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BIOLOGICAL ACTIVITY AND ALKALOID COMPOSITION OF *Chelidonium majus* L. GROWING AT THREE SITES IN THE EASTERN PART OF KUNGEY ALATAU RIDGE (KAZAKHSTAN)

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ABSTRACT

Chelidonium majus* L. (family Papaveraceae), widely known as greater celandine, is a valuable plant used in traditional medicine. The present study was aimed to compare the antioxidant and antimicrobial activities, as well as the alkaloid composition in tdifferent parts of *C. majus* collected from various locations in Kungei-Alatau region of Kazakhstan. The amount of total phenolic compounds and the antioxidant capability were determined by using Folin-Ciocalteu phenol reagent, radical (DPPH and ABTS) scavenging, and metal ion reducing (PFRAP) assays. Antimicrobial activity was evaluated by agar disc diffusion technique. The alkaloid composition was characterized by GC-MS. The root samples yielded the highest antioxidant and antimicrobial activity compared to the aerial parts. The root extracts yielded highest scavenging activity in DPPH (1745.37 ± 1.9 µmol TE mL^{-1}), ABTS (917.97 ± 10.43 µmol TE mL^{-1}), and PFRAP value (18.93 ± 0,14 mmol AAE mL^{-1}) as well as exhibited highest phenolic content (2617.62 ± 5.53 µg GAE mL^{-1}). The root extracts also showed largest inhibition zone (33.0 ± 0.82 mm) against *Bacillus subtilis*. A total of 26 alkaloids, including chelidonine, demethylchelerythrine, dihydro-chelerythrine, dihydrochelirubine, and dihydrosanguinarine, were identified. The results indicated that the roots of *C. majus* possess more biologically active component than its other plant parts.

Keywords: Alkaloids, antioxidant, antimicrobial activity, *Chelidonium majus*, GC-MS

INTRODUCTION

The natural compounds present in plants have since ages been used as a primary source of the compounds of pharmaceutical and medical interest. The antioxidant properties of medicinal plants have been exploited for developing innovative medications (Khatun *et al*., 2011). The secondary metabolites in plants have increasingly attracted the focus of researchers due to the vitality of structural configurations, and diversity in medicinal qualities (De Fátima *et al*., 2006). Therefore, there is a growing need to identify safe and effective natural products of therapeutic importance (Kavaz *et al*., 2023).

Kazakhstan is the home of around 6,000 plant species (Grudzinskaya *et al*., 2020) and the list of medicinal plant species in the country is constantly growing. Both conventional and alternative medicines utilize more than 150 plant species to treat a range of diseases. *Chelidonium majus* L.

commonly referred to as the greater celandine, is a member of Papaveraceae family and grows wild in Europe, North America, and Asia. *C. majus* is a perennial, branched, and sparsely hairy plant with its stem growing up to 1 m height. The plant contains alkaloids as its main active component that possess pharmaceutical properties (Colombo and Bosisio, 1996; Hiller *et al*., 1998). Currently, *C. majus* is recommended to treat numerous diseases including common dermatoses, viral infections and cancer (Gruenwald, 2004; Zuo *et al*., 2008).

The research on *C. majus* primarily focuses on exploiting the therapeutic qualities of its alkaloids (Kaminsky *et al*., 2006; Maji *et al*., 2015). The plant largely contains isoquinoline alkaloids (e.g., protopine, berberine, sanguinarine, coptisine, chelidonine and chelerythrine), phenolic acids and flavonoids (Colombo and Bosisio, 1996; Gilca *et al*., 2010). *C. majus* extracts and their purified components exhibit diverse biological actions, including analgesic, anti-inflammatory, antibacterial, choleretic, antitumoral, hepatoprotective and immunomodulatory properties, which align with their traditional usage. It is a key plant in contemporary phytotherapy and is applied orally and topically in a range of preparations (tinctures, gel, ointment, eye drops, liquid and dry extracts, herbal tea, etc.), either singly or in combination with other plants (Orvos *et al.*, 2015; Krizhanovska *et al*., 2021).

Many plants of family Papaveraceae exhibit significant antimicrobial properties against pathogens (Gerenčer *et al*., 2016) which is attributed to the presence of various alkaloids, like phthalide isoquinolines or tetrahydroprotoberberines, as well as various polyphenolic compounds (Cam *et al*., 2020; Zielińska *et al*., 2021). It is widely recognized that the cultivation conditions exhibit a substantial impact on the biologically active components of celandine (Jakovljevi´c *et al*., 2013; Zielinska *et al*., 2018). Hence, it is imperative to assess the environmental impact on the phytochemical characteristics of *C. majus* populations. The present study aimed to assess and compare the total phenolic contents, antioxidant potential and antimicrobial activity, and the alkaloid composition of aerial parts and roots of *C. majus* collected from three different locations in the Kungei Alatau region in Kazakhstan.

MATERIALS AND METHODS

Field study and plant material

The samples of *Chelidonium majus* were collected in June 2022 from three populations growing in the eastern part of the Kungei-Alatau Ridge. The first population was collected in the Kolsay territory [N1], the second in the Kayindy territory [N2], and the third in the Chet-Merki territory [N3], with the respective coordinates: N 42.5991 E 078.2007; N 42.5921 E 078.2791; and N0 43.0166 E 78.7442. The collected samples were air-dried at room temperature in shade and labelled after their authenticity was confirmed by Prof. N.M. Mukhitdinov from Al-Farabi Kazakh National University. The reference specimens were deposited in the Herbarium of the Botanical Garden in Astana under the numbers 6755, 6760, and 6753**,** respectively.

The three populations of *C. majus* growing in the eastern part of the Kungei-Alatau Ridge exhibited distinct soil characteristics. N1 (Kolsay) had thermoxeromorphic soils with high gravel content, a shallow humus horizon containing 1-2% humus, and an alkaline pH between 8 and 9. N2 (Kayindy) was characterized by mountain meadow alpine soils rich in organic matter (30-35% humus), with a dense root network and a moderately acidic pH level of 5-7. N3 (Chet-Merki) consisted of podzolized chernozems with a deep humus layer (17-18% humus) and a slightly acidic reaction (pH 6.4-7.0). The populations were located at distances ranging from 6.5 to 64.2 km from each other, covering various elevation gradients and microclimatic conditions within the eastern part of the Kungei-Alatau Ridge. The Kolsay and Kayindy populations are geographically close, with a distance of approximately 6.5 km between them, indicating potential ecological similarity. In contrast, the Chet-Merki population is significantly more distant, with 64.2 km separating it from Kolsay and 60.6 km from Kayindy, suggesting possible differences in ecological conditions and population isolation.

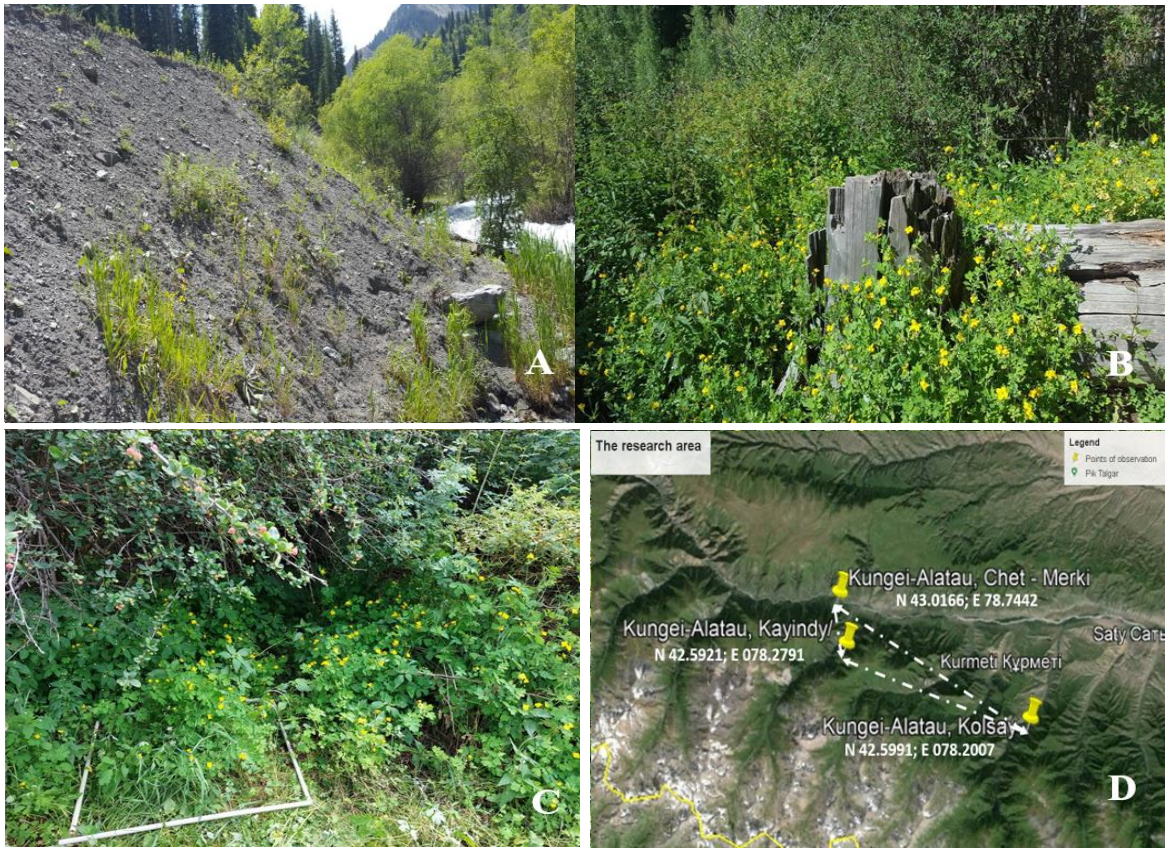

Fig. 1: The sites wherefrom *C. majus* samples were collected A) Kungei-Alatau, Kolsay, B) Kungei-Alatau, Kayindy, C) Kungei-Alatau, Chet-Merki and D) the satellite image obtained from Google Earth depicting the GPS coordinates of the study area

Chemicals and reagents

Methanol, petroleum ether, chloroform, acetonitrile, potassium persulfate, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2-azino-bis-3-ethylbenzothiozoline-6-sulfonic acid), Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), disodium hydrogen phosphate, potassium ferricyanide, trichloroacetic acid, sodium sulfate unhydrous were purchased from Sigma Aldrich; Folin-Ciocalteu's phenol reagent, sodium dihydrogen phosphate, ascorbic acid, ferric chloride and HCl were purchased from Merck, and sodium carbonate was purchased from Tekkim Lab. All the chemicals and reagents used in present study were of analytical grade.

Preparation of plant extracts

The roots and aerial parts of dried *C. majus* samples were divided and powdered by using a mortar and pestle. Then 2 g powdered material were mixed with 40 mL methanol and homogenized for 5 min at 8000 rpm using a homogenizer (HG-15D, Daihan Scientific). The mixture was sonicated for 30 min at 30°C in an ultrasonic bath (Sonorex, Bandelin), followed by centrifugation for 20 min at 4000 rpm and filtered through a filter paper (MN 615, Macherey Nagel). A rotary evaporator (HeiVap Value, Heidolph) was used to remove the solvent. Extract at a concentration of 200 mg mL^{-1} was prepared from the residue to assess the biological activities of the sample.

Total phenolic compounds

The total phenolic compounds were determined by using Folin-Ciocalteu reagent and methanolic extract. Gallic acid was used to develop a standard curve for calculating the total phenolic contents. The 1 mL Folin-Ciocalteu reagent was added to 100 µL extract and the mixture was agitated, followed by incubation at ambient temperature for 5 min. Then 1 mL of 7.5% Na_2CO_3 solution was added to

the mixture, and incubated for additional 90 min. The absorbance of sample was ascertained at 765 nm spectrophotometerically (Multiskan GO, Thermo). The quantity of total phenolic components was represented as µg gallic acid equivalents (GAE) mL^{-1} (Singleton *et al*., 1999).

DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed to measure the antioxidant potential of the plant extract. For this, a 0.1 mM DPPH solution was prepared in methanol and also a serial dilution of Trolox (10 µM-2 mM), and a serial dilution of extract (5 steps) were prepared as per Shimada *et al.* (1992). The 100 µL extract or Trolox solution, or methanol (control) was added to 2.9 mL DPPH solution, and the was mixture thoroughly agitated and allowed to settle at room temperature in dark for 15 min. The absorbance of the extract was measured at 517 nm spectrophotometrically (Shimada *et al.,* 1992). DPPH scavenging activity of extract was calculated using the formula:

$$SA\% = \frac{(ADPPH - ASample)}{ADPPH} \times 100$$

Wherein, SA% is the scavenging activity percentage of free radical scavenging activity, indicating the antioxidant capacity of the sample; ADPPH is absorbance of DPPH solution (control), and ASample is the absorbance of sample solution.

A standard curve was constructed according to the DPPH scavenging values of Trolox solutions, and the results expressed as µmol TE (Trolox equivalent) mL^{-1} extract.

ABTS assay

ABTS (2-azino-bis-3-ethylbenzothiozoline-6-sulfonic acid) is another stable radical that is frequently used to gauge how successfully anti-oxidative materials resist radicals. The 7.5 mM ABTS solution we combined with 2.45 mM potassium persulfate solution and incubated at ambient temperature in the dark for 12-16 h. $ABTS^{+}$ working solution was diluted to an absorbance of 0.700 ± 0.02 at 734 nm. Then 200 µL $ABTS^{+}$ working solution was mixed with both 10 µL extract and Trolox solutions in 96-well plates. The mixture was thoroughly shaken and allowed to settle for 6 min in dark (Cam *et al*., 2020). The absorbance of plate was measured at 734 nm and ABTS inhibition percentage of sample was calculated using the following formula:

$$ABTS\ Inhibition\ \% = \frac{(AABTS - ASample)}{AABTS} \times 100$$

Wherein ABTS Inhibition % represents the percentage of inhibition of ABTS radicals indicating the antioxidant activity of the sample; AABTS is the absorbance of ABTS solution without sample (control); and ASample is the absorbance of ABTS solution with sample.

A standard curve was constructed using % inhibition values of various concentrations of Trolox solution, and the results expressed as µmol TE mL^{-1} extract.

PFRAP assay

The rise in the absorbance of ferric ferrocyanide, a blue-colored complex with a maximum absorbance at 700 nm, is associated with the antioxidant activity of a sample (Xiao *et al*., 2020). In a 96-well plate, 30 µL of 0.2 M PBS (pH 6.6), 10 µL of varying concentration of extract or ascorbic acid solution as a standard, and 30 µL of 1% potassium ferricyanide solution were combined and incubated at 50°C for 20 min. Then, 30 µL of 10% trichloroacetic acid (TCA) solution, 20 µL ferric chloride and 100 µL distilled water were added to the wells. The absorbance of the plate was measured spectro-photometrically at 700 nm (Mokrani *et al*., 2016), compared with the standard curve prepared from ascorbic acid, and the results were expressed as mmol AAE mL^{-1} extract.

Antimicrobial activity

The antibacterial activity of *C. majus* extracts against various microorganisms was evaluated using the disk diffusion method (Taşkaya *et al.,* 2023). *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* SL1344, *Pseudomonas aeruginosa* DSMZ 50071, and

Bacillus subtilis DSMZ 1971, obtained from the Biotechnology Department Culture Collection of Niğde Ömer Halisdemir University, were used as test microorganisms. The bacterial strains (100 µL) were inoculated on Luria-Bertani (LB) broth medium, then 20 µL aliquots of *C. majus* extract at variable concentrations (200, 100, and 50 mg mL^{-1}) were transferred on sterile paper disks of 6 mm dia and incubated at 37°C for 24 h. The turbidity of microbial culture was adjusted to 0.5 McFarland standard. Following incubation, the widths or area of inhibition zones surrounding the paper disks were measured. Antibiotic discs with 30 µg vancomycin and 10 µg gentamicin were used as positive controls, while blank discs and solvents (ethanol or methanol,) were used as negative controls.

Extraction and characterization of total alkaloids

The total alkaloids from *C. majus* were extracted as per the method of Belyagoubi-Benhammou *et al.* (2019), with slight modifications. In brief, 13.5 mL petroleum ether was combined with 6.5 g powdered root or aerial parts of *C. majus*, and the mixture was incubated for 24 h at room temperature. After incubation, the mixture was centrifuged for 5 min at 4000 rpm, the supernatant discarded, and the precipitate dried at 37°C. The precipitate was then mixed with 25 mL methanol, homogenized for 3 min at 8000 rpm, and incubated for 30 min at 30°C in an ultrasonic bath. The homogenate was centrifuged for 10 min at 4000 rpm. The supernatant was filtered through a filter paper, and the filtrate evaporated on a rotary evaporator until it was completely dry at 40°C. The residue was then dissolved in 15 mL chloroform and acidified to pH 3 by adding a 5% HCl solution. The acidic layer was separated and extracted by adding 15 mL chloroform. This solution was then basified to pH 9 by adding 5% Na_2CO_3 solution. The chloroform layer was separated, evaporated to dryness, and recovered with 3 mL acetonitrile for GC-MS analysis. All extracts were dried by anhydrous Na_2SO_4 and filtered through 0,45 µm nylon filters (Macharey Nagel) before being injected to GC.

A Shimadzu QP2010 Ultra GC-MS equipment with a Restek Rxi-5MS column (30 m x 0.25 mm ID x 0.25 µm df) was used to characterize the total alkaloids of *C. majus* extracts. The operational conditions maintained were: 250°C temperature at the injection port; the AOC2-0i auto-sampler operating in split-less mode was used to inject 2 µL extract; a flow rate of 1.0 mL helium min^{-1} was employed as the carrier gas; following 2 min at 70°C, the oven temperature was raised to 200°C @ 25°C min^{-1} and maintained for 5 min, the temperature was then raised to 300°C @ 3°C min^{-1}. The interface and ion source temperatures were 250 and 230°C, respectively. All spectra were recorded in the electron impact (EI) mode. In full scan mode, the mass varied between 100 and 1000 m/z. The alkaloids were characterized by comparing the mass spectra of alkaloids derived from *C. majus* with those from the Flavor and Fragrance Natural and Synthetic Compounds Library (FFNSC 1.2) and the Wiley mass spectra library (W9N11).

Statistical analyses

All the above experiments were conducted in a completely randomized design with each treatment triplicated three times. One-way analysis of variance (ANOVA) and Tukey's post-hoc test were used to compare the means between groups at a significance level of $p<0.05$. The IBM Statistical Package for the Social Sciences (SPSS) program (Version 24.0) was used to evaluate the data.

RESULTS AND DISCUSSION

Antioxidant activities

PFRAP analysis showed that N1 root sample had maximum antioxidant capacity (18.93 ± 0.14 m mol AAE mL^{-1}). Root samples from N2 (18.39 ± 0.04 µmol TE g^{-1}) and N3 (18.03 ± 0.13a µmol TE g^{-1}) showed no significant difference ($p < 0.05$) and the activities were lower than N1 (Table 1). The antioxidant activity of *C. majus* is likely influenced by the extraction method and the specific plant section used. According to an analysis of the state of knowledge now available for determining the

anti-oxidative potential of *C. majus* extracts, the DPPH method is most frequently employed to evaluate antioxidative capabilities (Hădărugă *et al.,* 2009; Stef *et al.,* 2009; Maji *et al.,* 2015); while the FRAP approach is used less frequently (Then *et al.,* 2003; Ożarowski *et al.,* 2016). The plant's phenological stage and the analysis method impact the antioxidant activity. Studies conducted with *C. majus* samples suggest that strong antioxidant properties of plant extracts do not depend on their alkaloid and transition metal contents (Khodabande *et al*., 2017; Prabhudev *et al.,* 2023).

Alterations in soil pH affect antioxidant activity. Soil pH directly affects phytochemical content, and several methods can be used to measure the antioxidant capacity of plant products. A single method is usually insufficient to determine antioxidant activity. Reports suggest that an increase or decrease in soil pH may cause significant changes in total phenolic contents of plants and in their antioxidant capacity determined by DPPH, ABTS, and FRAP tests (Prabhudev *et al.,* 2023). Rahmonov *et al.* (2023) reported that soil pH affected the synthesis of antioxidant metabolites in *C. majus* growing in urban environments. This suggests that the soil pH where *C. majus* grows plays a decisive role in plant's antioxidant activity estimated by DPPH, FRAP, and ABTS methods.

Table 1: Total phenolic compounds and antioxidant activities of different parts of *C. majus* collected from three different locations in Kazakhstan

Location	TPC (µg GAE mL^{-1})	DPPH (µmol TE mL^{-1})	ABTS (µmol TE mL^{-1})	PFRAP (mmol AAE mL^{-1})
	Roots			
Kolsay (N1)	1075.95 ± 133.28^{a}	1735.85 ± 14.28^{b}	917.97 ± 18.06^{b}	18.93 ± 0.24^{b}
Kayindy (N2)	2617.62 ± 9.59^{c}	1711.11 ± 5.94^{a}	618.19 ± 48.39^{a}	18.39 ± 0.08^{a}
Chet-Merki (N3)	1589.56 ± 66.16^{b}	1745.37 ± 3.29^{b}	670.69 ± 42.53^{a}	18.03 ± 0.23^{a}
	Aerial parts			
Kolsay (N1)	833.73 ± 110.69^{a}	1734.9 ± 30.80^{b}	781.21 ± 25.21^{c}	4.56 ± 025^{b}
Kayindy (N2)	1420.12 ± 134.58^{b}	1689.21 ± 46.43^{a}	456.56 ± 23.57^{b}	6.02 ± 1.04^{b}
Chet-Merki (N3)	1040.12 ± 22.08^{a}	1664.46 ± 48.72^{a}	274.21 ± 30.55^{a}	1.36 ± 0.31^{a}

*TPC: Total phenolic compounds, DPPH: DPPH scavenging activity, ABTS: ABTS scavenging activity, PFRAP: Potassium ferri-cyanide reducing antioxidant power;

**The data in the table are expressed as means ± standard deviations of three independent measurements;

***Lower case letters in the same column indicate a significant difference between test groups at $p > 0.05$.

The total phenolic and antioxidant activities of the underground (root) and aboveground parts of *C. majus* are presented in Table 1. Antioxidant activity and total phenolic compounds were generally higher in root samples than in aerial samples. Of the three locations, the highest total phenol content of *C. majus* (using gallic acid as standard) was observed in N2 root sample (2617.62 ± 5.53 µg GAE mL^{-1}). Wojdylo *et al.* (2007) have reported that *C. majus* contain 2.09 mg GAE 100 g^{-1} dry weight. Flavonoids and phenolic compounds have previously been reported as the main constituents of *C. majus* extracts (Khodabande *et al*., 2017).

Phenolic compounds play a crucial role in plant interactions with various biotic and abiotic stresses and are known have antioxidant properties (Kulbat, 2016; Kumar *et al*., 2020; Nile *et al*., 2021). The concentrations of secondary metabolites such as phenolics in different parts of *C. majus* plant are influenced by the specific phenological stages of plant (Jakovljeví´c *et al*., 2013). Phenolic compounds present in plants accumulate at different rates, depending on the environmental conditions like temperature, humidity, and rainfall (Papageorgiou *et al.,* 2008).

DPPH assay is a fast and accurate method for assessing antioxidant activity. Spectrophotometric measurement of the rate of DPPH scavenging can be used to determine the antioxidant activity profile of the samples. In present study maximum DPPH radical scavenging activity was noticed in N3 root sample (1745.37 ± 1.9 µmol TE mL^{-1} extract). However, this value was not statistically significant as compared to the value obtained for N1 sample. The results revealed that *C. majus* extracts have a high antioxidant power which might provide protection to the plant against oxidative stress. Further, the

potent antioxidant activity of *C. majus* extract does not seem dependent on the alkaloid or transition metal content (Jakovljevi´c *et al*., 2013; Ikeuchi *et al*., 2013).

ABTS radical scavenging is another common antioxidant assay for plant extracts. It has been used for the plant extracts containing both lipophilic and hydrophilic substances (Saravanan *et al*., 2014). Using ABTS method, the antioxidant capacity of extracts was found to be lowest in the aerial parts of sample N3 (618.19 ± 27.94 μmol TE mL^{-1}) and highest in the root sample of N1 (917.97 ± 10.43 μmol TE mL^{-1}). The root extracts showed higher ABTS radical scavenging activity than aerial part extracts (Table 2).

The changes in soil pH affect the quality and quantity of phytochemicals (Ikeuchi *et al.*, 2013). The changes observed in the antioxidant activity in present study may also be due to pH changes in the soil structure in N1, N2, and N3 regions. It has been reported that FRAP and DPPH activities reach maximum levels in soybean grown at pH 6.5-7.5, while the activity of antioxidant enzymes is suppressed in extremely acidic or alkaline conditions (Liu *et al.*, 2021). However, some plants such as spinach may exhibit higher DPPH activity in slightly acidic soils compared to neutral pH, which is associated with plant species-specific metabolic adaptations (Chen *et al*., 2023).

Antimicrobial activity

The methanol extracts of roots and aerial parts of *C. majus* were assessed at various concentrations by disc diffusion method and inhibition zones for their antimicrobial activity against *E. coli, S. aureus, P. auroginosa, B. subtilis, S. typhimurium, C. michiganensis* and *C. albicans* (Tables 2 and 3).

Table 2: Antimicrobial activities (mm) of root extracts of *C. majus* collected from three different locations

Micro-organisms	Extract concentration (mg mL^{-1})								
	Kolsay (N1)			Kayindy (N2)			Chet-Merki (N3)		
	50	100	200	50	100	200	50	100	200
E. coli	nd	nd	16.67 ± 0.94	nd	nd	nd	nd	nd	nd
S. aureus	16.67 ± 0.94	19.67 ± 0.47	25.34 ± 0.47	17.67 ± 0.94	19.67 ± 0.47	22.67 ± 0.47	16.67 ± 0.47	19.67 ± 0.47	21.67 ± 1.25
P. aeruginosa	nd	nd	nd	nd	nd	nd	nd	nd	nd
S. typhimurium	nd	nd	nd	10.34 ± 0.47	13.00 ± 0.82	14.34 ± 0.47	nd	nd	nd
B. subtilis	24.34 ± 0.47	28.67 ± 0.94	33.0 ± 0.82	20.34 ± 0.47	25.34 ± 0.47	30.67 ± 0.47	24.67 ± 0.47	27.0 ± 0.82	29.34 ± 0.94
C. michiganensis	nd	nd	nd	nd	nd	nd	nd	nd	nd
C. albicans	13.67 ± 0.94	15.67 ± 0.47	18.34 ± 0.94	14.67 ± 0.47	16.67 ± 0.47	17.67 ± 0.47	13.67 ± 0.47	15.00 ± 0.0	16.0 ± 0.0

*nd: not detected; The data present the inhibition zone diameters (mm) as means ± standard deviations from three independent experiments

The highest antimicrobial activity was observed against *B. subtilis* with 33.0 ± 0.82 mm inhibition zone in N1 root extract used @ 200 mg mL^{-1} concentration, while the lowest activity was observed against *S. typhimurium* with 10.34 ± 0.47 mm inhibition zone by N2 root extract @ 50 mg mL^{-1} concentration. The root extracts of *C. majus* were effective at all concentrations against *S. aureus*, *B. subtili*s, and *C. albicans* with varying inhibition zone (Table 2). Only N1 root sample showed an inhibitory effect against *E. coli* with a 16.67 ± 0.94 mm inhibition zone at a concentration of 200 mg mL^{-1}. All the three test concentrations (50, 100, and 200 mg mL^{-1}) of N2 root sample were effective against *S. typhimurium*.

The highest antimicrobial activity of aerial part extracts was obtained against *B. subtilis* with a 24.67 ± 0.47 mm inhibition zone by the N3 sample at 200 mg mL^{-1} concentration, while the lowest activity was observed against *P. aeruginosa* with 7.34 ± 0.94 mm inhibition zone by the N2 root extract at 50 mg mL^{-1} concentration. The extracts obtained from aerial parts of *C. majus* caused inhibition zones against *S. aureus* and *B. subtilis* at all the test concentrations. Similar to root extracts, the N2

Table 3: Antimicrobial activities (mm) of the extracts from the aerial parts of *C. majus* collected from three different sites in the Eastern Kungei-Alatau Ridge in Kazakhstan

Micro-organisms	Extract concentration (mg mL^{-1})								
	Kolsay (N1)			Kayindy (N2)			Chet-Merki (N3)		
	50	100	200	50	100	200	50	100	200
E. coli	nd	nd	nd	nd	nd	nd	nd	nd	nd
S. aureus	11.7 ± 0.5	14.0 ± 0	16.3 ± 0.5	12.3 ± 0.5	14.3 ± 0.5	18.0 ± 0.0	13.7 ± 0.5	15.3 ± 0.9	17.0 ± 0.0
P. aeruginosa	nd	nd	nd	7.3 ± 0.9	9.3 ± 0.9	11.0 ± 0.8	nd	nd	nd
S. typhimurium	nd	nd	nd	nd	nd	nd	nd	nd	nd
B. subtilis	15.3 ± 0.5	17.7 ± 0.5	21.0 ± 0.0	14.7 ± 0.9	18.0 ± 0	20.3 ± 0.5	18.7 ± 0.9	21.3 ± 1.7	24.7 ± 0.5
C. michiganensis	nd	nd	nd	nd	nd	nd	nd	nd	nd
C. albicans	nd	nd	nd	nd	nd	nd	10.7 ± 0.5	12.3 ± 0.47	14.3 ± 1.3

*nd: not detected; **The data presents the inhibition zone diameters (mm) as means ± standard deviations (SD) of three independent experiments

aerial part samples formed an inhibition zone against *P. aeruginosa* at all tested concentrations. All the three concentrations (50, 100, and 200 mg mL^{-1}) of N3 sample were effective against *C. albicans* (Table 3). Methicillin-resistant *Staphylococcus* and multidrug-resistant *Enterococcus* strains were effectively inhibited by a glycoprotein isolated from *C. majus* (Fik *et al*., 1997). The antibacterial effect of *C. majus* is primarily due to the quaternary ammonium groups of isoquinoline alkaloids. *C. majus* milky juice has been used in folk medicine and homeopathy to treat viral warts and bacterial infections (Zielińska *et al.,* 2018). Some plant species in Papaveraceae family exhibit antibacterial effects. A recent study indicated that the most effective extracts against *S. aureus* and *Candida* spp. were obtained from three species of *Corydalis*, a member of Papaveraceae family (Zielińska *et al.,* 2021; Stefanowski *et al.,* 2021). The extracts from both aerial parts and roots exhibited high antibacterial activity.

Kokoška *et al*. (2002) examined 33 ethanolic extracts derived from 16 distinct plant species and found ethanolic aerial part extracts of *C. majus* were found ineffective against test bacteria. Numerous biological activities, including anti-inflammatory, antibacterial, immunomodulatory, and anticancer properties, was demonstrated by the crude extracts of *C. majus*. Alkaloids, including 8-hydroxyl, were effective against drug-resistant clinical yeast isolates and clinical isolates of methicillin-resistant *S. aureus* (MRSA) (Zuo *et al.,* 2011; Lee *et al.,* 2019).

Characterization of total alkaloids

GC-MS analyses of the roots and aerial parts of *C. majus* samples collected from three different sites (N1, N2, and N3) revealed the presence of several important alkaloids (Table 4). The compounds detected at their highest levels in roots and aboveground tissues were protopin, dihydrosanguinarin, and dihydrochelerythrin. Interestingly, the concentrations of these three alkaloids were significantly higher in root tissues than in the aboveground tissues. It was noteworthy that the above-ground samples from N2 site contained lower amounts of these compounds than the other sites.

Among the identified alkaloids, the analysis revealed clear tissue-specific distribution patterns. Only root extracts contained dihydrochelirubin, a substance typically found in root systems. In contrast, only aerial plant parts exhibited the accumulation of demethylchelerythrine, a derivative of benzophenanthridine. Examination of protoberberine class alkaloids showed a different distribution. canadine was limited to aerial tissue, whereas stylopine was found in both root and shoot systems. The plant extracts contained trace levels of several minor alkaloids such as tyramine, pseudolaudanin, and noroxyhydrastinin (Table 4).

The species belonging to the genus *Chelidonium* possess therapeutically significant alkaloids in their plant parts, particularly in latex. *Chelidonium* contains approximately 40 alkaloids including phenanthridine, protoberberine, protopine, quinolizidine, and aporphine (Golshan *et al*., 2021). Alkaloids have been extracted and identified in earlier studies (Kedzia *et al*., 2013; Hao *et al*., 2015; Stefanowski *et al.,* 2021). Isoquinoline alkaloids belong to this group and are biologically significant

Table 4: Alkaloid composition of *C. majus* extracts of root and aerial parts determined by GC-MS

S. No.	Chemical formula	Name		Roots						Aerial parts					
				Kolsay (N1)		Kayindy (N2)		Chet-Merki (N3)		Kolsay (N1)		Kayindy (N2)		Chet-Merki (N3)	
			MW	RT	RC	RT	RC	RT	RC	RT	RC	RT	RC	RT	RC
1.	$C_8H_{11}N$	Phenethylamine	121	5.86	1.07	5.87	0.88	-	-	-	-	-	-	-	-
2.	$C_{17}H_{18}N_2$	Amphetaminil	250	-	-	5.97	0.27	6.48	0.48	5.97	1.92	6.47	0.31	-	-
3.	$C_7H_{15}NO$	N-Ethyl-3-hydroxypiperidine	129	-	-	-	-	-	-	-	-	-	-	6.92	0.41
4.	$C_8H_{11}NO$	Tyramine	137	-	-	8.21	1.11	-	-	-	-	-	-	-	-
5.	$C_9H_{13}NO_2$	2-Hydroxy-4-methoxyphenethylamine	167	-	-	9.01	0.96	-	-	-	-	-	-	-	-
6.	$C_{13}H_{19}N$	N-(Isopentyl) phenethylamine	189	9.37	0.5	-	-	-	-	9.37	0.42	9.37	0.65	-	-
7.	$C_{11}H_{13}NO$	2,3a,4,5-Tetrahydro-1H-oxazolo[3,2-a]quinoline	175	-	-	-	-	-	-	10.13	0.33	10.13	0.42	-	-
8.	$C_{11}H_{15}NO_2$	N-Methyl-6-hydroxy-8-methoxy-1,2,3,4-tetrahydroisoquinoline	193	11.01	0.3	11.03	1.12	-	-	11.01	0.41	-	-	-	-
9.	$C_{11}H_{15}NO_2$	Salsoline	193	-	-	11.16	0.26	-	-	-	-	-	-	-	-
10.	$C_{24}H_{30}O_3$	Cannabinol PROP	366	11.39	0.62	11.39	0.81	11.39	1.09	11.39	1.25	11.39	2.5	11.39	1.28
11.	$C_{18}H_{21}NO_4$	Norreticuline	315	-	-	11.61	0.69	-	-	-	-	-	-	-	-
12.	$C_{13}H_{17}NO_4$	Isoquinoline-6-ol-1-carboxylic acid, 1,2,3,4-tetrahydro-7-methoxy-	251	11.68	5.69	11.68	3.54	11.68	2.88	11.68	0.78	11.67	2.35	11.68	0.39
13.	$C_{12}H_{15}NO_2$	Isoquinoline, 3,4-dihydro-6,7-dimethoxy-1-methyl-	191	12.07	0.32	11.99	0.28	-	-	-	-	-	-	-	-
14.	$C_{11}H_{13}NO_2$	3,4-Dihydro isoquinolin-7-ol, 6-methoxy-1-methyl-	191	12.09	0.82	12.08	1.48	12.09	0.84	-	-	12.08	1.62	-	-
15.	$C_{10}H_9NO_3$	Noroxyhydrastinine	191	17.07	0.53	-	-	-	-	17.06	5.69	17.05	2.21	17.06	1.83
16.	$C_{20}H_{25}NO_4$	Pseudolaudanine	343	36.08	0.68	36.08	0.76	-	-	36.08	1.37	-	-	-	-
17.	$C_{19}H_{17}NO_4$	Stylopine	323	-	-	40.69	0.31	-	-	40.69	2.23	40.70	3.32	40.70	1.61
18.	$C_{20}H_{21}NO_4$	Canadine	339	-	-	-	-	-	-	--	-	-	-	40.89	0.3
19.	$C_{20}H_{19}NO_5$	Protopine	353	41.45	12.89	41.49	28.41	41.44	17.13	41.44	25.97	41.42	9.94	41.45	28.9
20.	$C_{20}H_{15}NO_4$	Dihydrosanguinarine	333	44.67	26.56	44.67	24.02	44.62	20,76	44.59	28.05	44.57	6.11	44.59	17.03
21.	$C_{21}H_{19}NO_4$	Dihydrochelerythrine	349	45.18	13.31	45.19	14.82	45.16	102	45.14	7.6	45.14	2.14	45.14	2.79
22.	$C_{21}H_{21}NO_5$	(+-)-Corynoline	367	-	-	-	-	-	-	-	-	-	-	45.44	2.51
23.	$C_{20}H_{19}NO_5$	(.+-.)Chelidonine	353	45.55	2.61	45.52	1.93	45.46	1.16	45.46	2.76	-	-	-	-
24.	$C_{21}H_{17}NO_5$	Dihydrochelirubine	363	48.28	1.15	48.29	0.91	-	-	-	-	-	-	-	-
25.	$C_{19}H_{11}NO_4$	Norsanguinarine	317	49.95	2.31	49.95	1.52	-	-	-	-	-	-	-	-
26.	$C_{20}H_{15}NO_4$	Demethylchelerythrine	333	50.62	2.86	50.63	2.19	50.63	1.78	-	-	-	-	-	-

*MW: Molecular weight; RT: Retention time (min); RC.: Relative concentration (%)

significant (Grosso *et al*., 2014). *C. majus* is used to treat various liver disorders (Weiskirchen, 2016). The hepatoprotective and choleretic effects are attributed to hydroxycinnamic esters and dihydrochelerythrine (Zielinska *et al.,* 2018).

A significant class of small compounds containing nitrogen and benzophenanthridine alkaloids is categorized as isoquinoline alkaloids that are distinguished by a tetracyclic structural pattern (Bisai *et al.,* 2019). Dihydrosanguinarine, dihydrochelerythrine, chelidonine, and dihydrochelirubine are dihydrobenzophenanthridine alkaloids. Based on the degree of unsaturated skeleton, benzophenanthridine alkaloids isolated and identified from *C. majus* are further categorized into four structural types: dihydrobenzophenanthridine, hexahydrobenzophenanthridine, dihydrobenzophenanthridine, and benzophenanthrine quaternary amine (Bisai *et al*., 2019; Wei *et al*., 2020; Laines-Hidalgo *et al.,* 2022). Dihydrosanguinarine and dihydrochelerythrine were detected in both roots and

aerial plant parts collected from all three locations, whereas dihydrochelirubin was detected only in roots. This is consistent with the findings of Deng *et al.* (2017) and Li *et al*. (2024). Demethylchelerythrine, which belongs to the benzophenanthrine quaternary amine alkaloid group, was detected in aerial parts (Table 4). Protoberberine alkaloids are extensively distributed and constitute one of the largest classes of isoquinoline alkaloids. They are produced in plants via a sequence of intricate enzymatic processes that utilize tyrosine as a substrate (Liu *et al.,* 2023). This alkaloid consists of two fused isoquinoline rings, predominantly in hydrochloride form. In this study, stylopine and canadine were determined to be protoberberine alkaloids. Stylopine is reportedly present in whole plants, and only canadine in the aerial parts (Sárközi *et al.,* 2006; Bozhadze *et al.,* 2013).

Plant age, developmental stage, harvesting period, genotype and environmental conditions are some of the factors that influence the concentration of alkaloids in raw plant materials (Krizhanovska *et al.,* 2021). The production of related metabolites can substantially be inhibited by the detrimental consequences of perturbing the antioxidant-pro-oxidant equilibrium in plants (Jakovljevi´c *et al.*, 2013).

Studies showed that in *Chelidonium* phenolic compounds and alkaloids, specifically chelidonine, berberine, and protopine, exhibited choleretic activity and stimulated bile acid flow. The hepato-protective activity of this compound may be attributed to this mechanism (Golshan *et al.,* 2021). However, the primary issue with *Chelidonium* is the potential hepatotoxicity of plant due to the presence of alkaloids (Maji *et al.,* 2015; Stefanowski *et al.,* 2021). The toxicity of dried components of *Chelidonium* depends on its dosage. The European Medicines Agency has reported low toxicity when used at low doses. However, there is a risk of serious irreversible liver damage when used at high doses or over a long period. Additional research is required to ascertain the harmful consequences of regular drug administration and usage (EMA, 2011; Golshan *et al.,* 2021).

Conclusions The study revealed that a variety of parameters including plant developmental stage at sampling time, soil structure, soil pH, humidity, environmental conditions, geographical position, etc. influence the production and storage of distinct chemicals in distinct plant parts of *C. majus* collected from three locations in Kungei-Alatau region of Kazakhstan. *C. majus* possessed several alkaloids which have hepatoprotective and choleretic activity. Further, the roots exhibited higher antioxidant and antimicrobial activity, and contained higher levels of alkaloids and phenolic compounds than the aerial parts. Considering the effect of sampling location and environmental conditions on the production and storage of valuable secondary metabolites with antioxidant and antimicrobial activities, the studies on finding and characterizing more novel natural reagents should be promoted.

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