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Phytochemicals, bioactive compounds, and antimicrobial activities of *Ocimum basilicum*, *Teucrium polium*, *Cleome amblyocarpa*, and *Caralluma arabica* extracts: a comparative Omani study

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Abstract

The study aims to identify and quantify the phytochemical components of Ocimum basilicum, Teucrium polium, Cleome amblyocarpa, and Caralluma arabica extracts and to evaluate the antimicrobial activities of these Omani plants. The total phenolic content, flavonoid content, and tannin levels were quantified in both water and absolute ethanol extracts. The bioactive compounds present in the aerial parts of these plants were identified and characterized using liquid chromatography coupled with electrospray ionization mass. The antimicrobial properties were explored via the agar diffusion approach. The absolute ethanol extracts demonstrated higher phytochemical content compared to the water extracts for all plants. Ocimum basilicum revealed the highest quantities of total phenolic acids and flavonoids, followed by Teucrium polium, Cleome amblyocarpa, and Caralluma arabica. Quinic acid was detected in substantial quantities across all extracts, while three flavonoid compounds—1,3-di-O-caffeoylquinic acid, acacetin, and naringenin—were identified in all extracts, albeit in varying concentrations. Furthermore, the ethanolic extracts exhibited potent antimicrobial activity on the tested bacterial and fungal species. Staphylococcus aureus showed the highest sensitivity to Caralluma arabica extracts (22±0.1 mm). Staphylococcus aureus and Escherichia coli were the most vulnerable strains to Ocimum basilicum extracts (21±0.2 mm and 20±0.2 mm, respectively). Ocimum basilicum extracts demonstrated the best minimum inhibitory concentration (MIC: 1.28 mg/ml against Staphylococcus aureus and Salmonella enteritidis) and minimum bactericidal concentration (33.5 mg/ml against Salmonella enteritidis). Additionally, the Teucrium polium extract exhibited the lowest MIC (3.25 mg/ml) and minimum fungicidal concentration (17.28 mg/ml) against Fusarium spp. In conclusion, the aerial parts of Ocimum basilicum and Teucrium polium were rich in bioactive compounds, exhibited strong antimicrobial activity, and hold great potential for ethnomedicinal applications, warranting further investigation.

Keywords: *Ocimum basilicum*, *Teucrium polium*, *Cleome amblyocarpa*, *Caralluma arabica*, Phytochemicals, LC-ESI-MS analysis, Antimicrobial activity.

1. Introduction

Chronic health conditions are associated with cancer, which is typically treated with chemotherapy, radiation, and/or surgery [1, 2]. However, these treatments can lead to side effects such as neuropathy, myelosuppression, organ toxicity, and gastrointestinal disturbances, which negatively affect patients' quality of life [3]. There is growing interest in herbal medicine as an alternative for managing cancer and improving patient quality of life [4]. Herbal remedies are believed to inhibit tumor growth, prevent tumor recurrence, and reduce metastasis risk. Additionally, herbal therapies can alleviate the side effects of chemotherapy and radiotherapy as well as minimize complications after tumor removal surgery [3]. These approaches are favored for their non-toxic, renewable, and biodegradable qualities [4].

Herbal plants constitute a valuable reservoir of bioactive compounds, involving phenolic acids, flavonoids, and tannins, which impart antimicrobial and anticancer properties by scavenging free radicals [5, 6]. Additionally, these plants exhibit antioxidant activity, effectively neutralizing harmful species like superoxide anions, hydroperoxides, and hydroxyl radicals. To identify these bioactive compounds, plant material extraction is typically performed using solvents, such as water, methanol, acetone, or ethanol [7]. Both absolute ethanol and water have been shown to be effective and safe solvents for extracting polyphenols [6, 8]. Recent research has focused on evaluating wild plants native to tropical regions, such as *Ocimum basilicum*, *Teucrium polium*, *Cleome amblyocarpa*, and *Caralluma arabica* [9-14].

Basil (*Ocimum* spp.) grows naturally in the tropical and

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subtropical climates of South and Central America, Africa, and Asia. Basil has been widely used as a complementary treatment alongside conventional therapy [15]. Guardado et al. [12] and Zagoto et al. [16] demonstrated that basil could be used to treat kidney disorders, fever, menstrual irregularities, malaria, and the flu. Additionally, research by Al-Subhi [17] and Shahrajabian, et al. [18] emphasizes the medicinal value of basil extracts, highlighting their antioxidant, antidiabetic, anti-inflammatory, and anticancer potentials. Basil contains a variety of chemical compounds responsible for its distinct aroma and flavor. Vázquez-Fresno et al. [19] identified key compounds like methyl cinnamate, linalool, and estragole, while Ghasemzadeh et al. [20] noted additional bioactive compounds, including geraniol, limonene, methyl chavicol, eugenol, 1,8-cineole, and camphor. Varieties such as *Ocimum sanctum* are rich in phenolic compounds like isothymusin, flavonoids, caftaric acid, rosmarinic acid, vicenin, cirsimaritin, and apigenin [12, 21]. These compounds contribute to basil's medicinal properties, particularly the flavonoids in *Ocimum basilicum*, which enhance neutrophil phagocytic activity and exhibit immunostimulant effects [22]. Linalool, a prominent compound in basil's essential oil, has been proven to inhibit a diverse variety of microorganisms, especially Gram-positive bacteria [20, 23]. Furthermore, rosmarinic acid in basil interferes with DNA and protein synthesis, reducing DNA fragmentation and suppressing caspase-3 activation, which leads to inhibition of apoptosis [15].

Teucrium polium, a perennial herb reaching about 30 cm in height with a semi-woody structure, is recognized for its therapeutic potential in treating diseases such as diabetes and cancer [24]. Native to sandy and rocky regions across the Mediterranean, as well as parts of North Africa, Europe, Southeast Asia, and North America, *Teucrium polium* typically flowers twice a year, in June and September [25]. Recent studies have highlighted *Teucrium polium* extracts as a promising alternative for cancer treatment, owing to their various pharmacologic particularities, including antipyretic, anti-inflammatory, antitumor, antioxidant, hypolipidemic, and antiseptic effects [11, 24, 26]. These activities are attributed to *Teucrium polium*'s rich bioactive compound profile, which includes terpenoids, terpinen, flavonoids, β-pinene, saponins, tannins, caryophyllene oxide, asparagine, limonene, and sterols [24]. In addition to its standalone therapeutic effects, *Teucrium polium* has been shown to enhance the efficacy of other treatments, potentially overcoming drug resistance and increasing potency [24, 27]. Specifically, the combination of *Teucrium polium* with Tranilast has demonstrated potent activity by inhibiting angiogenesis in human umbilical vein endothelial cells, reducing cell viability, impairing endothelial cell migration, and promoting apoptosis [11, 23].

Cleome amblyocarpa Barr, a medicinal herb native to tropical and subtropical regions such as Oman and Tunisia, thrives in rocky, damp areas, red soils, fertile black soils, and regions impacted by wastewater in warm, temperate climates [5, 28]. Traditionally, extracts from this plant have been explored to cure various health concerns, including diabetes, rheumatic fever, and colic, owing to their antibacterial, antioxidant, analgesic, and cytotoxic properties. Phytochemical analyses of *Cleome amblyocarpa* have isolated and characterized bioactive compounds in the roots, stems, seeds, leaves, and flowers. These extracts contain both volatile and non-volatile compounds [5]. Notable non-volatile compounds include saponins, anthocyanins, fatty acids, glucosinolates, sterols, triterpenoids, alkaloids, and flavonoids [4, 13], while the primary volatile compounds are eugenol, ethyl 3-methylpentanoate, α-copaene, and β-caryophyllene [5].

Caralluma plants, from the Apocynaceae family, are being explored as alternatives to conventional cancer therapies due to their anticancer properties [9]. Native to rocky and mountainous regions like Oman, they contain bioactive compounds such as esterified polyhydroxypregnane glycosides and flavone glycosides, which contribute to their therapeutic effects. *Caralluma* extracts also serve as precursors to cardenolides [9] and are used in traditional medicine across Africa, the Middle East, and South Asia to treat various conditions [14, 29]. These plants are valued for their broad pharmacological activities [9, 10, 29].

Despite limited scientific reviews on the phytochemical composition, biological activities, and potential uses of four Omani herbal plants, including *Ocimum basilicum*, *Teucrium polium*, *Cleome amblyocarpa*, and *Caralluma arabica,* we aimed to estimate and compare, for the first time, the phytochemical composition, volatile compounds, and antibacterial and antifungal properties of the aerial parts of these plants.

2. Materials and methods

2.1. Solvents, chemicals, equipment

The study utilized various solvents and chemicals, sourced from Sigma Aldrich and Bio-Rad. Reagents such as Folin-Ciocalteu reagent, sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$, 5%), gallic acid ($C_7H_6O_5$), vanillin (4% in methanol), catechin ($C_{15}H_{14}O_6$), aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$), hydrochloric acid (HCl), ethanol, dimethyl sulfoxide (DMSO), sodium chloride (NaCl), and distilled water (H_2O) were supplied by Sigma-Aldrich. Additionally, specialized media and reagents, including Mueller Hinton Agar (MHA), sodium chloride (NaCl), distilled water (H_2O), Yeast Dehydrated Culture Medium (YDCM), chloramphenicol, cycloheximide, Mueller Hinton broth, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), and 0.1% Tween 80, were provided by the Faculty of Sciences, Gabes University, Tunisia. The current study required the following equipment: ultra-fast liquid chromatography (UFLC), a CO_2 incubator, a mass spectrometer, a UV-Vis spectrophotometer, and an electrical oscillator.

2.2. Plant materials

Various plant materials and extracts were used in the study. Plant collection was conducted in the South Ash Sharqiyah Governorate, Sultanate of Oman, between July and September 2023. The species collected included *Cleome amblyocarpa*, *Teucrium polium*, *Caralluma arabica*, and *Ocimum basilicum*. The plant specimens were authenticated by botanical specialist Professor Mustapha Gorai from the Higher Institute of Applied Biology of Medenine, University of Gabes (Tunisia). After being gathered, the plants were left to dry naturally in the shade at room temperature for several days. Once dried, the specimens were sealed in plastic containers and transferred to the research laboratory. The plant material was subsequently cut into small pieces, and the stems, leaves, and roots were tho-

roughly rinsed with distilled water [6]. Afterward, the cut specimens were oven-dried at 50°C for two days. Only the dried leaves and stems (excluding roots) were processed into a fine powder using an electric grinder.

2.3. Preparation of plant extracts

Plant extracts were prepared as we previously described [6]. In brief, 200 g of ground plant material was mixed with 800 ml of ethanol or distilled water in separate flasks to produce solvent-specific extracts. The resulting mixtures were transferred into sterile cell culture bottles, securely sealed, and placed on a rotator for 24 hours to ensure thorough mixing. After incubation, the extracts were filtered through Whatman filter paper to eliminate any solid deposits. The filtrates were subsequently centrifuged (4000 rpm for 10 minutes) to further clarify the extracts. The supernatants were then distributed into Petri dishes and allowed to evaporate at room temperature, facilitating the solvent removal. Finally, the concentrated extracts were freeze-dried at 4°C using a lyophilizer. The dried extracts were stored at 4°C until further laboratory analysis.

2.4. Phytochemical investigation of herbal plants

2.4.1. Total polyphenolic content

The total polyphenolic content (TPC) was measured using the method previously described by Cicco et al. [30]. In this procedure, 500 µl of distilled water, 125 µl of Folin-Ciocalteu reagent, and 125 µl of the herbal extract were mixed in a test tube. After allowing the mixture to react for 3 minutes, 1,250 µl of Na_2CO_3 (7%) was added to the tube. Subsequently, 3 mL of distilled water was added to adjust the final volume. The resulting mixture was incubated in the dark at room temperature for three hours. The absorbance of the solution was determined at 760 nm using a UV-Vis spectrophotometer. The results, conducted in triplicate, were expressed as milligrams of gallic acid equivalents per gram of dry plant extract (mg GAE/g).

2.4.2. Total flavonoid contents

The total flavonoid content (TFC) was determined using the aluminum chloride method. Briefly, 75 µl of $NaNO_2$ (5%) was mixed with 250 µl of the plant extract. After a 6-minute incubation at room temperature, 150 µl of $AlCl_3 \cdot 6H_2O$ solution was added to the mixture. Following an additional 5-minute incubation, 500 µl of NaOH solution was introduced. The final volume of the mixture was adjusted to 2,500 µl with distilled water. The flavonoid concentration in the resulting solution was measured spectrophotometrically at 430 nm. The results were expressed as milligrams of catechin equivalents per gram of dry plant extract (mg CE/g). The experiment was conducted in triplicate to ensure reliability [31].

2.4.3. Tannin contents

The method described by Abdessemed et al. [32] was employed with slight modification to estimate the tannin content. In this procedure, 300 µl of the plant extract was mixed with 3 mL of a 4% vanillin in methanol and 1.5 ml of HCl to determine the tannin concentration. After a 15-minute incubation period, the absorbance of the resulting solution was measured at 500 nm. The tannin content was expressed as milligrams of catechin equivalents per gram of dry plant extract (mg CE/g). The experiments were carried out in triplicate to ensure accuracy.

2.5. Liquid chromatography/electrospray ionization/mass spectrometry (LC-ESI-MS) analysis

The method outlined by Ayaz et al. [33] was employed, with slight modifications, to extract flavonoids and phenolic acids. Briefly, 0.5 g of powdered extracts containing phenolic acids and flavonoids was dissolved in 10 mL of ultra-pure water. For the herbal extracts, ethanol was employed as the solvent. After complete dissolution, the mixtures were shaken at room temperature for 24 hours. Before analysis, the samples were centrifuged at 4,000 rpm for 25 minutes, then filtered through a 0.45 µm Millipore filter. Finally, 5 µL of the filtered samples were injected into the system.

The LC–ESI–MS analysis was performed using an electrospray ionization source-equipped LCMS-2020 quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) operating in negative ionization mode.

A UFLC system was employed, consisting of an LC-20 AD XR binary pump, SIL-20AC XR autosampler, CTO-20AC column oven, and a Shimadzu DGU-20A 3R degasser, all connected online to the mass spectrometer. Prior to analysis, an Aquasil C18 guard column (10 mm × 3 mm, 3 µm, Thermo Electron) was paired with a 150 mm × 3 mm, 3 µm Aquasil C18 column (Thermo Electron, Dreieich, Germany). The mobile phase, consisting of solvent A (0.1% formic acid in H2O, v/v) and solvent B (0.1% formic acid in methanol, v/v), was subjected to a linear gradient elution: from 10% to 100% B over 0–45 minutes, then held at 100% B from 45 to 55 minutes. A re-equilibration time of 5 minutes was allotted between individual runs. The column temperature was maintained at 40°C, the injection volume was set at 5 µL, and the mobile phase flow rate was 0.4 mL/min. The Shimadzu Lab Solutions LC-MS software was used to process the spectra, which were monitored in Selected Ion Monitoring (SIM) mode [6]. Operating in negative ion mode, the mass spectrometer's parameters included a capillary voltage of -3.5 V, a nebulizing gas flow rate of 1.5 l/min, a dry gas flow rate of 12 L/min, a detector voltage set to 1.2 V, a desolvation line temperature of 250°C, a block source temperature of 400°C, and full scan spectra acquired over a range of 50-2000 m/z.

The identification of phenolic compounds was conducted by comparing their retention times and mass spectra with those of authentic standards. The chemical standards, including quinic acid, gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, trans-cinnamic acid, 4-O-caffeoylquinic acid, 1,3-di-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid, rosmarinic acid, salvianolic acid, catechin, epicatechin, acacetin, apigenin-7-O-glucoside, apigenin, cirsilineol, cirsiliol, hyperoside (quercetin-3-O-galactoside), luteolin-7-O-glucoside, luteolin, naringenin, naringin, quercitrin (quercetin-3-O-rhamonoside), quercetin, rutin, and silymarin, all with a purity of ≥99.0%, were obtained from Sigma Chemical Co. (St. Louis, MO, USA) [34].

2.6. Antimicrobial screening

2.6.1. Microbial strains and growth conditions

This study employed seven bacterial strains to evaluate the antibacterial properties of the ethanolic herbal extracts, encompassing both Gram-negative and Gram-positive bacteria. The Gram-negative bacteria included *Escherichia*

coli ATCC 25922, *Salmonella enterica* serotype Typhimurium, and *Klebsiella pneumoniae*. The Gram-positive bacteria included *Bacillus subtilis* JN 934392, *Bacillus cereus* JN 934390, *Micrococcus luteus*, *Staphylococcus aureus* ATCC 6538, and *Salmonella enterica* serotype Enteritidis ATCC 43972. Additionally, three fungal strains were used to assess the antifungal properties of the ethanolic herbal extracts: *Pythium catenulatum* AY598675, *Fusarium sp.* JX391934, and *Fusarium oxysporum* AB586994. All bacterial and fungal strains were clinically isolated and obtained from the Microbiology Department of Habib Bourguiba University Hospital in Sfax, Tunisia.

Mueller-Hinton Agar (MHA) was used to cultivate the test bacteria, which were placed in Petri dishes and incubated for 18 to 24 hours. After incubation, bacterial growth from the Petri dishes was transferred to 3 mL of MHA and incubated at 37°C with stirring at 200 rpm for 24 hours. For *Bacillus* species, the culture was maintained at 30°C. The study followed the methodology outlined by Hssouna et al. [35], using a final inoculum concentration of 10^7 colony-forming units (CFU)/mL of bacterial suspension to determine the minimum inhibitory concentration (MIC).

Fungal strains were cultivated on YDCM agar at 30°C for four days until the mycelial growth completely covered the plate. Subsequently, a spore suspension was prepared by extracting spores from the mycelial growth using 10 mL of sterile water supplemented with 0.1% Tween 80. The resulting inoculum was standardized to a concentration of 10^6 spores/mL and used to determine the minimum fungicidal concentration (MFC) [35].

2.6.2. Detection of antimicrobial activity by the agar diffusion method

The agar diffusion assay, as described by Hsouna et al. [35], was adapted to evaluate both antibacterial and antifungal activities. Briefly, a sterile Pasteur pipette was used to create a 6 mm well in MHA agar. A sterile swab was then used to inoculate the agar surface with a bacterial suspension (100 µl, adjusted to 10^7 CFU/mL) or a spore solution (100 µl, adjusted to 10^6 spores/mL). Next, 80 µL of each extract, prepared at a concentration of 125 mg/mL in DMSO, was added to each well, with DMSO serving as a negative control. The plates were incubated at 4°C for two hours to allow the extract to diffuse into the agar, as recommended by Trigui et al. [36]. After this incubation, the plates were further incubated at 37°C for 24 hours for bacterial strains and at 30°C for four to seven hours for fungal strains. Chloramphenicol was used as a standard antibacterial agent at a concentration of 15 µg/well. Cycloheximide was used as a standard antifungal agent at a concentration of 20 µg/well. The diameter of the inhibition zone around each well was measured to assess the antimicrobial properties of the extracts.

2.6.3. Determination of MIC and MFC by micro-dilution well method

The method described by Gulluce et al. [37] was adapted with minor modifications to determine the MIC, defined as the lowest concentration of ethanolic herbal extracts that inhibits visible microbial growth in a sterile 96-well microplate, each well with a capacity of 200 µL. A 125 mg/mL solution of each extract was prepared in DMSO, a solvent known for its antimicrobial properties [38]. Two-fold serial dilutions of the extracts were performed in the microplate wells, ranging from 0.98 to 125 mg/mL of extract in DMSO. Each well contained 100 µL of the extract solution and 10 µl of the microbial cell suspension, resulting in final inoculum concentrations of 10^7 CFU/mL for bacteria and 10^6 spores/ml for fungi. For fungal assays, 90 µL of Mueller-Hinton broth and yeast-dehydrated broth were added. The last well served as the positive-growth control, containing bacteria or fungi in the appropriate medium without extract. A negative control, consisting of DMSO alone without any extract, was also included. After homogenizing the contents, sterile plate covers were applied, and the plates were incubated for 24 hours at 37°C for bacterial cultures and three days at 30°C for fungal cultures.

As part of the microorganism viability assay, 25 µL of the 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) oxidation-reduction indicator was added to each well. The mixture was then incubated for 30 minutes at 37°C. Wells showing microbial growth inhibition remained clear after MTT incubation [35]. The MIC is defined as the lowest concentration of a substance required to prevent microorganism growth. The minimum bactericidal concentration (MBC) was determined by identifying the lowest concentration of the sample that showed no growth after adding 0.5 mg/mL of sterile distilled water containing thiazolyl-2,5-diphenyl-2H-tetrazolium bromide. After 48 hours of incubation at 37°C, this solution showed minimal turbidity and no detectable microbial growth. The MFC, defined as the lowest concentration that prevents mycelial growth [35], was determined by identifying the first wells showing no growth after 3 to 4 days of incubation at 30°C, followed by serial subculturing of 10 µL from each well onto YDCM plates to confirm the absence of viable colonies.

2.7. Statistical analysis

The experimental data were obtained from three replicates and are presented as means ± SEM. A one-way analysis of variance (ANOVA) was used to assess differences, and the Least Significant Difference (LSD) test was applied to identify significant changes and determine the effects at the 5% probability level.

3. Results

3.1. Phytochemical investigation of *Ocimum basilicum*, *Teucrium polium*, *Caralluma arabica*, and *Cleome amblyocarpa*

3.*1.1. Total phenolic, flavonoid, and tannin contents*

The total phenolic, flavonoid, and tannin contents were quantified using the Folin-Ciocalteu, aluminum chloride, and vanillin methods, respectively. The results are expressed as milligrams of gallic acid or catechin equivalents per gram of extract.

As detailed in Table 1, the ethanolic extracts of *Ocimum basilicum*, *Teucrium polium*, *Caralluma arabica*, and *Cleome amblyocarpa* exhibited higher phytochemical contents than the aqueous extracts. Specifically, the ethanolic extracts yielded 11.9±0.49, 11.5±0.4, 9.7±1.2, and 12.97±0.04, respectively, while the aqueous extracts yielded 8.53±0.2, 8.33±0.3, 6.1±0.4, and 7.13±0.1, respectively.

Furthermore, the ethanolic extracts exhibited higher levels of total phenolic acids and flavonoids compared

to the aqueous extracts (Table 1). In contrast, the tannin contents showed only minor variations between the ethanolic extracts (0.12±0.005, 0.18±0.03, 0.01±0.002, and 0.04±0.003, respectively) and the aqueous extracts (0.08±0.009, 0.09±0.01, 0.01±0.002, and 0.02±0.003, respectively).

No significant differences were observed between the yields of ethanolic and aqueous extracts ($p>0.05$). Additionally, the relationship between extract yields and the contents of total phenolic acids, flavonoids, and tannins was assessed. The p-values for total phenolic acids and flavonoid contents were both greater than 0.05, indicating no significant relationship. However, the p-value for tannin content was exactly 0.05, suggesting a significant borderline relationship.

3.2. Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) analysis

The phytochemical composition of the plants was analyzed using LC-ESI-MS to identify and quantify the phenolic and flavonoid constituents in the ethanolic extracts of *Ocimum basilicum*, *Teucrium polium*, *Caralluma arabica*, and *Cleome amblyocarpa*. The concentrations of these phytochemicals in ethanolic extracts are presented in parts per million (ppm) in Table 2. The LC-ESI-MS analysis revealed that each plant contained a distinct profile of compounds, with varying concentrations for each.

Ocimum basilicum contained 13 phytochemical compounds, including 4 phenolic acids and 9 flavonoids. As detailed in Table 2, rosmarinic acid was the most abundant phenolic acid in the ethanolic extract, with a concentration of 3,808.13 ppm, while protocatechuic acid was present at the lowest concentration (164.04 ppm). Among the flavonoids, 1,3-di-O-caffeoylquinic acid had the highest concentration (23,365.50 ppm), while *trans*-ferulic acid was detected in trace amounts (9.96 ppm). Overall, the ethanolic extract of *Ocimum basilicum* was rich in both phenolic and flavonoid compounds. However, certain compounds, such as apigenin-7-O-glucoside, cirsiliol, and cirsilineol, were not detected in this extract.

Based on the LC-ESI-MS analysis, the ethanolic extract of *Teucrium polium* contained fewer phytochemical compounds compared to the ethanolic extract of *Ocimum basilicum*. Specifically, *Teucrium polium* was composed of 3 phenolic acids and 6 flavonoids. As shown in Table 2, quinic acid was the most abundant compound, with a concentration of 1,161.69 ppm, while rosmarinic acid was present in much smaller amounts (130.04 ppm).

Table 3 also highlights several compounds detected across all four plant extracts, including quinic acid, 1,3-di-O-caffeoylquinic acid, acacetin, and naringenin, though their concentrations varied among the plants. Among these, 1,3-di-O-caffeoylquinic acid, a flavonoid, had the highest concentration in *Teucrium polium* (1,2920.28 ppm), followed by cirsiliol (5,915.94 ppm), acacetin (4,160.75 ppm), naringenin (893.42 ppm), cirsilineol (595.96 ppm), and apigenin (520.06 ppm).

Additionally, compounds such as caffeic acid, *trans*-ferulic acid, rutin, hyperoside (quercetin-3-O-galactoside), quercitrin (quercetin-3-O-rhamnoside), apigenin-7-O-glucoside, and luteolin were not detected in the ethanolic extract of *Teucrium polium*, as shown in Table 2.

As indicated in Table 2, the ethanolic extract of *Cleome amblyocarpa* contained one phenolic acid (quinic acid) and seven flavonoids, with varying concentrations of 1,3-di-O-caffeoylquinic acid, luteolin, cirsilineol, acacetin, apigenin, and naringenin. Several other phytochemicals, including protocatechuic acid and caffeic acid, were not detected in this extract.

In contrast, the ethanolic extract of *Caralluma arabica* contained a relatively limited number of phytochemicals, comprising one phenolic acid (quinic acid) and six flavonoids, with varying concentrations of compounds such as 1,3-di-O-caffeoylquinic acid, naringenin, hyperoside (quercetin-3-O-galactoside), apigenin-7-O-glucoside, acacetin, and *trans*-ferulic acid. Notably, apigenin-7-O-glucoside was exclusively found in the ethanolic extract of *Caralluma arabica*, while rutin and quercitrin (quercetin-3-O-rhamnoside), which were detected in the *Ocimum basilicum* extract, were absent in *Caralluma arabica*.

The final plant extract listed in Table 2 is *Caralluma arabica*, which contains the fewest phytochemical compounds, including one phenolic acid (quinic acid) and six flavonoids. The concentrations of these compounds, such as 1,3-di-O-caffeoylquinic acid, naringenin, hyperoside (quercetin-3-O-galactoside), apigenin-7-O-glucoside, acacetin, and *trans*-ferulic acid, varied. According to the same table, apigenin-7-O-glucoside was exclusively found in the ethanolic extract of *Caralluma arabica*, whereas rutin and quercitrin (quercetin-3-O-rhamnoside) were mainly present in the *Ocimum basilicum* extract.

3.3. Antimicrobial activities

The antimicrobial activities in this study were evaluated using the agar diffusion method against ten microorganisms, including seven bacteria and three fungi (Table 4). Antimicrobial potency was assessed both qualitatively and quantitatively by measuring the diameters of the inhibition zones and determining the MIC, MBC, and MFC. The bacterial and fungal activities were categorized as follows: sensitive (inhibition zone >18 mm), intermediate (inhibition zone between 13-17 mm), and resistant (inhibition zone <13 mm). Control growth inhibition tests, using chloramphenicol for bacteria and cycloheximide for fungi, were conducted in parallel with the plant extracts for comparative analysis.

As shown in Table 3, the results indicate that the ethanolic extracts of *Ocimum basilicum*, *Teucrium polium*, *Caralluma arabica*, and *Cleome amblyocarpa* exhibited stronger antibacterial activity than antifungal activity, as evidenced by larger inhibition zones against bacterial strains compared to fungal strains. For example, *Staphylococcus aureus* (a Gram-positive bacterium) displayed the greatest susceptibility, with the largest inhibition zones recorded in the ethanolic extracts of *Ocimum basilicum* (21±0.2 mm) and *Caralluma arabica* (22±0.1 mm), which were comparable to the chloramphenicol control (inhibition zone = 25.8±2.0 mm, $p<0.05$).

Other Gram-positive bacteria, including *Bacillus cereus*, *Bacillus subtilis*, and *Micrococcus luteus*, exhibited varying inhibition zones in response to different herbal extracts. *Bacillus cereus* and *Bacillus subtilis* were most susceptible to the ethanolic extract of *Teucrium polium*, with inhibition zones of 18±0.5 mm and 20±0.2 mm, respectively, while showing intermediate or resistant responses to the other extracts. Additionally, *Micrococcus luteus* demonstrated sensitivity to the ethanolic extracts of *Ocimum basilicum* and *Cleome amblyocarpa*, with inhi-

Table 1 Phytochemicals of ethanolic and water extracts of *Ocimum basilicum*, *Teucrium polium*, *Caralluma arabica* and *Cleome amblyocarpa.*

Plant name	Yields (%)		TPC (mg GAE/g)		TFC (mg CE/g)		Tannins content (mg CAT/g)	
	EE Yields	WE Yields	EE TPC	WE TPC	EE TFC	WE TFC	EE Tannin	WE Tannin
Ocimum basilicum	11.9±0.49	8.53+0.2	23.37±0.17	17.13±0.12	17.77±0.69	12.23±0.09	0.12±0.005	0.08±0.009
Teucrium polium	11.5±0.4	8.33±0.3	20.93±0.3	13.9±0.3	14.5±0.2	7.73±0.2	0.18±0.03	0.09±0.01
Caralluma arabica	9.7±1.2	6.1±0.4	14.8±0.09	9.43±0.08	9.33±0.2	7.17±0.04	0.01±0.002	0.01±0.002
Cleome amplyocarpa	12.97±0.04	7.13±0.1	11.47±0.09	7.4±00.2	8.13±0.06	3.8±0.09	0.04±0.003	0.02±0.003

The data are expressed as mean ± SEM (n = 3); EE: ethanolic extraction; WE: water extraction; TPC: total phenols content; TFC: total flavonoids content; GAE: gallic acid equivalent; CAT: catechin equivalent; CE: catechin equivalent. Significant difference expressed as probability value (p) <0.05.

Table 2 LC-EMI-MS analysis of ethanolic extracts of *Ocimum basilicum, Teucrium polium, Cleome amplyocarpa,* and *Caralluma arabica*.

Compound group	Compound name	*Ocimum basilicum*			*Teucrium polium*			*Cleome amplyocarpa*			*Caralluma arabica*		
		Rt	m/z	Cc (ppm)	Rt	m/z	Cc (ppm)	Rt	m/z	Cc (ppm)	Rt	m/z	Cc (ppm)
Phenolic acids	Quinic acid	2.13	191.00	570.96	1.94	191.00	1,161.69	2.24	191.00	110.69	2.36	191.00	5,037.01
	Rosmarinic acid	28.18	359.00	3,808.13	28.41	359.00	130.04	-	-	-	-	-	-
	Protocatechuic acid	6.58	153.00	164.04	6.57	153.00	336.42	-	-	-	-	-	-
	Caffeic acid	14.88	179.00	518.52	-	-	-	-	-	-	-	-	-
Flavonoids	1,3-di-O-caffeoylquinic acid	24.22	515.00	23,365.50	24.23	515.00	12,920.28	23.85	515.00	3,213.88	24.27	515.00	20,510.18
	Acacetin	43.17	283,00	578,64	43,11	283,00	4,160,75	42.99	283.00	94.48	43.03	283.00	29.44
	Naringenin	35.62	271.00	323.63	35.22	271.00	893.42	35.30	271.00	10.60	35.69	271.00	295.19
	Apigenin	36.36	269.00	189.42	36.55	269.00	520.06	36.44	269.00	13.93	-	-	-
	Hyperoside: quercetin-3-O-galactoside	26.34	463.00	82.29	-	-	-	-	-	-	26.11	463.00	36.85
	Trans-ferulic acid	23.79	193.00	9.96	-	-	-	-	-	-	23.90	193.00	13.28
	Cirsiliol	-	-	-	37.15	329.00	5,915.94	37.34	329.00	238.85	-	-	-
	Cirsilineol	-	-	-	40.78	343.00	595.96	40.83	343.00	183.29	-	-	-
	Luteolin	33.72	285.00	1,196.00	-	-	-	33.37	285.00	437.62	-	-	-
	Rutin	25.66	609.00	2,379.31	-	-	-	-	-	-	-	-	-
	Quercitrin: quercetin-3-O-rhamnoside	28.52	447.00	234.87	-	-	-	-	-	-	-	-	-
	Apigenin-7-O-glucoside	-	-	-	-	-	-	-	-	-	28.43	431.00	32.01

Cc: concentration, ppm: parts per million (ppm), Rt: Retention time, LC-EMI-MS: Liquid chromatography/electrospray/mass spectroscopy.

Table 3 Antimicrobial activities of Omani plant extracts against bacterial and fungal strains.

	Inhibition zone diameters (mm)				
	Ethanolic extracts				Control
Bacteria strains	***Ocimum basilicum***	***Teucrium polium***	***Caralluma arabica***	***Cleome amblyocarpa***	**Chloramphenicol**
Bacillus cereus	-	18±0.5[b]	-	-	26±1.0[a]
Bacillus subtili	15±0.3[b]	20±0.2[b]	11±0.5[b]	11±0.5[b]	24±0.0[a]
Staphylococcus aureus	21±0.2[b]	-	22±0.1[b]	-	25.8±2.0[a]
Micrococcus luteus	19±0.2[c]	11±0.3[a]	-	21±0.1c	22.5±0.5[a]
Salmonella enteritidis	15±0.3[a]	09±0.1[c]	10±0.33[a]	07±0.4a	16.5±0.5[a]
Escherichia coli	20±0.2[c]	18±0.3a	-	12±0.1c	23.5±0.5[a]
Klebsiella pneumonia	08±0.01[a]	15±0.2a	12±0.00[a]	07±0.00[a]	22±1.0[a]
Fungi strains					**Cycloheximide**
Fusarium oxysporum	12 ±0.2[a]	02 ±0.3[a]	-	06 ±0.0[a]	20 ± 2.0[a]
Fusarium sp	05 ±0.1[c]	17 ±0.1[c]	07 ±0.2[c]	07 ±0.2[c]	18± 1.5[a]
Pythium catenulatum	15.1 ±0.5[a]	10.1 ±0.2	05 ±0.5 [a]	04.1 ±0.3	17.5± 1.5[a]

The data are expressed as mean ± S.D (n=3). a, b, c: Different letters in the same rows indicate significant differences ($p<0.05$). Diameter of inhibition zones of extract including diameter of well 6 mm. Chloramphenicol was used as a standard antibacterial agent at a concentration of 15 µg/well. Cycloheximide was used as a standard antifungal agent at a concentration of 20 µg/well.

Table 4 Determination of MIC, MBC and MFC of plant extracts.

Extract of plants	**Concentration (mg/ml)**							
	Ethanolic extracts							
	Ocimum basilicum		***Teucrium polium***		***Caralluma arabica***		***Cleome amblyocarpa***	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Bacteria strains								
Bacillus cereus	-	-	3.45	38.7	-	-	-	-
Bacillus subtilis	6.47	39.2	1.47	33.5	9.41	38.8	9.39	39.5
Staphylococcus aureus	1.28	37.1	-	-	1.68	37.1	-	-
Micrococcus luteus	2.87	35.4	8.74	37.1	-	-	1.71	34.5
Salmonella enteritidis	1.28	33.5	9.15	37.8	9.87	38.5	8.78	38.5
Escherichia coli	1.34	35.5	2.69	36.5	-	-	8.73	38.7
Klebsiella pneumonia	9.26	38.5	1.37	34.5	3.37	36.5	8.83	37.8
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Fungi strains								
Fusarium oxysporum	4.67	17.85	8.25	21.45	-	-	6.25	21.47
Fusarium sp	8.24	21.85	3.25	17.28	8.41	22.48	7.38	18.79
Pythium catenulatum	3.62	17.73	7.68	19.75	6.53	20.87	8.68	23.85

MIC: minimum inhibition concentration; MBC: minimum bactericidal concentration; MFC: minimum fungicidal concentrations.

bition zones of 19±0.2 mm and 21±0.1 mm, respectively. Meanwhile, *Escherichia coli*, the only Gram-negative bacterium tested, responded to the extracts of *Ocimum basilicum* and *Teucrium polium* with inhibition zones of 20±0.2 mm and 18±0.3 mm, respectively, indicating sensitivity.

For antifungal activity, *Fusarium* spores and *Pythium catenulatum* exhibited intermediate inhibition zones in response to the ethanolic extracts of *Teucrium polium* and *Ocimum basilicum*, with values of 17±0.1 mm and 15.1±0.5 mm, respectively. These results were slightly lower than the inhibition zone observed with the cycloheximide control (20±2.0 mm, $p<0.05$). No antifungal activity was detected against *Fusarium oxysporum*, as this fungus was resistant to the four extracts investigated.

3.4. The potency activities of plant extract to inhibit or kill strains

The potency of plant extracts in inhibiting or killing microbial strains depends on the type and concentration of the extract, as indicated by the diameter of the inhibition zone, MIC, MBC, and MFC. As shown in Table 4, specific examples highlight the effectiveness of the extracts. For instance, the ethanolic extract of *Teucrium polium* was the only one capable of inhibiting the growth of *Bacillus cereus* at a concentration of 3.45 mg/mL, and it caused bacterial death at 38.7 mg/mL. Additionally, *Bacillus subtilis* exhibited varied responses, with all antibacterial extracts showing activity against the strain at certain concentrations. The ethanolic extract of *Ocimum basilicum* demonstrated the lowest MIC for *Staphylococcus aureus* and *Salmonella enteritidis* (1.28 mg/mL for both), while the lowest MBC was observed with the *Teucrium polium* extract for *Bacillus subtilis* and with the *Ocimum basilicum* extract for *Salmonella enteritidis* (33.5 mg/mL for both).

The diameter of the inhibition zone, along with the MIC, MBC, and MFC, depends on the type and concentration of the extract [6]. As shown in Table 4, specific examples illustrate these relationships. For instance, the ethanolic extract of *Teucrium polium* was the only extract capable of inhibiting the growth of *Bacillus cereus* at a concentration of 3.45 mg/ml, and it caused bacterial death at 38.7 mg/mL. Additionally, *Bacillus subtilis* exhibited distinct results among the Gram-positive bacteria tested, as all antibacterial extracts showed activity against the strain at specific concentrations. The ethanolic extract of *Ocimum basilicum* demonstrated the lowest MIC for both *Staphylococcus aureus* and *Salmonella enteritidis* (1.28 mg/mL for both), while the lowest MBC was observed with *Teucrium polium* extract for *Bacillus subtilis* and with *Ocimum basilicum* extract for *Salmonella enteritidis* (33.5 mg/mL for both).

For the fungal strains, the plant extracts exhibited strong inhibitory and eliminatory effects against most fungi, except *Fusarium oxysporum*, which was resistant to the *Caralluma arabica* antifungal extract. The lowest MIC and MFC rates were identified with the *Teucrium polium* extract, which inhibited *Fusarium* spore growth at concentrations of 3.25 mg/mL and 17.28 mg/mL, respectively (Table 4).

4. Discussion

Phenolic compounds are extensively distributed across various plant parts, with the aerial parts generally containing the highest concentrations of total phenols, flavonoids, and flavonols, and exhibiting stronger chelating activity compared to roots and seeds [5]. Herein, we explored the phytochemical constituents and biological properties of four Omani herbal plants: *Ocimum basilicum*, *Teucrium polium*, *Caralluma arabica*, and *Cleome amblyocarpa*.

Numerous solvents, such as water, methanol, acetone, and ethanol, exhibit different polarities that influence the solubility of specific metabolites, thereby affecting their biological activity. The polarity of ethanol (5.2) and the minimal water content in absolute ethanol enable effective extraction of metabolites from plant materials [7, 8]. In our study, both absolute ethanol and water were successfully employed to extract total phenolic acids, flavonoids, and tannins from the selected Omani plants. The extraction process involved continuous mixing of the sample and solvent for over 24 hours to enhance extraction efficiency [7]. Although effective in quantifying phenolic acids, flavonoids, and tannins, the Folin-Ciocalteu reagent can interact with vitamin C, aromatic amines, and saccharides [8]. UV-V spectrophotometry was used to measure phenolic acids and flavonoids, offering a simple, rapid, and high-quality method, though UV radiation may harm microorganisms [7, 8, 39].

Several studies have shown that a solvent's polarity correlates with its ability to dissolve a wide range of extracted components. Ethanol, with its moderate polarity, is especially effective for extracting both phenolic and non-phenolic compounds, such as glycosides, polyacetylenes, sterols, polyphenols, tannins, flavonoids, terpenoids, alkaloids, carbohydrates, and proteins. In the present study, ethanol extraction yielded the highest amounts, with *Cleome amblyocarpa* exhibiting the greatest yield (12.97±0.04), followed by *Ocimum basilicum* (11.9±0.49), *Teucrium polium* (11.5±0.4), and *Caralluma arabica* (9.7±1.2). These findings are consistent with those described in prior studies [8, 40]. Hikmawanti et al. [8] found that a 50% ethanol extract was optimal for extracting total phenolics and flavonoids, while Fadhila et al. [7] and Alhakim et al. [39] reported better yields with 70% to 80% ethanol. In contrast, Parikh and Kothari [40] suggested that methanol extracts were more effective for extracting plant compounds. Alcoholic solvents are generally favored for their superior ability to dissolve active compounds compared to water [7, 8]. Pure water is typically less effective in extracting certain compounds, such as rosmarinic acid [41]. However, Zourgui et al. [6] reported that, in some cases, aqueous extracts contained higher concentrations of bioactive compounds than their ethanolic counterparts. In our study, Tukey's post hoc test showed no significant differences between ethanol and water extraction yields ($p>0.05$), consistent with Gema et al. [42] but contrasting with Fadhila et al. [7]. Absolute ethanol, due to its safety, low toxicity, rapid extraction, and cost-effectiveness, was the preferred solvent for metabolite extraction in this study, aligning with previous research [7, 8].

The present study demonstrated that ethanolic extracts contained significantly higher levels of total phenolic and flavonoid compounds compared to water extracts, while both extraction methods yielded lower concentrations of tannins. These results agree with previous studies [6, 40, 43]. In contrast, Oonsivilai and Prasongdee [44] and Islam et al. [45] reported differing outcomes, with water extracts exhibiting the highest total phenolic content (353.93±1.65 mg/g), while ethanolic extracts showed the highest total

flavonoid content (557.12±14.27 mg/g). Various factors, including species, genotype, climate, location, and growing conditions, may impact the concentration of phenolic compounds in plants [46], as noted by Aburigal et al. [47]. Interestingly, these researchers reported that *Ocimum basilicum* plants from the Maldives had the highest total phenolic content, whereas those grown in Thailand exhibited the lowest [47]. In the present study, *Ocimum basilicum* exhibited the highest total phenolic content (23.32±0.17 mg GAE/g) and total flavonoid content (17.77±0.69 mg CE/g), followed by *Teucrium polium*, *Caralluma arabica*, and *Cleome amblyocarpa*.

Although tannins are regularly found in a wide range of plants and are recognized for their ability to neutralize free radicals and combat various pathogens [48], the Omani herbal extracts displayed relatively low concentrations of tannins. The highest tannin content was detected in the *Teucrium polium* extract, at 0.18±0.03 mg/g. In contrast, Zourgui et al. [6] reported a significantly higher tannin concentration in *Opuntia* species, with a value of 1.98±1.8 mg CAT/g.

In this study, LC-ESI-MS analysis complemented data from the Folin-Ciocalteu method to identify and quantify phenolic and flavonoid compounds in ethanolic extracts. LC-ESI-MS enables the rapid separation and characterization of metabolites by assessing the mass-to-charge ratio (m/z), isotopic patterns, Rt, and fragmentation spectra (MS2) [49, 50]. This technique allows differentiation of co-eluted compounds and precise identification through Rt variation, although overlapping fragmentation patterns and lack of structural details could complicate identification [6, 51]. Compounds are typically categorized as dominant, distinctive, trace, or absent [52]. Alhakim et al. [39] suggested comparing chromatograms of unknowns to known standards for enhanced identification. Phenolic compounds, characterized by aromatic structures and hydroxyl groups, and flavonoids, plant-derived pigments, are widely distributed across species [8].

Our comparative survey revealed that the ethanolic extract of *Ocimum basilicum* contained 13 compounds, comprising four phenolic acids and nine flavonoids, making it the most compound-rich extract among those analyzed. In contrast, *Teucrium polium* contained nine compounds (three phenolic acids and six flavonoids), ranking second in compound diversity. *Cleome amblyocarpa* placed third, with eight compounds, including one phenolic acid and seven flavonoids, while *Caralluma arabica* contained seven compounds, comprising one phenolic acid and six flavonoids [6].

This study identified rosmarinic acid as the predominant compound in *Ocimum basilicum* extract, consistent with previous reports. Notably, rosmarinic acid exhibited the highest RT, reflecting an efficient transition from polar to non-polar molecules, in agreement with Fadhila et al. [7]. In contrast, Prommajak et al. [46] reported a much higher concentration of rosmarinic acid in basil (13.25 ppm), compared to our study. Similarly, Chulova et al. [53] observed rosmarinic acid concentrations in basil ranging from 3.76 to 13.59 ppm.

We detected substantial amounts of protocathchuic acid in both *Ocimum basilicum* and *Teucrium polium*, and caffeic acid in *Ocimum basilicum*. These findings matched those of El-Azim et al. [54] and Prommajak et al. [46]. Additionally, all Omani extracts analyzed contained a common set of four compounds (one phenolic acid and three flavonoids) of which quinic acid, 1,3-di-O-caffeoylquinic acid, acacetin, and naringenin were found in notable concentrations. The highest concentration of quinic acid was recorded in *Caralluma arabica* (5,037.01 ppm). Quinic and caffeic acids are well-documented in various plants, including coffee beans, cinchona bark, and fruits like apples and peaches, due to their notable medicinal properties. These include radioprotective, anti-diabetic, anti-inflammatory, and anti-neuroinflammatory effects, as well as their roles in DNA repair, inhibition of NF-κB signaling, and neuroprotection [45, 55].

Phenolic compound identification was confirmed through comparative analysis of Rt, mass-to-charge ratios (m/z), molecular ions, and fragmentation patterns, utilizing a reference standards database [52, 56, 57]. For instance, quinic acid was detected in *Ocimum basilicum*, *Teucrium polium*, *Cleome amblyocarpa*, and *Caralluma arabica* at m/z 191, with varying Rt and concentrations, consistent with Vuković et al. [58] and Merghany et al. [59] findings.

Both *Ocimum basilicum* and *Teucrium polium* showed high levels of protocathchuic and rosmarinic acids, while only *Ocimum basilicum* contained caffeic acid, corroborating findings by El-Azim et al. [54] and Prommajak et al. [46]. A comparison of Rt revealed that Khlifi et al. [52] identified caffeic acid in *Cleome arabica* at m/z 179, with an Rt of 5.23 minutes, notably shorter than the 14.88 minutes observed in our study. This discrepancy may be attributed to variations in extraction methods and solvent interaction times [7, 58].

Overall, the Omani herbal extracts were valuable in phenolic and flavonoid compounds, with significant variation in both concentration and diversity, suggesting substantial therapeutic potential, particularly for treating infectious diseases. Considering the growing concern over bacterial resistance to synthetic antibiotics, there has been an increased focus on natural compounds as safer and more effective alternatives. Herbal extracts, especially those obtained using organic solvents, are known for their enhanced antimicrobial properties, attributed to their ability to extract both polar and non-polar bioactive compounds [5, 6]. Interestingly, our ethanolic herbal extracts demonstrated strong antimicrobial activity against all tested strains in a dose-dependent manner. However, some microbial strains showed weaker inhibition, likely due to lower concentrations of active compounds [6]. In fact, tannins, known for their antimicrobial properties by inhibiting bacterial growth through binding to microbial cells and depriving them of essential nutrients, are present only in small amounts in our extracts. Previous studies suggest that factors such as the hydrophobicity or solubility of compounds and the composition of microbial membranes can influence antimicrobial activity, with Gram-positive bacteria typically showing larger inhibition zones due to their less permeable peptidoglycan layer [6]. In contrast, our study observed varied inhibition patterns across both Gram-positive and Gram-negative bacteria.

Earlier research has revealed that *Cleome amblyocarpa* extracts exhibit dose-dependent antimicrobial activity against all tested strains [5]. However, in this study, *Cleome amblyocarpa* was ineffective against *Bacillus cereus* and *Staphylococcus aureus*, which contrasts with earlier findings. Similarly, although *Escherichia coli* is typically the most sensitive microorganism to antimicrobial

agents, its response varied in our study. For instance, the inhibition zone for *Escherichia coli* was consistent with previous reports for *Cleome amblyocarpa* extract (12±0.1 mm), but *Ocimum basilicum* and *Teucrium polium* extracts demonstrated stronger antimicrobial activity (20±0.2 mm and 18±0.3 mm, respectively). Furthermore, Pushpalatha et al. [60] observed significant bactericidal activity in *Psidium guajava* and *Syzygium aromaticum* extracts, with low MBC. In contrast, *Caralluma arabica* extract in our study showed no antimicrobial effect against *Escherichia coli*, which aligns with previous research [61, 62]. While Ginestra et al. [63] found weak activity in certain phytochemical fractions of *Opuntia ficus-indica*.

Regarding antifungal activity, the extracts were effective against all tested fungal strains, except for *Caralluma arabica*, which showed no activity against *Fusarium oxysporum*. This result aligns with findings by Zourgui et al. [6], who reported resistance of *Fusarium* species to *Opuntia* extracts. Among the tested extracts, *Teucrium polium* exhibited the lowest MIC and MFC values, measuring 3.25 mg/ml and 17.28 mg/ml, respectively, against *Fusarium* species. Overall, the extracts demonstrated robust antibacterial and antifungal activity, with *Ocimum basilicum* showing the most potent antibacterial effects, while *Teucrium polium* displayed the strongest antifungal activity, consistent with the findings of Pushpalatha et al. [60].

To the best of our knowledge, this study represents the first comprehensive comparative analysis of the metabolic profiles, antibacterial, and antifungal activities of four Omani wild herbal plants: *Ocimum basilicum*, *Teucrium polium*, *Cleome amblyocarpa*, and *Caralluma arabica*. Utilizing the Folin-Ciocalteu method, LC-ESI-MS analysis, and agar diffusion assays, we successfully identified key bioactive compounds, including phenolic acids and flavonoids, in these plants, which demonstrated significant antioxidant, antibacterial, and antifungal properties. Notably, *Ocimum basilicum* and *Teucrium polium* contained the highest concentrations of these bioactive compounds and exhibited the most potent antimicrobial effects. These findings suggest that these extracts hold great promise for further investigation and potential therapeutic applications. A deeper understanding of their diverse metabolites and interactions could pave the way for the discovery of novel pharmacological agents, offering new strategies for treating complex diseases such as cancer.

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Author contributions
JAM: Conceptualization, data collection, and data sorting. LZ: Conceptualization, Design, Resources, and Supervising the work. NM: Conceptualization, Visualization, Supervising the work and Paper Writing. All authors reviewed and approved the final version of the paper.

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The supplementary data will be available to the readers when they send the request.

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Conflict of interests
The authors declare that they have no competing interests.

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