Istanbul J Pharm 52 (1): 80-89
DOI: 10.26650/IstanbulJPharm.2022.1001685
Original Article

Evaluation of the medicinal potential of a traditionally important plant from Turkey: *Cerinthe minor* L.

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Cite this article as: Ersoy, E., Karahan, S., Boga, M., Cinar, E., Izgi, S., Mataraci Kara, E. ... Eroğlu Özkan, E. (2022). Evaluation of the medicinal potential of a traditionally important plant from Turkey: *Cerinthe minor* L. *İstanbul Journal of Pharmacy, 52*(1), 80-89. DOI: 10.26650/IstanbulJPharm.2022.1001685

ABSTRACT

Background and Aims: *Cerinthe minor* L. has been used for both culinary and medicinal purposes in Turkey. Since the plant has remained uninvestigated pharmacognostically, this study aims to provide data on the phytochemical profile and *in vitro* biological activities of the ethanol extract prepared from the aerial and root parts of *Cerinthe minor* L.

Methods: The phytochemical profile of the extract was determined by LC-MS/MS. Total phenolic and total flavonoid contents of *Cerinthe minor* ethanol extract were determined as pyrocatechol, and quercetin equivalents, respectively. Antioxidant activity of the extract was investigated by DPPH, and ABTS cation radical scavenging activity, and CUPRAC activity assays. Enzyme inhibitory activity assays were used to investigate anticholinesterase, antityrosinase, and antiurease activities. Cytotoxic potential of the plant was clarified by using the XTT method, and antimicrobial activity of the extract was established by the microbroth dilution technique.

Results: According to the LC-MS/MS results, 11 different constituents were present in the extract, and the major compounds were, malic acid (5392.18±60.93 µg/g extract), fumaric acid (4730.99±58.66 µg/g extract), and rosmarinic acid (2470.07±176.12 µg/g extract). Total phenolic and flavonoid contents were found as 10.39±0.73 µg PEs/mg extract, and 0.75±0.07 µg QEs/mg extract, respectively. The extract demonstrated moderate antioxidant activity, whereas no enzyme inhibitory activity was exerted against the tested enzymes. No cytotoxic activity was observed on human renal (A498, UO-31) or human colon (COLO205, KM12) cancer cell lines. The extract was shown to possess low-moderate antimicrobial activity against *C. tropicalis* with a MIC value of 78.12 µg/mL.

Conclusion: Having several pharmacologically valuable compounds, and not causing any toxicity on studied cell lines, *Cerinthe minor* is a plant that requires attention with its medicinal potential.

Keywords: *Cerinthe minor*, LC-MS/MS, Antioxidant, Cytotoxicity, Medicinal potential

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Submitted: 27.09.2021
Revision Requested: 28.10.2021
Last Revision Received: 03.11.2021
Accepted: 06.01.2022
Published Online: 28.04.2022

INTRODUCTION

Since time immemorial, people have used medicinal plants for the prevention and/or treatment of various diseases and ailments. Although the popularity of herbal medicines had been dramatically decreased in the western world with the development of synthetic medications in the last 200 years, the recent huge demand for plant-based therapeutic agents as primary health care in well-developed countries, and the sharp upward trend for using traditional remedies and natural-based products indicate the importance of research on medicinal plants. The massively increased consumption of phytotherapeutic agents has resulted in an estimate by the WHO that more than 80% of the population relies solely or largely on medicinal plants for health care (Schippmann, Leaman, & Cunningham, 2002).

The Anatolian peninsula, where modern Turkey is located, is an extraordinary location being estimated to contain more than 11000 taxa currently, and more importantly, 1280 of them were reported to be used traditionally for medicinal purposes (Dalar, Mukemre, Unal, & Ozgokce, 2018). Plausibly arising from the outstanding historical background of Turkey, a great number of plants are still in use by local inhabitants, according to recent surveys about traditional medicine practices. A recent study reported that 59% of people using traditional and complementary medicine in Turkey use herbal mixtures (Şimşek et al., 2017).

In efforts to discover new drugs, indigenous medicinal plants have always been essential resources, and therefore an ethnopharmacological approach that includes chemical analyses and investigation of biological activities of the plants play a vital role in this context. It should also be emphasized that traditionally used medicinal plants must fulfill the requirements on quality, efficacy, and safety to be evaluated as therapeutic agents in today's era (Süntar, 2020). In this day and age, several advanced instrumental techniques are being used to provide scientific evidence supporting the traditional use of an ethnobotanically important plant (Boğa et al., 2021a). Safety concerns about natural agents are a major issue, considering that concurrent use of herbal products with synthetic medications can reach up to 88% in the elderly population (Taneri, Akis & Karaalp, 2021).

Cerinthe minor L. (Boraginaceae) is among worth-mentioning traditionally used plants in Turkey. *C. minor* belongs to the tribe *Lithospermeae* Dumort, and genus *Cerinthe* L., which is known as a small genus that includes approximately ten species distributed in the circum-Mediterranean region and Central Europe, more specifically, from the Atlantic region of Morocco to the western parts of the Irano-Turanian region (Selvi, Cecchi, & Coppi, 2009).. As of today, four *Cerinthe* species (*C. minor, C. retorta* Sm.*, C. major* L.*, and C. glabra* Mill.), and two *C. minor* subspecies (*C. minor* subsp. *auriculata*, and *C. minor* subsp. *minor)* have been identified in Turkey (Güner, Aslan, Ekim, Vural, & Babaç, 2012, Jetter & Riederer, 1999; Zengin et al., 2016). *C. minor* and *C. minor* subsp. *auriculata* (Ten.) Domacare known as "Hışhış otu", and they are both most commonly consumed as food when cooked in different cities of Turkey, such as Tunceli, Batman and Kars (Doğan & Tuzlacı, 2015; Kadıoğlu et al., 2020; Yeşil & İnal, 2019). Furthermore leaves of *C. minor* are brewed as decoction and have been used to treat edema, and a mouthwash can be prepared with branches of *C. minor* subsp. *auriculata*, which has been used to treat gum wounds in folk medicine (Korkmaz & Karakuş, 2015; Kadıoğlu, Kadıoğlu & Sezer, 2021). Moreover, the traditional name of *C. minor* L. is "Cücegözü", in Iğdır province and "Gayebej, Gobel" in the Hasankeyf (Batman) district, where the plant has also been traditionally used for several ailments (Altundağ & Özhatay, 2009; Yeşil & İnal 2021).

Despite the fact that *C. minor* is a popular, commonly used medicinal plant in Turkey, interestingly enough, the plant has somehow remained "unexplored" pharmacognostically until now. To the authors' best knowledge, Zengin et al. (2016) conducted a study on *C. minor* subsp. *auriculata*, reporting the results of antioxidant, antityrosinase, anti-α-amylase, anticholinesterase, and anti-α-glucosidase activity assays of the plant, which is still the only report that provides data on the biological activities of the subspecies. Due to the constantly increasing popularity of herbal remedies, it has become unquestionably crucial and a primary task to shed more light on the chemistry and pharmacological activities of traditionally used medicinal plants which, for sure, requires extensive pharmacognostic research. With these efforts, it may be possible to provide solid scientific evidence of their efficacy, and over and above that, safety concerns related to these plants could be addressed. This is very important since the species have been shown to contain toxic alkaloids by previous studies (El-Shazly & Wink, 2014; Mroczek, Baj, Chrobok, & Glowniak, 2004). In efforts to discover natural and renewable resources to use in the pharmaceutical industry, gaining more knowledge about the toxicity potential of the plants is essential (Boğa et al., 2021a).

Bearing all this in mind, this study aimed to investigate the chemical constituents, and *in vitro* biological activities of *C. minor* collected from Kürecik-Malatya/Turkey. An LC-MS/MS analysis was conducted to determine the phytochemicals among 37 standards. Antioxidant, anticholinesterase, antityrosinase, antiurease, and antimicrobial activity assays were carried out on the extract obtained from aerial and root parts of the plant, also the cytotoxic potential of *C. minor* on COLO205 and KM12 colon, UO-31 and A498 renal, and high metastatic MG633, and low metastatic MG63 osteosarcoma cancer cell lines were detected by cell viability assay.

MATERIAL AND METHODS

Plant material

The aerial and root parts of *Cerinthe minor* were collected from Kürecik-Malatya, Turkey in April 2014. The authenticity of the plant was confirmed by Assoc. Prof. Dr. Yeter Yeşil Cantürk (Istanbul University, Faculty of Pharmacy, Pharmaceutical Botany Department). A voucher herbarium specimen was preserved in the Herbarium of the Faculty of Pharmacy of Istanbul University (ISTE number: 116048).

Preparation of plant extract

The aerial and root parts of the plant (40.82 g) were combined and macerated in ethanol (100 mL) for 24 hours at room tem-

perature (25 ± 2 °C) and the macerate was filtered out by using a Whatman no 1 filter paper. The same extraction process was triplicated. Then all filtrates were concentrated in a vacuum at 35 °C by using a rotaevaporator. The extract yields were weighted (1.52 g), afterwards, and the obtained extract was stored at -20 °C until the assessments. The stored filtrates were diluted with ethanol (250 mg/L) before LC-MS/MS analysis, then filtered out by a 0.2 µm microfiber filter. The yield percentages were calculated using the following formula: Extract yield % = R/S x 100 (where R; weight of dried plant and S; weight of plant raw sample).

Extraction yield (%) = 1.52 g / 40.82 g x 100 = %3.72

Chemicals and instruments

All compounds which were used for all assays were purchased from Merck (Germany), Sigma (Germany); and Fluka (Germany). All solvents used for the assays were of analytical grade.

Quantitative analysis of 37 compounds by LC-MS/MS

Quantitative analysis of 37 compounds (Table 1) was conducted by LC-MS/MS, using a previously developed and validated method (Yilmaz et al., 2018). The LC-MS/MS system was a Shimadzu brand, Nexera model UHPLC, which was coupled to a tandem MS instrument. To process the data obtained from LC-ESI-MS/MS, Shimadzu Lab Solutions software was used

Total phenolic and flavonoid contents of the extracts

The total phenolic content of *C. minor* ethanol extract was calculated as micrograms of pyrocatechol equivalents (PEs) by applying the method developed by Boğa et al. (2021b). The total flavonoid content of the extract was determined by applying the method designed by Moreno, Isla, Sampietro & Vattuone (2000). The results of this assay were expressed as micrograms of quercetin equivalents (QEs).

To calculate the total phenolic and flavonoid contents of the extract, the equations below were used:

Absorbance = 0.0409 pyrocatechol (µg) + 0.0495 (R^2 = 0.9975)

Absorbance = 0.0347 quercetin (µg) + 0.1174 (R^2 = 0.9992)

Antioxidant activity

Antioxidant activity of the ethanol extract of *C. minor* was investigated by using DPPH free radical scavenging, ABTS cation radical scavenging, and CUPRAC activity methods.

DPPH radical scavenging activity

The DPPH free radical scavenging activity of *C. minor* was determined by using a previously applied method (Ersoy, Özkan, Boğa & Mat, 2020). After the incubation period, the percentage inhibition of absorbance at 517 nm was calculated for each concentration relative to ethanol.

The following equation was used to calculate the DPPH free radical scavenging potential:

DPPH scavenging effect (Inhibition %) = ($A_{control}$ – A_{sample})/$A_{control}$ x 100

ABTS cation radical decolorization (scavenging) assay

The ABTS cation radical decolorization (scavenging) method previously modified by Boğa et al. (2016) was used to estimate the ABTS cation scavenging activity of the *C. minor* ethanol extract. After the incubation period, the absorbance was read at 734 nm using the microplate reader.

ABTS cation radical scavenging effect (Inhibition %) = (Acontrol – Asample)/Acontrol x 100

Cupric reducing antioxidant capacity (CUPRAC)

Cupric reducing antioxidant capacity (CUPRAC) activity of the *C. minor* ethanol extract was determined according to the method specified before by Ersoy et al. (2020). After the incubation period, the change in absorbance was measured at 450 nm by the microplate spectrophotometer. The results of the cupric reducing antioxidant capacity (CUPRAC) of the extract and standards were presented as concentration giving 0.5 absorbance values

Enzyme inhibition activity assays

Anticholinesterase inhibitory activity

To determine the anticholinesterase activity of the *C. minor* ethanol extract, a previously described method by Ersoy, et al. (2020) was applied. Following the incubation period, the absorbance of the mixture was measured at 412 nm. A well-known cholinesterase inhibitor, galantamine, was used as the positive control.

Tyrosinase inhibitory activity

Determination of the tyrosinase inhibition activity of the *C. minor* ethanol extract was performed according to the previously used method by (Ersoy, Özkan, Boğa, Yılmaz & Mat, 2019). The change in absorbance of the mixture was measured at 475 nm at 37 °C. For the positive control, kojic acid was used.

Urease inhibitory activity

The urease inhibitory activity of the *C. minor* ethanol extract was determined according to the protocol reported by Zahid et al. (2015). The change in absorbance of the mixture was measured at 630 nm, and thiourea was used for the positive control.

The following equation was used to calculate the enzyme inhibition %:

($A_{control}$ – A_{sample})/$A_{control}$ x 100

$A_{control}$ = Absorbance of the control

A_{sample} = Absorbance of the samples

Cell viability assays

Cytotoxicity assay on renal and colon cell lines

The cytotoxic potential of the *C. minor* ethanol extract was tested on human renal cancer cell lines UO-31 and A498, and human colon cancer cell lines COLO205 and KM12. All details of the XTT cytotoxic activity assay were performed according to a previous study conducted by (Eroğlu Özkan, Boğa, Yılmaz, Kara & Yeşil, 2020).

Metastatic potential assay

The metastatic potential of the *C. minor* ethanol extract on high (MG63.3) and low (MG63) metastatic potential human osteosarcoma cell lines was identified by using XTT assay, according to a previously applied method by (Eroğlu Özkan, Boğa, Yılmaz, Kara & Yeşil, 2020).

Antimicrobial activity

In vitro antimicrobial activity of *C. minor* ethanol extract was investigated by one of the most well-known antimicrobial susceptibility testing techniques, which is the microbroth dilution method. Ten different human pathogenic strains including *Staphylococcus aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Pseudomonasaeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153, *Candida albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, and *C. tropicalis* ATCC 750 were applied in this study. The microbroth dilution technique was applied according to the protocols described by the Clinical and Laboratory Standards Institute (1997, 2006, 2010). Serial two-fold dilutions ranging from 2500 to 1,2 µg/mL were prepared in Mueller Hinton Broth for the tested bacteria and RPMI-1640 medium for the yeast, respectively. The stock solutions of molecules also prepared in DMSO. The inoculum was prepared using a 4–6 h broth culture of each bacteria and 24 h culture of yeast strains adjusted to a turbidity equivalent of a 0.5 McFarland standard, diluted in broth media to give a final concentration of 5×10^5 CFU/mL for bacteria and 0.5×10^3 to 2.5×10^3 CFU/mL for yeast in the test tray. The MIC was defined as the lowest concentration of tested extracts giving complete inhibition of visible growth. Experiments were performed in triplicate. The following standard antibacterial and antifungal agents were used as standard compounds: Cefuroxime-sodium, cefuroxime, ceftazidime, amikacin, , and clotrimazole.

Statistical analysis

All measurements were performed in triplicate. The results were evaluated using a t-test with Microsoft Excel and expressed as mean ± standard deviation. Differences for all tests were considered significant at $p < 0.05$

RESULTS AND DISCUSSION

LC-MS/MS analysis

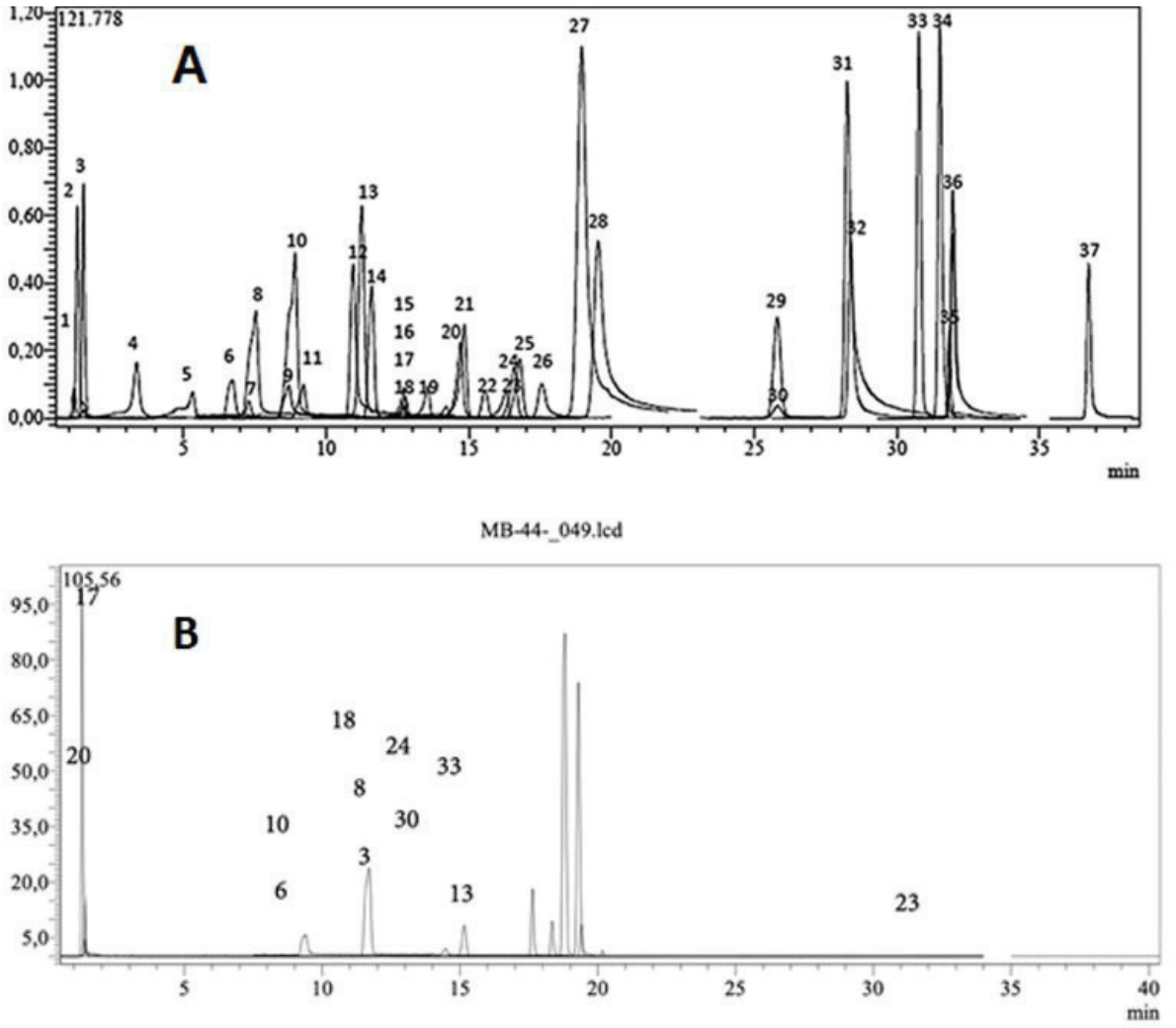
LC-MS/MS chromatograms of the standards (A) and the extract (B) were given in Figure 1. According to the results that are present in Table 1, 12 different constituents were present in the extract, and the major compounds were, malic acid (5392.18±60.93 µg/g extract), fumaric acid (4730.99±58.66 µg/g extract), rosmarinic acid (2470.07±176.12 µg/g extract), and 4-OH-Benzoic acid (1101.90±31.84 µg/g extract). The extract was also found to be rich in rutin (595.58±9.47 µg/g extract), and nicotiflorin (343.92±9.49 µg/g extract). Furthermore, *p*-coumaric acid (234.67±12.11 µg/g extract), caffeic acid (192.12±6.80 µg/g extract), isoquercitrin (193.07±2.57 µg/g extract), salicylic acid 21.37±0.70 µg/g extract), vanillin (2.23±0.06 µg/g extract) and naringenin (0.73±0.04µg/g extract) were detected in the extract.

Since this is the first report of the determination of the phenolic content of *C. minor* aerial parts and roots extracts to the authors' best knowledge, it is not possible to align all the research with the results of previous studies in comparison with the current one. However, there was a study carried out to investigate the secondary metabolites of the shoot and root extracts of the plant. Accordingly, allantoin, hydrocaffeic acid, and rosmarinic acid were found in the shoots extract, and allantoin and rosmarinic acid were found to be present in the roots extract of *C. minor* by high-performance capillary electrophoresis (Dresler, Szymczak, & Wójcik, 2017) Over and above that, there have been studies reporting the presence of pyrrolizidine alkaloids in *C. minor* extracts. Namely, 9-viridifloryl-retronecine monoester, 9-angeloyl-7-viridifloryl-retronecine, and 9-angeloyl-retronecine diester were identified in the leaves of *C. minor* (Mroczek, Baj, Chrobok, & Glowniak, 2004). Intermedine and lycopsamine are other pyrrolizidine alkaloids that were determined in *C. minor* extracts by further studies (El-Shazly & Wink, 2014). The cuticular wax of *C. minor* was also investigated by GC-mass spectrometry and GC-FTIR, whereas quantitation of individual compounds was revealed by GC-FID in a study performed by Jetter & Riederer (1999). A homologous series of 11 δ-lactones (1,5-alkanolides) was determined in cuticular waxes from the leaves of *C. minor*.

Malic acid, one of the major compounds determined in the studied extract, is a widely known organic acid that has many applications mainly in the food and beverage industry. It has been used as a taste enhancer and acidulant in many food products, also as a buffering and chelating agent in personal care formulations. Supplementarily, malic acid has further applications in the pharmaceutical industry. In particular, almotriptan malate is the active ingredient of a commonly used medication used for the treatment of migraines (Kövilein, Kubisch, Cai, & Ochsenreither, 2020). Besides, malic acid is effective against xerostomia by alleviating the dry mouth sensation of patients (Niklander et al., 2018). Last but not least, malic acid has been shown to possess protective effects on myocardial ischemia/reperfusion injuries, attributed to its anti-inflammatory and antiplatelet aggregation activities (Tang et al., 2013).

Fumaric acid was also found to be abundant in the *C. minor* extract. This finding can also be considered as worth mentioning since fumaric acid has important pharmacological activities. Speaking of which, fumaric acid esters have been licensed for the treatment of a chronic and progressive dermatological disease, psoriasis in Germany. Fumaric acid esters? Have been shown to be effective and safe for long-term clinical use as monotherapy or in combination (Atwan et al., 2015; Smith, 2017; Dickel, Bruckner & Altmeyer, 2018).

The studied *C. minor* extract was also shown to contain phenolic acids including rosmarinic and caffeic acids. Incidentally, there is an article pointing out the presence of rosmarinic acid in *Cerinthe* species. Although it does not specify which species that is, stating that the compound had been isolated from these plants, this could be interpreted as *Cerinthe* species are sources for rosmarinic acid (Khojasteh et al., 2020). A plethora of research has focused on the health benefits of phenolic acids, and accordingly, their properties such as antioxidant, antitumor, and anti-inflammatory activities are noteworthy (Al Jitan, Alkhoori, S, & Yousef, 2018).



3 – p-coumaric Acid, 6 – Caffeic Acid, 8- Salicylic Acid, 10- 4-OH-Benzoic Acid, 13- Rosmarinic Acid, 17- Fumaric Acid, 18- Vanillin, 20- Malic Acid, 23- Naringenin, 24- Rutin, 30- Isoquercitrin, 33- Nicotiflorin

Figure 1. LC-MS/MS chromatograms of the standards and the extract (A: standards, B: CM).

Determination of total phenolic and flavonoid contents

Total phenolic and flavonoid contents of the ethanol extract prepared from the whole plant aerial and root parts of *C. minor* were determined as pyrocatechol (PEs), and quercetin (QEs) equivalents, respectively. (y = 0.0409 pyrocatechol (mg) + 0.0495, R^2=0.9975 and y = 0.0347 quercetin (mg) + 0.1174 (R^2=0.9992).

The phenolic content of *C. minor* ethanol extract was calculated as 10.39±0.73 µg PEs/mg extract, and the flavonoid content of the extracts was calculated as 0.75±0.07 µg QEs/mg extract, which announces that the phenolic content of the extract is higher than its flavonoid content (Table 2). Considering the LC-MS/MS analysis results, it could be expected that the flavonoid content of the extract would not be high, therefore the results of the assays are consistent.

There has been only one study about the phenolic and flavonoid contents of subspecies, which is *C. minor* subsp. *auriculata*. Reportedly, the methanol extract of the plant was shown to contain 25.4 mg GAEs/g extract, the ethyl acetate extract contained 6.2 mg GAEs/g extract, and the aqueous extract of the plant had the highest amount of total phenolics with 46.6 GAEs/g extract. Regarding the total flavonoid contents of the studied three extracts, the ethyl acetate extract was the richest among them with 52.4 mg REs/g extract. The methanol extract was shown to contain 27.8 mg REs/g extract, and the aqueous extract had 24.6 mg REs/g extract total flavonoids. As it stands, total phenolics were expressed as gallic acid equivalents, and total flavonoids were expressed as rutin equivalents in the study. These findings along with the data provided by the current study suggest that *C. minor* is not a flavonoid-rich plant, or more likely the ethanol extract of the plant cannot be considered as a flavonoid-rich extract.

Determination of antioxidant activity

With the purpose of evaluating the antioxidant activity of plant extracts, it is crucial not to make conclusions based on only one single antioxidant method, considering that there are various oxidation aspects in the systems (Ersoy et al., 2020). Bearing this in mind, the antioxidant activity of the ethanol extract prepared from the aerial and root parts whole plant material of *C. minor* was conducted by using three comparative methods, which were DPPH free radical scavenging, ABTS cation radical

Table 1. LC-MS/MS results of *Cerinthe minor* extract.

	Analytes	RT[a]	Parent ion (m/z)	Daughter Ions	Ion. Mode	Quantification (µg analyte/g extract)[b]
						CM
1	Coumarin	17.40	147.05	91.0-103.2	Pos	N.D.
2	Hesperidin	12.67	610.90	303.1-465.1	Pos	N.D.
3	*p*-coumaric acid	11.53	162.95	119.25-93.25	Neg	234.67±12.11
4	*o*-coumaric acid	15.45	162.95	119.35-93.25	Neg	N.D.
5	Gallic acid	3.00	168.85	125.2-79.2	Neg	N.D.
6	Caffeic acid	8.80	178.95	135.2-134.3	Neg	192.12±6.80
7	Vanillic acid	8.57	166.90	152.25-108.25	Neg	N.D.
8	Salicylic acid	11.16	136.95	93.3-65.3	Neg	21.37±0.70
9	Quinic acid	1.13	190.95	85.3-93.3	Neg	N.D.
10	4-OH-Benzoic acid	7.39	136.95	93.3-65.3	Neg	1101.90±31.84
11	tr-Ferulic acid	12.62	192.95	178.3	Neg	N.D.
12	Chlorogenic acid	7.13	353.15	191.2	Neg	N.D.
13	Rosmarinic acid	14.54	359.00	161.2-197.2	Neg	2470.07±176.12
14	Protocatechuic acid	4.93	152.95	108.3	Neg	N.D.
15	Cinnamic acid	25.61	147.00	103.15-77.3	Neg	N.D.
16	Sinapinic acid	12.66	222.95	208.3-149.2	Neg	N.D.
17	Fumaric acid	1.48	115.00	71.4	Neg	4730.99±58.66
18	Vanillin	10.87	151.00	136.3-92.2	Neg	2.23±0.06
19	Pyrocatechol	6.48	109.00	108.35-91.25	Neg	N.D.
20	Malic acid	1.23	133.00	115.2-71.3	Neg	5392.18±60.93
21	Syringic acid	9.02	196.95	182.2-167.3	Neg	N.D.
22	Hesperetin	31.76	300.95	164.2-136.2	Neg	N.D.
23	Naringenin	30.68	270.95	151.2-119.3	Neg	0.73±0.04
24	Rutin	12.61	609.05	300.1-271.1	Neg	595.58±9.47
25	Quercetin	28.17	300.90	151.2-179.2	Neg	N.D.
26	Quercitrin	16.41	447.15	301.15-255.15	Neg	N.D.
27	Apigenin	31.43	268.95	117.3-151.2	Neg	N.D.
28	Chrysin	36.65	252.95	143.3-119.4	Neg	N.D.
29	Liquiritigenin	25.62	254.95	119.25-135.15	Neg	N.D.
30	Isoquercitrin	13.42	463.00	300.15-271.15	Neg	193.07±2.57
31	Cosmosiin	16.59	431.00	268.2-239.2	Neg	N.D.
32	Rhoifolin	16.11	577.05	269.2-211.15	Neg	N.D.
33	Nicotiflorin	14.68	593.05	285.1-255.2	Neg	343.92±9.49
34	Fisetin	19.30	284.95	135.2-121.25	Neg	N.D.
35	Luteolin	28.27	284.75	133.2-151.2	Neg	N.D.
36	Myricetin	18.72	317.00	179.15-151.25	Neg	N.D.
37	Kaempferol	31.88	284.75	255.1-117.3	Neg	N.D.

[a]RT: Retention time, [b]Values in µg/g (w/w) of plant extracts. N.D: not detected

scavenging, and CUPRAC activity methods. All samples were investigated by calculating the IC_{50} (for DPPH and ABTS cation radical scavenging assays) and $A_{0.5}$ (for CUPRAC activity assay) of antioxidant activity and comparing them by statistical analysis. The results are given in Table 2.

The extract showed moderate antioxidant activity according to the results of three conducted assays. The DPPH radical scavenging activity of the extract was calculated as IC_{50}: 155.17±0.41 µg/mL, whereas the ABTS cation radical scavenging activity of the extract, was found to be an IC_{50} value of 72.44±1.20 µg/mL. An $A_{0.5}$ value was calculated for the CUPRAC activity assay, which was 31.94±0.28 µg/mL.

Regarding the evaluation of the antioxidant capacity of medicinal plants, the typical approach has been based on analyzing correlations between antioxidant activity and the total phenolic content of an extract. It has been suggested that the extracts with higher total phenolic content are simply expected to be better antioxidants. However, this correlation is insufficient to reliably hand down a verdict, because the phenolic profiles, meaning the type of the phenolic compounds and also the relative amount of proportions of the phenolic compounds present in the extract may vary, and this affects the antioxidant activity directly (Jacobo-Velázquez & Cisneros-Zevallos, 2009). Not only that but also the extraction solvent along with the plant material, have been shown to have a huge influence on the antioxidant capacity. In a nutshell, there have been studies report-

Table 2. Total phenolic-flavonoid contents and antioxidant activities of *Cerinthe minor* extract*.

Samples	Phenolic content (μg PEs/mg extract)[a]	Flavonoid content (μg QEs/mg extract)[b]	DPPH Free Radical IC_{50} (μg/mL)	ABTS Cation Radical IC_{50} (μg/mL)	CUPRAC $A_{0.5}$ (μg/mL)
CM	10.39±0.73	0.75±0.07	155.17±0.41	72.44±1.20	31.94±0.28
BHA[c]	-	-	7.88±0.20	2.74±0.03	0.63±0.02
α-TOC[c]	-	-	16.30±0.79	10.20±0.05	13.38±0.07
BHT[c]	-	-	58.86±0.50	3.16±0.06	2.02±0.01

CM: *Cerinthe minor* extract; *Values expressed are means ± standard deviation of three parallel measurements ($p<0.05$); [a] PEs, pyrocatechol equivalents ($y = 0.0409x + 0.0495$, $R^2 = 0.9975$).; [b] QEs, quercetin equivalents ($y = 0.0347x + 0.1174$, $R^2 = 0.9992$); [c] Standard compounds

ing that extracts with higher total phenolic content ended up having lesser antioxidant effects than less phenolic-rich extracts (Terpinc, Čeh, Ulrih & Abramovič, 2012). *C. minor* may not be considered as a phenolic-rich, or more specifically a flavonoid-rich plant, according to the results of the current study. This may explain the comparatively moderate antioxidant activity results obtained from three antioxidant assays. Notwithstanding, since other parameters affect the total antioxidant capacity, further research on this issue is most definitely needed.

Enzyme inhibitory activities

The enzyme inhibitory potential of *C. minor* ethanol extract was evaluated by inhibitory activity on cholinesterase, tyrosinase, and urease enzymes. The results are given in Table 3.

Enzyme inhibitors decrease the bioactivity of the enzymes, which can play a vital role while correcting any metabolic imbalance, therefore there are many enzyme inhibitors available as medications on pharmacy shelves today (Boğa et al., 2021b). Apart from a very low antityrosinase activity (4.22±0.08% at 200 μg/mL concentration), *C. minor* ethanol extract did not exhibit any enzyme inhibitory activity according to the results of the current study.

The only study about the enzyme inhibitory activity of *C. minor* var. *auriculata* reported that only methanol and ethyl acetate extracts demonstrated low anticholinesterase and antityrosinase effects, whereas the water extract did not exhibit any enzyme inhibition activity towards these enzymes (Zengin et al., 2016). This situation indicates that the extraction solvent is very important in this context. Regarding antityrosinase activity, quinic acid and gallic acid are two molecules that have been proven to exert significant tyrosinase inhibitory effects. Together with this, few flavonoids containing 3′,4′-dihydroxyl substitution on ring B are known to inhibit the tyrosinase enzyme; (Şöhretoğlu, Sarı, Barut, & Özel, 2018; Ersoy et al., 2019). The absence of these compounds in the *C. minor* extract may illuminate the low antityrosinase activity. In connection therewith, quercetin, myricetin, myricitrin, and luteolin are among phenolic compounds with antiurease properties which were not present in the studied extract (Ersoy, et al., 2020). Expectedly, the extract did not exert any antiurease activity, either.

Cell viability assay

To investigate the cytotoxic properties of *C. minor* ethanol extract on COLO205 and KM12 colon, UO-31 and A498 renal, and high metastatic MG633, and low metastatic MG63 osteosarcoma cancer cell lines, a cell viability assay was carried out. The results a re presented in Table 4. Accordingly, the studied extract did not cause any significant changes in the growth of cancer lines.

Table 3. Acetylcholinesterase(AChE), butyrylcholinesterase (BChE), tyrosinase and urease enzymes inhibition (%) values of *Cerinthe minor* extract[a].

Samples	AChE	BChE	Tyrosinase	Urease
CM	NA	NA	4.22±0.08	NA
Galanthamine[b]	83.31±0.09	86.38±0.10	-	-
Kojic acid[b]	-	-	95.26±0.23	-
Thiourea[b]	-	-	-	88.61±1.16

CM: *Cerinthe minor* extract; [a] 200μg/mL; [b] Standard compounds; NA: Not Active

Table 4. Cytotoxic activity results of *Cerinthe minor* extract.

Extracts	2DAY-Renal (viability %)		2DAY-Colon (viability %)		META (viability %)	
	A498	UO31	COLO205	KM12	MG633	MG63
CM	93.74	>100	81.5	>100	99.17	93.81

CM: *Cerinthe minor* extract

Understanding the cytotoxic potential of *C. minor* can undoubtedly be considered as a crucial step in pharmacognostic research because the plant has been shown to contain pyrrolizidine alkaloids, as mentioned before (El-Shazly & Wink, 2014; Mroczek, Baj, Chrobok, & Glowniak, 2004). Plant-sourced pyrrolizidine alkaloids are known to be responsible for high hepatotoxic, genotoxic, cytotoxic, neurotoxic, and tumorigenic activities. Therefore , pyrrolizidine alkaloid-containing plants can be severely hazardous for humans and animals even through normal food intake, causing irreversible hepatic damage and cancer (Tamariz, Burgueño-Tapia, Vázquez, & Delgado, 2018). In the view of the foregoing, it was of utmost importance to investigate the cytotoxic potential of *C. minor*, since it has been consumed as food in several cities in Turkey. Our study has suggested that the plant can be comprehended as "safe" in terms of cytotoxicity on renal, colon, and osteosarcoma cell lines.

Antimicrobial activity

C. minor ethanol extract was screened for its antimicrobial activity against a standard panel of pathogenic bacteria and fungi, and the results are summarized in Table 5 (as MIC values). According to the obtained results, the extract was shown to possess low to moderate antimicrobial activity against *C. tropicalis* with a MIC value of 78.12 µg/mL, and low antimicrobial activity against *C. parapsilosis* with 312.5 µg/mL. This lack of high activity could be explained by the absence of an abundance of phenolic compounds in the extract, also the extract solvent could have affected the activity results.

Table 5. Antimicrobial activity results of *Cerinthe minor* extract.

	MIC Values (µg/mL)
Microorganisms	**CM**
Pseudomonas aeruginosa ATCC 27853	NA
Escherichia coli ATCC 25922	NA
Klebsiella pneumoniae ATCC 4352	NA
Proteus mirabilis ATCC 14153	NA
Staphylococcus aureus ATCC 29213	625
Staphylococcus epidermidis ATCC 12228	1250
Enterococcus faecalis ATCC 29212	625
Candida albicans ATCC 10231	NA
Candida parapsilosis ATCC 22019	312.5
Candida tropicalis ATCC 750	78.12

CM: *Cerinthe minor* extract; NA: No Activity.Standards; Cefuroxime-Na: 1.2 µg/ml for *S. aureus* ATCC 29213, Cefuroxime 9.8 µg/ml for *S. epidermidis* ATCC 12228, Amikacin 128 µg/ml for *E. faecalis* ATCC 29212, Ceftazidime 2.4 µg/ml for *P. aeruginosa* ATCC 27853, Cefuroxime-Na: 4.9 µg/ml for *E. coli* ATCC 25922 and *K. pneumoniae* 4352, Cefuroxime-Na 2.4 µg/ml for *P. mirabilis* ATCC 14153, Clotrimazole 4.9 µg/ml for *C. albicans* ATCC 10231, Clotrimazole 0.25 µg/ml for *C. parapsilosis* ATCC 22019, Clotrimazole 0.25 µg/ml for *C. tropicalis* ATCC 750 Amphotericin B 0.5 µg/ml for *C. parapsilosis* ATCC 22019, Amphotericin B 1 µg/ml for *C. tropicalis* ATCC 750. , Amphotericin B 0.5 µg/ml for *C. albicans* ATCC 10231

CONCLUSION

Cerinthe minor L. has a long history of being used in Turkey both for culinary and therapeutic purposes, traditionally. In spite of the fact that *C. minor* has been commonly consumed by people, the plant has remained uninvestigated pharmacognostically thus far. In order to evaluate the efficacy and safety of *C. minor*, it was crucial to identify the chemical constituents and to determine the pharmacologically important activities of the plant. In efforts to understand the properties of this plant in a better way, an LC-MS/MS analysis was performed, and the phytochemical profile of the plant was revealed by the current study. Antioxidant, anticholinesterase, antityrosinase, antiurease, and antimicrobial activity assays were carried out on the aerial and root parts whole plant ethanol extract, also the cytotoxic potential of *C. minor* on different cancer cell lines was detected by cell viability assay. In view of this, *C. minor* was reported to contain 11 different compounds, showed moderate antioxidant activity, whereas the extract did not demonstrate any enzyme inhibitory activities. More importantly, the extract was found to have no cytotoxic properties on the studied cancer cell lines, which was enlightening since the plant was shown to contain highly toxic pyrrolizidine alkaloids by previous studies. To conclude, *C. minor* was found to contain several biologically important compounds, a moderate antioxidant plant with no cytotoxicity on renal, colon or osteosarcoma cell lines. This study could be a major first step towards further research on *C. minor*, which is unquestionably required.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study-E.E.,M.B.,E.E.Ö.; Data Acquisition- E.E.,M.B.,E.E.Ö.,S.K.,E.Ç.,E.M.K.,Y.Y.C.; Data Analysis/Interpretation- E.E.,M.B.,E.E.Ö.,S.K.,E.Ç.,E.M.K.,Y.Y.C.; Drafting Manuscript- E.E.; Critical Revision of Manuscript- E.E.,M.B.,E.E.Ö.; Final Approval and Accountability- E.E.,M.B.,E.E.Ö.,S.K.,E.Ç.,E.M.K.,Y.Y.C.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors are thankful to the Research Fund of Dicle University (DUBAP: ECZACILIK.19.003) for the partial supports. LC-MS/MS analysis was performed by Dicle University Science and Technology Research and Application Center (DUBTAM), thanks to project number DUBAP: ECZACILIK.19.003 to pay the analysis fee.

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