



Phytochemical profiling and in vitro antimicrobial activity of selected *Centaurea L.* species extracts against multidrug-resistant pathogens

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ABSTRACT

Species belonging to the genus *Centaurea L.* (Asteraceae) are recognized for their richness in secondary metabolites and diverse biological activities. Preliminary phytochemical screening in this present investigation of major natural product groups, particularly terpenes and flavonoids. The antimicrobial property of *Centaurea hyalolepis* Boiss. and *Centaurea iberica* Trevir. ex Spreng. leaf extracts were investigated. Plant material was extracted via maceration (MAC) using methanol %80, and antimicrobial activity was measured using agar well diffusion method against *Escherichia coli*, MDR *Staphylococcus aureus* and *Candida albicans* ATCC. Four extract concentrations (100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml) were prepared using 5% Dimethyl sulfoxide as solvent. The result demonstrated that *C. hyalolepis* exhibited notable antibacterial and antifungal activities, with inhibition zones reaching 28 mm against *Escherichia coli* and MRD *Staphylococcus aureus* and 23 mm against *C. albicans* at highest concentration (400 mg/ml). Both species showed antibacterial effects. Among the tested samples; *C. iberica* showed the strongest antibacterial efficacy against MRD *S. aureus*, yielding an inhibition zone reached 33 mm at 400 mg/ml. In conclusion, these findings suggest that both species represent promising sources of natural antimicrobial agents, particularly due to their pronounced activity against multidrug-resistant *S. aureus*.

Keyword: antimicrobial activity, plant extraction, growth inhibition, multidrug-resistant.

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التحليل الكيميائي النباتي والنشاط المضاد للميكروبات في المختبر لمستخلصات أنواع مختارة من جنس القنطريون (*Centaurea L.*) ضد مسببات الأمراض متعددة المقاومة للمضادات الحيوية

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المخلص

تُعرف الأنواع التابعة لجنس *Centaurea L.* من الفصيلة النجمية (Asteraceae) بغناها بالمستقلبات الثانوية وتنوع أنشطتها الحيوية. وقد شمل التحري الكيميائي النباتي الأولي في هذه الدراسة الكشف عن المجموعات الرئيسية من المركبات الطبيعية ولا سيما التربينات والفلافونويدات. تمت دراسة الخصائص المضادة للميكروبات لمستخلصات أوراق *Centaurea hyalolepis* Boiss. و *Centaurea iberica* Trevir. ex Spreng. حيث استُخلصت المواد النباتية بطريقة النقع (Maceration) باستخدام الميثانول بتركيز 80%، كما تم قياس النشاط المضاد للميكروبات باستعمال طريقة الانتشار في الأغار بالحفر (Agar well diffusion method) ضد *Escherichia coli*، و *Staphylococcus aureus* المقاوم لعدة أدوية (MDR)، و *Candida albicans* ATCC، تم تحضير أربعة تراكيز من المستخلصات (100، 200، 300، و 400 ملغم/مل) باستخدام 5% من ثنائي ميثيل السلفوكسيد (Dimethyl sulfoxide) كذيب. أظهرت النتائج أن مستخلص النوع *C. hyalolepis* يمتلك نشاطاً ملحوظاً مضاداً للبكتيريا والفطريات، حيث بلغت مناطق التثبيط 28 ملم ضد *Escherichia coli* و MDR *Staphylococcus aureus*، و 23 ملم ضد *Candida albicans* عند أعلى تركيز (400 ملغم/مل). كما أظهر كلا النوعين تأثيرات مضادة للبكتيريا. ومن بين العينات المختبرة، أظهر *C. iberica* أقوى فعالية مضادة للبكتيريا ضد MDR *S. aureus*، إذ بلغت منطقة التثبيط 33 ملم عند تركيز 400 ملغم/مل. وفي الختام، تشير هذه النتائج إلى أن كلا النوعين يمثلان مصادر واعدة لعوامل طبيعية مضادة للميكروبات، خاصة بسبب نشاطهما الواضح ضد *Staphylococcus aureus* المقاوم لعدة أدوية.

الكلمات المفتاحية: النشاط المضاد للميكروبات، المستخلص النباتي، تثبيط النمو، متعدد المقاومة للمضادات الحيوية.

INTRODUCTION

Antimicrobial resistance recently became a global challenge, significantly reducing the efficacy of existing antibiotics and increasing the risk of treatment failure (Coast, Smith et al. 1996, Organization 2023). Poly-drug-resistant pathogens, including MDR *Staphylococcus aureus*, as well as opportunistic fungi for instance *Candida albicans* strains, pose serious challenges in clinical settings (Ventola 2015). In this context, medically important plants have been widely investigated as alternative antimicrobial compounds to inhibit the emergence and spread of multidrug-resistant microorganisms (Al-Snafi 2016). Owing to their extensive chemical diversity and long-standing use in traditional medicine, medicinal plants remain a beneficial source for the discovery of novel antimicrobial compounds (Newman and Cragg 2020).

Centaurea iberica, commonly known as Iberian star-thistle, is rich in diverse secondary metabolites, including lignins, terpenoids, anthocyanins, flavonoids, phenolic acids, stilbenes, and carotenoids (Arif, Küpeli et al. 2004). Previous studies have demonstrated that *C. iberica* exhibits a wide range of biological activities, as an example of antimicrobial, anticancer, antioxidant, and anti-inflammatory consequences (Koca, Süntar et al. 2009, Alper, Özay et al. 2021). Similarly, organic extracts of *Centaurea hyalolepis* have been assessed for their antimicrobial activity against diverse Gram-positive and Gram-negative bacterial strains (Ismail, Masi et al. 2024).

The antimicrobial potential of these species has received little scientific attention, particularly when using plant material collected from local environments, against multidrug-resistant bacteria and *Candida albicans* ATCC strains. Variations in regional and ecological conditions may lead to differences in phytochemical composition and, consequently, biological activity (Gou, Lu et al. 2023). Therefore, the existent study aimed to evaluate and compare the antimicrobial activeness of *C. hyalolepis* and *C. iberica* collected from the MSU (Mountain Sulaimani District) region of the Kurdistan Region, Iraq, against selected pathogenic microorganisms, including *Escherichia coli*, MDR *Staphylococcus aureus*, and *Candida albicans* ATCC.

MATERIALS AND METHODS

1. Plant collection

Samples were gathered from the field (Figure2) during the flowering period, from late March to June 2025, in the Mountain Sulaimani District (MSU) of the Kurdistan Region, Iraq (Figures 1). In this study, leaves of *Centaurea hyalolepis* and *Centaurea iberica* were harvested and stored in nylon bag, and herbarium data were noted for each sample, including collection site, date, collector's name, growth stage, ecological observations, and altitude of each locality. The samples were subsequently transported to the research laboratory, where a portion was dried and pressed for taxonomic



Figure 1: (A) Physiographic region and Districts of Iraq and **(B)** distribution of surveyed study species in (MSU)



Figure 2: Field pictures of *Centaurea hyalolepis* (A) and *Centaurea iberica* (B)

Leaf samples were carefully washed under running tap water to remove dust particles and surface impurities prior to further processing, then air dry under a shaded area at room temperature and ground using laboratory grinder. The powdered plant material was subjected to maceration using a

methanol (Bibi, Iqbal et al. 2024) which is a polar solvent with high solubility, is effective for extracting various polar compounds. In addition to phenolic compounds, lipids, fatty acids and terpenoids (Lee, Jayakody et al. 2024).

Methanol- water solvent system (80:20, v/v) at room temperature for 24 hours, with occasional shaking at 160 rpm. and filtered through Whatmann No.1 filter paper, The extracts were concentrated under reduced pressure using a rotary evaporator at 40°C and air-dried under a slipstream of air. The extracts were refrigerated at 4°C For additional investigations of antimicrobial activities analyses (Omokhua, Abdalla et al. 2018, Dhoubi, Manuguerra et al. 2020, Mahmood, Salih et al. 2023), Stock solutions were prepared by dissolving the sample in 5% of Dimethyl sulfoxide DMSO (Jaafreh, Khleifat et al. 2019) (2ml of sample dissolved in 5ml of DMSO 5% to achieve stock solution) serial dilutions were made to obtain concentrations ranging (300mg/ml, 200mg/ml, 100mg/ml); at least three biological replicates were operated for all experiment (Parker, Hamilton et al. 2018).

3. Phytochemical screening

Three reagents were used in the Phytochemical screening process: chloroform CHCl_3 , concentrated H_2SO_4 , and ferric chlorid FeCl_3 (Table 1).

Table1. Chemical tests used to identify active compounds in *Centaurea hyalolepis* and *C.iberica*

<i>Centaurea</i> <i>spp.</i>	Terpenoides			phenols		
	Detection method (Used reagent)	Detection result	reference	Detection method (Used reagent)	Detection result	reference
<i>C. hyalolepis</i>	Chloroform CHCl_3 and concentrated H_2SO_4	Erddish- brown color	Abdelhamed et al.,2020	FeCl_3	Appearance of a bluish green color	Kalaf et al.,2018
<i>C. iberica</i>	Chloroform CHCl_3 and concentrated H_2SO_4	Erddish- brown color	Abdelhamed et al.,2020	FeCl_3	Appearance of a bluish green color	Khalaf et al.,2018

3.1. Terpenoides Detection:

Terpenoids were qualitatively identified using the Salkowski test. In brief, five mL of the hydro-methanolic extract was added to a tube containing two mL of CHCl_3 , after that carefully titrated with three mL of concentrated H_2SO_4 to form a visible layer. The formation of a reddish-brown color indicates presence of terpenoids (Abdalla, Mustafa et al. 2020).

3.2 Phenols Detection

Phenols were detected by adding 3ml of hydro-methanol plant extract to 2ml of FeCl_3 1% solution. Bluish green color indicates phenolic compound presence (Khalaf, Alaa et al. 2018).

4. Antimicrobial activity

Antimicrobial activity was assessed against *E. coli*, *S. aureus*, and *C. albicans*. All microbial strains were procured from the Department of Biology College of Science and the Research Center of University of Sulaimani, Iraq.

Antibacterial and antifungal properties were calculated depending on well diffusion techniques (Rojas, Bustamante et al. 2003, Şahin, Karaman et al. 2003). Mueller-Hinton agar was used for bacterial cultivation, while for fungal cultivation, Sabouraud dextrose agar was used. Nearly 20 mL of melted Mueller–Hinton agar (45 °C) was poured into sterile Petri dishes and kept until solidify. Bacterial suspensions adjusted of equal value 0.5 McFarland standards (100 μL of 10^8 CFU/mL) (Mahmood, Salih et al. 2023). , and fungal suspensions (100 μL , 10^6 CFU/mL) were evenly spread onto the respective agar surfaces.

After drying the plates under aseptic conditions, different 6mm wells were made by sterile cork borer. About 100 μL of each prepared extract was added to the wells, then incubated at 37 °C for 24 hours and 48 hours for for bacterial and fungal tests respectively.

For antimicrobial measurement; 10 mg/mL Meropenem (10 mg/mL) was used as positive control for Gram-negative bacteria, and 30mg/mL Tetracycline for Gram-positive bacteria, 50mg/mL Ketoconazole for fungi was applied, where as 5% dimethyl sulfoxide solution served as the negative control. All experiments were done in triplicate. Antimicrobial activity was measured by calculating the inhibition zone diameters around the wells

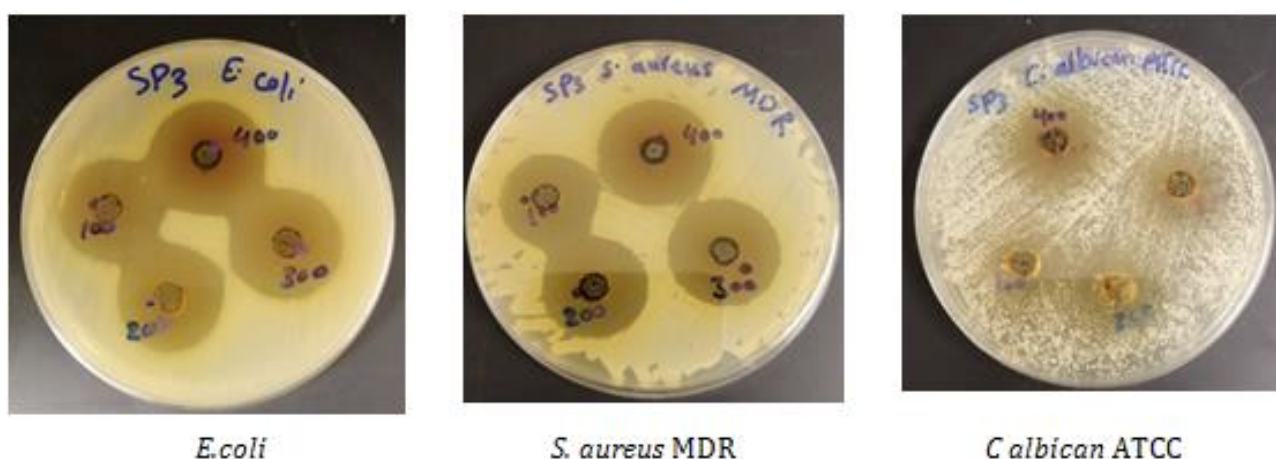


Figure 4. Effect of *C. hyalolepis* extract against examined microbial species.

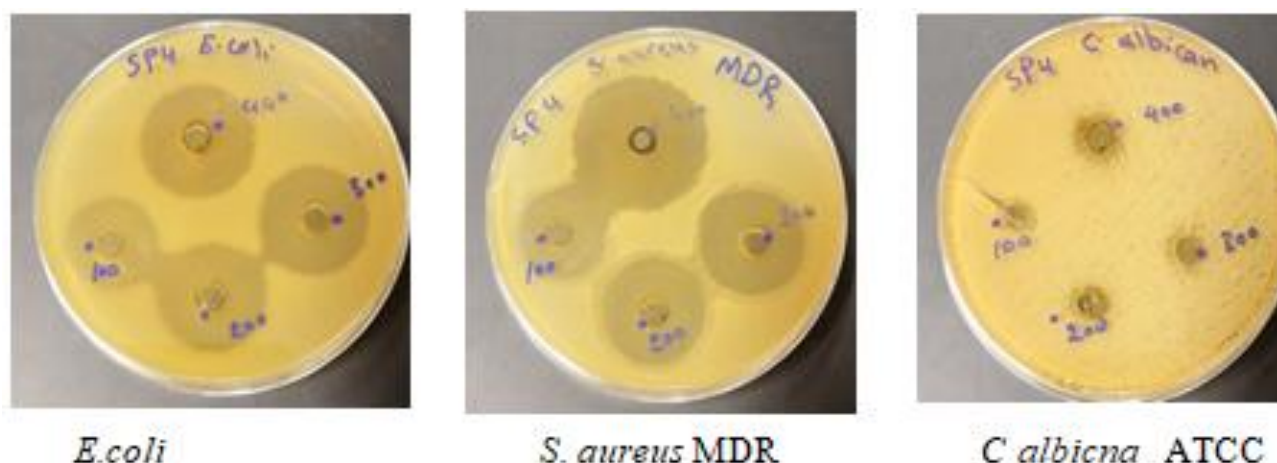


Figure 5. Effect of *C. iberica* extract against examined microbial species.

5 Statistical Analysis

Statistical analysis was done using R-Software depending on one-way ANOVA using.. Mean differences were evaluated by use Tukey's post hoc test(MacFarland 2011).

RESULTS AND DISCUSSION

1.Results

1.1 Chemical detection of active compounds

Phytochemical screening of the methanolic aqueous preparations from foliar material extracts of *Centaurea hyalolepis* and *Centaurea iberica* revealed the presence of terpenoids and Phenols (Table 1).

1.2 Anti-microbial activity of *C. hyalolepis* and *C. iberica* extracts

The hydro-methanolic leaf extracts of *Centaurea hyalolepis* and *Centaurea iberica* exhibited versus inhibitory effects of *Escherichia coli*, multidrug-resistant *Staphylococcus aureus* (MDR), and *Candida albicans* ATCC at concentrations of 100%, 200%, 300%, and 400 mg/mL, as determined by the diameter of the inhibition zones (mm)in comparison to the controls (Tables2 and 3).

Table 2: Antibacterial and Antifungal activities of *C. hyalolepis*

Extract	Concentration Mg/ml	Bacteria			Average concentration Average extract	Fungi		
		<i>E. coli</i>	<i>S. aureus</i>			<i>C. albicans</i>	Average concentration	Average extract
Hydro- sp3 Methanol	100m	A	B					
	g/ml	23.67	25.33	24.5	26.22 a	15	15	19.38 a
		ad	ae	a		a	a	
	200m	A	A	25.5		17.9	17.83	
	g/ml	25.33	25.7	a		b	b	

Control		ade	ae					
	300m	A	A	26.7	300mg/	20.33	20.33	
	g/ml	26.7	26.7	a	ml	c	c	
		ade	aef					
	400m	A	A		400mg/	23	24.33	
	g/ml	28.33	28	28.2	ml	d	d	
		ae	bf	b				
	Avera	A	A		Average			
	ge	26.08	26.4	26.23	fungi	19.38		
	bacter							
	ia							
	1/0.5	A	A	A	1/0.5	A		
	DMS	0	0	0	0	0		
	O	b	c	c	b			
	2/*M	A	B					
	RO				***KT	23	23	23
	**TE	*28	**15.3	21.65	21.	e	e	b
	T	ce	d	d	6			
				c				

Values represent the average of three replicates. Columns labeled with the same uppercase letters are not significantly different at $p \leq 0.05$. Similarly, columns sharing the same lowercase letter show on significant difference at the same probability level. .

*MRO= Meropenem **TET= Tetracycline ***KT= Ketoconazole

For *S. aureus*, the 400 mg/mL concentration of *C. iberica* resulted in a 33 mm inhibition zone compared to the control (15 mm) (Table 3), while *C. hyalolepis* at the same concentration produced a 28 mm inhibition zone versus the control (15.33 mm) (Table 2).

Regarding antifungal activity against *C. albicans*, *C. iberica* showed no inhibition at 100 mg/mL, while 200, 300, and 400 mg/mL concentrations produced inhibition zones of 10.66, 17.5, and 20.33 mm, respectively. *C. hyalolepis* exhibited inhibition zones of 15, 17.9, 20.33, and 23 mm at 100, 200, 300, and 400 mg/mL, respectively. Overall, the average inhibitory effects of *C. hyalolepis* were 26.22 mm against bacteria and 19.38 mm against fungi, while *C. iberica* showed averages of 27.31 mm and 12.12 mm, respectively (Tables 2 and 3).

Generally, no significant differences were notice between the 200 and 300 mg/mL concentrations, whereas significant differences were evident between the 100 and 400 mg/mL concentrations, suggesting a strong dose-dependent effect of both extracts.

Table 3. Antibacterial and Antifungal activities of *C. iberica*.

Extract	Concentration Mg/ml	Bacteria			Average concentration	Average extract	Concentration Mg/ml	Fung			Average concentration	Average extract
		<i>E. coli</i>	<i>S. aureus</i>					<i>C. i albicans</i>				
Hydro- sp3 methanol	100mg/ ml	A 22 a	B 26.7 a		24.35 a		100mg/ ml	A 0 a				
	200mg/ ml	A 24.6 7 bg	A 28.3 bg		26.47 b		200mg/ ml	A 10.66 b			10.66 b	
	300mg/ ml	A 25.7 cgo	A 28.8 ago		27.25 c	27.31 a	300mg/ ml	A 17.5 c			17.5 c	12.12 a
	400mg/ ml	A 29.3 3 dhp	B 33 chp		31.17 d		400mg/ ml	A 20.33 d			20.33 d	
	Average bacteria	A 25.4 3	b 29.2		27.32		Average fungi	A 0				
	1/0.5 DMSO	B 0 w	B 0 w		B 0 w	0	1/D.W	A 0 w				
	2/*MRO **TET	A *28 e	B **15. 3 e		21.65 e	21.6 b	***KT	A 23 e			A 23 e	23 B

Values represent the average of three replicates. Columns labeled with the same uppercase letters are not significantly different at $p \leq 0.05$. Similarly, columns sharing the same lowercase letter show on significant difference at the same probability level. *MRO= Meropenem **TET= Tetracycline ***KT= Ketoconazole.

2. Discussion

The growth of both Gram Positive and negative bacterial isolates can explain that bacteria can exhibit varying sensitivities to terpenoids and phenols. The antibacterial activity observed in this study is consistent with the use of hydro-methanol as an effective extraction solvent. Plants of the family Asteraceae, particularly the genus *Centaurea*, are recognized as rich sources of antimicrobial compounds (Khammar and Djeddi 2012).

In this study, the average inhibition zones of *C. hyalolepis* and *C. iberica* against the tested bacteria were 26.22 mm and 27.31 mm, respectively, which exceeded the average inhibition observed in control groups (21.65 mm) (Tables 2 and 3). This enhanced activity may be attributed to the existence of bioactive phytochemicals in *Centaurea hyalolepis* Boiss, *C. iberica* Trevir.ex spreng, including phenols and sesquiterpene lactones (STLs)(Erel, Demir et al. 2014, Ercan, Çalışkan et al. 2025). (Table 1). Additional factors that may influence the antimicrobial efficacy including the plant age and the part sample-specifically the leaves(Mugao 2024) .

Notably, *C. iberica* exhibited no measurable antifungal activity against *C. albicans* at the lowest concentration (100 mg/mL), which may reflect the high potency of the positive control, Ketoconazole (50 mg/mL), masking any inhibitory effects of the plant extract (Uno, Shigematsu et al. 1982). The methanolic extracts demonstrated activity against tested bacterial isolates, including *S. aureus*, and *E. coli* (Ercan, Çalışkan et al. 2025). Flavonoids have been reported to disrupt bacterial membrane permeability, interfere with porin proteins, and inhibit adhesion and biofilm formation, all of which are critical for bacterial survival and pathogenicity (Donadio, Mensitieri et al. 2021).

Phytochemical analyses revealed that both *C. hyalolepis* and *C. iberica* contain a wide range of secondary metabolites, in particular sesquiterpene lactones(STLp), essential oils, flavonoids, alkaloids, and lignans, which play a role in their antibacterial, cytotoxic, antioxidant, and anti-inflammatory activities(Al-Souz and Al-Newani 2024, Doğanay, Mertoğlu et al. 2025). Variability in the antimicrobial efficacy observed across samples may result from differences in the concentration of active compounds, likely influenced by environmental heterogeneity and regional ecological conditions, which can affect the phytochemical profile of the plants.

CONCLUSIONS

Presently, more than 5000 phytochemical compounds have been identified, however, their composition differs according to the plant species, *C. hyalolepis* and *C. iberica* . The leaves were

found to be rich in bioactive phytochemicals with notable therapeutic potential. Based on this, future studies should focus on isolating and identifying the specific bioactive compounds from the leaves of both species *C. hyalolepis* and *C. iberica* using different extraction methods and different techniques such as GC-MS (Gas Chromatography –Mass Spectrometry) to analyze quantitative and qualitative of chemical compounds, Green Chemistry and FTIR (Fourier Transform Infrared Spectroscopy) to determine the chemical structure, with bio-autography microdilution assays to assess biological effects, we gain a comprehensive understanding of the plant extract's impact on microbes.

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