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Noman, Omar M.

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A Phytochemical Analysis, Microbial Evaluation and Molecular Interaction of Major Compounds of *Centaurea bruguieriana* Using HPLC-Spectrophotometric Analysis and Molecular Docking

Omar M. Noman [1], Rashed N. Herqash [1], Abdelaaty A. Shahat [1], Syed Rizwan Ahamad [2], Hamza Mechchate [3,*], Abdulaziz N. Almoqbil [1] and Ali S. Alqahtani [1]

[1] Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia; onoman@ksu.edu.sa (O.M.N.); rherqash@ksu.edu.sa (R.N.H.); ashahat@ksu.edu.sa (A.A.S.); aalmoqbil@ksu.edu.sa (A.N.A.); alalqahtani@ksu.edu.sa (A.S.A.)
[2] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia; sarahamad@ksu.edu.sa
[3] Laboratory of Inorganic Chemistry, Department of Chemistry, University of Helsinki, P.O. Box 55, FI-00014 Helsinki, Finland
* Correspondence: hamza.mechchate@helsinki.fi

Abstract: Centaurea is one of the most important genera within the family Asteraceae. An investigation of the phytochemical composition of *Centaurea bruguieriana* using Gas-Chromatography coupled to Mass spectrometry (GC-MS) was performed. Antimicrobial activity was evaluated using the minimum inhibitory concentration method (MIC) and validated by molecular docking for the major compounds of the most active fraction (1,10-di-epi-cubenol and methyl 8-oxooctanoate) of *C. bruguieriana* against three bacterial receptors (TyrRS, DNA gyrase, and dihydrofolate reductase (DHFR)). Evaluation of antioxidant activity was conducted using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. High-performance liquid chromatography (HPLC) was used to identify and quantify the contents of major compounds from ethyl acetate fraction (luteolin 7-*O*-glucoside, chlorogenic acid, kaempferol and isorhamnetin). The antimicrobial activity test showed that the chloroform fraction was more active against all microbial strains. The results of the molecular docking of two major compounds from chloroform fraction showed that good affinities were made between 1,10-di-epi-cubenol and the three selected receptors (TyrRs: −6.0 Kcal/mol against −8.2 Kcal/mol obtained with clorobiocin (standard); DNA gyrase: −6.6 Kcal/mol against −9.1 Kcal/mole obtained with clorobiocin; DHFR: −7.4 Kcal/mol against −6.3 Kcal/mol obtained with SCHEMBL2181345 Standard). Antioxidant evaluation showed that the ethyl acetate fraction was the most active fraction in DPPH (IC_{50} 49.4 µg/mL) and ABTS (IC_{50} 52.8 µg/mL) models. HPLC results showed the contents of luteolin 7-*O*-glucoside (7.4 µg/mg), and chlorogenic acid (3.2 µg/mg). Our study demonstrated that *C. bruguierana* is a promising source of bioactive compounds.

Keywords: *C. bruguierana*; GC–MS; antioxidant; antimicrobial; HPLC–UV; molecular docking

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1. Introduction

Centaurea is one of the most important genera within the family Asteraceae [1], with more than 600 species. These taxa are thistle-like flowering plants that are distributed particularly in the Mediterranean region and West Asia [2]. Owing to their medicinal properties, various Centaurea species have been used extensively for the treatment of conditions such as peptic ulcers, diabetes, fever, coughs, common cold, intoxication treatments, and malaria [3]. According to numerous reports, these taxa are a potential source of natural antioxidants used in prevention and treatment of diseases in which reactive oxygen species are involved [4]. In addition, Centaurea species have shown various biological activities

including hepatoprotective, antimicrobial, anti-inflammatory, and cytotoxic activities which have been documented in several in vitro and in vivo studies [3,5–7]. Previous phytochemicals reports have indicated that Centaurea genera are rich in sesquiterpene lactones [8], flavonoids [9], flavonoid glycosides [10], lignans [11], alkaloids [12], triterpenes [13], and anthocyanins [9]. There is growing worldwide interest in the antioxidant ability of phenolics of this genus. Several studies have reported that many Centaurea species, such as *C. cyanus* L. [14], *C. bornmuelleri* Hausskn [15], exhibit remarkable antioxidants properties that allow for their use as a potential source of new nutraceutical products.

In course of our research of the genus Centaurea, we examined *Centaurea bruguieriana* (DC.) Hand.-Mazz. (Synonym *Centaurea phyllocephala* Boiss.) a Saudi Arabian plant, which was distributed in Najd province of Saudi Arabia [16]. *C. bruguieriana* has been reported to have various biological effects such as larvicidal activity on a malaria vector, a primary cytotoxic effect, anti-peptic ulcer activity, and a hypoglycemic effect on rats [17–21]. Molecular docking plays an important role in the rationale of drug design. In the field of drug discovery, molecular docking is a method to predict the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Molecular docking can be defined as an optimization problem, o describe the 'best-fit' orientation of a ligand that binds to a particular protein of interest [22,23].

Screening of phytocompounds through molecular modeling would help in building a stronger pipeline for in vitro and in vivo screening and reduce the chances of failure in clinical trials. Moreover, in silico studies could help in understanding the molecular mechanisms of action and targets of phytocompounds [24]. It is important to know that no studies have reported the phytochemical evaluation of *C. bruguieriana* with respect to antioxidant, and antimicrobial properties, or molecular docking for the major compounds responsible for such activities. Therefore, the aim of this study was to investigate the phytochemical constituents present of *C. bruguieriana* using GC-MS and HPLC analysis of two major compounds, luteolin 7-*O*-glucoside and chlorogenic acid, as well as the evaluation of antioxidant and antimicrobial activities along with molecular docking study.

2. Material and Method

2.1. Plant Material

Centaurea bruguieriana (DC.) Hand.-Mazz is an annual herb, 15–50 cm high, with purple spiny flowers and a white stem. It is found in northern Saudi Arabia Figure 1. It was collected in April 2019 from the Tamir area, about 140 km northwest of Riyadh. The plant was identified and authenticated by the taxonomist at the Herbarium Unit, College of Pharmacy, King Saud University. The voucher specimen (CB 4/2019) was deposited at the Herbarium of the Faculty of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Figure 1. *Centaurea bruguieriana*.

2.2. Preparation of Sample

The whole fruiting air-dried aerial parts (300 g) were ground to a coarse powder and subjected to maceration at room temperature using 1 L of 80% methanol and allowed to stand overnight for 2–3 days. This procedure was repeated until the plant material was exhausted and yielded a crude methanol extract. Then, the crude methanol extract was filtered and concentrated using a rotary evaporator (45 rpm and 40 °C) to obtain 52 g a dry methanol extract (CB. crude). After that, 45 g of this extract was suspended in water and fractionated by chloroform (CB. $CHCl_3$), ethyl acetate (CB. EtoAc), and *n*-butanol (CB. But.), respectively, using 300 mL and repeated three times for each solvent. The filtrates were concentrated using a rotary evaporator (45 rpm and 40 °C). The yield of the dried fractions was 0.86%, 27.56%, and 23.95% for the chloroform, ethyl acetate, and *n*-butanol) fractions, respectively. The dry fractions were transferred into separate vials and stored at −20 °C until use.

2.3. Phytochemical Analysis of Centaurea bruguieriana Chloroform Extract by GC-MS

The chemical constituents of *C. bruguieriana* extract were confirmed utilizing gas chromatography and a mass spectrometer (Turbomass, PerkinElmer, Waltham, MA, USA). The temperature program was fixed at 40 °C, followed by a 2 min hold, and then raised to 200 °C at a rate of 5 °C min^{-1}, which was then put on hold for 2 min. From 200 °C, the temperature was raised by 5 °C min^{-1} to 300 °C and held for another 2 min. *C. bruguieriana* chemical composition was determined by comparing the mass spectra obtained with the mass spectra from the National Institute of Standard and Technology Spectral library the Adams Library (Adams, 2007) [25] and the Wiley GC/MS Library [26].

2.4. Biological Studies

2.4.1. Antioxidant Activity

DPPH Radical-Scavenging Activity of *Centaurea bruguieriana*

Centaurea bruguieriana antioxidative activity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method as reported earlier by Fahad et al., 2020 [27–29]. Briefly, seven different concentrations of *C. bruguieriana* extract and fractions (10, 50, 100, 500 and 1000 µg/mL) were prepared. Thereafter, 0.5 mL of each concentration was mixed with 0.125 mL DPPH and 0.375 mL methanol and incubated for 0.5 h. Optical density was measured at λmax = 517 nm. Ascorbic acid was used as a positive control. Radical scavenging activity was calculated as following formula:

$$\% \text{ of radical scavenging activity} = (\text{Abs control} - \text{Abs extract}/\text{Abs control}) \times 100$$

Experiments were done in three replicates.

ABTS Radical Cation Scavenging Activity of *Centaurea bruguieriana*

(ABTS) radical cation scavenging activity of *C. bruguieriana* extract and fractions were conducted according to the method described earlier by Fahad et al., 2020 [29,30]. The percentage of antioxidant capacity for each extract was determined based on the reduction of ABTS absorbance by calculation using following formula [31].

$$\% \text{ of radical scavenging activity} = (\text{Abs control} - \text{Abs sample}/\text{Abs control}) \times 100$$

Experiments were done in three replicates.

2.4.2. Determination of the Antimicrobial Activity

Test Microorganisms

Two Gram-positive (*Staphylococcus aureus*, ATCC 25923; and *Enterococcus faecalis*, ATCC 29212), two Gram-negative (*Escherichia coli*, ATCC 25922; and *Proteus vulgaris*, ATCC 8427), and one *Candida albicans* (ATCC 60193) fungal strain were used in this investigation.

Minimum Inhibitory Concentrations

Centaurea bruguieriana extract and fractions were tested for their antimicrobial activity as described by Mann et al., 1998 [32]. Each extract was pipetted onto 96 well culture plates in two-fold serial dilutions (100 µL/well). All extracts were prepared in the appropriate broth medium to achieve concentrations ranging from 2000 to 31.2 mg/mL. The suspensions of 100 µL and 1106 CFU/mL bacteria and fungus were then added and incubated for 24 and 72 h, respectively, at the appropriate temperatures. The minimum inhibitory concentration (MIC) for no detectable bacterial or fungal growth was found. Five microliters were taken from the wells and placed in agar plates since there was no growth. They were then incubated for 24 or 72 h to achieve the lowest bactericidal/fungicidal concentrations (MBC/MFC).

2.5. Molecular Docking

2.5.1. Preparation of the Ligands

The Spatial Data File (SDF) of 1,10-di-epi-cubenol (CID: 12046149; Figure 2) and methyl 8-oxooctanoate (CID: 535040; Figure 2) were obtained from PubChem. The SDF file was converted to the Protein Data Bank, Partial Charge (Q), & Atom Type (T) (PDBQT) format using AutoDock Tools v1.5.6 (Scripps Research, CA, USA). For the final ligand preparation Gasteiger partial charges were added, rotatable bonds were defined and the nonpolar hydrogen atoms were merged.

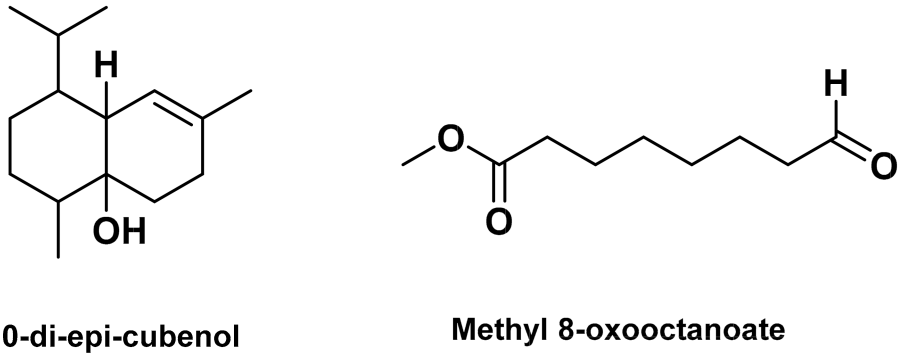

Figure 2. Major compounds of *Centaurea bruguieriana* from chloroform extract.

2.5.2. Preparation of the Receptors

Three receptors were selected (Tyrosine-tRNA ligase (TyRS)), DNA gyrase and Dihydrofolate reductase (DHFR) to perform the docking study based on their well-known role in the bacterial growth and survival. Each receptor's Protein Data Bank (PDB) file was obtained from the protein data bank website. The receptors' X-ray crystal structures were selected for their completeness, resolution, and compatibility with our study goal. Details of the selected receptors are described in Table 1. Before performing the analysis, Discovery Studio Visualizer v 19.1.0 (BIOVIA, San Diego, United states (windows software)) was used to begin the preparation of the receptors by removing water molecules and heteroatoms. Later, AutoDock Tools were used to add Gasteiger charges and polar hydrogen atoms to the structure. For the final preparation for molecular docking, the final file was converted to the PDBQT format.

Table 1. Description of the studied receptors.

Receptor	PID	Resolution (Å)	Classification
TyRS	1jij	3.20 Å	Ligase
DNA gyrase	1KZN	2.30 Å	Isomerase
DHFR	3fyv	2.20 Å	Oxidoreductase

2.5.3. Simulation

The grid box size for each receptor was determined using AutoDock Tools (1jij: center X = −11.668, Y = 19.196, Z = 92.023 and size X = 102 Y = 126, Z = 126; 1KZN: center X = 23.792, Y = 19.108, Z = 43.258 and size X = 98 Y = 92, Z = 86; 3fyv: center X = 24.477, Y = 11.603, Z = 38.439 and size X = 108 Y = 126, Z = 126). Docking simulations for the Ligands and three receptors were performed using AutoDock Vina. The simulation's exhaustiveness was set to 24, and 20 different poses were generated. The protein-ligand complexes were visualized using Discovery Studio Visualizer. The active site was determined based on the co-crystalized ligand with the receptors and the Discovery Studio Visualizer screening.

2.6. HPLC of the Ethyl Acetate Fraction of C. bruguieriana and Chromatographic Conditions

The biomarkers, luteolin 7-*O*-glucoside, chlorogenic acid, kaempferol and isorhamnetin were purchased from Phytolab (Vestenbergsgreuth, Germany). The analysis was performed by HPLC with UV detection (Alliance 2695 Separations Module, Waters Instruments, Inc., Milford, MA, USA) using a reverse-phase C18 column (Pinnacle C18 column, 250 × 4.6 mm, 5 µm, Shimadzu, Kyoto, Japan). The mobile phase was composed of different proportions of (A) 0.5% acetic acid in ultra-pure water (acidified water), and (B) acetonitrile and methanol (60:40, *v*/*v*) using a flow rate of 1 mL/min. The optimized gradient program was as follows: 0–5 min (0–55% B), 5–10 min (55–65% B), 10–20 min (65–90% B), 20–30 min (90–100% B), and 30–35 min (0% B). Samples were injected into the system at 20 µL. The detection was performed at a single wavelength (280 nm) and processed using EMPOWER software, version 2.

3. Results

3.1. Centaurea bruguieriana Chloroform Extract Chemical Composition

Twenty phytocompounds were identified in the *C. bruguieriana* chloroform extract by GC-MS. The active phytocompounds with their retention times, molecular formulas, molecular weights, and concentrations (%) are presented in Table 2 and Figure 1. The identified compounds are represented in order of their elution on the HP Innowax column. Methyl 8-oxooctanoate (17.6%) was the primary constituent, and 1,10-di-epi-cubenol was the secondary constituent (13.4%) (Figure 3).

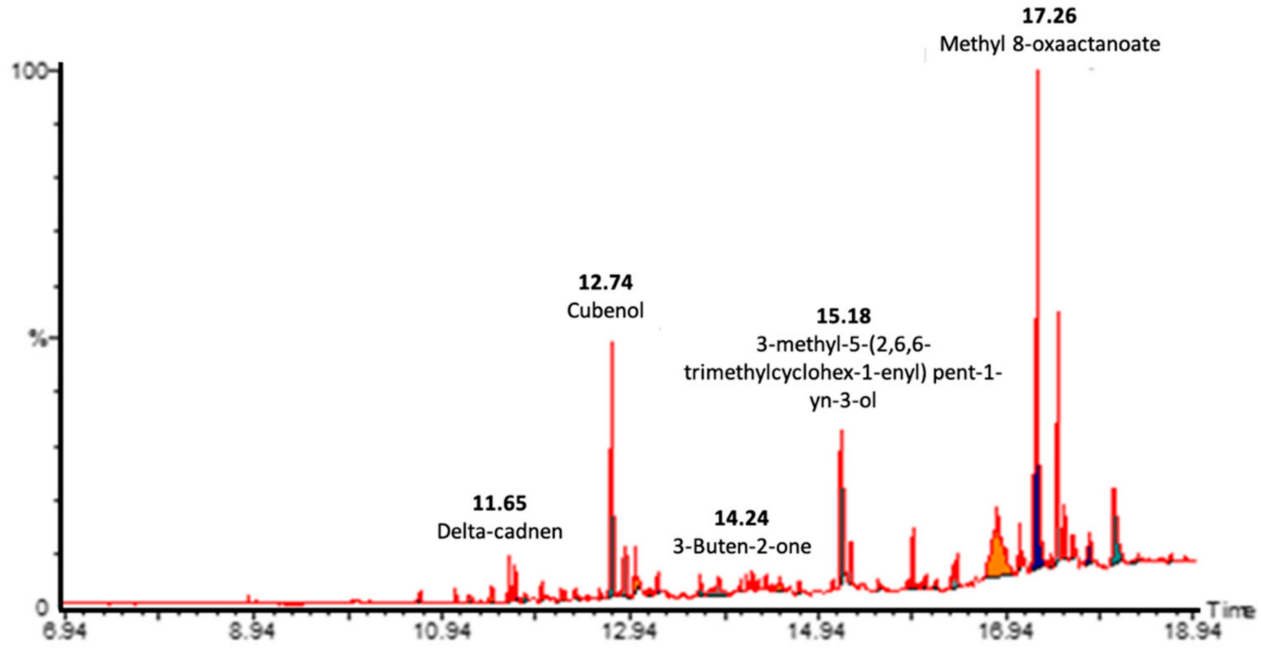

Figure 3. GC-MS Chromatogram of *Centaurea bruguieriana* chloroform extract.

Table 2. GC-MS analysis of *Centaurea bruguieriana* chloroform extract.

Compound Name	Chemical Formula	Molecular Weight (g/mol)	RT (min)	Area%
Trans-caryophyllene	$C_{15}H_{24}$	204.35	10.70	0.600
Alpha-humulene	$C_{15}H_{24}$	204.35	11.09	1.430
Delta-cadinene	$C_{15}H_{24}$	204.35	11.65	2.530
(1s)-cis-calamenene	$C_{15}H_{22}$	202.33	11.71	3.640
Beta-cedrene	$C_{15}H_{24}$	204.35	11.82	0.700
Nerolidol	$C_{15}H_{26}O$	222.37	11.99	0.640
Caryophyllene oxide	$C_{15}H_{24}O$	220.35	12.36	1.13
Humuladienone	$C_{15}H_{24}O$	220.35	12.62	0.330
1,10-di-epi-Cubenol	$C_{15}H_{26}O$	222.37	12.74	13.480
Tau-muurolol	$C_{15}H_{26}O$	222.37	12.89	5.32
Shyobunol	$C_{15}H_{26}O$	222.37	13.22	1.210
7-acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo [4.3.0] nonane	$C_{15}H_{26}O_2$	238.37	13.68	2.150
Citronellyl acetate	$C_{12}H_{22}O_2$	198.3	14.19	0.600
14.38	$C_{15}H_{28}O_2$	240.38	14.38	0.700
Ethyl heptadecanoate	$C_{19}H_{38}O_2$	298.5	14.53	4.140
3-methyl-5-(2,6,6-trimethylcyclohex-1-enyl) pent-1-yn-3-ol	$C_{15}H_{24}O$	220.35	15.18	11.500
Ethyl palmitate	$C_{18}H_{36}O_2$	284.5	15.28	5.01
Phytol	$C_{20}H_{40}O$	296.5	16.08	1.360
7-oxabicyclo [2.2.1] heptane	$C6H_{10}O$	98.14	16.84	12.140
Methyl 8-oxooctanoate	$C_9H_{16}O_3$	172.22	17.26	17.610

3.2. Antimicrobial Study

The antimicrobial effect resulting from the *C. bruguieriana* extract and fractions (CB. Crude, CB.$CHCl_3$, CB.EtoAC, and CB.But) in terms of MIC and MBC/MFC are displayed in Table 3. The chloroform fraction exhibited stronger antimicrobial activity than the other plant extract and fractions, the most sensitive strains being the Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis* (MIC: 78.13 µg/mL). Moreover, both Gram-positive bacteria were also sensitive to the ethyl acetate fraction of *C. bruguieriana*-positive bacteria (MIC: 156.25 µg/mL). The MBC or MFC values were approximately two times higher than the MICs (Table 3).

Table 3. Minimal inhibitory concentrations (MIC), minimal bactericidal concentrations (MBC), and minimal fungicidal concentrations (MFC) of the crude extracts of *Centaurea bruguieriana*.

	Activity	*S. aureus*	*E. faecalis*	*E. coli*	*P. vulgaris*	*C. albicans*
			(µg/mL)			
CB. Crude	MIC	312.5	312.5	625	625	156.25
	MBC	625	625	1250	1250	-
	MFC	NT	NT	NT	NT	312.5
CB. $CHCl_3$	MIC	78.13	78.13	312.5	312.5	39.1
	MBC	156.25	156.25	625	625	-
	MFC	NT	NT	NT	NT	78.13
CB. EtoAC	MIC	156.25	156.25	312.5	312.5	78.13
	MBC	312.5	312.5	625	625	-
	MFC	NT	NT	NT	NT	156.25

Table 3. *Cont.*

	Activity	*S. aureus*	*E. faecalis*	*E. coli*	*P. vulgaris*	*C. albicans*
CB. But	MIC	312.5	312.5	625	625	156.25
	MBC	625	625	1250	1250	-
	MFC	NT	NT	NT	NT	312.5
Gentamycin	MIC	7.8	7.8	3.9	3.9	NT
	MBC	15.6	15.6	7.8	7.8	NT
Nystatin	MIC	NT	NT	NT	NT	3.5
	MFC	-	-	-	-	7.0

3.3. Molecular Docking

The results of the affinities between the selected receptors and molecules from the chloroform fraction are presented in Table 4. Clorobiocin was selected as positive control to compare the results of the molecules with the TyrRS and DNA gyrase receptors, while SCHEMBL2181345 was used to compare against the DHFR. From the results are summarized in Table 4. Good affinities were made between 1,10-di-epi-cubenol and the three selected receptors (TyrRS: −6.0 Kcal/mol against −8.2 Kcal/mol obtained with Clorobiocin (Figure 4); DNA gyrase: −6.6 Kcal/mol against −9.1 Kcal/mol obtained with Clorobiocin (Figure 5); DHFR: −7.4 Kcal/mol against −6.3 Kcal/mol obtained with SCHEMBL2181345 (Figure 6). As for methyl 8-oxooctanoate, poor affinities were obtained against all selected receptors (TyrRS: −3.5 Kcal/mol against −8.2 Kcal/mol obtained with Clorobiocin (Figure 4); DNA gyrase: −4.4 Kcal/mol against −9.1 Kcal/mol obtained with Clorobiocin (Figure 5); DHFR: −4.4 Kcal/mol against −6.3 Kcal/mol obtained with SCHEMBL2181345 (Figure 6).

Table 4. Affinity results of the selected receptors and molecules from the chloroform fraction.

Compounds	Affinities (Kcal/mol)		
	TyrRS	DNA gyrase	DHFR
1,10-di-epi-cubenol	−6.0	−6.6	−7.4
Methyl 8-oxooctanoate	−3.5	−4.4	−4.4
Clorobiocin	−8.2	−9.1	-
SCHEMBL2181345	-	-	−6.3

3.4. Antioxidant Activity of Centaurea bruguieriana

The scavenging activity of the CB. Crude, CB. EtoAC, CB. $CHCl_3$, and CB. fractions varied (Table 5). The chloroform fraction of *C. bruguieriana* showed the highest antioxidant activity with IC_{50} 49.4 µg/mL in the DPPH scavenging activity test and IC_{50} 52.8 µg/mL in the ABTS test. However, the crude extract of *C. bruguieriana* (CB. Crude) showed the weakest antioxidant activity with IC_{50} 143.4 µg/mL in the DPPH scavenging activity test and IC_{50} 123.6 µg/mL in the ABTS test. Therefore, the major compounds of the ethyl acetate fraction (luteolin 7-*O*-glucoside and chlorogenic acid) were tested for their antioxidant potential and their activity was confirmed.

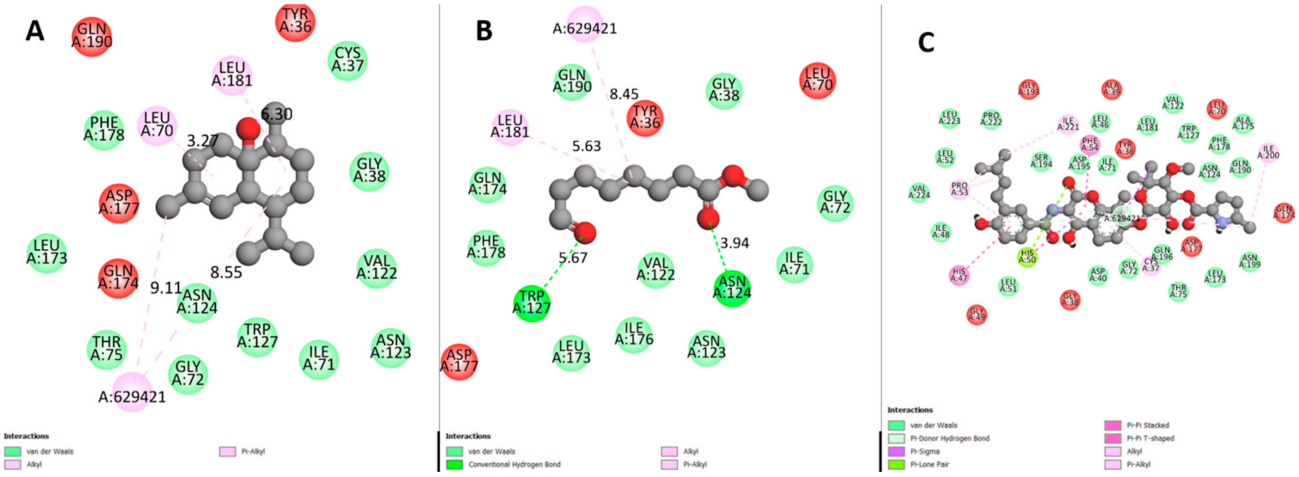

Figure 4. Two-dimensional scheme of TyrRS interaction with the tested ligands (**A**): 1,10-di-epi-cubenol; (**B**): Methyl 8-oxooctanoate; (**C**): Clorobiocin (control). Dotted lines: Distances between ligand atoms and receptor amino acids.

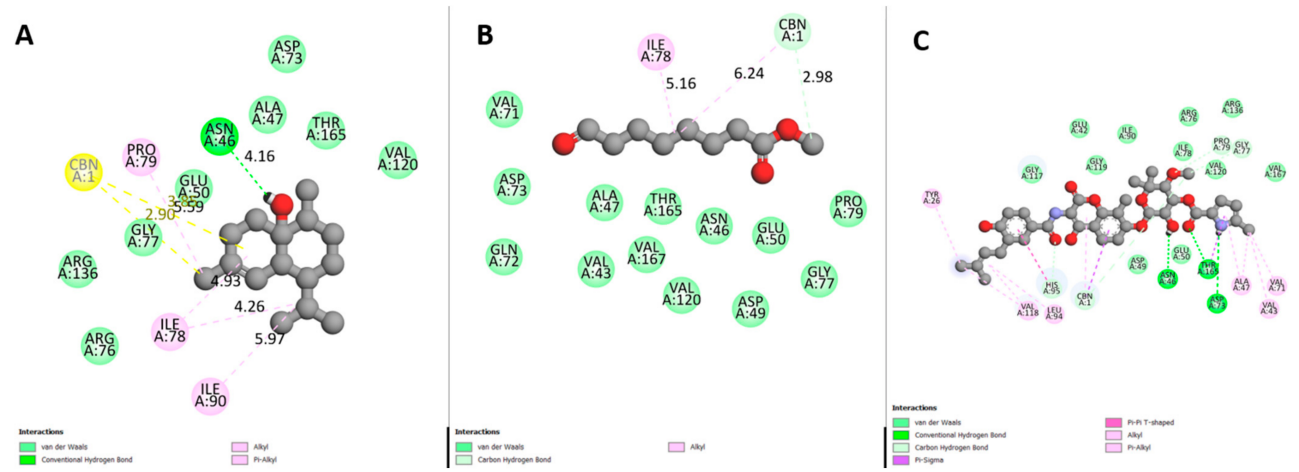

Figure 5. Two-dimensional scheme of DNA gyrase interaction with the tested ligands, (**A**): 1,10-di-epi-cubenol; (**B**): Methyl 8-oxooctanoate; (**C**): Clorobiocin (control). Dotted lines: Distances between ligand atoms and receptor amino acids.

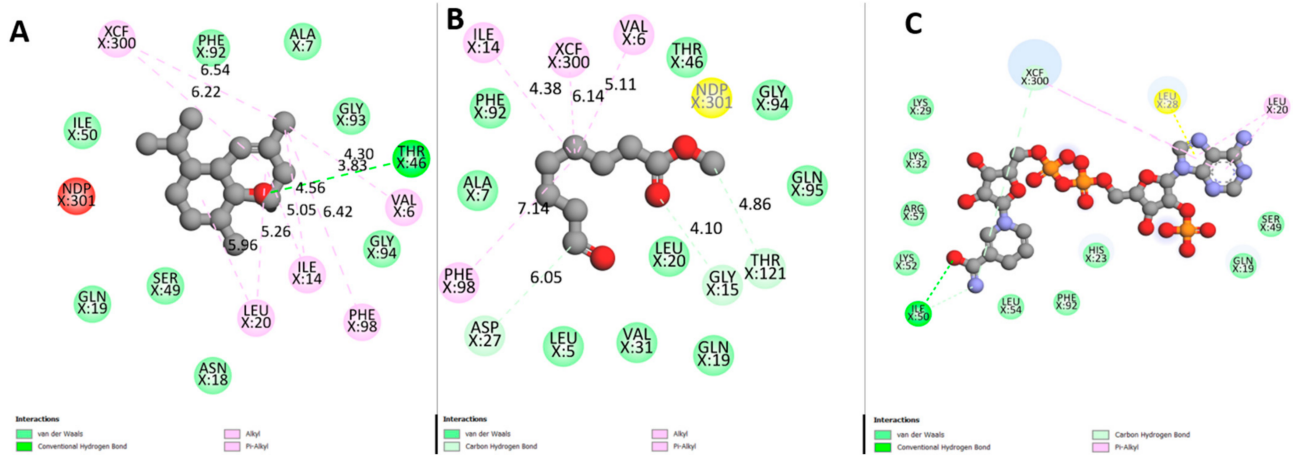

Figure 6. Two-dimensional scheme of DHFR interaction with the tested ligands, (**A**): 1,10-di-epi-cubenol; (**B**): Methyl 8-oxooctanoate; (**C**): SCHEMBL2181345 (control). Dotted lines: Distances between ligand atoms and receptor amino acids.

Table 5. IC_{50} Scavenging activities of *Centaurea bruguierana* extract and fractions.

Sample	IC_{50} ABTS	IC_{50} DPPH
CB. Crude	123.6 ± 0.9 µg/mL	143.4 ± 1.2 µg/mL
CB. CHCl3	91.5 ± 1.6 µg/mL	86.1 ± 1.3 µg/mL
CB. EtoAC	52.8 ± 2.6 µg/mL	49.4 ± 1.6 µg/mL
CB. But	88.4 ± 1 µg/mL	50.6 ± 1.9 µg/mL
Ascorbic Acid	7.4 *** ± 1.2 µg/mL	8.9 *** ± 0.8 µg/mL

Results expressed as mean ± SD, experiment done in 3 replicates; *** $p < 0.001$ compared to all samples.

3.5. HPLC Analysis of the Ethyl Acetate Fraction

The chemical structures of the two biomarkers are illustrated in Figure 7. HPLC chromatograms of *C. bruguieriana* ethyl acetate extract and standard solutions of the analyzed compounds are shown in Figure 8. Outcome analysis showed that the contents of luteolin 7-*O*-glucoside and chlorogenic acid were 7.4 and 3.2 µg/mg, respectively. The method was validated according to the guidelines set by International Conference on Harmonization [33]. The following features of validation were evaluated: linearity, quantitation limits, intra and inter-day precision, and recovery.

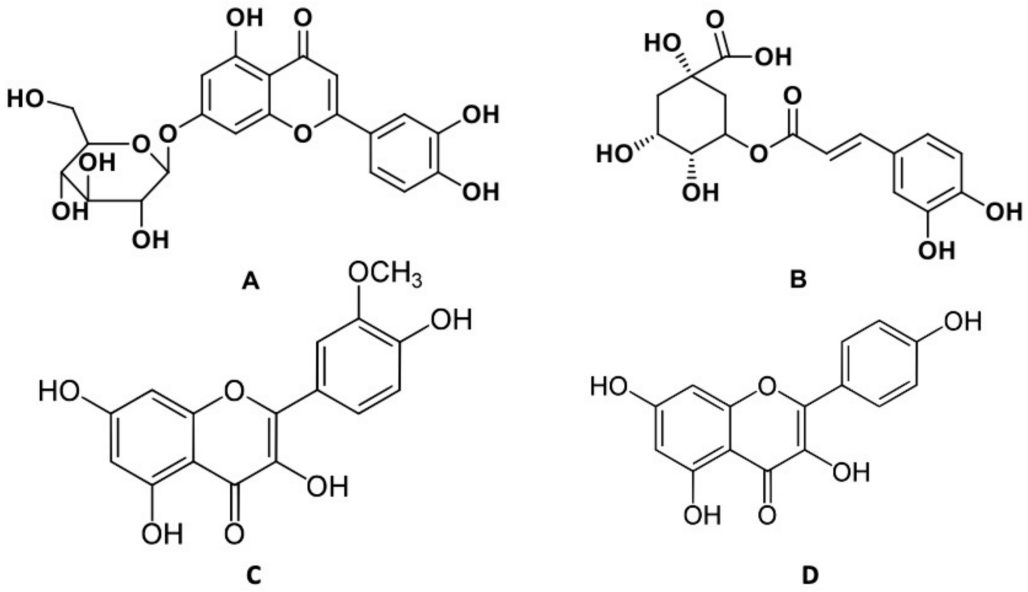

Figure 7. Chemical structure of biomarkers luteolin 7-*O*-glucoside (**A**), chlorogenic acid (**B**), isorhamnetin (**C**), kaempferol (**D**).

Linearity was evaluated by building external calibration curves for each compound using a working standard solution containing the two phenolic compounds. Each concentration of the mixed standard solution was injected in triplicate, and regression parameters were calculated. The coefficients of correlation (r^2) of calibration curves were ≥0.997 which confirms the linearity of the method. The sensitivity of the method was estimated by calculating the limits of detection (LOD) and quantification (LOQ) of the two analytes based on the calibration curve. The LOD was 0.171 and 0.159 µg/mL for chlorogenic acid and luteolin 7-*O*-glucoside, respectively, and the corresponding LOQ values were 0.518 and 0.481 µg/mL (Table 6).

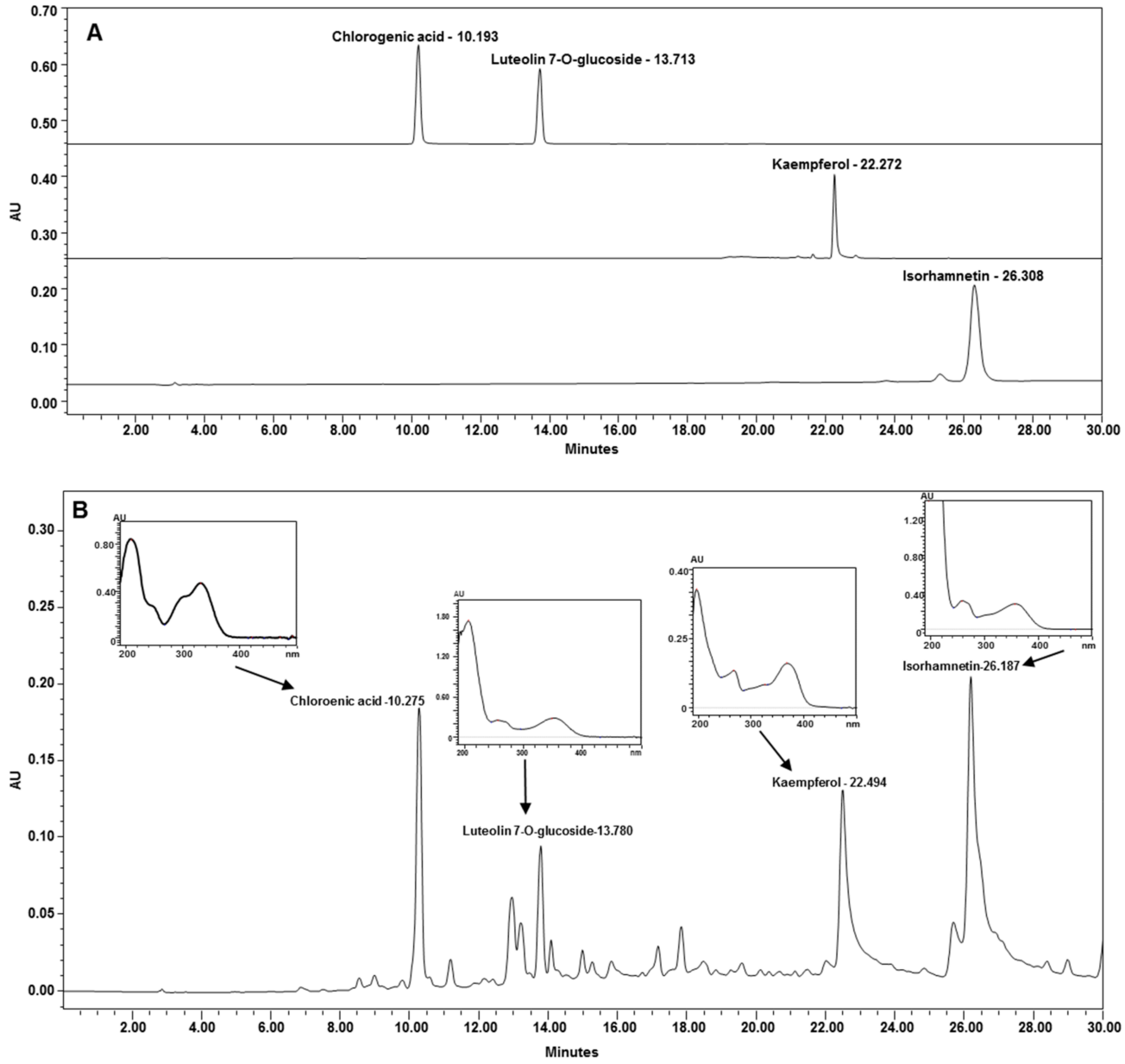

Figure 8. HPLC chromatograms of (**A**) standard phenolic compounds and (**B**) *Centaurea bruguieriana* ethyl acetate extract (inserts show chromatographic UV spectra of the main phenolic compounds).

Table 6. Calibration parameters and sensitivity data for the polyphenols using the proposed HPLC method.

Compound	Retention Time (min)	Range (μg/mL)	Linearity (r^2)	LOD (μg/mL)	LOQ (μg/mL)
Chlorogenic acid	10.25 ± 0.03	0.20–25.00	0.998	0.171	0.518
luteolin 7-*O*-glucoside	13.88 ± 0.05	0.20–25.00	0.997	0.159	0.481
Kaempferol	22.37 ± 0.04	0.20–25.00	0.997	0.161	0.483
Isorhamnetin	26.18 ± 0.03	0.20–25.00	0.998	0.175	0.544

4. Discussion

Plants produce and store a diverse range of phytoconstituents in variable amounts, necessitating the use of a variety of analytical procedures to assess them [34]. *C. bruguieriana*

is a medicinally significant plant that contains a variety of volatile components along with nonvolatile constituents.

In the investigation of unknown plant components, GC-MS plays a crucial role. Plant materials are often complex, making GC-MS an excellent choice for their examination due to its high sensitivity and selectivity. Compounds are ionized and their mass numbers are measured using GC-MS. This adds to the structure of these profiles by providing more information [35]. A total of 20 compounds were identified in the extract. The findings indicated that there was a presence of 3 methyl 8-oxooctanoate (17.6%) and 1,10-di-epi-cubenol (13.4%) as the major compounds in the chloroform extract of *C. bruguieriana*.

Flavonoid composition has long been known in the Asteraceae family. Apigenin, luteolin, and their glucosides, particularly 7-glycosides, are the most frequent flavonoids. Polyphenols, especially flavonoids, have sparked a lot of interest due to their widespread distribution in plants, biological (including antioxidant) activity, and physiological impacts [36]. Therefore, in our study, in the group of phenolic compounds, chlorogenic acid, luteolin 7-*O*-glucoside, kaempferol and isorhamnetin were specifically identified and quantified in the ethyl acetate fraction. For the first time, quantitative study of these two chemicals was carried out in this species. The phytochemical chlorogenic acid is well-known and well-described. Chlorogenic acid is an ester of caffeic and quinic acids with a broad range of antioxidant, anti-inflammatory, anti-HIV, anti-HBV, mutagenesis suppression, and carcinogenesis properties in vivo and in vitro [37–42]. Luteolin 7-*O*-glucoside also has pharmacological effects, such as reducing superoxide production in neutrophils [43]. In lipopolysaccharide-induced macrophage RAW 264 cells, the protein expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX) was inhibited by the action of luteolin [44]. Therefore, the presence of different phenolic compounds such as chlorogenic acid, luteolin 7-*O*-glucoside, kaempferol, and isorhamnetin could be responsible for the antioxidant activity. Similarly, Lockowandt et al. found [45] a significant association between phenolic acid concentration and antioxidant activity in *C. cyanus* plant sections (edible flowers and non-edible portions); however, this class of phenolic compounds was not found in our investigation. Lahneche et al. [46] reported that the n-butanol extracts of *C. sphaerocephala* exhibited higher antioxidant activity than other extracts. They were likewise more abundant in total phenolics and flavonoids. Furthermore, numerous additional studies have examined the antioxidant activity of Centaurea species using various assays, revealing the potential involvement of different phytochemicals in such activities as well as the prospective application of these species in human diet as antioxidant agents [2,14,47–50]. Several studies, on the other hand, have found antibacterial activity for several Centaurea species in a species and pathogen-dependent manner [51]. Sesquiterpenes and lactones have been identified as the primary chemicals responsible for these actions [52–54].

Because of growing resistance to conventional antibiotics, bacterial infection has been a constant and unrelenting danger to human health. As a result, most of the research has focused on the development of novel antibacterial medicines that target a variety of important bacterial survival mechanisms [55].

Tyrosyl-tRNA synthetase (TyrRS), a member of the aminoacyl-tRNA synthetase family, may recognize information such as concurrent tRNA molecules and amino acid structures, which are required for translating coded information into protein structures in nucleic acids. As a result, finding and using tyrosyl-tRNA synthetase inhibitors might be a viable strategy for controlling these disorders in people [56]. DNA gyrase is a type II topoisomerase that decreases topological strain in an ATP-dependent manner when double-stranded DNA is unwound in front of the advancing replication fork by elongating RNA-polymerase or by helicase. Inhibiting gyrase's ATPase activity prevents the formation of negative supercoils in DNA and confines the chromosome in a positively supercoiled state, which might affect cell physiology and division [57,58]. Folate, on the other hand, is required by rapidly proliferating cells to produce thymine. Bacteria need DHFR (dihydrofolate reductase) to thrive and reproduce; hence, inhibitors selective for bacterial vs. host DHFR have found use as antibacterial drugs [59]. DHFR inhibitors are widely used to treat fungal, bacterial,

and mycobacterial diseases, as well as malaria and other protozoal illnesses. Several compounds have been identified and several medications have been introduced to the market throughout the years [60]. The presence of 1,10-di-epi-cubenol could be responsible for antimicrobial activity, which is supported by the molecular docking of this compounds. As we mentioned earlier, good affinities were made between 1,10-di-epi-cubenol and the three selected receptors (TyrRS: −6.0 Kcal/mol against −8.2 Kcal/mol obtained with Clorobiocin; DNA gyrase: −6.6 Kcal/mol against −9.1 Kcal/mol obtained with Clorobiocin; DHFR: −7.4 Kcal/mol against −6.3 Kcal/mol obtained with SCHEMBL2181345.

Our study revealed that the chloroform fraction of *C. bruguieriana* showed the highest antibacterial activity against Gram-positive bacteria. Similarly, Tekeli et al. reported that Centaurea species are effective against *E. coli*, *S. aureus*, and *B. cereus* [51], while 'Ciri'c et al. [52] showed that zuccarinin was the most effective sesquiterpenes lactone isolated. These in vitro results confirm the in silico results obtained with the chloroform fraction where the two major compounds, 1,10-di-epi-cubenol and Methyl 8-oxooctanoate, inhibited three of the receptors involved in bacterial growth.

5. Conclusions

As alternative medications, plant products are attracting a great deal of interest. *C. bruguieriana* growing in Saudi Arabia possesses excellent antioxidant and antibacterial properties, according to our research. The antimicrobial activity of the chloroform fraction was confirmed by a molecular docking study that showed comparable affinities of 1,10-di-epi-cubenol against three receptors TyrRS, DNA gyrase and DHFR. Therefore, the presence of different phenolic compounds, such as chlorogenic acid, luteolin 7-*O*-glucoside, kaempferol, and isorhamnetin, could be responsible for the antioxidant activity of the ethyl acetate fraction when it is compared to the other fractions. Therefore, extensive research is needed to optimize and estimate other compounds from the leaves to develop novel functional products.

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