative lyophilized bacteria, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enterica* ATCC 19430, *Klebsiella pneumoniae* ATCC 10031 and *Salmonella typhimurium* ATCC 14028,

were obtained from the Iranian Research Organization for Science and Technology, Persian Type Culture Collection, Tehran, Iran. They were cultured in MHA medium and incubated at 37 ° C for 18–24 h.

Antibacterial Susceptibility Tests

Pre-evaluation of the antibacterial activity of the extracts was performed using the Well-Plate method.[34] The wells (8mm diameter) were made in MHA medium, streaked with a saline suspension of microorganisms with turbidity equivalent to a 0.5 McFarland standard. The wells were filled with 100 µl of different concentrations of extracts, including 500, 250, 125, and 62.5 mg/ml dissolved in distilled water (DW) or DW and Tween 80 (D/T) (80:20), and the solvents were used as negative controls. Plates were incubated at 37 °C for 18-24 h. The test was repeated three times and the average diameters of the inhibition zones for each concentration were reported.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC values of the extracts were determined using the broth microdilution method, according to CLSI (Clinical Laboratory Standardization Institute) guidelines.[34,35] Inoculums with turbidity equivalent to 0.5 McFarland standard (1.5×10^8 CFU/ml) were prepared by making a direct broth suspension of isolated colonies selected from 24 h of cultured bacteria on an MHA plate. Each extract was dissolved in MHB medium to make concentrations equal to 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.95, 0.97, and 0.48 mg/ml (One row was considered for evaluation of extract sterility). The bacterial suspensions corresponding to 0.5 McFarland were diluted 20 times with sterile normal saline. 10 µl of each bacterial suspension was added to each well of the microplate. A column without herb extracts was considered a growth control. The microplates were incubated at 37 °C for 24 h. The MIC was determined to be the lowest extract concentration that inhibits the visible growth of microorganisms. Each test was performed in triplicate.

Determination of Minimum Bactericidal Concentration (MBC)

To confirm MICs and establish MBC, 20 µl of each well with no visible growth was added to the MHA medium, and plates were incubated at 37 °C for 24 h. MBC was defined as the lowest concentration of extract at which no bacterial growth was observed. Each experiment was performed in triplicate.[36]

Antimalarial Assay

The antimalarial effect of the herbal extracts was evaluated using the ITHD (Inhibition Test of Heme Detoxification) method.[37] Briefly, the samples were dissolved in DMSO at 200 µg/ml. A 120 µg/ml hemin solution in DMSO was prepared and diluted with acetate buffer (1 M, pH 4.8) to 60 µg/ml. Tween 20 was diluted with distilled water to 0.012 g/L. A 96-well microplate was filled with sample solution (DMSO), three times diluted Tween 20, and hemin, respectively, with a ratio of 2:9:9. Additionally, to eliminate sample matrix residual, a control was prepared without hemin under the same conditions. Chloroquine diphosphate was used as a positive control and DMSO (sample solvent) was added to negative control wells. After 24 h of incubation of the plates at 60 °C, their absorbance was measured with an ELISA reader adjusted to 405 nm.

Total Phenolic Content Assay

The total amounts of phenolic compounds in each extract were determined using the Folin-Ciocalteu colorimetric method.[38] The calibration curve was obtained with gallic acid (Merck, Germany) as the standard. Phenolic compounds in the extracts were analyzed in triplicate and calculated according to the line diagram equation. Total phenolic contents were expressed as a percentage of gallic acid equivalents in dry extract matter.

Total Flavonoid Content Assay

The modified Woisky and Salatino[39] $AlCl_3$ colorimetric method was used to measure the total flavonoid content. A standard curve was prepared using rutin in an ethanol (80%) solution. Standard solutions were prepared by dissolving 10 mg of rutin in ethanol and diluting to 100, 50, and 25 µg/ml. Then, 0.5 ml of each standard solution was mixed with 0.1 ml of potassium acetate (1M), 0.1 ml of aluminum chloride (10%), 1.5 ml of ethanol (95%), and 2.8 ml of distilled water. The same amount of distilled water was replaced by 10% aluminum chloride in the blank. After 30 minutes of incubation at room temperature, the mixture's absorbance was measured with a UV spectrophotometer (Shimadzu, Japan). To determine the flavonoid content, 0.5 ml of methanol extract was reacted with $AlCl_3$.

Antioxidant Activity Assay

The sample's ability to eliminate free radicals was measured using stable diphenyl-1-picrylhydrazyl (DPPH*) radicals by adding 1 ml of 0.3 mM DPPH* solution to samples provided in a 2.5 ml volume at concentrations of 6.25-400 µg/ml (in 90% alcohol) by serial dilution. These tubes were then incubated at 25 °C for 30 minutes, and their absorbance values were measured at 517 nm using a UV-Vis spectrophotometer.[40,41] Gallic acid and rutin were used as positive controls. The DPPH* percentage inhibition (I_{DPPH*} %) was calculated using the following formula:

$$I_{DPPH}(\%) = \left[\frac{A_C - (A_S - A_B)}{A_C}\right] \times 100$$

The A_C, A_S, and A_B respectively belong to the absorbance

of the control, sample, and blank sample, respectively.

Statistical Analysis

GraphPad Prism software is used to report the results as mean ± standard deviation (SD). $p \leq 0.05$ was considered to be statistically significant.

Results

Chemical Composition of Essential Oil

The outcome of *B. prostrata* and *C. album* hydro-distillation was 1.2 and 0.5 ml of pale yellow oils, respectively. They had 26 and 14 identified compounds, which accounted for 85.2% and 66.3% of the total identified compounds, respectively (Table 1). *B. prostrata* contained five monoterpenes, four sesquiterpenes, and four diterpenes, while *C. album* contained one monoterpene, two diterpenes, and one steroid (Table 2).

Table 1. Chemical Compounds of *C. album* and *B. prostrata* all Parts Essential Oil Analyzed by GC/MS

Compound	RT	Content (rel. %)		Biological Activity
		B. prostrata	*C. album*	
Tanshindiol A	9.11	-	3.3	NR[1]
Decanal	11.26	3.1	-	NR
2,6-Dihydroxy benzoic acid	14.15	2.1	-	NR
2,3-Dihydro furan	14.57	0.9	-	NR
α − Cyclocitral	14.88	-	1.7	NR
Pinocamphone	14.93	1.2	-	Epileptigenic and Neurotoxic*
Thymohydroquinone	16.20	0.5	-	Antibacterial, Antiperiodontic, Antistreptococcic
Carvacryl acetate	18.15	1.1	-	NR
Pentadecane	20.92	0.4	-	NR
Perhydrofarnesyl acetone	21.17	3.8	-	NR
Geranyl acetone	22.34	3.7	-	Besides its perfume,[70] it has a sedative effect.*
β-Ionone	23.28	17.6	13.0	Antibacterial, Anticariogenic,[71] Antitumor, Hypocholesterolemic,[72] Cytochrome-P450 Inducer*
2-Butyl-2-octenal	23.66	1.4	7.8	NR
Curdione	23.92	1.7	-	Antitumor (Cervix cancer),[73] Antileukopenic[74]
2,6,10-Trimethyl tetradecane	24.21	0.7	-	NR
Isolongifolene	25.77	2.7	-	NR
Nonadecane	27.24	2.4	1.6	NR
Cuparene	28.12	1.4	-	Anti-inflammatory*
(E)-Phytol	28.89	4.3	-	NR
2-(Octadecyloxy) ethanol	29.77	-	1.5	NR
Steroid**	30.04	-	2.6	NR
Stearyl alcohol	32.52	-	0.8	NR
Isophytol	33.22	5.9	-	NR
Farnesyl acetone A	34.57	1.3	-	NR
Methyl palmitate	34.95	4.3	8.9	NR
Oleic acid	36.14	5.4	-	Hypocholesterolemic,[75] 5-Alpha-Reductase-Inhibitor, Anti-inflammatory, Antileukotriene-D4, Antialopecic*
Linolenic acid	38.76	4.9	1.7	Antiaggregant, Hypotensive, Immunostimulant, Lymphocytogenic,[76] Antimenorrhagic, Antiprostatitic,[77] Prostaglandin-Synthesis-Inhibitor[78]
Neophytadiene	39.37	13.1	18.2	Antibacterial[47]
Methyl stearate	39.60	-	3.3	NR
Heptacosane	39.85	-	0.4	NR
n-Nonadecane	41.60	0.6	-	NR
n-Heneicosane	43.62	0.5	-	NR
Docosane	47.22	0.2	-	NR
16,17-Didehydrocuran	47.55	-	1.5	NR
Total compounds		**85.2%**	**66.3%**	

[1]NR: not reported. *The biological effects are based on Dr. Duke's phytochemical and botanical databases.[79] ** This steroid could not be accurately identified with the mass spectrophotometer used in this study.

Table 2. *C. album* and *B. prostrata* Essential Oils Chemical Content Classification

Compounds	Contents (%)	
	B. prostrata	***C. album***
Aliphatic	4.7	1.6
Terpenes	59.1	35.5
• Monoterpene	24.2	13.0
• Sesquiterpenes	9.5	0
• Diterpenes	25.4	19.9
• Triterpenes	0	2.6
Other Compounds	21.4	29.2
Total	85.2	66.3

Yield of Extraction

The extraction yield of *C. album* and *B. prostrata*, respectively, was 12.91 and 9.62% w/w.

Antibacterial Activity

Inhibition Zone Diameters

The pre-evaluation of the antibacterial activity of samples

using the cup-plate technique is presented in Table 3. In all cases, the inhibition diameter increased with increasing concentration. *C. album* extract showed antibacterial activity against *P. aeruginosa*, *S. epidermidis*, and *B. subtilis* at all examined concentrations, while this plant remarkably inhibited *P. aeruginosa*, *S. typhimurium*, *E. coli*, and all tested Gram-positive species at 500 mg/ml. On the other hand, *B. prostrata* extract was effective at this concentration only against *S. aureus*, *S. epidermidis*, *K. rhizophila*, *S. typhimurium*, *P. aeruginosa*, and *S. enterica*. The highest activities of the *C. album* and *B. prostrata* zones of inhibition were 33.33 ± 2.1 mm and 20.33 ± 1.1 mm, respectively, recorded against *P. aeruginosa* at 500 mg/ml.

MIC

The results of the *C. album* and *B. prostrata* extracts minimum inhibitory concentration determination are shown in Table 4. According to this table, *E. coli* and *P. aeruginosa* were the most sensitive species to *C. album* at 62.5 mg/ml, and the least sensetive was *K. rhizophila* at 250 mg/ml. On the other hand, *K. rhizophila* was the most sensitive species to *B. prostrata* at 31.25 mg/ml, and then *S. epidermidis* and *P. aeruginosa* at 62.5 mg/ml. Other bacteria species were inhibited at 125 mg/ml of each extract.

MBC

MBC values of *C. album* and *B. prostrata* extracts are shown in Table 4. *C. album* extract was only effective against Gram-negative species at 500 mg/ml; however, *B. prostrata* was effective against both Gram-positive and Gram-negative species. The most sensitive species to *B. prostrata* was *S. epidermidis* at 62.5 mg/ml.

Table 3. The Mean (±SD) of Inhibition Zone Diameter of Methanol Extracts of *C. album* and *B. prostrata* in Gram-Negative and Positive Bacteria (mm) Based on the Cup-Plate Technique, the Test Was Carried out in Triplicate

Bacteria	The extracts concentrations (mg/ml)							
	C. album				***B. prostrata***			
	62.5	125	250	500	62.5	125	250	500
E. coli	-	-	-	11.7 ± 2.0	-	-	-	-
P. aeruginosa	18.0 ± 0.0	20.3 ± 1.1	24.0 ± 1.7	33.3 ± 2.1	16.3 ± 1.2	17.3 ± 1.2	19.7 ± 0.6	20.3 ± 1.1
S. enterica	-	-	-	-	12.0 ± 1.0	15.3 ± 0.6	16.0 ± 0.0	16.7 ± 0.6
K. pneumoniae	-	-	-	-	-	-	-	-
S. typhimurium	-	-	-	15.7 ± 1.5	-	-	-	18.7 ± 0.6
S. aureus	-	-	9.7 ± 0.6	10.7 ± 1.1	-	-	-	12.0 ± 0.0
S. epidermidis	9.3 ± 0.6	10.0 ± 0.0	11.0 ± 0.0	15.0 ± 0.0	13.0 ± 0.0	14.3 ± 1.1	15.7 ± 0.6	17.0 ± 0.0
K. rhizophila	-	-	-	11.0 ± 1.7	10.3 ± 0.6	10.7 ± 0.6	12.0 ± 0.0	12.0 ± 1.0
B. subtilis	9.7 ± 0.6	11.0 ± 1.0	12.0 ± 1.0	12.3 ± 06	-	-	-	-
B. cereus	-	-	-	10.0 ± 0.0	-	-	-	-

Table 4. The Mean of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Methanolic Extracts of *C. album* and *B. prostrata* Against Different Types of Bacteria (mg/ml), Test Was Carried out in Triplicate

Bacteria	The extracts concentrations (mg/ml)			
	MIC		MBC	
	C. album	***B. prostrata***	***C. album***	***B. prostrata***
S. aureus	125	125	ND*	500
S. epidermidis	125	62.5	ND	62.5
K. rhizophila	250	31.25	ND	500
B. subtilis	125	125	ND	500
B. cereus	125	125	ND	500
E. coli	62.5	125	500	250
P. aeruginosa	62.5	62.5	500	125
S. enterica	125	125	500	125
K. pneumoniae	125	125	500	500
S typhimurium	125	125	500	500

*ND: Not Determined

Antimalarial Activity

In this method, if the percentage of the Inhibition Test of Heme Detoxification (ITHD) was more than 90%, the assay would be considered positive, whereas values less than 90% indicated a negative result. The ITHD results of the two extracts show that they do not have antimalarial activity with this mechanism, whereas their chloroquine assay was positive.

Total Phenol and Flavonoid Content

The total phenol and total flavonoid results were represented as gallic acid equivalent and rutin equivalent in dry extract matter in Table 5.

Table 5. Total Phenol (TP) and Total Flavonoid (TF) Content of *C. album* and *B. prostrata* Methanolic Extracts

Plants	Contents (%)	
	TP[1]	TF[2]
C. album	4.22	3.31
B. prostrata	4.67	4.26

[1]Total Phenolic Content; [2]Total Flavonoid Content

Antioxidant Activity

The antioxidant properties of *C. album* and *B. prostrata*

were evaluated using the DPPH method. After processing the data, the final results (Figure 1) demonstrated that *B. prostrata* (IC_{50} = 134.4 µg/ml) has better DPPH radical scavenging capability than *C. album* (IC_{50} = 153.1 µg/ml).

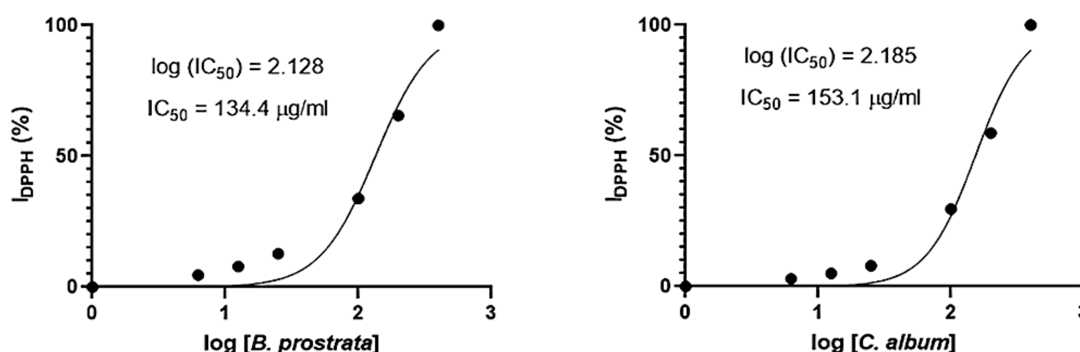

Figure 1. DPPH Inhibition Percentage Diagrams of *B. prostrata* and *C. album* Methanol Extracts.

Discussion

There is a growing trend in numerous countries to document the therapeutic application of medicinal herbs for their pharmacological attributes. According to the WHO, almost 25% of prescription medications are derived from plants.[42] In this study, we determined the phytochemical contents of both *C. album* and *B. prostrata* and assessed the biological activity of these plants. The phytochemical evaluation revealed that the essential oil of *C. album* and *B. prostrata* collected in Iran contained 14 and 26 different compounds, respectively. They had six compounds in common, Neophytadiene, *β*-Ionone, Methyl palmitate, linolenic acid, nonadecane, and 2-butyl-2-octenal, which together accounted for more than 40% of *B. prostrata*'s and 50% of *C. album*'s essential oil content. *β*-Ionone (a rose ketone) in *B. prostrata* and Neophytadiene in *C. album* were the most abundant compounds in each essential oil, and both showed to have significant antioxidant[43,44] and antibacterial activity.[45,46] The antibacterial activity of Neophytadiene is attributed to its ability to disrupt bacterial cell membranes and interfere with metabolic processes within the bacteria.[47] It assumed that the *β*-Ionone is involved in the disruption of bacterial cell membranes, leading to increased permeability and eventual cell lysis.[48] The essential oil of *B. prostrata* and its analysis have been done for the first time in this paper, therefore there is no recent study to compare. In a study of the essential oil composition of *C. album* subsp. *Striatum* analyzed, which represented 97.09% of the total oil,[49] which was much higher than our studied plant. Yıldız et al. evaluated the essential oil composition of *Onosma bulbotrichum* and *Onosma isaurica*, where the neophytadiene was one of their main constituents.[50] Neophytadiene has also been observed in *Jatropha curcas*.[51] Additionally, GC-MS results indicated that terpenes were the most predominant chemical group presented in the essential oil of both *C. album* and *B. prostrata* (35.5% and 59.1%, respectively). Accordingly, Drioua et al. reported that terpenoids are the significant compounds of the aqueous *C. ambrosioides* extract.[2]

Phenolic compounds and flavonoids are responsible for most bioactivities, such as antioxidant and antimicrobial activity (through disruption of both microbial-cell membrane[52] and energy metabolism[53] and inhibition of nucleic acid synthesis[54]) and UV radiation absorption to protect herbs against external and internal stresses.[55,56] Based on the results, *B. prostrata* contained these compounds more than *C. album*; besides, it has shown better antioxidant and antibacterial results. According to Seitimova et al.,[57,58] in two different studies, the flavonoid content of *B. prostrata* harvested from Almaty (Kazakhstan) was measured, and both results were less than the Iranian ones in this research. The DPPH method was used to study the antioxidant activity of the extracts of these two species. The calculated IC_{50} for *C. album* was considerably higher than *B. prostrata*, which specified that *B. prostrata* is a relatively suitable antioxidant. This is the first study to survey and represent the antioxidant activity of *B. prostrata.* However, Lone et al.[59] reported fewer IC_{50} and Nowak et al.[60] represent a higher percentage of antioxidant activity for *C. album*, but Amodeo et al.[61] and Amodeo et al.[61] have reported less antioxidant activity than our results for *C. album*. These varied findings could be owing to the different chemical composition of this herb, diverse growth, and ecological conditions.

Natural sources, especially herbs, are always in a prime position to discover new active pharmaceutical components. Given that ADR is a global growing concern, addressing this issue with herbal compounds is logical. While our tested extracts did not show antimalarial activity using the ITHD method, Parveen et al. reported the positive antimalarial effects of various *C. album* extracts using the MTT method.[62] On commonly affecting bacteria, *B. prostrata* was more efficient in inhibiting *P. aeruginosa*, *S. epidermidis*, *S. enterica*, and *K. rhizophila*, although *C. album*'s suppressive

effects on *P. aeruginosa* were significantly greater than *B. prostrata*. The bactericidal activity of *B. prostrata* on *S. epidermidis* was notable, while *C. album* had a better effect on *B. subtilis*, *B. cereus*, and *E. coli*. A higher presence of phenols, flavonoids, and β-Ionone in *B. prostrata* could explain the noteworthy bactericidal activity against gram-positive bacteria compared to *C. album*. Saini et al. testified to the antibacterial activity of different extract types of *C. album* using the disc diffusion method against *B. subtilis*, *E. coli*, and *P. aeruginosa*. The methanol extract displayed the highest antibacterial activity against all tested strains.[63] Külcü et al. surveyed to determine the antibacterial potential of *C. album* hexane, chloroform, and ethanol extracts using disc diffusion and broth dilution methods. The ethanol extract was the strongest against *E. aerogenes* (MIC: 128 µg/ml), while the weakest activity was observed with the chloroform extract against *S. aureus* (MIC: 1024 µg/ml).[29]

Hussain et al.[64] showed that different concentrations of methanol extract of indigenously cultivated *C. album* (from Faisalabad, Pakistan) could inhibit the growth of both *E. coli* and *S. aureus*, with inhibitory zones much larger than our findings. Akbaş et al.[65] demonstrated the antimicrobial effects of *C. album* (from Kars, Türkiye) and found that while the minimum inhibitory concentration (MIC) for both *E. coli* and *S. aureus* was similar to our results, they recorded a much lower MIC for *S. aureus*, *B. subtilis*, and *B. cereus*. Imran et al.[66] reported that *B. prostrata* harvested from Pakistan had a stronger effect on *S. aureus*, *E. coli*, and *B. subtilis*. In this study, the *C. album* collected from Iran showed better effects in the examination compared to the Indian *C. album* in Sing et al.[67] and the Turkish *C. album* in Korcan et al.,[68] highlighting the influence of different ecological settings on the phytochemical components and biological activity of a plant species. Genetic variability of the plant, environmental factors, and microbial resistance differences between regions may also contribute to the variation in methanolic extract activity against different bacterial strains. Matotoka et al. demonstrated that acetone leaf extracts of *C. bispinosa*, *S. peterisana*, G. *volkensii*, and *C. glabrum* had broad-spectrum antibacterial activities against *S. aureus*, *E. coli*, *P. aeruginosa*, and *M. smegmatis*.[69] The primary limitation of the present study was that the anticancer effects of these extracts against various cancer cell lines were not evaluated. While antibacterial activity was assessed against various species, more serious and resilient species should be exposed to the investigated herbal extracts. Furthermore, the antifungal activity of these plants needs to be determined. Nevertheless, the outcomes of this study would help us to design further studies with a focus on specific anticancer and antimicrobial activity.

Conclusion

In this research, the results revealed that *C. album* and *B. prostrata* methanol extracts have suitable antioxidant and antibacterial activity. This is likely due to the presence of phenols and flavonoids in these extracts. Additionally, some antibacterial compounds were found in the essential oils. The promising biological activity of *C. album* and *B. prostrata* suggests that they could be used as medicinal plants and pharmaceutical agents. These findings encourage further biochemical and pharmacological studies, including investigations into their anticancer potential and antibacterial efficacy against resistant bacterial strains.

Authors' Contributions

EK: Experimental analysis, preparation of the manuscript draft, and data interpretation; RM: experimental analysis, preparation of the manuscript; FM: Study supervision and revision of the manuscript; AM: Study supervision and revision of the manuscript; ES: Original idea presentation, study design, study supervision, experimental analysis, data interpretation and revision of the manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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