Global NEST Journal, Vol 26, No X, pp 1-10

Phytochemical profile by LC-MS/MS, total phenolic content and antioxidant properties of *erodium guttatum* from algeria

Ismahan O.[1*], Souheila S.[1*], Jihane B.[1], Bensouici C.[2], Badis A.[1] and Bouacha A.[1]

[1]Research Laboratory of Interaction, Biodiversity, Ecosystems and Biotechnology, Faculty of Sciences, University 20 August 1955 Skikda, Algeria

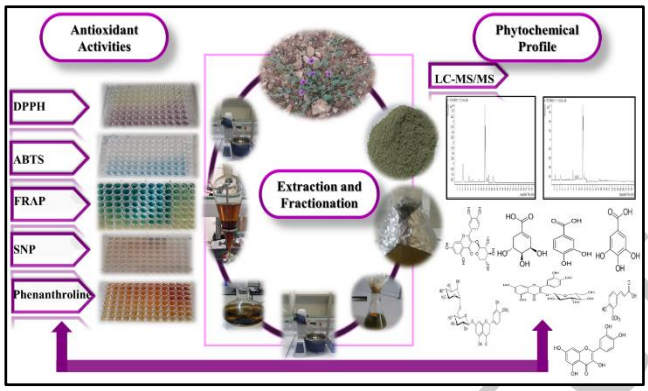
[2]Biotechnology Research Center (CRBT), Ali Mendjli New Town UV 03, B.P E73, Constantine 25016, Algeria.

Received: 16/07/2024, Accepted: 17/09/2024, Available online: 08/10/2024

*to whom all correspondence should be addressed: e-mail: shsouheila@yahoo.fr

https://doi.org/10.30955/gnj.06464

Graphical abstract



Abstract

The present study aimed to evaluate the phytochemical composition and the antioxidant potential of the crude extract and its various fractions which are n-hexane, chloroform, ethyl acetate, and n-butanol of the aerial part of *Erodium guttatum (E. guttatum)*. Total flavonoid and phenolic content have been estimated using the trichloroaluminum colorimetric and Folin-Ciocalteu method. The phytochemical profile was investigated in various polar (ethyl acetate) and nonpolar (chloroform) fractions, using LC-MS/MS technique. The antioxidant activities were also assessed using in vitro assays such as the DPPH, ABTS, FRAP, SNP and Phenanthroline. The obtained results showed that the ethyl acetate fraction had the highest phenolic and flavonoid content (332.76 ± 1.10 µg GAE/mg and 192.07± 1.26 µg QE/mg), respectively. Moreover, LC-MS/MS analysis of the chloroform and ethyl acetate fractions revealed the presence of 16 and 14 compounds, respectively, the main compounds in the chloroform fraction being shikimic acid (13.708 mg/g), hesperidin (0.356 mg/g), isoquercitrin (0.282 mg/g). and in the ethyl acetate fraction, shikimic acid (3.989 mg/g), quercetin 3-xyloside (2.082 mg/g), and gallic acid (0.881 mg/g). The Antioxidant studies revealed that the ethyl acetate fraction exhibited significantly superior activity with IC_{50} of 2.37±0.02 µg/ml and 1.49±0.02 µg/ml against DPPH and ABTS radicals, respectively. Additionally, the fraction showed $A_{0.5}$ values of 2.98±0.07µg/ml, 3.62±0.15µg/ml, and 1.23±0.01 µg/ml against FRAP, SNP and Phenanthroline, respectively. *E. guttatum* may serve as a promising resource for antioxidants, warranting further exploration of its potential in medicine or as a dietary supplement due to its components with potential pharmacological benefits.

Keywords: E. guttatum, crude, fractions; poly phenols, LC-MS-MS, antioxidant activities

1. Introduction

For a long time, people have used plants to treat various illnesses. Actually, these traditional medicines are used in the pharmaceutical industry to search a novel chemical compounds produced from plants (Salmerón-Manzano *et al*., 2020; Eshete and Molla, 2021).

Plants, in general, are a suitable source for producing a wide range of natural antioxidants, so they provide an effective defense against oxidative stress through their secondary metabolites, particularly phenolic and flavonoid components (Amzad Hossain & Shah, 2015). Natural antioxidants are receiving increasing attention as they play a protective role in food and pharmaceutical products by preventing or delaying toxic oxidation reactions and neutralising free radicals, thereby maintaining their quality and extending their shelf life. In addition, they delay or prevent the negative effects of oxidative stress associated with several diseases such as cancer, cardiovascular disease, inflammation, and neurodegenerative diseases (Gulcin, 2020; Khiya *et al*., 2021). Due to their unique chemical structures characterised by aromatic properties, highly compatible systems, and numerous hydroxyl groups; polyphenols act as effective electron and hydrogen atom donors (Sequeira & Poppitt, 2017). This enables them to combat free radicals and other reactive oxygen species (ROS) (Rudrapal *et al*., 2022). More than 3,000 species of plants are inventoried in Algeria. This abundance is a result of the country's climate variability and its strategic geographical location in the Southern Mediterranean Basin (Benarba *et*

al., 2015; Miara *et al*., 2018) making it an important center for plant biodiversity (Naili *et al*., 2010). *Erodium*, belonging to the Geraniaceae family, with over 63 species, are particularly prevalent in Mediterranean regions (Fiz *et al*., 2006). *Erodium* is used in traditional medicine to treat a wide range of health problems such as gastrointestinal disorders, urinary inflammations, diabetes, circulatory system disorders, blood pressure disorders, pneumonia, constipation, and hemorrhage, effective for healing wounds, burns, and coughs, as well as relieving various skin conditions such as eczema (Munekata *et al*., 2019). The diverse uses of this plant genus are linked to the richness of its phytochemical compounds, which has led to a focus of research on analysing their biochemical composition, paving the way for the discovery of new bioactive compounds, Jin *et al*. (2020); Bilić *et al*. (2020) and Samet *et al*. (2022) have documented the presence of various phenolic constituents in numerous *Erodium* species. These include hydroxycinnamic acids, such as ferulic acid, p-coumaric acid, rosmarinic acid, and caffeic acid, as well as hydroxybenzoic acids, including gallic acid, protocatechuic acid, gentisic acid, vanillic acid, p-hydroxybenzoic acid, and salicylic acid. Additionally, these studies have identified also the flavonoids such as flavonol glycosides (isoquercitrin, rutin, hyperoside) and flavonol aglycones (quercetin, kaempferol, isorhamnetin), flavones (luteolin and its derivatives), along with tannins such as catechin, gallotannins (Methyl gallate 3-O-β-D-glucopyranoside, (–) 3-O-Galloylshikimic acid), ellagitannins (geraniin, corilagin), and brevifolin and its derivatives.

Erodium guttatum (Desf) Willd is a seasonal plant that grows in the Mediterranean region throughout North Africa, Palestine, and southern Spain (Mrabti *et al*., 2021). Traditionally, it has long been used in Algeria to treat gastrointestinal disorders (Abdelkrim *et al*., 2006). There is little information in the literature on the composition and pharmacological properties of this species. A study conducted by Mrabti *et al*. (2021) revealed by phytochemical screening that this species, collected in Morocco, contains phenolic compounds, notably a dominance of flavonoids, followed by tannins and anthraquinones, and Significant levels of polyphenols and flavonoids were observed, and a wide range of mineral elements was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES), additionally, considerable antioxidant activity was noted. A correlation between the chemical contents and biological activities of this species and others collected in Tunisia was established by Hamza *et al*. (2018).

Our study focuses on the plant *Erodium guttatum* harvested in the northeastern region of Batna, known as "Hchichet kol Blia". The main objective of this research is to highlight the quantitative characteristics, specifically the total quantity of phenol and flavonoids, Furthermore, investigate the antioxidant capacity of crude extract and their fractions of the aerial part from the plant through five tests (DPPH, ABTS, FRAP, SNP, and Phenanthroline), and this is the first study of this species conduct chemical analysis using LC-MS/MS on diverse, polar and nonpolar fractions of the aerial portion of the plant to enhance scientific knowledge about this botanical species.

2. Materials and methods

2.1. Sample collection

The fresh aerial parts (leaves, stems and flowers) of *Erodium guttatum* (Fig.1) growing in the wild were collected in April 2021 from Batna; a region located in the northeast of Algeria (35°44'27.2"N 6°38'15.4" E). The plant was identified by Pr Sakhraoui Nora, a botanist in the Department of Natural and Life sciences at Skikda University. Samples were gently cleaned, dried in the dark and powered.

2.2. Extraction and fractionation

Two hundred (200) g of powder were used for extraction by an ultrasound-assisted extraction technique. The extraction process was carried out using an ultrasound probe (vibracell 75186 sonicator) with the determination of solvent concentration (70%), sonication time (30min) and amplitude (70%). The solvent was evaporated using a rotary evaporator (37°C) to yield a crude extract, which was dissolved in 200 mL of distilled water and partitioned successively with n-hexane, chloroform (CHCl3), ethyl acetate (EtOAc), and n-butanol (n-BuOH). Finally, the organic solvents were evaporated, and the residues were stored until used for biological and phytochemical tests.

2.3. Determination of total phenolic content (TPC)

To determine the total phenolic content (TPC) of the crude extract and the four fractions, a spectrophotometric method using the Folin-Ciocalteu reagent was employed (Müller *et al*., 2010). Gallic acid was used as a reference standard to construct the calibration curve. Briefly, 20 µL µL volume of plant extract/ fraction was combined with 100 µL of Folin-Ciocalteu reagent (1:10), neutralised with 75 µL of sodium carbonate solution (7.5%, w/v) each added to a 96-well microplate. A blank was prepared in the same way, replacing the extract with the solvent used (methanol). The reaction mixture was kept in the dark at room temperature for 2 h, followed by a reading at 765 nm. TPC were determined using a linear regression equation derived from the gallic acid standard curve. Total phenolic content was calculated as the mean ± SD (n = 3) and expressed as µg gallic acid equivalent per mg of extract.

2.4. Determination of flavonoid content (TFC)

The TFC of the various samples was determined using the microplate test reported by Topçu *et al*. (2007) With a slight modification. The creation of a compound between flavonoids and Al3+ is essential to the process. 50 µL of extract solution (1 mg/mL), 130 µL of methanol, 10 µL of aluminum nitrate (10%) and finally 10 µL of potassium acetate (1M) was added to each well, similarly, a blank was produced by replacing the extract with the solvent used, then the plate was kept incubating for 40 minutes followed by a reading at 765 nm. TFC were determined using a linear regression equation derived from the quercetin standard curve. Total flavonoid content was

calculated as the mean ± SD (n = 3) and expressed as µg of quercetin equivalent per mg of extract.

2.5. Liquid chromatography-electrospray ionization-mass spectrometry analysis LC-ESI-MS/MS analysis

The chloroform and ethyl acetate fractions of the aerial part of *E. guttatum* were subjected to LC-ESI-MS/MS analysis after preparation according to the method described by Erenler *et al*. (2023) An Agilent Technologies 1260 Infinity II, 6460 Triple Quad Mass spectrometer with positive and negative electrospray ionization (ESI) mode were used. The chromatographic separation was performed using the Poroshell 120 SB-C18 column (3.0 × 100 mm, I.D., 2.7 µm) column oven, with a binary solvent system water (A) and methanol (B), both consisting of 0.1% formic acid and 5 mM ammonium formate for the mobile phase. The gradient program was adjusted as: (75% A-25% B) at 3 min, (50% A-50% B) at 12 min, (10% A-90% B) at 16 min, (10% A-90% B) at 21 min and (97.5% A-2.5% B) at 24 min. The injection volume was 5.12 µl, and the flow rate was calibrated at 0.40 mL/min. The conditions adopted are as follows: The column temperature was 40°C, the drying and nebulizing flow of nitrogen gas was 08 L/min, and the pressure was 15 psi, in addition to the capillary voltage and temperature, which were fixed at 4000 V and 350 °C, respectively. All standards used in this analysis are presented in Table 2.

2.6. Evaluation of antioxidant capacity

2.6.1. DPPH free radical-scavenging assay

The DPPH (1, 1-Diphenyl-2-Picrylhydrazyl) assay for free radical scavenging (Blois, 1958) with some modifications was performed. 40µL of extracts or standards (Ascorbic acid, BHA, BHT and Trolox) at several concentrations were combined with 160µL of DPPH solution in methanol. After that, the mixture was incubated in the dark at room temperature for 30 min. Methanol was used as a control. The absorbance was measured at 517 nm using a 96-well microplate reader.

Lower absorbance of the reaction mixture indicates a Greater scavenging action of free radicals DPPH and the percentage of scavenging activity of the crude extract and its fractions on DPPH radical was determined by applying the following equation:

The % inhibition of DPPH = [(Ao-As)/Ao] × 100

Ao represents the control's absorption and As the tested extract solution's absorption.

The concentration required for the sample to scavenge 50% of the initial DPPH free radicals is indicated by the IC_{50} value. And was employed to contrast the antioxidant extracts' quality.

2.6.2. ABTS scavenging assay

ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)]: With a few adjustments, this test was carried out using the Re *et al*. (1999) procedure.

Equal parts of an aqueous ABTS (7 mM) and potassium persulfate (2.45 mM) solution were reacted to create the ABTS stock solution. The mixture was allowed to sit in the dark for 16 hours before being used.

diluted stock solution in methanol, the ABTS working solution was prepared, with an absorbance of 0.70 at 734 nm. 160 µL of the ABTS solution was then combined with 40 µL of extracts at varying concentrations. The combinations were incubated in the dark for 10 min. at room temperature. Methanol was used as a control. Ascorbic acid, BHA, BHT and Trolox were used as standards.

Absorbance was measured at 734 nm using a 96-well microplate reader. ABTS scavenging ability was expressed as IC_{50} (µg/mL) and the following formula was used to determine the percentage of inhibition (I%) associated with each extract's trapping activity on ABTS.

The % inhibition of ABTS= [(Ao-As)/ Ao] × 100.

Ao represents the control's absorption and As the tested extract solution's absorption.

2.6.3. Ferric reducing antioxidant power assay (FRAP)

the methodology of (Oyaizu, 1986) was followed to determine the reducing power activity, with minor adjustments to accommodate the microplate reader. In brief, 10 µl of the different concentrations of crude extract and its fractions were combined with 40 µl of phosphate buffer (0,2 M, pH 6,6) and 50 µl of potassium ferricyanide (1%), and the plate was incubated for 20 min at 50°C. Subsequently, 10% trichloroacetic acid (50 µL), distilled H_2O (40 µL), and 0.1% ferric chloride FeCl3 (10 µL) were added. After reading the absorptions at 700 nm, the concentration corresponding to the absorption at 0.50 nm was estimated as $A_{0.5}$ (µg/mL). The reducing power of the different extracts was contrasted with the standard (Ascorbic acid, BHA and BHT).

2.6.4. Silver nanoparticle assay (SNP)

According to the protocol described by Özyürek *et al*. (2012), the antioxidant capacity of the crude extract/plant fractions was assessed using the silver ion (Ag+) reduction method. To create spherical silver nanoparticles, 50 ml of silver nitrate (AgNO3 (1.0 mM)) was heated for 10 minutes. Next, 5 ml of trisodium citrate (1%) was added dropwise until a pale-yellow colour was obtained. The mixture was then allowed to cool at room temperature Approximately 130 ml of SNP solution and 50 ml of distilled water were added to 20 µL of different doses of plant extract/fractions dissolved in methanol, and after incubation at 25°C for 30 min, the absorbance was measured at 423 nm.

The concentration that corresponded to the absorption at 0.50 nm was estimated as $A_{0.5}$ (µg/mL), and the results were compared to those of Ascorbic acid and Trolox.

2.6.5. Phenanthroline assay

The phenanthroline activity was performed using Szyd\lowska-Czerniak *et al*. (2008) protocol. 10 µl of each sample at varying concentrations, 50 µl of ferric chloride FeCl3 (0.2%), 30 µl of phenanthroline (0.5%) solution, and 110 µl of methanol, were combined. The resulting mixtures were incubated at 30 °C for 20 minutes. The results were compared to the antioxidants standard Trolox and Ascorbic acid and were expressed as

absorbance $A_{0,5}$ µg/mL, which indicated the concentration producing 0.5 absorbance. The absorbance was measured at 510 nm using a 96-well microplate reader.

2.7. Statistical analysis

All experiments were performed in triplicate and results were presented as mean value ±SD, and IBM SPSS Statistics, version 25, one-way ANOVA was used to analyze the data and find significant differences at $p<0.05$. The Tukey test was then performed. The correlation between total phenolic content and antioxidant activities was ascertained using Pearson's correlation coefficient (r).

3. Results end discussion

3.1. Extraction

The yields obtained from various polar and nonpolar fractions derived from 69.69 g (34.84%) of the crude methanolic extract of *E. guttatum's* aerial parts (**Table 1**) exhibited a wide range, spanning from 1.02% to 15.85%, with the n-butanol fraction yielding the highest at 15.85%, succeeded by the ethyl acetate 11.42% then the chloroform fraction 1.37%. In contrast, the n-hexane fraction yielded the lowest 1.02%. Our results can be attributed to a clear correlation between solvent polarity and extraction yield due to the increased solubility of chemical compounds, particularly phenolic compounds in polar solvents compared to nonpolar solvents. (Do *et al*., 2014; Alara *et al*., 2021).

3.2. Total phenolic and flavonoid content

The total phenolic content (TPC) and the total flavonoid content (TFC) in the crude extract and selected fractions (n-hexane, chloroform, ethyl acetate, n-butanol) of *E. guttatum* are shown in Table 1. The results indicated a wide and significant TPC variation ranging from 21.38 to 332,76 µg GAE /mg extract. The ethyl acetate fraction showed the highest TPC (332,76 ± 1.10 µg GAE /mg), followed by the n-butanol fraction (324,52 ± 0.34 µg GAE /mg) then the crude extract (310,97 ± 3,94 µg GAE /mg) after that the chloroform fraction (121.74 ± 0.13 µg GAE /mg). In contrast, the n-hexane fraction had the lowest total phenol content (21.38 ± 0.38 µg GAE /mg). The results demonstrated that the TFC varied also significantly from 1.22 to 192,07µg QE/mg extract. The maximum amount found in the ethyl acetate fraction (192,07± 1.26 µg QE/mg extract).

To our knowledge, no studies have been conducted on this plant using fractional extraction of the crude hydroalcoholic extract, so we only compared the crude extract. Therefore, our results are close to those of (Mrabti *et al*., 2021) where the TPC and TFC values of the crude extract from *E. guttatum* of Moroccan origin were 279.71 ± 0.31 mg GAE/g extract and 118.58 ± 0.14 mg QE/g extract, respectively. Similarly, our results were better than those reported by (Hamza *et al*., 2018) where TPC and TFC values for the crude extract of *E. guttatum* from Tunisian origin as 124 ± 6 mg GAE/g extract and 52 ± 2.3 mg QE/g extract, respectively.

Table 1. Yield, TPC and TFC Values of crude Extract and its fractions from *E. guttatum*

Extracts and fractions	Yield (%)	TPC (µg GAE/mg)	TFC (µg QE/mg)
Crude extract	34.84	310.97 ± 3,94^{c}	71.32 ±1.67^{b}
n-BuOH	15.85	324.52 ± 0.34^{d}	93.67 ± 1.4^{c}
EtOAc	11.42	332.76 ± 1.10^{e}	192.07± 1.26^{d}
CHCl3	1.37	121.74 ± 0.13^{b}	3.01 ± 0.13^{a}
n-hexane	1.02	21.38 ± 0.38^{a}	1.22 ± 0.8^{a}

Data are expressed as mean ± S.D. (n= 3); (µg GAE/mg): µg of gallic acid equivalent per mg of plant extract; (µg QE/mg): µg of quercetin equivalent per mg of plant extract. The values with different superscripts (a, b, c, d, e) in the same columns are significantly different ($p < 0.05$).

3.3. Phytochemical profile by LC-MS/MS

The different components present in the aerial part of the *E. guttatum* plant after using 33 standards provided quantification data comprehensively presented in Table 2.

The polar and nonpolar fractions showed a similar profile in terms of phytoconstituents. The LC-MS/MS methods identified 16 components in the chloroform fraction and 14 components in the ethyl acetate fraction: hydroxybenzoic acid derivatives (shikimic acid, gallic acid, protocatechuic acid and salicylic acid), cinnamic acid derivatives (o-coumaric acid, trans-ferulic acid and chlorogenic acid), flavonols (quercetin), flavonol glycosides (quercetin 3-xyloside, Kaempferol-3-glucoside and isoquercitrin), flavanones (hesperidin), flavones (luteolin), phenolic aldehydes (vanillin and hydroxybenzaldehyde), ester-bound phenolic acids (Protocatechuic ethyl ester),and finally an alkaloid (capsaicin). Notably, vanillin, Protocatechuic ethyl ester, and Capsaicin, which were absent in the ethyl acetate fraction and present in the chloroform fraction, and Kaempferol-3-glucoside was present in the ethyl acetate fraction, but not in the chloroform fraction. Additionally, there were variations in the concentration of the components between the fractions.

In the ethyl acetate fraction, compounds with the highest quantitative abundance were shikimic acid (3.989 mg/g), quercetin 3-xyloside (2.082 mg/g), gallic acid (0.881 mg/g), and isoquercitrin (0.704 mg/g). Conversely, hydroxybenzaldehyde (0.004 mg/g) was present in the lowest amounts. However, in the chloroform fraction, the phenolic components with the highest quantitative presence were shikimic acid (13.708 mg/g), hesperidin (0.356 mg/g), isoquercitrin (0.282 mg/g), and trans-ferulic acid (0.179 mg/g). capsaicin (0.00008 mg/g) was identified as the least abundant in this fraction.

Both fractions contain a significant amount of shikimic acid, which agrees with (Ljoljić Bilić *et al*., 2022). Recent

investigations have extensively examined the biological activities in both in vitro and in vivo settings and proved the anti-inflammatory, antiviral, antibacterial, hypolipidemic, skin, bone, and neuroprotective effects, as well as antidiabetic and antioxidant effects of shikimic acid (Gandhi *et al*., 2023). Our results showed that gallic acid emerged as one of the most prevalent constituents in *guttatum* species, evident in polar fractions, Bilić *et al*. (2020) observed similar results. They identified gallic acid as a predominant component in *E. cicutarium*, with concentrations ranging from 0.679 to 2.310 mg/g in aqueous and methanolic extracts from four Croatian sites. They also, found that, of the 85 chemicals identified, the majority (24 of them) were gallic acid derivatives. Additionally, Cüce *et al*. (2022) have reported that gallic acid was one of the main phenolic acids present in *E. hendrikii* in glycoside (2450.51 nmol/g) or ester-bound form (527.05 nmol/g). Moreover, gallic acid has been shown to have a variety of positive effects, including anti-inflammatory, anti-cancer, antioxidant, antibacterial, cardioprotective, neuroprotective, and gastroprotective properties (Fernandes *et al*., 2022). Our extracts contain other phenolic acids at considerable concentrations such as Protocatechuic acid, o-coumaric acid, Salicylic Acid, and Trans-ferulic acid. These findings are confirmed by (Bakari *et al*., 2018) and (Cüce *et al*., 2022). Although, certain phenolic acids are absent in our extracts like caffeic acid which was present in the hydroalcoholic extracts of flowers and leaves of the plant *E. glaucophyllum* (Bakari *et al*., 2018), and in the aqueous and methanolic extracts of *E. cicutarium* collected from four sites in Croatia (Bilić *et al*., 2020), whereas Cüce *et al*. (2022) found that the most abundant phenolic acid in the crude extract of *E. hundrikii* was caffeic acid. On the other hand, Quercetin-3-d-xyloside, also known as reynoutrin, emerged as the predominant flavonoid in the aerial part of our plant within the polar fraction. This natural flavonoid was commonly found in the fruits and leaves of various plants, but its presence in the genus *Erodium* has yet to be identified. Despite, (Bilić *et al*., 2020) had expanding knowledge by identifying eight glycoside derivatives of quercitrin in *E. cicutarium* and research indicates that reynoutrin exhibits antioxidant properties and may possess antiviral (Rehman *et al*., 2018; Butkevičiūtė *et al*., 2020). Many studies also have reported that reynoutrin significantly improved cardiac function suppressed the death of cardiomyocytes, reduced the release of inflammatory agents, lowered oxidative stress, and lessened myocardial fibrosis (Yang *et al*., 2021) and prevented the induction of breast cancer from progressing (Yüksel *et al*., 2022). Our extract contains Hesperidin which, was the most prevalent flavonoid in the nonpolar portion (0.356 mg/g). These findings are consistent with those of Cüce *et al*. (2022) who also noted that the primary flavonoid in the crude extract is hesperidin in *E. hundriiki*, and according to research, hesperidin has a wide range of biological properties, including the potential to prevent diabetes, cancer, hyperlipidemia, ulcers, atherosclerosis and liver protection. It may also possess anti-inflammatory, antimicrobial, antifungal, antioxidant, antihypertensive and antiallergic effects (Li *et al*., 2018). Furthermore, there are other flavonoids present in the aerial part of *E. guttatum*, which are known by their biological effects such as quercetin, isoquercitrin, and luteolin, and they have been confirmed by Çelikler Özer *et al*. (2020) and Berber *et al*. (2022). Rutin is recognized as one of the best natural antioxidants in its class and is renowned for its diverse pharmacological activities, including anti-inflammatory, antibacterial, antiviral, antiprotozoal, antitumoral, cytoprotective, antiplatelet, vasoactive, antihypertensive, antispasmodic, hypolipidemic and antiallergenic properties (Patel & Patel, 2019). While it is absent from the aerial part of the *E. guttatum* plant, it is present in various species belonging to the *E. genus, such as E. glaucophyllum, E. cicutarium, E. arborescens, E. chium, E. crassifolium, E. laciniatum, E. malacoides, E. moschatum, E. neuradifolium, E. oxyrrhynchum, E. pulverulentum, E. touchyanum* (Saleh *et al*., 1983; Çelikler Özer *et al*., 2020). Generally, The variations in the presence, absence, or concentration of different phenolic compounds observed in various studies can be due to several possible reasons including plant species, growth conditions, extraction technique, chromatography type, measurement, and susceptibilities to degradation (Pająk *et al*., 2014).

Free radical scavenging activity was also determined using the ABTS radical. According to the results, we can say that the ABTS radical was inhibited by all of the standards and tested samples with the highest ABTS scavenging activity was found in the ethyl acetate fraction with an IC 50 value of 1.49 µg/mL. In contrast, the n-hexane fraction exerted the lowest ABTS scavenging capacity with an IC_{50} of 9.81 µg/mL. Nevertheless, compared with standards, BHA, BHT, ascorbic acid, and Trolox, the crude extract and all its polar fractions tested showed significantly higher ABTS radical scavenging activity ($P < 0.05$).

According to the FRAP assay, electron-donating antioxidants reduce the ferric complex 2,4,6-tripyridyl-s-triazine (Fe3+-tripyridyltriazine) to a blue-colored ferrous form (Fe2+-tripyridyltriazine) and the reaction medium's increased absorbance indicates an increase in the tested extracts' power. FRAP antioxidant activity was detected in all samples examined, including the standards, indicating that both polar fractions, EtOAc and BUOH, exhibit a significant ferric reducing power remarkably superior to that of the standards with A0.5 values for EtOAc and BUOH are 2.98±0.07 and 5.04±0.01 µg/mL, respectively, significantly exceeding those determined for the crude extract 9.53±0.07µg/mL and nonpolar fractions of hexane and chloroform (15.93 ± 0.62and 11.58 ± 0.22 µg/mL, respectively).

The crude extract and all fractions gave good results in the phenanthroline test compared to the standards, as did the silver nanoparticle test; in this case, too, the polar extracts, especially the ethyl acetate fraction (1.23 ± 0.01µg/mL, 3.62 ± 0.15µg/mL respectively), gave better results significatively ($p<0.05$) than the nonpolar fraction.

Table 2. Phytochemical profile determined by LC-MS-MS of CHCl3 and EtOAc fractions of *E. guttatum*

Nº	Analyte	RT	Concentration mg/g		Ion Source	Ion Transitions	Ion Mode	R2	LOQ (ug/L)	LOD (ug/L)	Linearity Range (ug/L)
			CHCL3	EtOAc							
1	Shikimic acid	1.336	13.708	3.989	ESI	173.0 -> 93.1	Negative	0.9992	78.7970	20.3983	75-4800
2	Gallic acid	3.166	0.098	0.881	ESI	169.0 -> 125.1	Negative	0.9986	18.5862	7.1674	31.25-500
3	Protocatechuic acid	5.467	0.024	0.154	ESI	153.0 -> 109.0	Negative	0.9969	13.1729	3.1564	15.625-250
4	Epigallocatechin	6.870	ND	ND	ESI	307.0 -> 139.0	Positive	0.9995	3.8750	2.0903	12.5-200
5	Catechin	6.935	ND	ND	ESI	288.9 -> 245.1	Negative	0.9946	7.5013	1.7055	343.750-5500
6	Chlorogenic acid	7.467	0.013	0.010	ESI	353.0 -> 191.0	Negative	0.9981	25.9023	11.5890	31.25-500
7	Hydroxybenzaldeyde	7.783	0.043	0.004	ESI	121.0 -> 92.0	Negative	0.9993	12.8651	4.9742	15.625-250
8	Vanillic acid	7.852	ND	ND	ESI	167.0 -> 151.8	Negative	0.9958	1424.2132	219.0421	1250-20000
10	vanillin	8.662	0.014	ND	ESI	153.0 -> 125.0	Positive	0.9949	40.5411	14.5885	62.5-1000
11	Syringic acid	8.437	ND	ND	ESI	197.1 -> 181.8	Negative	0.9990	857.3388	358.5000	1250-20000
12	Caffeic Acid	7.847	ND	ND	ESI	178.9 -> 135.1	Negative	0.9994	24.1620	6.9205	31.25-500
13	Caffeine	8.419	ND	ND	ESI	195.0 -> 137.9	Positive	0.9986	15.4959	6.8099	18.75-300
14	o-coumaric acid	9.315	0.014	0.015	ESI	163.0 -> 119.1	Negative	0.9996	7.9973	4.0164	15.625-500
15	Salicylic acid	9.769	0.078	0.042	ESI	137.0 -> 93.1	Negative	0.9981	82.9646	47.6695	112.5-1800
16	Taxifolin	9.733	ND	ND	ESI	304.8 -> 258.9	Positive	0.9938	23.5110	11.0294	37.5-600
17	Resveratrol	9.772	ND	ND	ESI	229.0 -> 107.0	Positive	0.9910	13.5575	4.5806	18.75-300
18	Polydatine	9.615	ND	ND	ESI	390.9 -> 228.9	Positive	0.9987	1.8411	1.1471	7.8125-125
19	trans-ferulic acid	9.986	0.179	0.036	ESI	193.1 -> 133.9	Negative	0.9950	11.5276	6.1184	31.25-1000
20	Sinapic acid	10.335	ND	ND	ESI	223.1 -> 208.0	Negative	0.9972	4.9652	1.9437	125-2000
21	Scutellarin	11.054	ND	ND	ESI	462.8 -> 286.8	Positive	0.9978	4.0013	3.1346	9.375-300
22	p-coumaric acid	11.536	ND	ND	ESI	163.0 -> 119.0	Negative	0.9987	1.5416	3.5348	31.25-500
23	Protocatehuic ethyl ester	11.410	0.007	ND	ESI	181.0 -> 107.9	Negative	0.9996	24.9201	14.5610	15.625-1000
24	Hesperidin	11.320	0.356	0.176	ESI	611.0 -> 302.9	Positive	0.9957	17.6753	4.1396	31.25-500
25	Isoquercitrin	11.398	0,282	0.704	ESI	464.9 -> 302.8	Positive	0.9982	11.2680	9.9382	18.75-300
26	Rutin	12.336	ND	ND	ESI	611.0 -> 302.8	Positive	0.9980	240.6720	59.5597	125-2000
27	Quarcetin-3-xyloside	12.105	0.156	2.082	ESI	432.7 -> 299.5	Negative	0.9900	69.4059	18.7126	125-2000
28	Kaempferol-3-glucoside	12.845	ND	0,027	ESI	448.8 -> 286.9	Positive	0.9997	4.5238	1.1609	7.8125-125
29	quercetin	14.417	0.020	0.149	ESI	300.8 -> 151.0	Negative	0.9964	16.9127	4.6558	27.5-440
30	Fisetin	13.150	ND	ND	ESI	287.0 -> 137.0	Positive	0.9954	44.3662	10.8961	15.625-250
31	Baicalin	17.143	ND	ND	ESI	446.8 -> 270.9	Positive	0.9991	3.0988	0.5276	15.625-250
32	Chrysin	14.524	ND	ND	ESI	254.9 -> 153.0	Positive	0.9989	0.1338	0.0737	1.5625-25
33	trans-cinnamic acid	14.331	ND	ND	ESI	149.0 -> 131.1	Positive	0.9999	22.0279	11.1853	31.25-500
34	Morin	15.828	ND	ND	ESI	302.8 -> 153.0	Positive	0.9981	0.5284	0.1253	1.5625-50
35	Baicalein	17.025	ND	ND	ESI	271.0 -> 123.0	Positive	0.9988	0.9631	0.5955	1.5625-25
36	Luteolin	17.421	0.049	0.067	ESI	285.0 -> 133.1	Positive	0.9962	21.4535	20.0000	31.25-500

37	Biochanin A	17.918	ND	ND	ESI	284.9 -> 151.9	Positive	0.9963	0.7333	0.1475	1.5625-25
38	Daidzein	14.187	ND	ND	ESI	254.9 -> 128.0	Positive	0.995	420	140	530-26500
39	Capsaicin	18.043	0.00008	ND	ESI	306.1-> 137.0	Positive	0.998	172	86	9700-97000
40	Diosgenin	23.54	ND	ND	ESI	415.0-> 271.0	Positive	0.996	1000	330	10000-60000

Rt: Retention time, LOQ: Limit of quantification, LOD: Limit of detection, ND: Not detected.

Table 3. Antioxidant activity of crude extract and its fractions of *Erodium guttatum* by DPPH, ABTS, reducing power, phenanthroline, and silver nanoparticle (SNP) assays

Extracts and fractions	**DPPH IC_{50} (µg/ml)**	**ABTS IC_{50} (µg/ml)**	**RP $A_{0.5}$ (µg/ml)**	**SNP $A_{0.5}$ (µg/ml)**	**Phenanthroline $A_{0.5}$ (µg/ml)**
Crude extract	3.74 ±0.03[b]	1.60±0.02[a]	9.53±0.07[c]	11.26±0.24[c]	2.21±0.04[b]
n-BuOH	4.84± 0.06[c]	1.65±0.01[a]	5.04±0.01[b]	6.30±0.19[b]	1.41±0.11[a]
EtOAc	2.37± 0.02[a]	1.49±0.02[a]	2.98±0.07[a]	3.62±0.15[a]	1.23±0.01[a]
CHCL3	32.29±0.03[f]	6.89±0.02[c]	11.58 ± 0.22[d]	37.96±0.14[e]	3.86± 0.01[d]
n-hexane	37.99±0.24[g]	9.81±0.02[e]	15.93 ± 0.62[e]	20.32±0.09[d]	7.78±0.03[f]
BHA	15.74±0,47[e]	7.54±0.67[d]	8.41±0.67[c]	NT	NT
BHT	6.55±0,59[d]	1.55±0.26[a]	>50[f]	NT	NT
Ascorbic acid	4.39±0.01[c]	3.04±0.05[b]	9.01±1.46[c]	7.14±0.05[b]	3.08±0.02[c]
Trolox	5.12±0.21[c]	3.21±0.06[b]	NT	34.17±1.23[e]	5.21±0.27[e]

Table 4. Correlation analysis between phenolic compounds and antioxidant activity of crude extract and its fraction from *E. guttatum*

	DPPH	**ABTS**	**FRAP**	**SNP**	**Phenanthroline**	**TPC**	**TFC**
DPPH	1						
ABTS	.987**	1					
FRAP	.872**	.889**	1				
SNP	.816**	.720**	.686**	1			
Phenanthroline	.903**	.955**	.923**	.536*	1		
TPC	-.989**	-.999**	-.908**	-.741**	-.955**	1	
TFC	-.833**	-.795**	-.909**	-.806**	-.756**	.818**	1

***. La corrélation est significative au niveau 0.01, *. La corrélation est significative au niveau 0.05.*

This excellent antioxidant capacity may be due to the high content of phenolic components, particularly in polar extracts/fractions, which appear to be efficient hydrogen and electron donors, and due to their ideal structural chemistry, Additionally, as a biological activity requires the consideration of chemical synergy, we should not ignore the presence of other small phenolic compounds (Khiya *et al*., 2021), as well as to the presence of major components known for their anti-oxidant power such hydroxybenzoic acid derivatives (gallic acid, protocatechuic acid, salicylic acid), cinnamic acid derivatives (o-coumaric acid, trans-ferulic acid, chlorogenic acid) (Razzaghi-Asl *et al*., 2013), flavonoids and its derivatives (kaempferol, quercetin) (Burda & Oleszek, 2001).

The concentration at 50% inhibition and the concentration at 0.50 absorbance, respectively, are referred to IC50 and A0.50 values. By using a linear regression analysis, the IC_{50} and $A_{0.50}$ values were determined and expressed as mean ± SD (n =3). The values in the same columns that have different superscripts (a, b, c, d, e, f, or g) differ significantly ($p < 0.05$). BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, NT: not tested.

3.4. Correlations between total phenolic and flavonoid contents and antioxidant capacities

Pearson correlation analysis was used to examine the relationships between the levels of phenolic compounds (TPC and TFC) and antioxidant capacities (DPPH, ABTS, FRAP, SNP, and phenanthroline). The results are presented in **Table 4**. However, the relationship between the content of phenolic compounds and the antioxidant properties of this plant has not been examined in any study so far. The present study showed remarkably strong and positive correlations between antioxidant activities, the highest being between DPPH and ABTS ($r = 0.987$, $P < 0.01$), and ABTS and phenanthroline ($r = 0. 955$, $P < 0. 01$), there was also a moderate correlation between SNP and ABTS and FRAP ($r = 0.720$, $P < 0.01$), ($r = 0.686$, $P < 0.01$) respectively, and a small correlation was found between SNP and phenanthroline ($r = 0.536$, $P < 0.05$). In addition, a strong positive correlation was reported between TPC and TFC ($r = 0.818$, $P < 0.01$), however, strong and significant negative correlations were found between phenolic and flavonoid compounds and antioxidant activities, while the strongest correlation was between TPC and ABTS ($r = - 0.999$, $P <0.01$) and between TFC and FRAP ($r = - 0.909$, $P < 0.01$). A moderate correlation was found between TPC and SNP ($r = - 0.741$, $P < 0.01$). Phenolic compounds contribute significantly to the antioxidant activities of this species and thus may play an essential role in its beneficial effects**.**

4. Conclusion

The findings from this research indicate that both the crude extract and different fractions of *E. guttatum* exhibit significant antioxidant properties in vitro. The quantitative composition of phenolic and flavonoid compounds is particularly intriguing, especially in the crude extract and polar fractions. LC-MS/MS analysis unveiled the presence of various phenolic acids, and flavonoids recognized in the literature for their pharmacological effects. However, additional investigations are warranted to isolate and characterize the bioactive compounds responsible for the antioxidant potential. Subsequent in vivo studies are essential to assess their efficacy in disease prevention.

References

Abdelkrim C., Belboukhari N., Karima S. and Hacini S. (2006). Plants of Algerian semi-arid regions used for the treatment of gastro-intestinal disorders. *Algerian Journal of Arid region*, **5**, 07.

Alara O.R., Abdurahman N.H. and Ukaegbu C.I. (2021). Extraction of phenolic compounds: A review. *Current Research in Food Science*, ***4*****,** 200–214.

Amzad Hossain M. and Shah M.D. (2015). A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant Merremia borneensis. *Arabian Journal of Chemistry*, **8**(1), 66–71.

Bakari S., Hajlaoui H., Daoud A., Mighri H., Ross-Garcia J.M., Gharsallah N. and Kadri A. (2018). Phytochemicals, antioxidant and antimicrobial potentials and LC-MS analysis of hydroalcoholic extracts of leaves and flowers of *Erodium glaucophyllum* collected from Tunisian Sahara. *Food Science and Technology*, ***38***, 310–317.

Benarba B., Belabid L., Righi K., Bekkar A.A., Elouissi M., Khaldi A. and Hamimed A. (2015). Ethnobotanical study of medicinal plants used by traditional healers in Mascara (North West of Algeria). *Journal of Ethnopharmacology*, **175**, 626–637.

Berber D., Toksöz O., Türkmenoğlu İ. and Sesal C. (2022). A review on antimicrobial activities of some culinary herbs and spices against Staphylococcus aureus. *Frontiers in Life Sciences and Related Technologies*, **3(2)**, 75–85.

Bilić V.L., Gašić U., Milojković-Opsenica D., Nemet I., Rončević S., Kosalec I. and Rodriguez J.V. (2020). First Extensive Polyphenolic Profile of *Erodium cicutarium* with Novel Insights to Elemental Composition and Antioxidant Activity. *Chemistry & Biodiversity*, **17(9)**, e2000280.

Blois M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, **181(4617)**, 1199–1200.

Burda S. and Oleszek W. (2001). Antioxidant and Antiradical Activities of Flavonoids. *Journal of Agricultural and Food Chemistry*, **49(6)**, 2774–2779.

Butkevičiūtė A., Liaudanskas M., Kviklys D., Gelvonauskienė D. and Janulis V. (2020). The qualitative and quantitative compositions of phenolic compounds in fruits of Lithuanian heirloom apple cultivars. *Molecules*, **25(22)**, 5263.

Çelikler Özer Ö., Baki Kekilli E., ERDOĞAN ORHAN İ. and KAHRAMAN A. (2020). Erodium L'HER. (dönbaba/iğnelik). *Türk Farmakope Dergisi*, **5(1)**.

Cüce M., Colak N., Zeljković S.Ć., Tarkowski P., Strnad M., Gruz J., İmamoğlu K.V. and Ayaz F.A. (2022). An evaluation of volatiles and phenolic compounds in conjunction with the antioxidant capacity of endemic endangered species of Erodium hendrikii Alpinar. *South African Journal of Botany*, **149**, 458–467.

Do Q.D., Angkawijaya A.E., Tran-Nguyen P.L., Huynh L.H., Soetaredjo F.E., Ismadji S. and Ju Y.-H. (2014). Effect of extraction solvent on total phenol content, total flavonoid

content, and antioxidant activity of Limnophila aromatica. *Journal of Food and Drug Analysis*, **22(3)**, 296–302.

Erenler R., Atalar M.N., Yildiz İ., Geçer E.N., Yildirim A., DemiRtas İ. and Alma M.H. (2023). Quantitative analysis of bioactive compounds by LC-MS/MS from Inula graveolens. *Bütünleyici ve Anadolu Tıbbı Dergisi*, **4(3)**, 3–10.

Eshete M.A. and Molla E.L. (2021). Cultural significance of medicinal plants in healing human ailments among Guji semi-pastoralist people, Suro Barguda District, Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, **17(1)**, 61.

Fernandes T.A., Antunes A.M.M., Caldeira I., Anjos O., de Freitas V., Fargeton L., Boissier B., Catarino S. and Canas S. (2022). Identification of gallotannins and ellagitannins in aged wine spirits : A new perspective using alternative ageing technology and high-resolution mass spectrometry. *Food Chemistry*, **382**, 132322.

Fiz O., Vargas P., Alarcón M.L. and Aldasoro J.J. (2006). Phylogenetic Relationships and Evolution in <I>Erodium</I> (Geraniaceae) based on <I>trnL-trnF</I> Sequences. *Systematic Botany*, **31(4)**, 739–763.

Gandhi G.R., Vasconcelos A.B.S., Antony P.J., Montalvão M.M., de Franca M.N.F., Hillary V.E., Ceasar S.A. and Liu D. (2023). Natural sources, biosynthesis, biological functions, and molecular mechanisms of shikimic acid and its derivatives. *Asian Pacific Journal of Tropical Biomedicine*, **13(4)**, 139.

Gulcin İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of Toxicology*, **94(3)**, 651–715.

Hamza G., Emna B.-H., Yeddes W., Dhouafli Z., Moufida T.S. and El Akrem H. (2018). Chemical composition, antimicrobial and antioxidant activities data of three plants from Tunisia region : Erodium glaucophyllum, Erodium hirtum and Erodium guttatum. *Data in brief*, **19**, 2352–2355.

Jin L., Fayvush G., Khutsishvili M., Atha D. and Borris R.P. (2020). Phytochemical Investigation of *Erodium oxyrrhynchum* M. Bieb. *Natural Product Communications*, ***15*(6)**, 1934578X2093085.

Khiya Z., Oualcadi Y., Gamar A., Berrekhis F., Zair T. and Hilali F. E. (2021). Correlation of Total Polyphenolic Content with Antioxidant Activity of Hydromethanolic Extract and Their Fractions of the *Salvia officinalis* Leaves from Different Regions of Morocco. *Journal of Chemistry*, **2021**, e8585313.

Li W., Kandhare A.D., Mukherjee A.A. and Bodhankar S.L. (2018). Hesperidin, a plant flavonoid accelerated the cutaneous wound healing in streptozotocin-induced diabetic rats : Role of TGF-ß/Smads and Ang-1/Tie-2 signaling pathways. *EXCLI Journal*, ***17***, 399–419.

Ljoljić Bilić V., Gašić U.M., Milojković-Opsenica D., Rimac H., Vuković Rodriguez J., Vlainić J., Brlek-Gorski D. and Kosalec I. (2022). Antibacterial Fractions from Erodium cicutarium Exposed—Clinical Strains of Staphylococcus aureus in Focus. *Antibiotics*, **11(4)**, Article 4.

Miara M.D., Bendif H., Ait Hammou M. and Teixidor-Toneu I. (2018). Ethnobotanical survey of medicinal plants used by nomadic peoples in the Algerian steppe. *Journal of Ethnopharmacology*, **219**, 248–256.

Mrabti H.N., Doudach L., Kachmar M.R., KHALI Z., MRABTI N.N., BENRAHOU K.B., HARRAQUI K., ZENGİN G., BOUYAHYA A. and FAOUZI M.E.A. (2021). Phenolic content, antibacterial, antioxidant, and toxicological investigations ofErodium guttatum (Geraniaceae) collected from the Northeast of Morocco. *Turkish Journal of Botany*, **45(8)**, 739–749.

Müller L., Gnoyke S., Popken A.M. and Böhm V. (2010). Antioxidant capacity and related parameters of different fruit formulations. *LWT-Food Science and Technology*, ***43*(6)**, 992–999.

Munekata P.E.S., Alcántara C., Collado M.C., Garcia-Perez J. V., Saraiva J.A., Lopes R.P., Barba F.J., do Prado Silva L., Sant'Ana A.S., Fierro E.M. and Lorenzo J.M. (2019). Ethnopharmacology, phytochemistry and biological activity of Erodium species : A review. *Food Research International (Ottawa, Ont.)*, **126**, 108659.

Naili M.B., Alghazeer R.O., Saleh N.A. and Al-Najjar A. Y. (2010). Evaluation of antibacterial and antioxidant activities of Artemisia campestris (Astraceae) and Ziziphus lotus (Rhamnacea). *Arabian Journal of Chemistry*, ***3*(2)**, 79–84.

Oyaizu M. (1986). Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese journal of nutrition and dietetics*,***44*(6)**, 307–315.

Özyürek M., Güngör N., Baki S., Güçlü K. and Apak R. (2012). Development of a Silver Nanoparticle-Based Method for the Antioxidant Capacity Measurement of Polyphenols. *Analytical Chemistry*, **84(18)**, 8052–8059.

Pająk P., Socha R., Gałkowska D., Rożnowski J. and Fortuna T. (2014). Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food Chemistry*, ***143***, 300–306.

Patel K. and Patel D.K. (2019). The Beneficial Role of Rutin, A Naturally Occurring Flavonoid in Health Promotion and Disease Prevention: A Systematic Review and Update. In *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases* (457–479). Elsevier.

Rajan S., Mahalakshmi S., Deepa V., Sathya K., Shajitha S. and Thirunalasundari T. (2011). *ANTIOXIDANT POTENTIALS OF PUNICA GRANATUM FRUIT RIND EXTRACTS*. **3**(3).

Razzaghi-Asl N., Garrido J., Khazraei H., Borges F. and Firuzi O. (2013). Antioxidant Properties of Hydroxycinnamic Acids : A Review of Structure- Activity Relationships. *Current Medicinal Chemistry*, **20(36)**, 4436–4450.

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M. and Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, **26(9-10)**, 1231–1237.

Rehman S., Ashfaq U.A., Ijaz B. and Riazuddin S. (2018). Anti-hepatitis C virus activity and synergistic effect of Nymphaea alba extracts and bioactive constituents in liver infected cells. *Microbial pathogenesis*, **121**, 198–209.

Rudrapal M., Khairnar S.J., Khan J., Dukhyil A.B., Ansari M.A., Alomary M.N., Alshabrmi F.M., Palai S., Deb P.K. and Devi, R. (2022). Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases : Insights Into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action. *Frontiers in Pharmacology*, **13**, 806470.

Saleh N.A.M., El-karemy Z.A.R., Mansour R.M.A. and Fayed A.-A. A. (1983). A chemosystematic study of some geraniaceae. *Phytochemistry*, ***22*(11)**, 2501–2505.

Salmerón-Manzano E., Garrido-Cardenas J.A. and Manzano-Agugliaro F. (2020). Worldwide research trends on medicinal plants. *International journal of environmental research and public health*, **17(10)**, 3376.

Samet S., Ayachi A., Fourati M., Mallouli L., Allouche N., Treilhou M., Téné N. and Mezghani-Jarraya R. (2022). Antioxidant and antimicrobial activities of Erodium arborescens aerial part

extracts and characterization by LC-HESI-MS2 of its acetone extract. *Molecules*, **27(14)**, 4399.

Sequeira I.R. and Poppitt S.D. (2017). Unfolding Novel Mechanisms of Polyphenol Flavonoids for Better Glycaemic Control : Targeting Pancreatic Islet Amyloid Polypeptide (IAPP). *Nutrients*, **9(7)**, 788.

Szyd\lowska-Czerniak A., Dianoczki C., Recseg K., Karlovits G. and Sz\lyk E. (2008). Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. *Talanta*, **76(4)**, 899–905.

Topçu G., Ay M., Bilici A., Sarıkürkcü C., Öztürk M. and Ulubelen A. (2007). A new flavone from antioxidant extracts of Pistacia terebinthus. *Food chemistry*, **103(3)**, 816-822.

Yang W., Tu H., Tang K., Huang H., Ou S. and Wu J. (2021). Reynoutrin Improves Ischemic Heart Failure in Rats Via Targeting S100A1. *Frontiers in Pharmacology*, **12**.

Yüksel T.N., Bozgeyik E. and Yayla M. (2022). The effect of quercetin and quercetin-3-d-xyloside on breast cancer proliferation and migration. *Journal of Basic and Clinical Health Sciences*, **6(2)**, 569–578.