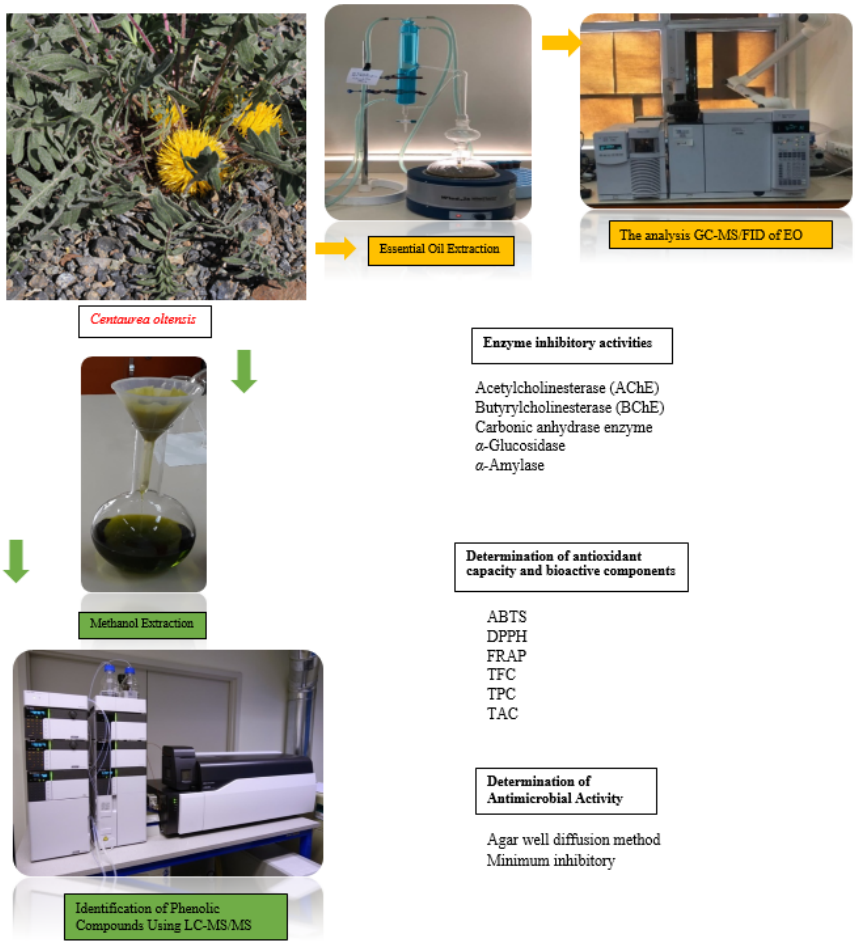
Chemical Profiling of Essential Oils and Evaluation of Enzyme Inhibition, Antioxidant, and Antimicrobial Activities in Both Methanol Extract and Essential Oils of *Centaurea oltensis*

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DOI: 10.15376/biores.21.4.11085-11114

GRAPHICAL ABSTRACT



Chemical Profiling of Essential Oils and Evaluation of Enzyme Inhibition, Antioxidant, and Antimicrobial Activities in Both Methanol Extract and Essential Oils of *Centaurea oltensis*

İmdat Aygül [a,*] and Berfin Akşit [b]

The phytochemical composition and biological activities of *Centaurea oltensis*, an endemic species in Türkiye, were studied. GC–MS/FID analysis identified 128 compounds representing 99.2% of the EO, with decanoic acid (15.1%), α-pinene (7.9%), and sabinyl acetate (5.4%) as the principal constituents. GC–MS/FID analysis identified 128 compounds representing 99.2% of the essential oil (EO), with decanoic acid (15.1%), α-pinene (7.9%), and sabinyl acetate (5.4%) as the principal constituents. LC–MS/MS analysis of the methanol extract (ME) revealed high concentrations of ferulic acid (2410 µg/g dw) and chlorogenic acid (1170 µg/g dw), together with caffeic, salicylic, and 4-hydroxybenzoic acids. The ME exhibited potent carbonic anhydrase-II inhibition (IC_{50} = 0.028 µg/mL) and significant α-glucosidase inhibition (IC_{50} = 0.255 mg/mL). The EO showed strong acetylcholinesterase and butyrylcholinesterase inhibitory activities, with IC_{50} values of 0.0145 µg/mL and 0.204 µg/mL, respectively. Antioxidant assays, including DPPH, ABTS, and FRAP, demonstrated superior activity of the ME, correlating with its higher phenolic and flavonoid contents. The EO displayed remarkable antibacterial activity against *Staphylococcus aureus* (MIC = 0.02 mg/mL) and moderate antifungal activity against *Candida albicans*. Overall, *C. oltensis* demonstrated considerable chemical diversity and promising antioxidant, antimicrobial, neuroprotective, and enzyme inhibitory potential for future pharmacological and therapeutic applications.

DOI: 10.15376/biores.21.4.11085-11114

Keywords: Centaurea oltensis; Enzyme inhibition; Phenolic compounds; Antioxidant activities; Antimicrobial activities; Essential oil

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INTRODUCTION

In the southwest, center, and east of Türkiye, there are representatives of the genus *Centaurea* L. (Asteraceae). In addition, this genus includes roughly 500 to 600 annual, biannual, and perennial herbaceous plants that are found worldwide, but exclusively north of the equator. The majority of these plants are found in the Eastern Hemisphere, with the Middle East serving as the center of diversification (Djeddi *et al.* 2011; Carev *et al.* 2022). With 112 endemic species out of 181 total, *Centaurea* has one of Türkiye's highest rates of endemism. Türkiye is one of the gene centers of this genus, as evidenced by the high endemism ratio (Yayli *et al.* 2005; Aydin *et al.* 2013; Kilic 2013). In *Centaurea* species,

essential oils are commonly obtained from aerial organs, particularly flowers, leaves, and stems, although their yield and chemical composition may vary considerably among plant organs, species, developmental stages, and environmental conditions. Flowers and leaves are often of particular interest because of their abundance of secretory structures and volatile secondary metabolites. Therefore, the selection and clear identification of the plant organs used for essential-oil extraction are important for both chemical characterization and comparison among *Centaurea* species.

The majority of terpenes and terpenoids found in EO are naturally occurring combinations that are derived from fragrant and medicinal plants. Each species or subspecies has a different essential oils chemical makeup, which is unique to that species. Several approaches must be used in order to identify the various components of complicated combinations, such as terpenes and terpenoids in essential oils. Gas chromatography–mass spectrometry (GC–MS), one of the most widely used techniques for analyzing the composition of essential oils, enables the identification of the particular natural compounds present in an essential oil by comparing their mass spectra and relative retention times/indices (Yayli *et al.* 2005).

According to earlier chemical research on the genus *Centaurea*, the most distinctive and consistently significant ingredients are the sesquiterpene lactones. Triterpenes, steroids, hydrocarbons, polyacetylenes, flavonoids, anthocyanins, lignans, and alkaloids are some of the additional secondary metabolites found in plants in this genus (Kilic 2013).

Phytopharmaceuticals from *Centaurea* species are recognized to have both positive and negative effects on human health. Research on herbal therapy has grown in emerging nations as a means of reviving long-standing customs and as a substitute for urban health issues. Therefore, with the increasing popularity of traditional medicine as an alternative type of health treatment, the screening of plants for active chemicals has become highly significant. A number of *Centaurea* members biological actions, including cytogenetic, antimicrobial, antifungal, antiulcerogenic, antioxidant, antiviral, antiplasmodial, antiprotozoal, anti-colon cancer, and cytotoxic activity, have been documented elsewhere. Additionally, folk medicine uses the aerial parts of numerous *Centaurea* species as antibiotics, diuretics, choleretics, antibacterials, antipyretics, antidiarrheics, and anti-inflammatory agents (Kilic 2013; Carev *et al.* 2022).

Given the considerable interspecific variation in essential-oil and phytochemical composition previously reported among *Centaurea* species, it was hypothesized that *C. oltensis* may possess a distinctive phytochemical profile and exhibit significant biological activities. Specifically, the high phenolic and terpenoid content of *C. oltensis* was expected to contribute to strong antioxidant, antimicrobial, and enzyme inhibitory properties. Therefore, this study was designed to test whether the EO and ME of *C. oltensis* demonstrate distinct compositional characteristics and multifunctional bioactivities that could support their potential use as natural therapeutic and preservative agents. Compared with previous studies on other *Centaurea* species, the significance of the present work lies in the integrated evaluation of the chemical composition and multiple biological activities of *C. oltensis*. By combining essential-oil profiling, phenolic characterization, enzyme inhibition, antioxidant, and antimicrobial analyses, this study provides a broader assessment of the phytochemical and biological potential of this endemic species and contributes to the comparative understanding of chemical and functional diversity within the genus *Centaurea*.

EXPERIMENTAL

Plant Material

In this study, samples of *Centaurea oltensis* were collected from the Bayburt province (Türkiye), Kop Pass, near the Coşan Mountain and the border of Erzincan (coordinates: 39°58'37"N, 40°22'17"E, altitude: 2220 m, date: 28 June 2024, Habitat: roadsides, sandy places). Figure 1 illustrates the habitus, flowers, and leaves of the plant. A total of 1000 g of plant material was collected for analysis. The taxonomic identification of the plant was verified by Assoc. Prof. Abdurrahman Sefalı from the Department of Primary Education, Faculty of Education, Bayburt University, Bayburt, Türkiye. The collected sample was deposited in the Herbarium of Bingöl University, where it was assigned the reference number BIN Sefalı 1049a.

Morphologically, *C. oltensis* is a perennial herb bearing solitary or up to six capitula. Scapes are sessile or up to 2 cm long. Rosette leaves are conspicuously covered with simple hairs and are pinnatipartite to pinnatifid or sublyrate, with linear to lanceolate lateral segments, occasionally up to 15 pairs, and a slightly larger, triangular terminal segment. Phyllaries bear brownish-yellow, lanceolate, usually curved appendages, and the florets are golden yellow. Achenes are 4 to 5 mm long, with a pappus 1 to 2 mm long. The species typically occurs on rocky slopes.



Fig. 1. *Centaurea oltensis*: Habitus and capitula with appendages

Extraction Procedure

Essential oil extraction

A 500 g portion of the collected aerial parts (stems, leaves, and flowers) was dried in the shade and finely ground. After homogenization and sieving through a 250-µm screen, 100 g of the processed aerial plant material was used for essential-oil extraction. Using a modified Clevenger apparatus with both internal and exterior cooling systems, the extraction was performed by hydrodistillation at 100 °C. To maintain its integrity, the resultant EO was filtered through a 0.45- µm membrane, dissolved in hexane, and stored in amber-colored vials (Oz *et al.* 2023, 2025).

Extraction with methanol

A 3 L, 320 W ultrasonic bath (Bandelin Ultrasonic Bath) was used for the extraction procedure. First, 50 mL of a 30% aqueous methanol (MeOH) solution was added to 10 g of the ground plant material after weighing. The mixture was extracted using ultrasonic assistance for 60 min at a controlled temperature of 40 °C. To separate the plant extracts, the solution was centrifuged at 4000 rpm for 10 min after being filtered twice through Whatman No. 1 filter paper. The final extract was obtained by carefully transferring the resulting supernatant to a beaker and completely evaporating the methanol–water mixture at 40 °C (Oz *et al.* 2023). The dried extract was dissolved in hexane, filtered through a 0.45 µm membrane, and securely stored in amber-colored vials to preserve its integrity (Oz *et al.* 2023).

Enzyme Inhibitory Activities of the Extracts

Activity assay for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes

The Ellman method was used to spectrophotometrically evaluate the activity of the AChE and BChE enzymes. Acetylthiocholine iodide, a thioester, is utilized as a substrate in this experiment rather than acetylcholine. AChE hydrolyzes it enzymatically, releasing thiocholine, which then interacts with DTNB (5,5'-dithio-bis-(2-nitrobenzoic acid)). The production of TNB (5-thio-2-nitrobenzoic acid), a yellow-colored molecule produced by this reaction, is tracked by measuring absorbance at 412 nm (Ellman *et al.* 1961). The process is the same for BChE, with the exception that the substrate is butyrylthiocholine iodide. At different inhibitor doses, enzyme activities were measured and the % activity was computed. Lineweaver–Burk plots were used to extract IC_{50} values from the inhibition curves (Lineweaver and Burk 1934).

Carbonic anhydrase enzyme activity assay

Using *p*-nitrophenyl acetate as the substrate, this approach uses the esterase activity of carbonic anhydrase to hydrolyze it producing *p*-nitrophenol or *p*-nitrophenolate, both of which absorb at 348 nm. The dissociation of a proton from the phenolic OH group has no effect on the measurement because these two forms show the same absorbance at this wavelength (Kandel *et al.* 1970; Armstrong *et al.* 1966; Landolfi *et al.* 1997).

α-Glucosidase enzyme activity assay

α-Glucosidase activity was measured using *p*-nitrophenyl-*α*-D-glucopyranoside (*p*-NPG) as the substrate, in accordance with the procedure outlined by Akmese *et al.* (2025). Absorbance at 405 nm was used to track the enzymatic process. Plotting the proportion of residual activity against inhibitor concentration allowed for the evaluation of inhibitory effects at different concentrations. The obtained inhibition curves were analyzed using the Lineweaver–Burk model to determine the IC_{50} values (Lineweaver and Burk 1934).

α-Amylase enzyme activity assay

A modified version of the Caraway–Somogyi iodide/potassium iodide (IKI) method described by Kilinc *et al.* (2025) was used to evaluate the *α*-amylase inhibitory activity. Briefly, 25 µL of each sample was added to a 96-well microplate, followed by 50 µL of *α*-amylase solution prepared in phosphate buffer (pH 6.9, containing 6 mM NaCl). After incubation at 37 °C for 10 min, 50 µL of a 0.05% starch solution was added. In parallel, an enzyme-free blank was prepared. The plate was then incubated for an additional 10 min at 37 °C. The reaction was stopped by adding 25 µL of 1 M HCl, after which 100

µL of IKI reagent was added to develop the color. Absorbance was measured at 630 nm using a microplate reader. Acarbose served as the reference inhibitor, and IC_{50} values were calculated to express the inhibitory potential of the samples.

Determination of Phenolic Profiles Using LC-MS/MS Analysis

A Thermo Scientific Dionex Ultimate 3000 UHPLC system in conjunction with a TSQ Quantum Access Max tandem mass spectrometer was used to conduct the LC-MS/MS analysis. The liquid chromatography system has a column compartment, dual pump, autosampler, and degasser. A C18 reversed-phase Inertsil ODS HYPERSIL analytical column (250 mm × 4.6 mm, 5 µm) was used for the chromatographic separation, which was kept at a steady temperature of 30 °C.

Phase A was ultrapure water with 0.1% formic acid, while phase B was methanol. These two substances made up the mobile phase. The following gradient elution program was used: 0% B for 0 to 1 min, 95% B for 1 to 22 min, 95% B for 22 to 25 min, and 100% B for 25 to 30 min. 34 min was the allotted run time, including re-equilibration. The flow rate was set at 0.7 mL/min, and the injection volume was 20 µL.

This mobile phase composition and gradient program were chosen after a number of optimization trials to guarantee efficient ionization and separation of the target phenolic chemicals. This LC-MS/MS method was used to examine the phenolic compounds indicated in Tables 2 and 3. A chromatogram of the standard phenolic compounds is also included. The protocol created by Kayir *et al.* (2023) served as the model for the analytical technique used in this investigation.

Determination of Antioxidant Capacity

Several techniques were used to assess the antioxidant capacity of the methanol extract and essential oil of *Centaurea oltensis*. The FRAP assay was used to analyze the ferric ion's (Fe^{3+}) reducing capability, and the ABTS and DPPH assays were used to evaluate its capacity to neutralize free radicals. Additionally, the total antioxidant capacity (TAC), total flavonoid content (TFC), and total phenolic content (TPC) were measured in order to quantify the extract's bioactive composition. The antioxidant qualities of the extract and its underlying bioactive components were thoroughly understood thanks to these analyses.

The ferric ion reducing antioxidant power (FRAP) of the methanol extracts and essential oil was measured using the method described by Fidan *et al.* (2023). First, 500 µL of distilled water was used as the blank, and a FRAP reagent was created. The same conditions were used to process standard solutions (250 µL). A calibration curve ($y = 0.0115x + 0.0521$, $R^2 = 0.998$) produced from $FeSO_4$ solutions was used to calculate the samples' FRAP values. The entire iron-reducing ability was represented by the results, which were given as milligrams of $FeSO_4$ equivalents per 100 grams of material.

The method outlined by Kobya *et al.* (2024) was used to assess the ABTS radical scavenging activity. Methanol (150 µL) was utilized as the blank in an ABTS solution. The sample extract and standard ascorbic acid solutions (150 µL) underwent the same processing. Using a spectrophotometer, the absorbance of the resultant mixes was determined at 734 nm. The ABTS cation radicals' scavenging activity was measured using a calibration curve made from Trolox standards ($y=-0.0144x+0.615$, $R^2 = 0.997$). According to Eq. 1, the results were reported as mg of ascorbic acid equivalents (AAE) per 100 g, mg of Trolox equivalents per gram, and the percentage of free radical scavenging.

$$Inhibition\ (\%) = \frac{(Control\ Absorbance - Sample\ Absorbance)}{Control\ Absorbance} \times 100 \quad (1)$$

The ME and EO extracted from the plant were tested for their ability to scavenge DPPH radicals using the method described by Yilmaz *et al.* (2023). This method involved mixing the extract with solutions of 2,2-diphenyl-1-picrylhydrazyl (DPPH) at specific quantities. After giving the mixtures a thorough vortex, they were allowed to sit at room temperature for 30 min in the dark. Following the incubation period, the samples' absorbance at 517 nm was evaluated. Equation 1 was used to calculate the DPPH radicals' % inhibition. The findings were presented as the percentage of free radical scavenging activity and as milligrams of ascorbic acid (AA) equivalents per 100 grams (based on the calibration curve y=-0.0093x+0.945, R^2 = 0.998).

Following the procedure described by Gedikli *et al.* (2024), a molybdate reagent was used to assess the TAC of the plant's ME and EO. Pure water (250 µL) was used as the blank in this process instead of the sample. A spectrophotometer was used to measure the reaction mixtures' absorbance at 695 nm. First, 500 µL aliquots of the standard solutions were made and treated in the same way. Using a calibration curve (y=0.0022x-0.057, R^2 = 0.998) produced from ascorbic acid standards, the TAC content in the methanol extract was measured in milligrams of gallic acid (GA) equivalents per gram (Gedikli *et al.* 2024).

The Yilmaz *et al.* (2023) technique was used to calculate the TFC of the EO and ME. A spectrophotometer was used to measure the reaction mixture's absorbance at 506 nm, using 500 µL of distilled water as the blank. The same procedure was applied to standard catechin solutions made in ethanol. TFC values were calculated using the calibration curve y=0.0036x+0.0168 (R^2 = 0.999) and reported as mg catechin equivalents (CAE) per 100 g of sample.

The Folin-Ciocalteu reagent was used to assess the TPC, a crucial bioactive component in the ME and EO, in accordance with the protocol described by Gedikli *et al.* (2024). The reaction mixture was prepared, vortexed completely, and then allowed to sit at room temperature for 120 min in the dark. At 760 nm, the mixture's absorbance was then measured. 3.7 mL of water, 500 µL of methanol, 100 µL of Folin-Ciocalteu reagent, and 600 µL of a 10% sodium carbonate (Na_2CO_3) solution were combined to create a blank. The phenolic content of the samples was measured using a calibration curve (y=0.0052x+0.0074, R^2 = 0.997) created from GA standards, and expressed as milligrams of GA equivalents (GAE) per 100 grams.

Determination of Antimicrobial Activity

Agar diffusion method

Essential oils from *C. oltensis* were evaluated for their antibacterial activity using a modified agar diffusion method based on CLSI criteria (Clinical & Laboratory Standards 2017). The test organisms included gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 9634), gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922), and the yeast *Candida albicans* ATCC 18804. Fresh microbiological cultures were grown on Müller-Hinton Agar (MHA), and they were standardized to 0.5 McFarland turbidity (about 1.5 x 10^8 CFU/mL) using 0.9% sterile saline. The inoculum was uniformly spread across MHA plates using sterile swabs.

Sterile 5.5 mm disks were used to cover the inoculated agar surfaces, and 15 µL of each sample (25 mg/mL in DMSO) was applied. To allow for compound diffusion, plates were kept at 4 °C for two h. DMSO served as the negative control, while nalidixic acid (512 µg/mL) and chloramphenicol served as the positive controls. Following diffusion, bacterial and yeast plates were incubated for 24 and 48 h at 37 and 28 °C, respectively. The inhibitory zones were measured using a digital caliper. Each experiment was conducted in triplicate, and the results were statistically analyzed.

Determination of MIC and MBC values

Centaurea oltensis essential oils' minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values against particular pathogens were calculated in accordance with CLSI criteria (Clinical & Laboratory Standards 2017). Using Müller-Hinton broth (MHB) as the culture medium, MIC testing was carried out in sterile 96-well plates using the broth microdilution method. Double-strength MHB was included in the first wells, and normal MHB was added to the following wells. Sample solutions (25 mg/mL) were added to the first wells following successive dilutions in order to reach an initial concentration of 12.5 mg/mL. Positive controls included chloramphenicol (256 µg/mL in ethanol) and nalidixic acid. First, 10 µL of a microbiological suspension standardized to 0.5 McFarland turbidity were added to each well as an inoculant. Negative controls were wells that contained either medium alone or medium with extracts. For 24 h, plates were incubated at 37 °C for bacteria, and for 48 h, plates were incubated at 28 °C for fungus. The lowest concentration that totally prevented observable growth was known as the minimum inhibitory concentration, or MIC.

The MBC values of essential oils of *C. oltensis* against particular infections were calculated in accordance with CLSI recommendations (Clinical & Laboratory Standards 2017). Using Müller-Hinton Broth (MHB) as the culture medium, MIC testing was carried out in sterile 96-well plates using the broth microdilution method. Double-strength MHB was included in the first wells, and normal MHB was added to the following wells. Sample solutions (25 mg/mL) were added to the first wells following successive dilutions in order to reach an initial concentration of 12.5 mg/mL. Positive controls included chloramphenicol (256 µg/mL in ethanol) and nalidixic acid. Ten microliters of a microbiological suspension standardized to 0.5 McFarland turbidity were added to each well as an inoculant. Negative controls were wells that contained either medium alone or medium with extracts. Plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. The MIC was defined as the lowest concentration that completely inhibited visible growth.

To estimate MBC, 50 µL samples from wells corresponding to MIC, 2×MIC, and 4×MIC were spread onto MHA plates using a Drigalski spatula. The plates were then incubated in the same way. After incubation, colonies were counted, and the MBC was calculated by selecting the lowest concentration that resulted in a decrease of at least 99.9% in viable cells.

GC–MS/FID Analysis of the Essential Oil

Helium (>99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min to inject 1 µL of the EO solution with a 1:5 split ratio for GC–MS/FID analysis. The analyses were performed using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass spectrometer (GC–MS/FID; Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an HP-5 apolar capillary column (30 m × 0.32 mm, 0.25 µm film

thickness). The injector, ion source, and quadrupole temperatures were maintained at 250 °C, 230 °C, and 150 °C, respectively. The GC oven temperature was initially held at 60 °C for 2 min, then increased to 240 °C at a rate of 3 °C/min. The FID detector was operated at 250 °C with hydrogen and air flows of 35 mL/min and 350 mL/min, respectively. Mass spectra were acquired in the range of 45 to 450 m/z with a solvent delay of 4.0 min (Oz *et al.* 2023). By comparing their mass spectra with reference data from the NIST and Wiley libraries, the constituents of essential oils were identified. Furthermore, in accordance with earlier research, the compounds' identities were verified using the Kovats indices (Adams 2007).

RESULTS AND DISCUSSION

Enzyme Inhibitory Activities

With low IC_{50} values underscoring their potential, the enzyme inhibitory results of *Centaurea oltensis* ME and EO demonstrate exceptional bioactivity. ME inhibited CA-II very well (IC_{50} = 0.0283 µg/mL), but acetazolamide (0.0067 µg/mL) was still more effective. Since glaucoma, epilepsy, and similar conditions can be effectively treated with carbonic anhydrase inhibitors (Supuran 2016), the ME may be a viable natural source of CA-II inhibitors because of its high phenolic content (Poulsen and Davis 2013).

In terms of cholinesterase inhibition in Table 1, the EO was less effective than tacrine (0.038 µg/mL), although it showed better activity against AChE (IC_{50} = 0.0145 µg/mL) than the ME (0.111 mg/mL). This is in accordance with other research showing that EO's terpenoids and volatile chemicals work well as cholinesterase inhibitors (Loizzo *et al.* 2008; Mukherjee *et al.* 2007). The importance of lipophilic components was further shown by the EO's greater BChE inhibition (IC_{50} = 0.204 µg/mL) in comparison to the ME's (0.422 mg/mL). The EO of *C. oltensis* may be a viable natural neuroprotective candidate because both AChE and BChE inhibitors are important for treating Alzheimer's disease (Darvesh *et al.* 2003; Greig *et al.* 2001).

The ME exhibited considerable *α*-glucosidase inhibition (IC_{50} = 0.255 mg/mL) with respect to carbohydrate-hydrolyzing enzymes, whereas the EO remained inactive. Despite having a lower activity than acarbose (IC_{50} = 0.059 µg/mL), the presence of this inhibition points to possible antidiabetic benefits associated with flavonoids and phenolics (Tadera *et al.* 2006; Van de Laar *et al.* 2005). The ME (IC_{50} = 18.52 mg/mL) and EO (IC_{50} = 11.20 mg/mL) showed comparatively weaker *α*-amylase inhibition than acarbose (33.20 µg/mL). This finding is consistent with previous studies reporting that plant extracts generally show greater inhibitory activity against *α*-glucosidase than against *α*-amylase (Tadera *et al.* 2006).

Overall, the findings show that *C. oltensis* has strong enzyme inhibitory qualities, especially when it comes to cholinesterase and CA-II inhibition. The EO works better against cholinesterase, while the ME seems more promising for inhibiting carbonic anhydrase. According to Khammar and Djeddi (2012), these results are consistent with prior publications on bioactive chemicals in *Centaurea* species and similar taxa, indicating that both fractions merit more phytochemical and pharmacological research. Isolating the accountable secondary metabolites and validating the therapeutic potential *in vivo* should be the main goals of future research.

Table 1. Enzyme Activity Results of *C. Oltensis* ME and EO

	CA-II IC_{50}	ACHE IC_{50}	BCHE IC_{50}	α-Glucosidase IC_{50}	α-Amylase IC_{50}
ME	0.0283±0.00028 µg/mL	0.111± 0.0021 mg/mL	0.422± 0.023 mg/mL	0.255±0.0098 mg/mL	18.52±0.26 mg/mL
EO	0.172±0.0042 µg/mL	0.0145±0.00021 µg/mL	0.204± 0.0035 µg/mL	No inhibition observed	11.20±0.43 mg/mL
Asetazolamid	0.0067 ±0.0002 µg/mL	-	-	-	-
Tacrine	-	0.038 ±0.001 µg/mL	0.0085±0.0003 µg/mL	-	-
Acarbose	-	-	-	0.059 ±0.002 µg/mL	33.20±0.48 µg/mL

Values are expressed as mean ± standard deviation (SD) of three replicate measurements (n = 3).

Phenolic Compounds

Figures 2 and 3 present the chromatograms of the 33 standards used in the analysis and the chromatogram of the ME obtained from the aerial parts of *C. oltensis.* This comprehensive analysis, conducted using LC-MS/MS, highlights the extract's rich phenolic profile, providing detailed insights into its chemical composition.

The current study indicates that *C. oltensis*'s aerial parts have a distinct phenolic profile. Despite the absence of most flavonoids, these sections have a particularly high phenolic acid content (Table 2). This suggests that the species may have a distinct phenolic metabolic pathway compared to other species in the genus *Centaurea*. The most common of these compounds (2410 µg/g dw) was ferulic acid, which is an essential part of the species' phenolic composition. This observation highlights *C. oltensis* as a species particularly rich in ferulic acid.

In *C. oltensis*, chlorogenic acid represents the second most abundant phenolic component, with a concentration of 1170 µg/g dw, highlighting a notable species-specific enrichment. Although chlorogenic acid has been regularly reported in other *Centaurea* taxa, the relatively high levels observed in *C. oltensis* suggest that genetic factors may strongly influence phenolic composition within the genus. For instance, *Centaurea gigantea* contains chlorogenic acid as a dominant phenolic in its aerial parts, where it exhibits significant anti-colon cancer activity (Shoeb *et al.* 2007). Similarly, *Centaurea paphlagonica* harbors chlorogenic acid and its methyl ester in the flowers and aerial tissues, with the latter showing antiproliferative activity against cancer cell lines (Tufekci *et al.* 2023). In *Centaurea bruguieriana*, chlorogenic acid has been quantified at 3.2 µg/mg in the ethyl acetate fraction, further confirming its consistent occurrence across the genus (Noman *et al.* 2022). Taken together, these findings indicate that chlorogenic acid is a ubiquitous but variably abundant phenolic in *Centaurea* taxa. Its presence contributes substantially to the phytochemical profile and pharmacological potential of the genus, underscoring both its taxonomic relevance and its importance as a bioactive compound with potential applications in health, nutrition, and biotechnology.

Caffeic acid (79.97 µg/g dw) and 4-hydroxybenzoic acid (79.80 µg/g dw) were among the compounds found in moderate amounts. Both substances are known to contribute to the genus's phenolic diversity. In the genus *Centaurea*, caffeic acid has frequently been reported as one of the dominant phenolic acids, whereas 4-hydroxybenzoic

acid is considered a marker of phenolic variation across species. For instance, in *C. solstitialis*, caffeic acid was identified as the most abundant phenolic compound (24.1 µg/g extract), while 4-hydroxybenzoic acid was present at 2.08 µg/g (Alper *et al.* 2021). Similarly, *C. paphlagonica* was shown to contain caffeic acid, 3,4-dihydroxybenzoic acid, and various caffeic acid derivatives (Tufekci *et al.* 2023). In polyherbal mixtures involving *C. erythraea*, both caffeic acid and *p*-hydroxybenzoic acid, along with their derivatives, were also detected (Petrovič *et al.* 2023). Additional studies reported the presence of 4-hydroxybenzoic acid in *C. urvillei* and *C. jacea* while *C. horrida* growing in Lebanon contained caffeic acid together with flavonoids such as fisetin, hispidulin, and quercetin (Boukhary *et al.* 2017). These findings confirm that both compounds, although varying in concentration among species, play a central role in shaping the antioxidant and cytotoxic activities attributed to *Centaurea* (Khan *et al.* 2021; Pavlikova 2022).

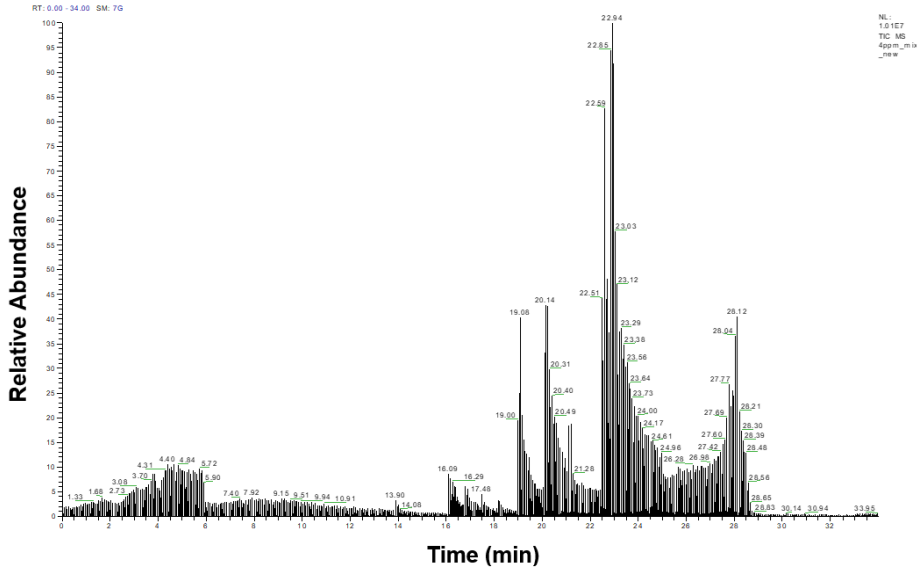


Fig. 2. Chromatogram of standard phenolic compounds analyzed by LC/MS/MS

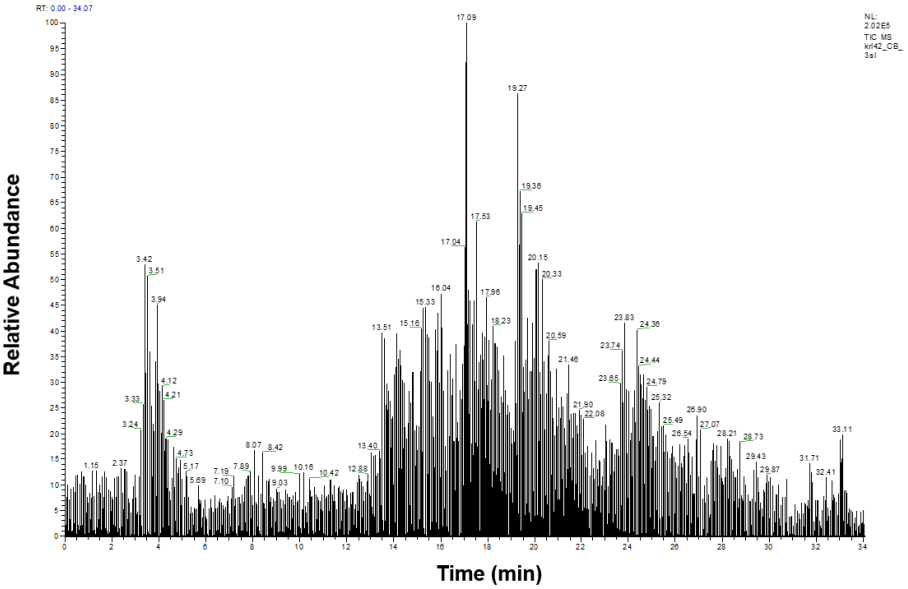


Fig. 3. Chromatogram of extracts of aerial parts of *Centaurea oltensis* by LC/MS/MS

Furthermore, significant concentrations of protocatechuic acid (56.15 µg/g dw), salicylic acid (56.65 µg/g dw), and protocatechuic aldehyde (28.53 µg/g dw) were found. Previous observations of salicylic acid in *C. behen* and protocatechuic acid in *C. cyanus* (Bahadori *et al.* 2020) indicate that these phenolics are common constituents across multiple *Centaurea* species. Together with the frequently reported caffeic acid and 4-hydroxybenzoic acid profiles in the genus (Alper *et al.* 2021), these findings underscore a shared yet quantitatively variable phenolic backbone that likely contributes to the antioxidant and cytotoxic capacities noted for *Centaurea* extracts. Beyond chemotaxonomic value, protocatechuic acid and protocatechuic aldehyde have been associated with broad pharmacological actions—including antioxidant, anti-inflammatory, and cytoprotective effects—supporting their functional relevance when present at moderate-to-high levels (Song *et al.* 2020; Zhang *et al.* 2021). Overall, the relative abundances of these acids and aldehydes vary among species, highlighting intra-generic chemical diversity with potential implications for bioactivity and standardization of *Centaurea*-derived preparations (Alper *et al.* 2021). The profile was further complicated by compounds that were present at lower amounts, such as luteolin-7-glucoside (9.32 µg/g dw), vanillin (9.68 µg/g dw), and rosmarinic acid (34.1 µg/g dw). Particularly, luteolin derivatives have been found in *C. cyanus* and *C. stoebe*, suggesting that *C. oltensis* and other genus members have similarities in phenolic variety.

Interestingly, *C. oltensis* did not contain any of the phenolic compounds that are frequently described in many *Centaurea* species, such as catechin, epicatechin, rutin, quercetin, oleuropein, naringenin, taxifolin, resveratrol, and ellagic acid. Other congeners, including *C. horrida*, *C. castriferrei*, *C. parviflora*, *C. lycaonica*, and *C. glastifolia*, have consistently shown flavonoids such as quercetin, apigenin, luteolin, kaempferol, and chlorogenic acid (Boukhary *et al.* 2017; Gozcu *et al.* 2024; Hechaichi *et al.* 2023). The absence of these compounds in *C. oltensis* highlights the uniqueness of its phenolic profile and may be explained by both methodological and biological factors. Variations in extraction method, solvent type, or analytical technique can substantially affect phenolic detection (Zengin *et al.* 2018), while genetic and environmental influences such as climate, soil characteristics, and altitude are also known to modulate secondary metabolite synthesis in *Centaurea* species (Salachna *et al.* 2021). This pattern underscores the remarkable chemical diversity within the genus and suggests that *C. oltensis* may represent a chemotaxonomically distinctive lineage.

Overall, ferulic acid and chlorogenic acid predominate in *C. oltensis'*s phenolic profile, which is reinforced by the moderate and low amounts of a number of phenolic acids and aldehydes. Although there are clear similarities in the types of compounds when compared to other *Centaurea* species, significant quantitative differences show that *C. oltensis* has distinct phytochemical properties. These results offer fresh perspectives on the phytochemical uniqueness of *C. oltensis* and advance our knowledge of the chemical diversity within the genus. Taken together, these findings indicate that the phenolic profile of *C. oltensis* is particularly remarkable for its exceptionally high levels of ferulic and chlorogenic acids, while the presence of compounds such as protocatechuic acid, salicylic acid, 4-hydroxybenzoic acid, and rosmarinic acid further underscores the species' chemical diversity. Comparative evaluations with other *Centaurea* taxa reveal clear qualitative and quantitative distinctions, confirming the phytochemical uniqueness of *C. oltensis*. This distinctiveness not only highlights its significance for chemotaxonomic classifications but also points to its potential value in biological activity, pharmacological applications, and natural product research, warranting further detailed investigations in future studies

Table 2. The Amount of Phenolic Compound in the Extracts of Aerial Parts of *C. oltensis*

No	R.T.	Phenolic Compound	µg/g dw	Equation	R^2	Linear Range
1	7.30	Pyrogallol	n.d.	Y = 29126.1*X	0.9976	0.25-8 ppm
2	9.24	Gallic Acid	n.d	Y = -6517.23+17957.3*X	0.9985	0.25-8 ppm
3	11.16	Protocatechuic Acid	56.15±2.11	Y = -2848.85+18509.7*X	0.9980	0.25-8 ppm
4	12.03	Protocatechuic Aldehyde	28.53±1.12	Y = 96774+449088*X	0.9995	0.25-8 ppm
5	12.05	Sesamol	n.d	Y = 109779+125863*X	0.9994	0.25-8 ppm
6	12.48	Gentisic Acid	n.d	Y = 11742.1*X	0.9941	0.25-8 ppm
7	12.54	Catechin	n.d	Y = 35635.4+249444*X	0.9989	0.25-8 ppm
8	13.69	Chlorogenic Acid	1173.75±5.99	Y = 184688*X	0.9994	0.25-8 ppm
9	13.95	Epicatechin	n.d	Y = 51887.5+354890*X	0.9995	0.25-8 ppm
10	14.32	Caffeic Acid	79.97±3.12	Y = -5962.19+92187.9*X	0.9988	0.25-8 ppm
11	14.67	Vanillin	9.68 ±0.34	Y = 10281+371448*X	0.9991	0.25-8 ppm
12	15.20	Syringaldehyde	n.d	Y = 304599*X	0.9988	0.25-8 ppm
13	15.75	Taxifolin	n.d	Y = -116.631+1476.29*X	0.9934	0.25-8 ppm
14	*15.89*	*p*-Coumaric Acid	n.d	Y = 18470.2+92906.8*X	0.9980	0.25-8 ppm
15	16.16	Ferulic Acid	2409.71±15.65	Y = 5566.9+20085.9*X	0.9937	0.25-8 ppm
16	16.28	Vitexin	n.d	Y = 2.10386e+006*X	0.9986	0.25-8 ppm
17	16.59	Salicylic Acid	56.65±2.61	Y = 473736+1.04668e+006*X	0.9919	0.25-8 ppm
18	16.61	4-Hydroxybenzoic Acid	79.802±2.96	Y = 452696+1.20893e+006*X	0.9993	0.25-8 ppm
19	16.81	Hesperidin	n.d	Y = 14796.7*X	0.9955	0.25-8 ppm
20	17.04	Rosmarinic Acid	34.06 ±1.11	Y = -1023.86+3266*X	0.9949	0.25-8 ppm
21	17.07	Syringic Acid	n.d	Y = 5846.34+11932*X	0.9946	0.25-8 ppm
22	17.11	Oleuropein	n.d	Y = -10850.9+64250*X	0.9983	0.25-8 ppm
23	17.33	Luteolin-7-Glucoside	9.32±0.63	Y = 675594*X	0.9950	0.25-8 ppm
24	17.60	Rutin	n.d	Y = -11643.8+48198.3*X	0.9976	0.25-8 ppm
25	17.62	Resveratrol	n.d	Y = 137913+991721*X	0.9995	0.25-8 ppm
26	18.66	Cinnamic Acid	n.d	Y = 853918*X	0.9982	0.25-8 ppm
27	19.20	Naringenin	n.d	Y = 1.4263e+007*X	0.9976	0.25-8 ppm
28	19.42	Ellagic_acid	n.d	Y = -2260.64+12027.3*X	0.9926	0.25-8 ppm
29	19.86	Quercetin	n.d	Y = 178145+930034*X	0.9975	0.25-8 ppm
30	21.25	Apigenin	n.d	Y = 695319*X	0.9996	0.25-8 ppm
31	22.70	Chrysin	n.d	Y = 1.18637e+007*X	0.9978	0.25-8 ppm
32	22.83	Galangin	n.d	Y = 104168*X	0.9968	0.25-8 ppm
33	22.96	Flavone	n.d	Y = 9.19684e+006+3.14052e+007*X	0.9976	0.25-8 ppm

n.d.: not detected, d.w.: dry weight. X represents the concentration of the standard analyte (ppm), and Y represents the corresponding LC–MS/MS peak area used to construct the calibration curve. “n.d.” indicates not detected

FTIR Analysis

As shown in Fig. 4, the FTIR spectrum of the methanol extract of *C. oltensis* exhibited several characteristic absorbance bands, confirming the chemically diverse nature of the extract. The broad and intense band at 3252 cm^{-1} was attributed to hydrogen-bonded O–H stretching vibrations, indicating the presence of phenolic and alcoholic hydroxyl groups. The weak band at 2932 cm^{-1} corresponded to aliphatic C–H stretching vibrations. The prominent band at 1587 cm^{-1} was assigned mainly to aromatic C=C stretching and/or asymmetric carboxylate vibrations, whereas the band at 1397 cm^{-1} may be associated with O–H bending, aliphatic C–H deformation, and symmetric carboxylate stretching (Coates 2000; Stuart 2004).

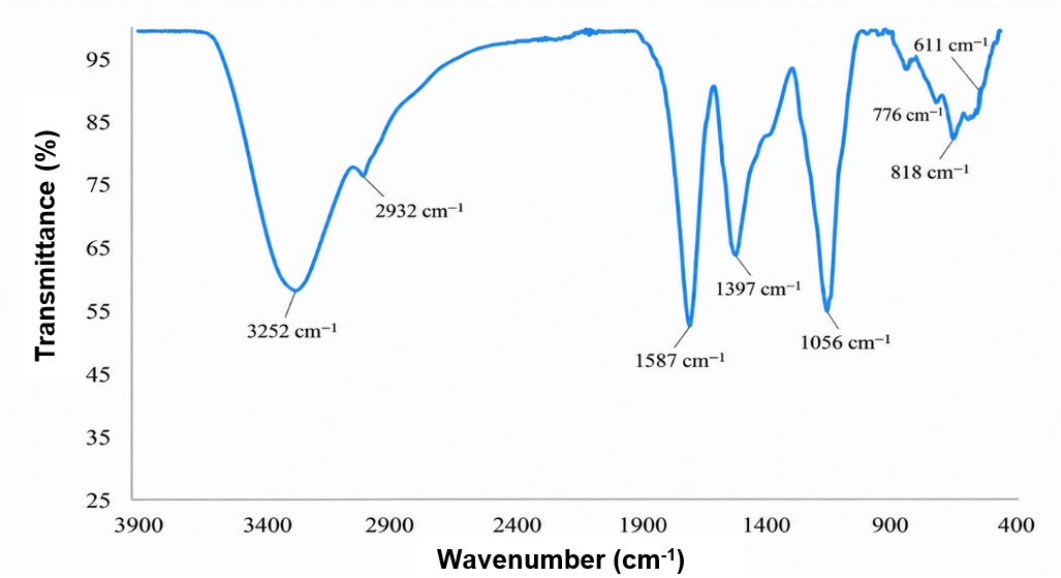


Fig. 4. FTIR spectrum of the methanol extract obtained from the aerial parts of *Centaurea oltensis*

The strong absorbance band at 1056 cm^{-1} was consistent with C–O stretching vibrations of alcohols, phenols, ethers, or glycosidic structures. Bands observed at 818 and 776 cm^{-1} were attributable to out-of-plane aromatic C–H bending, while the lower-frequency band at 611 cm^{-1} was associated with skeletal and ring-deformation vibrations. Overall, the FTIR profile indicates that the methanol extract is rich in hydroxylated aromatic and oxygen-containing constituents. These spectral features support the LC–MS/MS findings, particularly the predominance of ferulic and chlorogenic acids and the presence of other phenolic acids, including caffeic, protocatechuic, salicylic, and 4-hydroxybenzoic acids (Socrates 2001; Stuart 2004).

Antioxidant Capacity and Bioactive Component

The present study provides a comparative evaluation of the antioxidant activity and bioactive components of the ME and EO of *C. oltensis*. The findings clearly demonstrate significant differences between the two extracts, which can be attributed to their chemical composition, extraction efficiency, and solubility of bioactive compounds.

The DPPH assay is widely used to assess the hydrogen-donating ability of compounds, which directly reflects their capacity to neutralize free radicals. Although the EO exhibited a higher DPPH value (83.8 mg AAE/100 g), the ME displayed a much lower IC_{50} value (172 mg/mL) compared to the EO (674 mg/mL), suggesting that the ME is more

efficient at lower concentrations (Table 3). This result is consistent with the notion that lower IC_{50} values correspond to stronger antioxidant activity (Prior *et al.* 2005). Ascorbic acid, used as a positive control, exhibited an even lower IC_{50} value (92.6 µg/mL), confirming its exceptional radical scavenging efficiency.

The ABTS assay, which measures the ability of antioxidants to quench the ABTS radical cation, also highlighted significant differences between the two extracts. The ME (975 mg TRE/100 g) exhibited a nearly fivefold stronger scavenging activity compared to the EO (205 mg TRE/100 g) (Table 3). These findings are in line with previous reports indicating that extracts rich in polar phenolic compounds exhibit superior ABTS radical scavenging activity (Re *et al.* 1999). Moreover, the FRAP assay demonstrated the superior electron-donating ability of the ME (74.1 mg $FeSO_4$/100 g) relative to the EO (17.2 mg $FeSO_4$/100 g), supporting earlier findings that high ferric ion reducing power correlates with phenolic content (Benzie and Strain 1996).

The antioxidant assays strongly correlate with the bioactive composition of the extracts. The ME was found to contain significantly higher levels of TPC (281 mg GAE/100 g) compared to the EO (49.9 mg GAE/100 g). Phenolic compounds are widely acknowledged as major contributors to antioxidant activity because of their ability to donate hydrogen atoms and electrons to stabilize free radicals (Huang *et al.* 2006). Similarly, the TFC of the ME (33.4 mg CAE/100 g) exceeded that of the EO (20.7 mg CAE/100 g). Flavonoids not only act as radical scavengers but also possess metal chelation properties, which reduce oxidative stress by limiting the availability of transition metals that catalyze free radical formation (Middleton *et al.* 2000).

Interestingly, the TAC values of both extracts were relatively close (364 mg AAE/100 g for ME and 353 mg AAE/100 g for essential oil). This suggests that while phenolics and flavonoids contribute substantially to antioxidant activity, other non-phenolic compounds present in the EO may also play a role in its antioxidant potential.

Similar observations have been reported in other aromatic plants, where terpenoids and other volatile constituents contribute to moderate antioxidant activity despite lower phenolic content (Bakkali *et al.* 2008).

The superior performance of the ME can largely be explained by solvent polarity. Methanol is a highly effective solvent for extracting polar compounds such as phenolics and flavonoids, which are largely responsible for antioxidant activity (Dai and Mumper 2010). In contrast, essential oils obtained through hydrodistillation or steam distillation are primarily composed of volatile terpenoids, which may not exhibit as strong antioxidant effects as phenolics. Therefore, the differences in activity between the two extracts are directly linked to their chemical profiles.

The antioxidant values observed in the ME of *C. oltensis* are comparable to those reported for other members of the *Centaurea* genus, where methanol extracts have consistently shown higher phenolic content and stronger radical scavenging activity compared to essential oils (Kedare and Singh 2011). This consistency suggests that methanolic extracts of *Centaurea* species can serve as promising natural sources of antioxidants for food and pharmaceutical industries. Essential oils, while less effective in radical scavenging assays, may provide complementary benefits through antimicrobial and anti-inflammatory properties, as previously demonstrated (Bakkali *et al.* 2008).

Table 3. Antioxidant Activity and Bioactive Component Profile of *C. Oltensis* ME and EO

Antioxidant Activity Levels						
	EO and ME Yield	DPPH			ABTS	FRAP
	g/100 g	mg AAE/100 g	Inhibition %	IC_{50} (mg/mL)	mg TRE/100 g	mg $FeSO_4$ / 100 g
ME	9.28±0.19[a]	73.27±3.02[b]	63.81±0.47[b]	171.74±1.44[b]	974.76±05.72[a]	74.10±0.39[a]
EO	0.17±0.02[b]	83.85±1.49[a]	62.02±0.32[c]	674.19±5.28[a]	204.65±2.02[b]	17.16±0.50[b]
AA			98.32±0.01[a]	92.65±0.11[c] µg/mL		
Bioactive Components						
	TPC mg GAE/100g		TFC mg CAE/100g		TAC mg AAE/100g	
ME	280.62±3.44[a]		33.44±3.18[a]		364.17±8.72[a]	
EO	49.87±0.34[b]		20.67±0.33[b]		352.59±0.29[b]	

Different letters within the same column in the table indicate significant statistical differences in pairwise comparisons ($p<0.05$). n:3. Duncan's test was applied. AAE: Ascorbic Acid Equivalent, TRE: Trolox Equivalent, GAE: Gallic Acid Equivalent, CAE: Catechin Equivalent

Overall, the results indicate that the methanol extract of *C. oltensis* has strong potential as a natural antioxidant source due to its high phenolic and flavonoid content. Such properties make it suitable for incorporation into functional foods, nutraceuticals, and natural preservatives in the food industry. The essential oil, despite its lower antioxidant efficiency, may still be valuable in pharmaceutical or cosmetic formulations where antimicrobial or anti-inflammatory effects are desired. Future studies could focus on optimizing extraction conditions, identifying specific active compounds, and exploring the synergistic effects of combining methanol extracts with essential oils to enhance bioactivity.

Antimicrobial Activities

The 30% ME and EO of *C. oltensis* were tested against gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*), gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*), and the yeast *Candida albicans*, with chloramphenicol and nystatin used as reference standards (Table 4).

The EO showed strong antimicrobial activity against gram-positive strains. For *Staphylococcus aureus*, the inhibition zone was 14.4 mm, with an MIC of 0.02 mg/mL and MBC of 0.05 mg/mL, representing the lowest MIC among all tested microorganisms. In comparison, chloramphenicol exhibited superior potency (DDT = 19.0 mm, MIC = 0.001 mg/mL, MBC = 0.005 mg/mL). Similarly, *Enterococcus faecalis* was sensitive to the EO (DDT = 14.2 mm, MIC = 0.07 mg/mL, MBC = 0.14 mg/mL), whereas *Bacillus cereus* showed inhibition of 15.0 mm with MIC 0.14 mg/mL and MBC 0.28 mg/mL. These results clearly indicate that the EO is more active against gram-positives, consistent with earlier findings for *Centaurea sessilis* and C. *armena*, where essential oils exhibited higher efficacy against gram-positive bacteria (Yayli *et al.* 2005). The activity is generally attributed to lipophilic sesquiterpenes such as *β*-caryophyllene, spathulenol, and caryophyllene oxide, which destabilize bacterial membranes through synergistic mechanisms (Carev *et al.* 2023).

Against gram-negatives, EO activity was comparatively weaker. *Escherichia coli* was inhibited at 13.3 mm (MIC = 0.14 mg/mL, MBC = 0.28 mg/mL), while *Klebsiella pneumoniae* showed an inhibition zone of 13.2 mm (MIC = 0.48 mg/mL, MBC = 0.56 mg/mL).

Pseudomonas aeruginosa was the least sensitive (DDT = 12.2 mm, MIC = 0.28 mg/mL, MBC = 0.56 mg/mL). These findings are in agreement with reports that gram-negative bacteria are less susceptible to plant essential oils due to their outer membrane structures (Yayli *et al.* 2005). Nonetheless, the ability of lipophilic EO components to disrupt membrane integrity provides moderate inhibitory effects. A noteworthy observation is that the methanol extract of *K. pneumoniae* presented an unusual profile, with MIC 3.20 mg/mL but a lower MBC (0.41 mg/mL), suggesting either bactericidal effects at reduced concentrations or possible experimental variability that requires further study.

The EO exhibited moderate antifungal activity against *C. albicans*, with DDT 13.4 mm, MIC 0.57 mg/mL, and MBC 1.13 mg/mL. By contrast, nystatin produced markedly stronger inhibition (DDT = 27.2 mm, MIC 0.008 mg/mL, MBC 0.016 mg/mL). The antifungal activity of the EO is likely linked to terpenoids that interfere with fungal cell wall integrity and ergosterol biosynthesis (Serra *et al.* 2018). Comparable results have been reported for other *Centaurea* species: the methanol extract of *C. baseri* inhibited *Candida utilis* (MIC = 60 µg/mL) (Kose *et al.* 2016); the EO of *C. pulcherrima* showed activity against *C. albicans* (Kahriman *et al.* 2012); and *C. solstitialis* EO displayed potent antifungal effects attributed to its high germacrene D content (Carev *et al.* 2016).

In contrast, the ME exhibited very weak antimicrobial activity, with inhibition zones ranging from 6.80 to 7.73 mm, MIC values of 0.20 to 3.25 mg/mL, and MBC values of 0.41 to 6.50 mg/mL. The limited activity can be explained by the extract's chemical profile, which is dominated by polar phenolic acids and flavonoids. These compounds possess antioxidant capacity and only modest antimicrobial properties compared to the more membrane-active, lipophilic sesquiterpenes in the EO (Albayrak *et al.* 2017; Sharonova *et al.* 2021).

Taken together, the EO of *C. oltensis* demonstrated remarkable antimicrobial potential, particularly against gram-positive bacteria (*S. aureus*, *E. faecalis*, *B. cereus*) and moderate antifungal activity against *C. albicans*. Activity against gram-negatives was limited but meaningful, in agreement with earlier studies on *C. pumilio* EO, which showed inhibitory effects even against multidrug-resistant strains (Naeim *et al.* 2020). The observed activities across *Centaurea* species are thought to arise from synergistic interactions among multiple terpenoid constituents (Carev *et al.* 2023).

These findings support the potential of *C. oltensis* EO as a complementary or alternative antimicrobial agent in contexts of rising antibiotic resistance. However, further *in vivo* research and detailed phytochemical isolation are necessary to identify the key active components and evaluate their clinical applicability.

Table 4. Antimicrobial Activities of *Centaurea oltensis* ME and EO

	MeOH %30			EO			Chloramphenicol			Nystatin		
	DDT (mm)	MIC mg/mL	MBC mg/mL	DDT (mm)	MIC mg/mL	MBC mg/mL	DDT (mm)	MIC mg/mL	MBC mg/mL	DDT (mm)	MIC mg/mL	MBC mg/mL
Bacillus cereus ATCC 9634	7.73	1.63	3.25	14.95	0.14	0.28	21.12	0.04	0.07	NT	NT	NT
	±0.15	±0.03	±0.12	±017	±0.02	±0.10	±0.16	±0.01	±0.01			
Escherichia coli ATCC 25922	7.43	0.81	1.63	13.34	0.14	0.28	19.88	0.02	0.07	NT	NT	NT
	±0.12	±0.04	±0.09	±0.10	±0.022	±0.04	±0.14	±0.01	±0.01			
Klebsiella pneumoniae ATCC 13883	6.80	3.20	0.41	13.18	0.48	0.56	29.88	0.02	0.07	NT	NT	NT
	±0.10	±0.07	±0.11	±0.25	±0.04	±0.10	±0.17	±0.01	±0.01			
Pseudomonas aeruginosa ATCC 27853	7.33	1.63	3.25	12.18	0.28	0.56	10.51	0.02	0.07	NT	NT	NT
	±0.06	±0.10	±0.01	±0.10	±0.03	±0.06	±0.14	±0.01	±0.01			
Staphylococcus aureus ATCC 25923	7.37	0.41	0.41	14.39	0.02	0.05	18.99	0.001	0.005	NT	NT	NT
	±0.12	±0.05	±0.02	±0.19	±0.01	±0.01	±0.11	±0.001	±0.001			
Enterococcus faecalis ATCC 29212	7.67	0.20	0.41	14.23	0.07	0.14	11.12	0.018	0.035	NT	NT	NT
	±0.06	±0.01	±0.03	±0.19	±0.01	±0.01	±0.12	±0.001	±0.001			
Candida albicans ATCC 18804	7.47	3.25	6.50	13.40	0.57	1.13	NT	NT	NT	27.21	0.008	0.016
	±0.12	±0.02	±0.05	±0.10	±0.10	±0.02				±0.12	±0.001	±0.001

The discs have a diameter of 5.5 mm. Samples: 25 mg/mL, Antibiotic: ±0.51 mg/mL. 15 µL were pipetted onto each disc, n = 3. DDT: Disk Diffusion Test

GC-MS/FID Conditions for Essential Oil Analysis

The EO yield calculated as a result of the hydrodistillation process carried out with *C. oltensis* (100 g dried aerial parts) was determined as 0.17±0.03% w/w. Djeddi *et al.* (2011) reported that the yields of essential oils from *Centaurea pullata*, *C. grisebachii* subsp. *grisebachii* and *C. affinis* were approximately 0.03%, 0.01% and 0.02% (v/w) on a dry weight basis, respectively. By hydrodistillation with clevenger type apparatus, EO yields of 0.25% and 0.18% (v/w) on dry weight basis were obtained from whole *Centaurea sessilis* and *C. armena* plants, respectively (Yayli *et al.* 2005). When the EO yield obtained in the study is compared, it can be said that it is more or similar to some *Centaurea* species.

The structures of 128 natural compounds identified in the analyzed EO were determined by comparing their retention times and mass spectra with those of compounds in the NIST and Wiley libraries using a GC-MS/FID instrument, as well as with data reported in the literature. The structures of two compounds were not determined. 99.2% of the compounds identified in the EO analysis were identified. The GC-MS/FID analysis results of *C. oltensis* EO are shown in Table 5.

The main components of the EO were determined to be 15.1% decanoic acid, 7.93% *α*-pinene, and 5.40% sabinyl acetate. Some terpene and terpenoid compounds, whose structures are identified and listed in Table 4, are *α*-pinene, *p*-cymene, dihydroedulane-I, *α*-copaene, and limonene; some aldehyde compounds are duraldehyde, nonanal, and hexanal; some alcohol compounds are nonanol, dihydromyrcenol, and 1-decanol; and some ester compounds are sabinyl acetate and 4-tert-butylcyclohexyl acetate.

A total of 53 compounds were identified, representing 93.6% of the EO of *C. pullata*. Oxygenated monoterpenes (45.2%) were the dominant components, among which caryophyllene oxide (38.5%) was the main compound. Other major components were hydrocarbons (36.5%), aldehydes (16.3%), followed by alkanes and alkenes (16.1%). Twenty-six different substances were identified, representing 82.4% of *C. grisebachii* subsp. *grisebachii*; oxygenated sesquiterpenes were the dominant components with 47.3%, among which spathulenol (12.2%) and *β*-eudesmol (10.1%) were the main components, followed by hydrocarbons (28.6%), and 6,10,14-trimethyl pentadecan-2-one (12.9%) was found as the main compound. The EO of *C. affinis* evidenced 62 compounds, constituting 92.9% of the total extract, with oxygenated sesquiterpenes (15.5%) being detected as the dominant components (Djeddi *et al.* 2011).

Table 5. GC-MS/FID Analysis Results of The EO Obtained from *C. Oltensis*

No	RT	RI	LRI	Compound Class	Compound	% Ratio
1	4.186	671	671	Hydrocarbon	3-Methyl-hexane	0.09
2	4.392	688	687	Hydrocarbon	cis-1,3-Dimethyl-cyclopentane	0.04
3	4.537	700	700	Other	2-Ethyl-furan	0.50
4	4.982	719	719	Hydrocarbon	Methylcyclohexane	0.32
5	6.832	799	799	Aldehyde	Hexanal	1.23
6	7.575	820	825	Hydrocarbon	Hexamethylcyclotrisiloxane	0.07
7	8.597	850	850	Aldehyde	(*E*)-2-Hexenal	0.52
8	10.103	892	895	Aldehyde	(*Z*)-4-Heptenal	0.80
9	10.417	901	901	Aldehyde	Heptanal	0.18
10	11.159	919	924	Other	2,3-Dimethylpyridine	0.10
11	11.733	933	933	Monoterpene	**α-Pinene**	**7.93**
12	12.324	947	947	Monoterpene	Camphene	1.19
13	12.563	953	953	Monoterpene	Verbenene	0.27
14	12.796	959	959	Aldehyde	Benzaldehyde	0.23
15	13.377	973	973	Monoterpene	Sabinene	0.41
16	13.495	976	976	Monoterpene	*β*-Pinene	1.06
17	13.829	984	984	Ketone	2,3-Octanedione	0.10
18	14.133	991	991	Monoterpene	*β*-Myrcene	2.03
19	14.295	995	995	Ketone	6-Methyl-5-hepten-2-one	2.38
20	14.612	1003	1003	Aldehyde	Octanal	0.53
21	15.193	1017	1017	Monoterpene	*α*-Terpinene	0.19
22	15.534	1025	1025	Monoterpene	*p*-Cymene	3.82
23	15.713	1029	1029	Monoterpene	Limonene	2.87
24	16.111	1038	1038	Monoterpene	*cis-β*-Ocimene	0.28
25	16.34	1044	1044	Aldehyde	Benzeneacetaldehyde	0.20
26	16.533	1048	1048	Monoterpene	*trans-β*-Ocimene	0.13
27	16.985	1059	1059	Monoterpene	γ-Terpinene	0.69
28	17.177	1063	1063	Ketone	1-Phenyl-ethanone	0.22
29	17.296	1066	1066	Ketone	Acetophenone	0.02
30	17.552	1072	1072	Alcohol	Dihydromyrcenol	1.78
31	17.680	1075	1075	Monoterpenoid	Linalool oxide	0.08
32	18.028	1084	1085	Ketone	8-Nonen-2-one	0.19
33	18.254	1089	1089	Monoterpene	α-Terpinolene	0.36

No	RT	RI	LRI	Compound Class	Compound	% Ratio
34	18.44	1093	1093	Ketone	(*E,E*)-3,5-Octadien-2-one	0.16
35	18.652	1098	1098	Monoterpenoid	Tetrahydrolinalool	0.35
36	18.906	1104	1104	Aldehyde	Nonanal	1.28
37	19.314	1114	1114	Monoterpenoid	α-Fenchol	0.57
38	19.692	1123	1127	Monoterpenoid	1-Terpinenol	0.23
39	19.830	1127	1127	Monoterpenoid	*α*-Campholenal	0.45
40	19.938	1129	1129	Monoterpene	Allo-Ocimene	0.09
41	20.036	1132	1135	Alcohol	3,7-Dimethyl-1-octen-3-ol	0.06
42	20.370	1140	1139	Monoterpenoid	Pinocarveol	0.59
43	20.620	1146	1146	Monoterpenoid	Camphor	0.23
44	20.765	1150	1150	Monoterpenoid	3-Methylcamphenilanol	0.71
45	21.113	1158	1158	Monoterpenoid	Isoborneol	0.19
46	21.201	1160	1160	Aldehyde	(*E*)-2-Nonenal	0.15
47	21.353	1164	1164	Monoterpenoid	Pinocarvone	0.42
48	21.518	1168	1168	Monoterpenoid	p-Mentha-1,5-dien-8-ol	1.04
49	21.700	1172	1172	Alcohol	Nonanol	2.37
50	21.964	1179	1179	Monoterpenoid	Terpinen-4-ol	1.17
51	22.190	1184	1181	Monoterpenoid	Safranal	1.17
52	22.524	1192	1192	Monoterpenoid	*α*-Terpineol	1.95
53	22.747	1198	1198	Monoterpenoid	Myrtenol	0.45
54	22.865	1201	1201	Monoterpenoid	Safranal	0.25
55	23.061	1206	1206	Aldehyde	Decanal	0.70
56	23.314	1212	1214	Alcohol	*p*-tert-Butylcyclohexanol	0.70
57	23.628	1220	1220	Monoterpenoid	*trans*-Carveol	0.23
58	23.810	1225	1225	Other	Benzothiazole	0.05
59	23.972	1229	1229	Monoterpenoid	Citronellol	0.04
60	24.093	1232	1229	Monoterpenoid	*p*-Cymen-8-ol	0.11
61	24.468	1241	1234	Aldehyde	3-Isopropylbenzaldehyde	0.08
62	24.620	1245	1245	Monoterpenoid	Carvone	0.77
63	25.015	1255	1255	Monoterpenoid	*cis*-Citral	0.32
64	25.319	1263	1264	Ester	**Sabinyl acetate**	**5.40**
65	25.535	1268	1260	Ketone	Thymoquinone	0.05
66	25.680	1272	1272	Alcohol	1-Decanol	0.95
67	25.936	1279	1279	Monoterpenoid	Carvacrol	0.17

No	RT	RI	LRI	Compound Class	Compound	% Ratio
68	26.274	1287	1285	Monoterpenoid	*trans*-Bornyl acetate	0.13
69	26.399	1290	1290	Monoterpenoid	Dihydroedulan IIA	0.28
70	26.567	1295	1295	Monoterpenoid	Dihydroedulane-I	3.60
71	26.797	1301	1300	Ester	*trans*-Pinocarvyl acetate	0.39
72	27.033	1307	1307	Aldehyde	Undecanal	0.39
73	27.337	1315	1316	Ester	4-tert-Butylcyclohexyl acetate	0.54
74	27.445	1318	1323	Aldehyde	Mesitaldehyde	1.01
75	28.553	1340			Unidentified compounds	0.42
76	28.856	1356	1365	Ester	2-methylpropanoic acid-2,2-dimethyl-1-(2-hydroxy-1-methylethyl)-propyl ester	0.18
77	29.048	1361	1364	Aldehyde	Duraldehyde	3.18
78	29.325	1368	1368	Oil acid	**Decanoic acid**	**15.12**
79	29.588	1375	1375	Ester	Propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester	0.05
80	29.680	1378	1378	Sesquiterpene	α-Copaene	3.37
81	29.970	1386	1386	Monoterpenoid	*trans*-*β*-Damascenone	0.20
82	30.263	1394	1395	Ketone	2-Dodecanone	0.13
83	30.405	1397	1400	Hydrocarbon	Tetradecane	0.15
84	30.523	1401	1404	Other	Diphenyl ether	0.10
85	30.631	1404	1404	Other	Methyleugenol	0.09
86	30.756	1407	1407	Aldehyde	Dodecanal	0.12
87	31.276	1422	1422	Sesquiterpene	Caryophyllene	0.25
88	31.506	1429	1429	Monoterpenoid	*α*-Ionone	0.16
89	31.637	1432	1435	Sesquiterpene	*α*-Cedrane	0.19
90	32.326	1452	1452	Monoterpenoid	*trans*-Geranylacetone	0.09
91	33.055	1473	1473	Alcohol	1-Dodecanol	0.20
92	33.217	1477	1477	Sesquiterpene	*γ*-Selinene	1.30
93	33.345	1481	1481	Monoterpenoid	*α* Isomethyl ionone	0.10
94	33.440	1484	1484	Sesquiterpene	*α*-Curcumene	0.81
95	33.568	1487	1487	Monoterpenoid	*trans*-β-Ionone	0.32
96	33.916	1497	1500	Hydrocarbon	Pentadecane	1.13
97	34.138	1504	1504	Sesquiterpenoid	Dihydro-*β*-agarofuran	0.24
98	34.307	1509	1509	Sesquiterpene	*β*-Bisabolene	0.18
99	34.557	1516	1516	Sesquiterpene	Germacrene D	0.10
100	34.685	1520	1522	Monoterpenoid	*α*-Methyl-ionone	0.13
101	35.168	1535	1534	Ester	Isoamyl salicylate	0.38

No	RT	RI	LRI	Compound Class	Compound	% Ratio
102	35.505	1545	1540	Sesquiterpene	7-epi-*α*-Selinene	0.12
103	36.356	1570	1573	Sesquiterpenoid	1,5-Epoxysalvial-4(14)-ene	0.34
104	36.481	1574	1574	Ester	Amyl salicylate	0.20
105	36.731	1582	1582	Sesquiterpenoid	Spathulenol	0.45
106	36.920	1587	1587	Sesquiterpenoid	Caryophyllene oxide	2.03
107	38.054	1622	1622	Sesquiterpenoid	*α*-Cadinol	1.50
108	38.266	1629	1628	Ketone	Benzophenone	0.16
109	38.874	1648	1645	Aldehyde	*α*-Amylcinnamaldehyde	0.33
110	39.063	1654	1650	Ester	Methyl dihydrojasmonate	0.35
111	39.687	1674	1675	Alcohol	1-Tetradecanol	0.34
112	39.799	1678	1682	Ester	Hexyl salicylate	0.51
113	40.855	1712	1712	Aldehyde	Pentadecanal	0.34
114	41.071	1719	1716	Other	1,1,3-Trimethyl-3-phenylindane	0.17
115	41.502	1733			Unidentified compounds	0.36
116	41.824	1744	1727	Sesquiterpenoid	Valerenal	0.08
117	41.949	1748	1747	Aldehyde	*α*-Hexylcinnamaldehyde	0.05
118	42.337	1761	1756	Sesquiterpenoid	Ambrox	0.79
119	42.766	1775	1770	Sesquiterpenoid	Acetyl cedrene	0.34
120	43.390	1796	1800	Hydrocarbon	Octadecane	0.31
121	44.730	1843	1843	Sesquiterpenoid	Hexahydrofarnesyl acetone	0.09
122	45.060	1854	1850	Sesquiterpenoid	Galaxolide	0.92
123	45.456	1868	1868	Ester	Diisobutyl phthalate	0.29
124	46.752	1913	1924	Ester	Butyl isobutyl phthalate	0.29
125	46.836	1916	1916	Sesquiterpenoid	(*E,E*)-Farnesyl acetone	0.16
126	48.007	1956	1957	Oil acid	Hexadecanoic acid	0.03
127	48.078	1958	1959	Ester	Dibutyl phthalate	0.04
128	49.148	1995	2000	Hydrocarbon	Eicosane	0.07
Total						100.00

RT: Retention Time, **RI**: Experimental Kovats Retention Index, **LRI**: Literature Kovats Retention Index

Yayli *et al.* (2005) determined that the major components of the EO of *C. sessilis* were *β*-eudesmol (12.4%), caryophyllene oxide (10.0%), phytol (6.4%), spathulenol (4.9%) and 6,10,14-trimethyl-2-pentadecanone (3.1%). The major components of the EO of *C. armena* were *β*-eudesmol (19.3%), calarin (10.3%), 6,10,14-trimethyl-2-pentadecanone (5.7%) and *β*-caryophyllene (5.4%). The chemical composition of the EO of dried aerial parts of *C. iberica*, *C. solstitialis* subsp *solstitialis*, and *C. virgata* was analyzed using the HS-SPME/GC-MS extraction technique and the GC-MS system. Thirty-nine, forty-two, and forty-two components were identified, representing 90.8%, 91.6%, and 92.5% of the oil, respectively. The main compounds of *C. iberica*, *C. solstitialis* subsp. *solstitialis* and *C. virgata* were determined as germacrene D (20.3%), caryophyllene oxide (10.7%), *β*-caryophyllene (10.5%), *β*-eudesmol (15.5%), bicyclogermacrene (14.2%), spathulenol (11.3%) and germacrene D (21.4%), *β*-caryophyllene (16.5%), caryophyllene oxide (9.5%), respectively (Kilic 2013). The predominant chemical compounds in the EO obtained by hydrodistillation from air-dried aerial parts of *C. scabiosa* using a Clavenger apparatus were heptacosane (19.6%), caryophyllene oxide (10.90%), alloaromadendrene epoxide (10.27%), *β*-cyperone (8.16%), and *α*-bisabolol (4.99%) (Carev *et al.* 2022).

The chemical classification, number of compounds, main components, and percentage amounts of the compounds identified in *C. oltensis* EO are shown in Table 6.

When the chemical classification results of the compounds identified in the EO obtained from *C. oltensis* are examined in Table 6, it can be seen that the 128 compounds identified were divided into 12 groups.

When the chemical component classes identified in the EO were evaluated, it was apparent that the most abundant compound class in terms of percentage was monoterpenes at 21.3%, with *α*-pinene as the main component. Other compound classes included monoterpenoids at 16.5%, with dihydroedulane-I as the main component; oil acids at 15.2%, with decanoic acid as the main component; and aldehydes at 11.3%, with duraldehyde as the main component. The most abundant compound classes in these classes were monoterpenoids (31), aldehydes (18), monoterpenes (14), esters (12), and sesquiterpenoids (11). There was a total of 64 compounds in the essential oil samples and 51.1% were terpene or terpenoid class compounds (Table 6).

Sesquiterpenoids are the main components of *C. sessilis* and *C. armena* essential oils at 35.1% and 34.8%, respectively. A total of 28 components and 59.4% and 15 components and 70.7% of terpenes or terpenoid class compounds were found in the EO samples, respectively (Yayli *et al.* 2005). Carev *et al.* (2022) grouped the chemical compounds of the EO extracted from *C. scabiosa* as terpenes (43.7%), including deoxygenated sesquiterpenes (1.23%), sesquiterpenoids (41.1%), and diterpenes (1.41%); and non-terpene compounds, including hydrocarbons (31.1%), aldehydes (3.67%), acids (7.00%), esters (4.03%), and other compounds (0.68%).

Table 6. Chemical Classification, Number of Compounds, Main Components, and Percentage Amounts of the Compounds Identified in *C. oltensis* EO

Compound Classification	Compound Number	Main Components	Ratio (%)
Monoterpenes	14	α-Pinene	21.34
Monoterpenoids	31	Dihydroedulane-I	16.47
Oil acids	2	Decanoic acid	15.15
Aldehydes	18	Duraldehyde	11.34
Esters	12	Sabinyl acetate	8.64
Sesquiterpenoids	11	Caryophyllene oxide	6.95
Sesquiterpenes	8	α-Copaene	6.33
Alcohols	7	Nonanol	6.40
Ketones	9	6-Methyl-5-hepten-2-one	3.41
Hydrocarbons	8	Pentadecane	2.19
Others	6	2-Ethyl-furan	1.01
Unidentified compounds	2		0.78
Total	**128**		**100.00**

Comparison of the volatile components identified in studies of essential oils from *C. oltensis* and other *Centaurea* species reveal similar compounds. Sesquiterpenoids are prominent among the chemical classes of volatile components in these studies, and monoterpenes predominate in this study. The differences in the chemical classification and volatile compound amounts identified can be attributed to many factors, including species differences, climatic conditions, different growing locations, natural diversity, collection times, storage conditions, and analysis parameters (Öz *et al.* 2023).

Further investigations involving compound isolation, mechanistic studies, and *in vivo* validation are recommended to elucidate the therapeutic potential and industrial applicability of this endemic Turkish species.

Compared with previous studies on *Centaurea* species, the present findings indicate that *C. oltensis* may have potential for multiple biological applications rather than a single functional property. Previous studies have demonstrated the antioxidant, antimicrobial, cytotoxic, and enzyme inhibitory potential of different *Centaurea* species, whereas the present study integrates these functional evaluations with detailed volatile and phenolic profiling. In particular, the strong cholinesterase inhibitory activity of the EO suggests potential relevance to further research on neuroprotective applications, while the CA-II and α-glucosidase inhibitory activities and antioxidant capacity of the ME indicate additional potential for pharmacological and nutraceutical investigations. Moreover, the antimicrobial activity of the EO, particularly against *Staphylococcus aureus*, supports its further evaluation as a source of natural antimicrobial agents. However, these potential applications should be considered preliminary, and further isolation, mechanistic, toxicity, and *in vivo* studies are required before practical therapeutic or industrial applications can be proposed.

CONCLUSIONS

1. The present study provides the first comprehensive chemical and biological characterization of *Centaurea oltensis*. Gas chromatography-mass spectroscopy/flame ionization detector (GC–MS/FID) analysis revealed a complex essential oil (EO) composition dominated by decanoic acid, *α*-pinene, and sabinyl acetate, while liquid chromatography – tandem mass spectrometry (LC–MS/MS) profiling of the methanol extract (ME) demonstrated exceptionally high levels of ferulic and chlorogenic acids. These findings confirm that *C. oltensis* possesses a distinctive phenolic and terpenoid signature compared with other *Centaurea* taxa.
2. Biological activity assays indicated that both extracts exhibit notable pharmacological potential. The ME showed remarkable carbonic-anhydrase and *α*-glucosidase inhibitory effects and superior antioxidant activity, directly associated with its rich phenolic and flavonoid content. Conversely, the EO demonstrated pronounced acetylcholinesterase (AChE) and butylcholinesterase (BChE) inhibition, together with strong antibacterial and moderate antifungal properties, suggesting possible neuroprotective and antimicrobial applications.
3. Overall, *C. oltensis* represents a valuable natural source of bioactive compounds with multifunctional properties relevant to the development of antioxidant, antimicrobial, and enzyme-inhibitory agents.

ACKNOWLEDGEMENTS

This study is a part of a master thesis entitled “Determination of Antioxidant, Antimicrobial, Anticholinesterase, Antiglaucoma and Antidiabetic Activities of Methanol Extract and Essential Oils of Centaurea Oltensis Sosn Plant” prepared by Berfin AKŞİT under the supervision of Assistant Professor Dr. İmdat AYGÜL in Gümüşhane University.

Conflict of Interest

The authors report no conflicts of interest.

Data Availability Statement

The original contributions presented in this study are included in the article.

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Article submitted: May 8, 2026; Peer review completed: August 8, 2026; Revised version received and accepted: September 8, 2026; Published: September 25, 2026.
DOI: 10.15376/biores.21.4.11085-11114