Food Analytical Methods
https://doi.org/10.1007/s12161-025-02818-7

RESEARCH

Extraction Process and Phenolic Profiling of *Glebionis Coronaria*: Insight into Antioxidant and Cytotoxic Activities with ADME-Based Therapeutic Potential

Larbi Derbak[1] · Hamdi Bendif[2] · Radia Ayad[3] · Khellaf Rebbas[4] · Ibrahim Demirtas[5] · Ilyas Yildiz[6] · Fehmi Boufahja[2] · Stefania Garzoli[7]

Received: 6 March 2025 / Accepted: 8 May 2025

Abstract

This study sought to maximize the extraction of *Glebionis coronaria*'s total phenolic content (TPC) and total flavonoid content (TFC), determining the best conditions for separating these beneficial substances. Liquid–liquid partitioning was carried out after extraction using solvents of different polarities, such as n-butanol, ethyl acetate, and chloroform. While antioxidant activity was evaluated using DPPH, ABTS, reducing power, phenanthroline, and silver nanoparticle assays, TPC and TFC were measured using the Folin-Ciocalteu and $AlCl_3$ techniques, respectively. Cytotoxic effects were evaluated against two cancer cell lines (CAPAN-1 and dld-1) as well as a healthy cell line (L929), and the ethanolic extract's substance composition was examined using LC–ESI–MS/MS. The optimal TPC and TFC extraction parameters were found to be X_1 (48 h), X_2 (70% ethanol), and X_3 (30 mL/g). The n-butanol fraction showed significant antioxidant activity (IC_{50}: 13.89 µg/mL for DPPH, 29.18 µg/mL for ABTS) and had the highest TPC (325.33 mg GAE/g dw) and TFC (112.5 mg QE/g dw). Additionally, it demonstrated a considerable ability to chelate metals. Twelve bioactive compounds were identified in the ethanolic extract, with chlorogenic acid being the main component (422.47 µg/g). Cytotoxicity testing revealed significant effects against CAPAN-1 cells, reducing viability by 38.53% at 1 mg/mL. An ADME model provided insights into the pharmacokinetics and bioavailability of the identified compounds, highlighting their therapeutic applications.

Keywords *Glebionis coronaria* · Polyphenols · Flavonoids · Antioxidant activity · LC–ESI–MS/MS · Bioactive compound

✉ Stefania Garzoli
stefania.garzoli@uniroma1.it

Larbi Derbak
larbi.derbak@univ-msila.dz

Hamdi Bendif
hlbendif@imamu.edu.sa

Radia Ayad
radia.ayad@univ-jijel.dz

Khellaf Rebbas
khellaf.rebbas@univ-msila.dz

Ibrahim Demirtas
ibrahim.demirtas@igdir.edu.tr

Ilyas Yildiz
ilyas.yildiz@igdir.edu.tr

Fehmi Boufahja
faboufahja@imamu.edu.sa

[1] Department of Natural and Life Sciences, Faculty of Sciences, University of M'Sila, University Pole, Road Bordj Bou Arreiridj, 28000 M'Sila, Algeria

[2] Biology Department, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), 11623 Riyadh, Saudi Arabia

[3] Laboratory of Phytochemistry and Pharmacology, Department of Chemistry, Faculty of Exact Sciences and Informatics, University of Jijel, 18000 Jijel, Algeria

[4] Laboratory of Agro-Biotechnology and Nutrition in Arid and Semi-Arid Areas, Ibn Khaldoun University, 14000 Tiaret, Algeria

[5] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ondokuz Mayıs University, Samsun, Turkey

[6] Research Laboratory Application and Research Center (ALUM), Foundation of the Faculty of Health Sciences, Nutrition and Dietetics Department, Igdır University, Igdır 76000, Turkey

[7] Department of Chemistry and Technologies of Drug, Sapienza University, 00185 Rome, Italy

Published online: 24 May 2025

Introduction

Glebionis coronaria (L.) Cass. Ex Spach, commonly known as crown daisy, garland chrysanthemum, or edible chrysanthemum, is a prominent medicinal and dietary plant of the *Asteraceae* family (Grigoraș et al. 2019; Wijaya et al. 2020). Its name, derived from the Greek "chryos" (gold) and "anthemon" (flower), meaning "golden flower," refers to its bright yellow blooms, while the Latin "*coronaria*" refers to its coroniform structure (Grigoraș et al. 2019). Native to the Mediterranean region, *G. coronaria* has spread globally and is cultivated in northern India, Turkey, and East Asia, where it is valued not only for its ornamental properties but also for its medicinal and culinary applications (Mahdi et al. 2023). Historically, *G. coronaria* has held a prominent place in traditional medicine. Various parts of the plant, including its extracts and essential oil, are used in Mediterranean countries for their hepatoprotective, antioxidant, anti-angiogenic, antitumor, nematocidal, insecticidal, and antimicrobial properties (Ivashchenko et al. 2019). Similarly, in East Asia, including China, Korea, Japan, and India, *G. coronaria* is consumed as a dietary product and used therapeutically to treat ailments such as syphilis, gonorrhea, chronic constipation, and stomach disorders. Its leaves are consumed as edible greens, while its flowers are used to make herbal teas (Ivashchenko 2017; Wang et al. 2016). The plant's dietary significance is further reflected into its nutritional profile, which has a low fat content, and is rich in proteins and antioxidant compounds such as beta-carotene, vitamin C, tannins, and essential minerals (Ivashchenko et al. 2019). In recent years, scientific interest in *G. coronaria* has intensified due to its diverse pharmacological properties and rich phytochemical profile. Studies have identified a wide array of bioactive compounds, particularly polyphenols and flavonoids, which have been associated with its potent anti-inflammatory, antioxidant, and anticancer properties (Sulas et al. 2017; Singh and Khanna 2021). Polyphenols and flavonoids have attracted considerable attention as natural therapeutic agents, leading to the development of advanced extraction techniques aimed at isolating these compounds without compromising their bioactivity (Ameer et al. 2017; Dzah et al. 2020). Despite the extensive investigation into the medicinal value of *G. coronaria* (Sulas et al. 2017; Ivashchenko et al. 2019; Wijaya et al. 2020; Mahdi et al. 2023), a comprehensive examination of phytochemical profile and bioactivity of the same species growing in Algeria is still lacking. To this aim, here the aerial parts of *G. coronaria* were exhaustively extracted using a sequential method with solvents at different polarities. Total phenol (TPC) and total flavonoid contents (TFC) were assessed, and the phenolic profile of the most enriched fraction was investigated using LC–ESI–MS/MS. The same extracts underwent evaluation of antioxidant and antiproliferative activities on cancer and healthy cell lines. Finally, the bioactive compounds detected in *G. coronaria* extracts were subjected to an in silico ADME evaluation: this computational model provided critical insights into the pharmacokinetic properties of the identified molecules, assessing their absorption, distribution, metabolism, and excretion profiles.

Materials and Methods

Reagents and Chemicals

The reagents and solvents used in this study were of analytical grade, including Folin-Ciocalteu reagent (FCR), DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), quercetin, α-tocopherol, silver nanoparticles, methanol (≥ 99.9%), ethanol (≥ 99.9%), chloroform (≥ 99%), ethyl acetate (≥ 99%), and n-butanol (≥ 99%). All chemicals were obtained from Merck (St. Louis, USA).

Plant Material

The aerial flowering parts of *G. coronaria* (Fig. 1) were harvested during March 2024 from wild populations in M'sila province, northeastern Algeria. Taxonomic identification

Fig. 1 Illustration of *Glebionis coronaria* collected in Hammam Dalaa (M'sila), March 2024, Photo K. Rebbas

was confirmed according to Quezel and Santa (1962) and POWO (2024), by Prof. K. Rebbas at M'sila University. The plant samples were washed with distilled water, dried at ambient temperature of 25 °C in a dark, ventilated space, ground into fine powder, and stored at 4 °C for future analysis. A voucher specimen, assigned the identification code N° KR0015, has been deposited in the personal herbarium of Prof. K. Rebbas.

Extracts Preparation

Optimization of TPC and TFC Extraction

The maceration technique was applied for optimizing the TPC and TFC extraction from the aerial parts of *G. coronaria*, using a solvent mixture of ethanol and water. The extraction was carried out at a constant temperature of 25 °C. A face-centered central composite design was employed to create surface response plots, with three factors studied including extraction time (X_1), ethanol concentration (X_2), and liquid–solid ratio (X_3). TPC and TFC were the response variables. The optimized conditions are listed in Table 1.

Extraction and Fractionation

A sample of 50 g of *G. coronaria* powder was macerated at room temperature, filtered, and the resulting extract was evaporated at 45 °C under reduced pressure using a Büchi Rotavapor R-215 (Büchi Labortechnik AG, Switzerland). The extraction was performed using optimized conditions determined by response surface methodology (X_1, 48.95 h of extraction time, X_2, 70.22% of ethanol concentration, and X_3, 30 mL/g of solvent-to-material ratio). These parameters were selected to maximize the recovery of total phenolic content and biological activity from the aerial parts of the plant. The resulting dry extract was dissolved in 100 mL of distilled water heated to 100 °C, followed by sequential liquid–liquid extraction with solvents with increasing polarity (chloroform, ethyl acetate, n-butanol). For each extraction step, 100 mL of the respective solvent was added to the aqueous solution, and the mixture was left to stand overnight at room temperature to allow sufficient time for the partitioning of compounds from the aqueous phase into the organic solvent. Each extraction step was carried out in duplicate to ensure consistency. After extraction, the organic phases were collected and concentrated using a rotary evaporator at 45 °C under reduced pressure.

Table 1 The experimental face-centered central composite design

Parameters	Parameters levels		
	− 1	0	1
[a]X_1 (h)	24	48	72
[b]X_2 (%)	50	70	90
[c]X_3 (mL/g)	10	20	30

[a]X_1, extraction time (h)
[b]X_2, solvent concentration
[c]X_3, liquid–solid ratio

TPC and TFC

Total Phenolic Content

Based on the methodology described by Müller et al. (2010), a modified Folin-Ciocalteu reagent test was used to quantify the TPC of *G. coronaria* extracts. Twenty microliters of each extract (1 mg/mL) was mixed in a 96-well microplate with 75 µL of a 7.5% sodium carbonate solution and 100 µL of FCR that had been diluted 1:9 with distilled water for this test. The mixes were incubated at room temperature in the dark for 2 h. The assay was conducted in triplicate to ensure accuracy and reproducibility. With values ranging from 0 to 200 µg/mL, the absorbance was measured at 765 nm, and the TPC was computed using a linear regression equation ($y = 0.0033x + 0.1043$, $R^2 = 0.998$) derived from a gallic acid standard curve.

This was conducted in triplicate.

Total Flavonoid Content

According to Topçu et al. (2007), the aluminum chloride colorimetric test was used to measure the TFC of *G. coronaria* extracts. Fifty microliters of each extract (1 mg/mL) was combined with 130 µL of methanol, 10 µL of 10% aluminum nitrate, and 10 µL of 1 M potassium acetate in a 96-well microplate. After 45 min of room temperature incubation, the mixes' absorbance at 415 nm was measured. Each measurement was carried out in triplicate to enhance data accuracy and reproducibility. Using the same parameters, a quercetin standard curve was created with values ranging from 0 to 50 µg/mL. The linear regression equation ($y = 0.0041x$, $R^2 = 0.998$) was used to get the TFC values.

LC–ESI–MS/MS Analysis

The Griffith et al. (2019) procedure was slightly modified for the LC–ESI–MS/MS analysis of the ethanolic extract of *G. coronaria*. In a 2 mL Eppendorf tube, 50 mg of the ethanolic extract was first dissolved in a solution that included 1 mL of methanol and n-hexane each. After vortexing for 2 min at 4 °C using a Bioprep-24 homogenizer, the mixture was centrifuged using a Hettich Universal 320 R centrifuge (Germany) for 10 min at 15,033 × g. The methanol layer was separated after centrifugation and diluted 1:9 with distilled water. Prior to analysis, a 5.12 µL injection volume

of the produced solution was filtered using a Captiva premium syringe filter (nylon membrane, 25 mm diameter, 0.45 µm pore size). Utilizing an Agilent 1260 Infinity II LC system connected to an Agilent 6460 Triple Quadrupole Mass Spectrometer with electrospray ionization source, the chromatographic separation and analysis were carried out. A reversed-phase Agilent Poroshell 120 EC-C18 analytical column (100 mm × 3.0 mm, 2.7 µm) kept at 25 °C was used to achieve separation. The mobile phase was made up of acetonitrile (0.1% formic acid, eluent B) and water (5 mM ammonium formate, eluent A) at an isocratic ratio of 75:25. The process was carried out at a flow rate of 0.5 mL/min for 30 min. Using the Multiple Reaction Monitoring (MRM) technique and Agilent Mass Hunter Software for compound identification and quantification, data was acquired in both positive and negative ionization modes (ESI^+ and ESI^-, respectively). In order to improve the fragmentation and transmission of ions, optimized collision energy were used. The following settings were established for the instrument: capillary voltage of 4000 V, gas temperature of 350 °C, and nitrogen gas flows of 11 mL/min for nebulization and 15 mL/min for drying (Köktürk et al. 2022). Following the procedures outlined by Yilmaz (2020), the method's validation included evaluating important analytical parameters such the Limit of Detection (LOD), Limit of Quantification (LOQ), and Linearity Range.

Antioxidant Activity

Ninety-six-well microplates were used for all investigations, and a PerkinElmer EnSpire Multimode Plate Reader (Waltham, MA, USA) was used to measure absorbance. All experiments were conducted in triplicate to ensure reproducibility and accuracy of the results. The IC_{50} and $A_{0.5}$ values (µg/mL) were determined by nonlinear regression analysis. For IC_{50}, the concentration of the extract required to inhibit 50% of the maximum activity was calculated by fitting the data to a sigmoidal dose–response curve. Similarly, $A_{0.5}$ represents the concentration needed to achieve 50% of the maximal activity. The data points from different concentrations were plotted, and a curve-fitting method was applied to estimate these values accurately. The model used was selected based on its ability to best describe the experimental data, ensuring a reliable and precise estimation. The mean values reported are based on three independent experiments.

DPPH Radical Scavenging Assay

The antioxidant activity of *G. coronaria* extracts was assessed using the DPPH free radical scavenging method, following the procedure of Blois (1958). Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) served as positive controls for comparison.

ABTS Cation Radical Scavenging Assay

The antioxidant activity of *G. coronaria* extracts was evaluated using the ABTS radical scavenging method, adapted from Re et al. (1999). BHT and BHA were used as standards.

Ferric Reducing Antioxidant Power Assay

The reducing power of *G. coronaria* plant extracts was assessed using the method described by Oyaizu et al. (1986). Ascorbic acid was used as a positive control to benchmark the reducing ability of the plant extracts.

Phenanthroline-Based Iron Reducing Assay

The iron reducing potential of *G. coronaria* plant extracts was evaluated following the method of Szydlowska-Czerniak et al. (2008). Butylated hydroxyanisole (BHA) was used as a positive control to validate the assay.

Silver Nanoparticle Assay

The antioxidant capacity of *G. coronaria* extracts was determined using the silver ion (Ag^+) reduction method described by Ozyurek et al. (2012). Ascorbic acid and Trolox were included as positive controls to compare the reducing power of the plant extracts.

Antiproliferative Activity

Two cancer cell lines (CAPAN-1 pancreatic cancer cells and dld-1 colorectal adenocarcinoma cells) and a normal cell line (L929 fibroblasts) were acquired from Prof. Dr. Mustafa Türk's lab at Kırıkkale University (Türkiye) in order to assess the antiproliferative activity of *G. coronaria* ethanolic extract. Multi-well and culture plates from Corning, USA, were used in all cell culture methods. The antiproliferative activity of *G. coronaria* ethanolic extract was evaluated following Karan and Erenler (2018) and Aydin et al. (2016). After seeding 10×10^3 cells per well in 96-well plates and incubating for 24 h to allow cell adherence, the cells were treated with various concentrations (1, 0.75, 0.5, and 0.25 mg/mL) of the ethanolic extract prepared in complete DMEM medium (Sigma-Aldrich, USA). After 24 h of treatment, the medium was replaced with 100 µL of MTT solution (1 mg/mL in PBS; Sigma-Aldrich, USA), and the plates were incubated for an additional 2–2.5 h at 37 °C in the dark. Formazan crystals formed by viable cells were dissolved using 100 µL of isopropanol (Merck, Germany) per well, and absorbance was measured at 570 nm using a microplate reader (Thermo Scientific Multiskan GO, USA). No commercial MTT kit was used; all reagents were

prepared manually using high-purity analytical-grade chemicals from Sigma-Aldrich and Merck. Cell viability (%) was calculated using the formula:

$$Cell\ viability\,(\%) = \frac{As}{Ac} \times 100$$

where, *As* and *Ac* represent the absorbance of treated and control cells, respectively.

Bioavailability and Pharmacokinetic Characteristics

The bioavailability of the compounds, influenced by physicochemical properties like molecular size, lipophilicity, polarity, and solubility, was assessed through in silico analyses as outlined by Mhadhbi et al. (2023). Additionally, the druggability and pharmacokinetic profiles of the phytochemicals identified in *G. coronaria* were evaluated, focusing on their absorption, distribution, metabolism, and excretion (ADME) characteristics (Derbak et al. 2024).

Statistical Analysis

Multiple regression analysis was performed using Minitab Release 19 (Minitab Inc., State College, PA, USA), while response surfaces were generated with Statistica 10 (StatSoft, France). Analysis of variance (ANOVA) was utilized to assess the impact of various factors on the responses. The adequacy of the statistical models was confirmed by partitioning the residual sum of squares into pure error and lack-of-fit components, and the coefficient of determination (R^2) was calculated to evaluate the fit of the models. Optimization procedures were conducted using Minitab Release 19. Statistical significance was determined through ANOVA ($p < 0.05$). Differences in treatment means were further analyzed using Tukey's multiple range test to identify significant variations among groups.

Table 2 The face-centered central composite design matrix and corresponding response values for the extraction of *G. coronaria*

Entry	X_1 (h)[a]	X_2 (%)[b]	X_3 (mL/g)[c]	[d]TPC (mg GAE[f]/g dw[g])	[e]TFC (mg EQ[h]/g dw)
1	24	50	10	55.58	21.3
2	24	50	30	113.92	31.4
3	24	70	20	75.27	36.5
4	24	90	10	55.51	31.32
5	24	90	30	82.68	40.95
6	48	50	20	118.21	28.8
7	48	70	10	67.19	25.1
8	48	70	20	107.2	37.05
9	48	70	20	107	36.5
10	48	70	20	107.27	36.88
11	48	70	20	107.3	37.1
12	48	70	20	107.27	37
13	48	70	20	107.3	36.9
14	48	70	30	113.21	41.63
15	48	90	20	88.83	36.4
16	72	50	10	54.55	18.6
17	72	50	30	104.6	28.03
18	72	70	20	93.3	37.27
19	72	90	10	62.59	32.43
20	72	90	30	92.23	42.8
[i]R^2				**0.957**	**0.965**

CCDC face-centered central composite design

[a]X_1, extraction time

[b]X_2, solvent concentration

[c]X_3, liquid–solid ratio

[d]TPC, total phenolic content

[e]TFC, total flavonoid content

[f]GAE, gallic acid equivalents

[g]dw, dry weight

[h]QE, quercetin equivalents

[i]R^2, determination coefficient

Results and Discussion

Optimization of Extraction

The influence of maceration extraction parameters, namely extraction time, solvent concentration, and liquid–solid ratio, on the yield of TPC and TFC from the aerial parts of *G. coronaria* was systematically investigated. Response surface methodology (RSM) was utilized to optimize the extraction conditions, employing a face-centered central composite design (CCCD) matrix. Each response variable was precisely quantified as part of the experimental design, measuring TPC for polyphenols and TFC for flavonoids. The response values from the 20 tests conducted are presented in Table 2.

The powdered extracts of *G. coronaria* exhibited significant variations in their total phenolic and flavonoid content during the maceration extraction process using ethanol as solvent and varying extraction parameters. The values ranged from 54.55 to 118.21 mg GAE/g dw for TPC, and from 18.6 to 42.8 mg QE/g dw for TFC. Regression analysis was conducted on the experimental data to determine the significance of the model coefficients in relation to the extraction of phenolic and flavonoid compounds. Table 2 indicates R^2 values of 0.957 and 0.965 for TPC and TFC, respectively, reflecting a significant correlation between the experimental results and the model for the selected

parameters. The interaction among extraction time, solvent concentration, and liquid–solid ratio in the extraction of phenolic and flavonoid compounds is described by Eqs.1 and 2 respectively.

$$TPC = -44.642 + 2.636X_1 - 0.522X_2 + 9.266X_3 - 0.031\ {X_1}^2 + 0.004\ {X_2}^2 - 0.119\ {X_3}^2 + 0.007X_1X_2 - 0.003X_1X_3 - 0.032X_2X_3 \quad (1)$$

$$TFC = -38.895 - 0.282X_1 + 1.436X_2 + 1.69X_3 + 0.001\ {X_1}^2 - 0.009\ {X_2}^2 - 0.029\ {X_3}^2 + 0.002X_1X_2 + 0.00004X_1X_3 + 0.0003X_2X_3 \quad (2)$$

The results of the multiple regression analysis are consistent with the findings from the surface plot analysis, as demonstrated in Figs. 2 and 3. Figures 2a and 3a illustrate the effects of extraction duration and ethanol concentration on TPC and TFC, respectively. The extraction duration emerges as a significantly important quadratic variable, in addition to its positive linear effect. The findings indicate that TPC levels reached their maximum at ethanol concentrations ranging from 50 to 65% over a time interval of 45 to 60 h, while TFC levels peaked at ethanol concentrations between 80 and 90% within a time range of 25 to 72 h. In fact, the integration of water with organic solvents creates a moderately polarized environment that facilitates the interaction between the plant matrix and the solvent used for extraction (Paniwnyk et al. 2009). Figures 2b and 3b illustrate the effects of extraction time and liquid–solid ratio on TPC and TFC, respectively. TPC reached its peak after 55 h, with the liquid–solid ratio having a significant influence at < 1 g/30 mL. The highest TFC concentration was observed at 25 h, with a liquid–solid ratio of < 1 g/18 mL. Figures 2c and 3c further depict how ethanol concentration and liquid–solid ratio influence TPC and TFC. The optimal extraction of TPC occurs at ethanol concentrations below 60%, while TFC is maximized at concentrations exceeding 80%. The liquid–solid ratio is a key factor in optimizing extraction, as it influences solvent penetration and compound diffusion. Adequate solvent volume facilitates hydration and swelling of the plant matrix, promoting better solute diffusion (Cacace et al. 2003; Hemwimon et al. 2007). However, excessive solvent use can dilute target compounds and increase energy costs for solvent removal (Chadni et al. 2022). These findings facilitated the identification of optimal extraction conditions using a

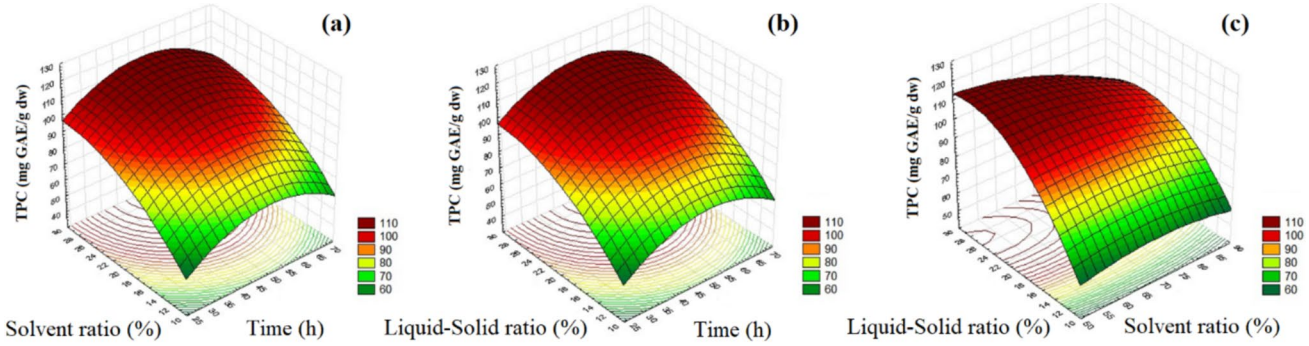

Fig. 2 (panels **a**, **b** and **c**) Response surface plots illustrating the interaction of maceration parameters on total phenolic content

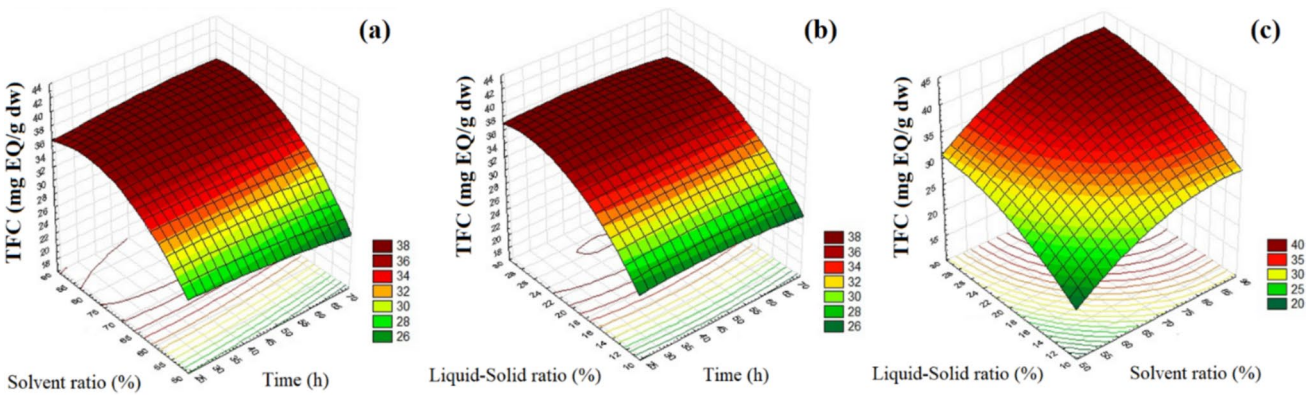

Fig. 3 (panels **a**, **b** and **c**) Response surface plots indicating the interaction of maceration parameters on total flavonoid content

desirability function, ranging from 0.95 to 1, with 1 representing the best outcome. To validate these findings, three independent replicates were performed under the optimized parameters determined by RSM. The results, presented in Table 3, confirm the effectiveness of these conditions in maximizing polyphenol and flavonoid recovery.

TPC and TFC

The *G. coronaria* extracts were analyzed to determine their total phenolic and flavonoid contents using the Folin-Ciocalteu assay and an aluminum chloride colorimetric method, respectively. Results are presented in Table 4.

The highest concentrations of TPC and TFC were found in the n-butanol extract (325.33 mg GAE/g dw and 112.5 mg QE/g dw, respectively), followed by the ethyl acetate and ethanolic extracts. Statistical analysis using ANOVA and Tukey's post hoc test confirmed significant differences in TPC and TFC levels across the *G. coronaria* extracts ($p < 0.05$). These findings clearly indicate effective separation during the liquid–liquid extraction process. The findings of this study align closely with those reported by Belhachat et al. (2023), who identified TPC concentrations in the ethanolic extract of a *G. coronaria* Algerian species with value of 164.785 mg GAE/g dw and TFC levels with value of 21.86 mg CE/mg dw. Furthermore, Mahdi et al. (2023) reported that the water extract had the highest concentrations of TPC (260.57 mg GAE/g dw) and TFC (87.83 mg QE/g dw) for a *G. coronaria* Egyptian species. The methanolic extract followed, with TPC and TFC values of 190.33 mg GAE/g dw and 31.82 mg QE/g dw, respectively. In contrast, the non-polar petroleum ether extract presented the lowest concentrations, with TPC values of 34.21 mg GAE/g dw and TFC values of 11.15 mg QE/g dw. The differences observed between the TPC and TFC values obtained in this study and those reported in the literature may be attributed to several factors, including the geographical location of the plant species, environmental conditions, plant age, harvest timing, and extraction methods (e.g. solvent and duration). Each of these factors can significantly influence the bioactive constituents present in the plant (Kalemba et al. 2024). The findings of this study confirm that an increase in solvent polarity enhances the recovery of phenolic and flavonoid compounds from plant materials (El Aanachi et al. 2020). Solvents, as n-butanol and ethyl acetate, are particularly effective for extracting polyphenols due to their greater affinity for these compounds compared to non-polar solvents (Srief et al. 2023). Although n-butanol may yield quantities of polyphenols and flavonoids that are similar to or slightly lower than those obtained with ethanol, its use can be limited due to its toxicity and unpleasant odor (Naczk and Shahidi 2004). Conversely, the lower extraction efficiency of phenolic and flavonoid compounds using chloroform and non-polar solvents can be attributed to the reduced solubility of these compounds in low-polarity solvents (Zerrouki et al. 2022).

Table 3 Optimal conditions and associated predicted and experimental values for the responses analyzed

Optimum extraction parameters		
X_1 (h)[a]	X_2 (%)[b]	X_3 (mL/g)[c]
48.95	70.22	30.00
Response variables TPC[d] (mg GAE[f]/g dw[g])		
Predicted	Experimental	
114.306	117.21 ± 1.12	
Response variables TFC[e] (mg QE[h]/g dw)		
Predicted	Experimental	
39.422	40.90 ± 0.31	

The values are expressed as the mean ± standard deviation (SD), derived from three independent replicates

[a]X_1, extraction time

[b]X_2, solvent concentration

[c]X_3, liquid/solid ratio

[d]TPC, total phenolic content

[e]TFC, total flavonoid content

[f]GAE, gallic acid equivalents

[g]dw, dry weight

[h]QE, quercetin equivalents

Table 4 Phenolic and flavonoid content of *Glebionis coronaria* extracts

Extract	TPC[a] ± SD[b] (mg GAE[c]/g dw[d])	TFC[e] ± SD (mg QE[f]/gdw)
EtOH	171.16 ± 1.32[e]	31.89 ± 0.54[e]
$CHCl_3$	13.66 ± 0.11[f]	3.21 ± 0.01[f]
AcoEt	299.33 ± 2.12[g]	99.5 ± 0.54[g]
n-BuOH	325.33 ± 2.98[h]	112.5 ± 0.75[h]

The values reported are from three independent analyses. The unrelated characters (a, b, c, or d) denote significative variations between the values ($p < 0.05$)

[a]TPC, total phenolic content

[b]SD, standard deviation

[c]GAE, gallic acid equivalents

[d]dw, dry weight

[e]TFC, total flavonoid content

[f]QE, quercetin equivalents

LC–ESI–MS/MS Characterization of Ethanol Extract

Table 5 presents the chemical compounds detected in the ethanolic extract of *G. coronaria* by LC–ESI–MS/MS analysis and prepared using extraction conditions optimized via response surface methodology. The final parameters were optimized (X_1, 48.95 h of extraction time, X_2, 70.22% of

Table 5 Identification and quantification of phenolic compounds in the ethanolic extract of *Glebionis coronaria* via LC–ESI–MS/MS analysis

No	Compound	Concentration (µg/g)	[a]RT (min)	Ion source	Ion transitions	Ion mode	[b]R^2	[c]LOQ (µg/L)	[d]LOD (µg/L)	Linearity range (µg/L)
1	Protocatechuic acid	41.7038	5.897	ESI	153.0—> 109.0	Negative	0.997	13.173	3.151	15.625–250
2	Chlorogenic acid	422.47	7.584	ESI	353.0—> 191.0	Negative	0.998	25.902	11.589	31.25–500
3	Hydroxybenzaldeyde	2.1992	7.9236	ESI	121.0—> 92.0	Negative	0.999	12.865	4.9742	15.625–250
4	Caffeic acid	30.8552	8.0313	ESI	178.9—> 135.1	Negative	0.999	24.34	6.9505	31.25–500
5	o-coumaric acid	9.3929	9.377	ESI	163.0—> 119.1	Negative	0.999	7.578	4.0983	15.625–500
6	Salicylic acid	13.2204	9.753	ESI	137.0—> 93.1	Negative	0.998	82.465	47.669	112.5–1800
7	Protocatechuic ethyl ester	2.2708	11.575	ESI	181.0—> 137.0	Negative	0.997	14.0	4.1	15.625–250
8	Hesperidin	148.74	11.616	ESI	611.0—> 302.9	Positive	0.996	17.687	4.1396	31.25–500
9	Isoquercitrin	3.6443	11.656	ESI	464.9—> 302.8	Positive	0.998	11.268	9.9382	18.75–300
10	Kaempferol-3-glucoside	1.7989	13.198	ESI	448.8—> 286.9	Positive	0.999	4.5238	1.1609	7.8125–125
11	Trans-cinnamic acid	3.4441	14.289	ESI	149.0—> 131.1	Positive	0.999	22.132	11.185	31.25–500
12	Quercetin	11.039	14.838	ESI	300.8—> 151.0	Negative	0.996	16.834	4.6558	27.5–440

[a]RT: retention time

[b]R^2: coefficient of determination

[c]LOD/[d]LOQ (µg/L): Limit of detection/quantification

ethanol concentration, and X_3, 30 mL/g of solvent-to-material ratio). A total of 12 compounds were identified based on their retention times and characteristic ion transitions.

The findings from the LC–ESI–MS/MS analysis are in agreement with the measurements of TPC and TFC obtained from the Folin-Ciocalteu assay and the aluminum chloride method, respectively, highlighting the considerable abundance of phenolic and flavonoid compounds in *G. coronaria*. In fact, the aerial parts of *G. coronaria* are known to contain numerous flavonoids and polyphenolic compounds, which play a crucial role in helping the plant cope with both biotic and abiotic stresses (Wan et al. 2017). The prominent concentration of these compounds further suggests their essential contribution to the therapeutic properties of the extract. In fact, the extraction of various phenolic compounds at significant concentrations is supported by the implementation of optimized extraction methods, which established the optimal conditions necessary for enhanced quantitative and qualitative yields, thereby maximizing the recovery of valuable phytochemicals. The results presented in Table 5 revealed chlorogenic acid as the most abundant compound with a concentration of 422.47 µg/g, followed by hesperidin (148.74 µg/g). Several studies (Chuda et al. 1998; Hosni et al. 2013; Sulas et al. 2017; Kellal et al. 2023; Belmamoun et al. 2024) have confirmed the presence of chlorogenic acid in the aerial parts of *G. coronaria*. Notably, Kellal et al. (2023) highlight the prominence of this compound as a key chemical constituent of the plant, illustrating its potential significance in the plant's biological activity and therapeutic applications. In fact, chlorogenic acid is an ester of caffeic acid with quinic acid, found in a variety of fruits and vegetal species, and has a notable antioxidant potential (Sulas et al. 2017). Furthermore, it exerts antibacterial, hepatoprotective, cardioprotective, anti-inflammatory, antipyretic, neuroprotective, anti-obesity, antiviral, antimicrobial, and antihypertensive properties (Singh et al. 2023). Additionally, this phenolic compound serves as a potent free radical scavenger and exhibits stimulatory effects on the central nervous system (Naveed et al. 2018). The findings of this study align with numerous studies that have identified the presence of bioactive compounds such as *o*-coumaric acid, protocatechuic acid, quercetin, caffeic acid, hydroxycinnamic acid, and kaempferol in *G. coronaria* or other species within its genus (Farag et al. 2015; Sulas et al. 2017; Kandylis 2022; Youssef et al. 2023; Belmamoun et al. 2024). Kandylis, (2022) identified protocatechuic acid as one of the major compounds present in *G. coronaria* flowers, while quercetin was reported as a predominant compound in other species within the same genus. However, Belmamoun et al. (2024) identified *o*-coumaric acid and chlorogenic acid as the predominant compounds in the methanolic extract of *G. coronaria* leaves, accounting for 9.55% and 6.00% of the total identified compounds, respectively. The differences in the identified compounds may arise from variations in plant parts, extraction methods, or environmental conditions, which are known to significantly influence the phytochemical composition of plant species (Altemimi et al. 2017). In fact, these compounds have demonstrated notable therapeutic effects in previous research (Patel et al. 2018; Naveed et al. 2018). In particular, hesperidin has been recognized for its antioxidant and anticancer properties (Aggarwal et al. 2020) and exhibits inhibitory effects on neurodegenerative diseases (Hajialyani et al. 2019). In addition, protocatechuic acid is notable for its significant antioxidant and anticancer activities (Patel et al. 2018). The presence of these components imparts therapeutic properties to the plant, thereby encouraging further investigation into the potential benefits of these extracts (Fig. 4).

Biological Activities

Antioxidant Activity

The antioxidant activity of *G. coronaria* extracts was assessed using two proton transfer methods (DPPH and ABTS) and three electron transfer methods (FRAP, phenanthroline, and SNP). The evaluation of antioxidant activity is essential for determining the potential biological applications of *G. coronaria*, as oxidative stress is implicated in various pathological conditions. Given that phenolic and flavonoid compounds are major contributors to antioxidant mechanisms (Treml et al. 2016; Benabderrahim et al. 2019), assessing their activity provides valuable insight into the therapeutic potential of the plant. The results measured in terms of IC_{50} or $A_{0.5}$ are presented in Table 6, and compared against various reference standards to match each assay's redox mechanism, ensuring accurate benchmarking of radical scavenging and electron transfer activities.

Despite exhibiting lower antioxidant activity compared to standard references, *G. coronaria* extracts demonstrated promising free radical scavenging potential. The n-butanol extract showed the highest activity among the tested fractions, with IC_{50} values of 13.89 µg/mL and 29.18 µg/mL in the DPPH and ABTS assays, respectively, approaching the efficacy of BHT. Additionally, its performance in the phenanthroline and SNP assays (with $A_{0.5}$ values of 7.23 µg/mL and 171.24 µg/mL, respectively) highlights its ability to engage in multiple antioxidant mechanisms. Furthermore, the ethyl acetate extract exhibited the highest antioxidant activity in the FRAP assay, with an $A_{0.5}$ value of 29.16 µg/mL, highlighting its potent ferric-reducing ability compared to the standard α-tocopherol. The variation in antioxidant activity among fractions can be attributed to the differing polarity values of the solvents used in this study (Derbak et al. 2024). The significant antioxidant activity observed through various methods can be attributed to the substantial

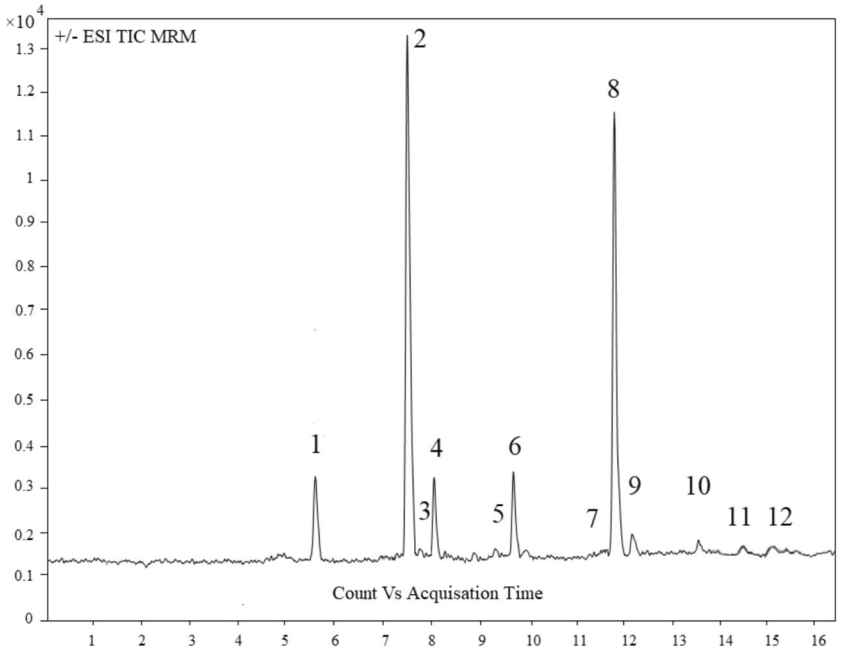

Fig. 4 Chemical profile of the ethanolic extract of *Glebionis coronaria*, characterized by LC–ESI–MS/MS. 1—Protocatechuic acid, 2—Chlorogenic acid, 3—Hydroxybenzaldehyde, 4—Caffeic acid, 5—o-Coumaric acid, 6—Salicylic acid, 7—Protocatechuic ethyl ester, 8—Hesperidin, 9—Isoquercitrin, 10—Kaempferol-3-glucoside, 11—trans-Cinnamic acid, 12—Quercetin

Table 6 In vitro antioxidant activity of *Glebionis coronaria* extracts

Extracts and standards	DPPH IC_{50} (μg/mL)	ABTS IC_{50} (μg/mL)	FRAP $A_{0.5}$ (μg/mL)	Phenanthroline $A_{0.5}$ (μg/mL)	SNP $A_{0.5}$ (μg/mL)
EtOH	108.79 ± 0.65[a]	67.46 ± 2.13[a]	192.71 ± 1.01[a]	>200	>400
$CHCl_3$	>800	350.14 ± 0.45[b]	>200	120.31 ± 1.42[a]	>400
AcoEt	21.14 ± 0.19[b]	30.46 ± 0.23[c]	29.16 ± 0.17[b]	27.86 ± 0.56[b]	295.32 ± 1.53[a]
n-BuOH	13.89 ± 0.12[c]	29.18 ± 0.17[d]	34.16 ± 0.21[c]	7.23 ± 0.23[c]	171.24 ± 1.65[b]
BHA	6.35 ± 0.13[d]	12.11 ± 0.10[e]	NT	1.04 ± 0.14[d]	NT
BHT	12.61 ± 0.34[e]	1.28 ± 0.05[f]	NT	2.24 ± 0.06[e]	NT
α-Tocopherol	NT	NT	34.43 ± 0.50[c]	NT	NT
Ascorbic acid	NT	NT	6.50 ± 0.50[d]	NT	7.14 ± 0.12[c]
Trolox	NT	NT	NT	NT	34.17 ± 1.03[d]

The values within the columns, denoted by unrelated characters (a, b, c, d, e, or f), exhibit significant differences ($p < 0.05$)

BHT butylhydroxyltoluene, *BHA* butylhydroxyanisole, *NT* not tested

quantities of phenolic and flavonoid compounds detected using the Folin-Ciocalteu and aluminum chloride methods. These compounds were further identified and quantified for the ethanolic extract through LC–ESI–MS/MS analysis, highlighting their crucial role in contributing to the antioxidant properties of the plant extracts. Indeed, the phenolic and flavonoid compounds are considered among the most promising and quantifiable phytochemical constituents within plant secondary metabolites (Al-Owaisi et al. 2014). In fact, chlorogenic acid exhibits strong radical scavenging and metal-chelating properties, comparable to ascorbic acid (Rojas-González et al. 2022; Nakatani et al. 2000). In addition, hesperidin and caffeic acid further enhance antioxidant capacity, as confirmed by in vitro assays (Lou et al. 2014; Khan et al. 2016). Additionally, *G. coronaria* contains beta-carotene, a precursor of vitamin A, known for its role in reducing oxidative stress (Ivanshchenko et al. 2019). The combined action of these bioactive molecules contributes to the plant's ability to mitigate oxidative stress-related diseases.

Anti-Cancer Activity

Oxidative stress is strongly associated with the development of various degenerative diseases, including cancer and age-related disorders (Akash et al. 2014). To further explore the potential biological applications of *G. coronaria*, its ethanol extract was evaluated for antiproliferative activity against CAPAN-1 (pancreatic cancer), dld-1 (colorectal adenocarcinoma), and L929 (fibroblast) cell lines. CAPAN-1 and

dld-1 were selected as oxidative stress plays a key role in the progression of pancreatic and colorectal cancers, making antioxidant-rich extracts potential candidates for therapeutic investigation (Mohammed et al 2023; Cancemi et al. 2023). L929 fibroblasts were included as a non-cancerous control to assess selective cytotoxicity. The concentration range used in this assay (0.25 to 1 mg/mL) was chosen based on preliminary screening results, which showed that lower concentrations did not elicit measurable cytotoxic effects. This range allowed for accurate estimation of the IC_{50} values while encompassing both sub-effective and near-maximal responses, ensuring a reliable dose–response evaluation. The results are detailed in Table 7 and Fig. 5.

The ethanolic extract of *G. coronaria* demonstrated a cytotoxic activity against the CAPAN-1 pancreatic cancer cell line, reducing cell viability to 38.53% at a concentration of 1 mg/mL (Table 7). This suggests a potential therapeutic effect against pancreatic cancer. Interestingly, the extract showed minimal cytotoxicity toward the dld-1 colorectal adenocarcinoma cell line, with cell viability percentages exceeding 100%. Furthermore, the L929 fibroblast cell line exhibited significantly increased viability, indicating that the extract may have a protective or proliferative effect on normal cells. These findings suggest that, at the tested concentration, *G. coronaria* exhibits promising therapeutic potential against pancreatic cancer, as it demonstrates selective cytotoxicity toward cancer cells while maintaining higher viability in normal cells. This selective effect may provide a valuable avenue for developing targeted treatments that minimize damage to healthy tissues, thus enhancing the safety and efficacy of cancer therapies. In fact, medicinal plants hold considerable promise as innovative anticancer and antiproliferative therapies, largely due to their robust anticarcinogenic and chemoprotective properties. The bioactive compounds within these plants demonstrate reduced toxicity, offering effective pathways for cancer prevention

Table 7 Cytotoxic effect of *Glebionis coronaria* ethanolic extract on the tested cell lines

Concentration (mg/mL)	Cell viability (%)		
	CAPAN-1	dld-1	L929
1.0	38.53 ± 1.20^{a}	105.08 ± 1.87^{a}	215.64 ± 3.45^{a}
0.5	183.25 ± 2.87^{b}	145.18 ± 2.14^{b}	353.71 ± 6.35^{b}
0.25	186.52 ± 3.15^{c}	199.69 ± 3.14^{c}	376.24 ± 5.78^{c}
0.125	189.06 ± 3.65^{d}	224.76 ± 4.25^{d}	375.85 ± 5.21^{d}
Extract IC_{50} (mg/mL)	0.97	> 1	> 1
Doxorubicin IC_{50} (µg/mL)	16.34	18.19	18.85
Control absorbance	0.242 ± 0.001	0.239 ± 0.002	0.122 ± 0.002

Different letters (a, b, c, or d) signify statistically significant differences between the values ($p < 0.05$)

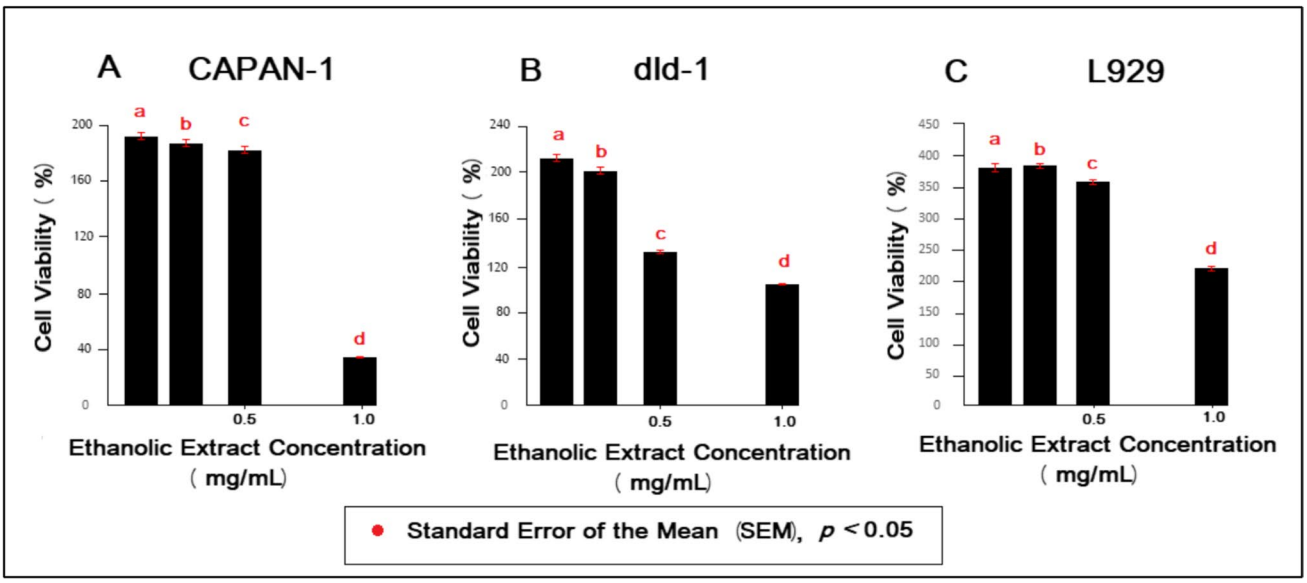

Fig. 5 (panels **a**, **b** and **c**) Cytotoxic effect of *Glebionis coronaria* ethanolic extract against CAPAN-1, dld-1 and L929 cell lines. Different letters (a, b, c, or d) signify statistically significant differences between the values ($p < 0.05$)

and treatment (Gezici and Sekeroglu 2002). In the current investigation, the TPC and TFC determination, supported by LC–ESI–MS/MS analysis for the ethanolic extract, demonstrated that *G. coronaria* is rich in bioactive compounds with promising anticancer and antiproliferative properties. In fact, polyphenols have demonstrated potential in cancer prevention by mitigating the damaging effects of free radicals on cells, primarily through their scavenging abilities. Their diverse chemical structures contribute to their versatility in neutralizing free radical activities, thus reducing oxidative stress to levels that prevent harm to cellular DNA and the synthesis of regulatory proteins essential for coordinated cellular functions (Brglez Mojzer et al. 2016; Dzah et al. 2020). These compounds have the capacity to influence and regulate various biochemical processes and mechanisms involved in carcinogenesis. Furthermore, they function as biological response modifiers, enhancing immune system activity and protecting cells from free radical-induced damage (Niedzwiecki et al. 2016). Experimental evidence also suggests that polyphenols inhibit cancer progression, as they can slow the advancement of malignancy and promote the repair of damaged cells (Tabrez et al. 2013). Furthermore, polyphenols demonstrate selective protection for normal cells, exhibit cytotoxicity toward cancer cells, and modulate growth factor-receptor interactions and cell signaling pathways that regulate the survival of healthy cells and the apoptosis of malignant cells (Brglez Mojzer et al. 2016). Additionally, chlorogenic acid, identified as the major compound by LC–ESI–MS/MS analysis, has been reported to exhibit antiproliferative activity against a range of cancer cell lines, including MDA-MB-231, T47D, 4 T1, EMT6, BT-549, MCF-7, MCF-10 A, and the pancreatic cancer cell lines PANC-1 and AsPC-1, through mechanisms of action that include inducing apoptosis, causing mitochondrial dysfunction, and modulating pathways involving Bcl-2, caspase-9, and other related factors (Gupta et al. 2022). The findings of this study are consistent with those reported by Lee et al. (2002), who demonstrated the antiproliferative effects of sesquiterpene lactones, specifically dihydrochrysanolide derivatives isolated from *G. coronaria*, against human cell lines A549, PC-3, and HCT-15. This concordance reinforces the potential anticancer activity of *G. coronaria*. Therefore, further investigations are essential to thoroughly evaluate the therapeutic potential of this plant and its bioactive compounds in cancer treatment.

Pharmacokinetics and Bioavailability Evaluation

Pharmacokinetic and bioavailability evaluation are frequently investigated to prevent drug failures during later

Table 8 ADME characteristics of compounds identified from *Glebionis coronaria*

Entry	Bioactive molecules of *G. coronaria* ethanolic extract											
	1	2	3	4	5	6	7	8	9	10	11	12
Physicochemical characteristics and lipophilicity												
Molecular weight	154.12	354.31	122.12	180.16	164.16	138.12	182.17	610.56	464.38	448.38	148.16	302.24
No. Rotatable bonds	1	5	1	2	2	1	3	7	4	4	2	1
No. H-bond acceptors	4	9	2	4	3	3	4	15	12	11	2	7
No. H-bond donors	3	6	1	3	2	2	2	8	8	7	1	5
TPSA (Å2)	77.76	164.75	37.3	77.76	57.53	57.53	66.76	234.29	210.51	190.28	37.3	131.36
Consensus Log Po/w	0.65	− 0.38	1.17	0.93	1.4	1.24	1.4	− 0.72	− 0.25	− 0.25	1.79	1.23
Bioavailability, pharmacokinetics, and druglikeness evaluation												
GI absorption	High	Low	High	High	High	High	High	Low	Low	Low	High	High
BBB permeant	No	No	Yes	No	Yes	Yes	Yes	No	No	No	Yes	No
CYP1 A2 inhibitor	No	No	No	No	No	No	No	No	No	No	No	Yes
CYP2 C19 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No
CYP2 C9 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No	No	No	No	No	No	Yes
CYP3 A4 inhibitor	Yes	No	No	No	No	No	No	No	No	No	No	Yes
log Kp (cm/s)	− 6.42	− 8.76	− 6.09	− 6.58	− 5.86	− 5.54	− 6.1	− 10.12	− 8.88	− 8.52	− 5.69	− 7.05
Bioavailability score	0.56	0.11	0.55	0.56	0.85	0.85	0.55	0.17	0.17	0.17	0.85	0.55
Synthetic accessibility	1.07	4.16	1	1.81	1.85	1	1.57	6.34	5.32	5.29	1.67	3.23

TPSA topological polar surface area, *Log P* lipophilicity, *GI* gastrointestinal, *BBB* blood-brain barrier

1—Protocatechuic acid, 2—Chlorogenic acid, 3—Hydroxybenzaldehyde, 4—Caffeic acid, 5—o-Coumaric acid, 6—Salicylic acid, 7—Protocatechuic ethyl ester, 8—Hesperidin, 9—Isoquercitrin, 10—Kaempferol-3-glucoside, 11—trans-Cinnamic acid, 12—Quercetin

development phases. The 12 bioactive molecules identified in *G. coronaria* ethanolic extract through LC–ESI–MS/MS analysis display a range of physicochemical characteristics that are essential for drug design (Table 8). Compounds such as hydroxybenzaldeyde (MW 122.12 Da, TPSA 37.3 $Å^2$) and trans-cinnamic acid (MW 148.16 Da, TPSA 37.3 $Å^2$) possess relatively low molecular weights and TPSA, aligning well with Lipinski's Rule of Five, which supports good membrane permeability and oral bioavailability (Lipinski et al. 1997). Conversely, larger molecules, such as hesperidin (MW 610.56 Da, TPSA 234.29 $Å^2$) and isoquercitrin (MW 464.38 Da, TPSA 210.51 $Å^2$), exhibit high TPSA and molecular weight, which may restrict passive diffusion across biological membranes, resulting in their observed low GI absorption (Varma et al. 2005). The lipophilicity of these compounds, indicated by their consensus Log Po/w, further influences their absorption potential. Trans-cinnamic acid, with a Log Po/w of 1.79, suggests a balance between lipophilicity and hydrophilicity, supporting good absorption, while chlorogenic acid, with a negative Log Po/w of − 0.38, indicates a hydrophilic nature, which may reduce membrane permeability and contribute to low GI absorption. Moreover, the GI absorption profile highlights that most molecules, including protocatechuic acid, hydroxybenzaldeyde, caffeic acid, o-coumaric acid, salicylic acid, protocatechuic ethyl ester, trans-cinnamic acid, and quercetin, exhibit high absorption potential. Considering, o-coumaric acid, with a moderate MW (164.16 Da) and TPSA (57.53 $Å^2$), fulfills criteria that favor high GI absorption. Molecules with low GI absorption, such as hesperidin (high MW and TPSA), are typically more challenging to formulate for oral administration due to reduced permeability through the intestinal membrane (Aungst 1993). For therapeutic applications, these findings indicate that while most compounds are viable for oral delivery, alternatives may be needed for those with low GI absorption scores (e.g., hesperidin, isoquercitrin, and kaempferol-3-glucoside). In addition, only a subset of molecules, specifically hydroxybenzaldeyde, o-coumaric acid, salicylic acid, protocatechuic ethyl ester, and trans-cinnamic acid, demonstrate BBB permeability. This feature is especially relevant for molecules intended for systemic therapies where limited CNS exposure is beneficial, as it reduces the risk of central side effects (Upadhyay 2014). Salicylic acid, for instance, with a Log Po/w of 1.24 and a low TPSA (57.53 $Å^2$), supports BBB penetration. On the other hand, high TPSA molecules like hesperidin (234.29 $Å^2$) show poor BBB permeability, indicating limited CNS distribution. Indeed, minimal inhibition across cytochrome P450 enzymes was observed, with only protocatechuic acid and quercetin showing CYP3 A4 inhibition. CYP3 A4, a key enzyme in hepatic metabolism, plays a significant role in drug-drug interactions (Pal and Mitra 2006). Quercetin, showing inhibitory activity for both CYP2D6 and CYP3 A4, suggests a higher risk for metabolic interactions than other compounds, which might be relevant for polypharmacy considerations. Most compounds do not inhibit CYP450 enzymes, suggesting a favorable metabolic profile with potentially lower risks of drug-drug interactions, aligning with favorable

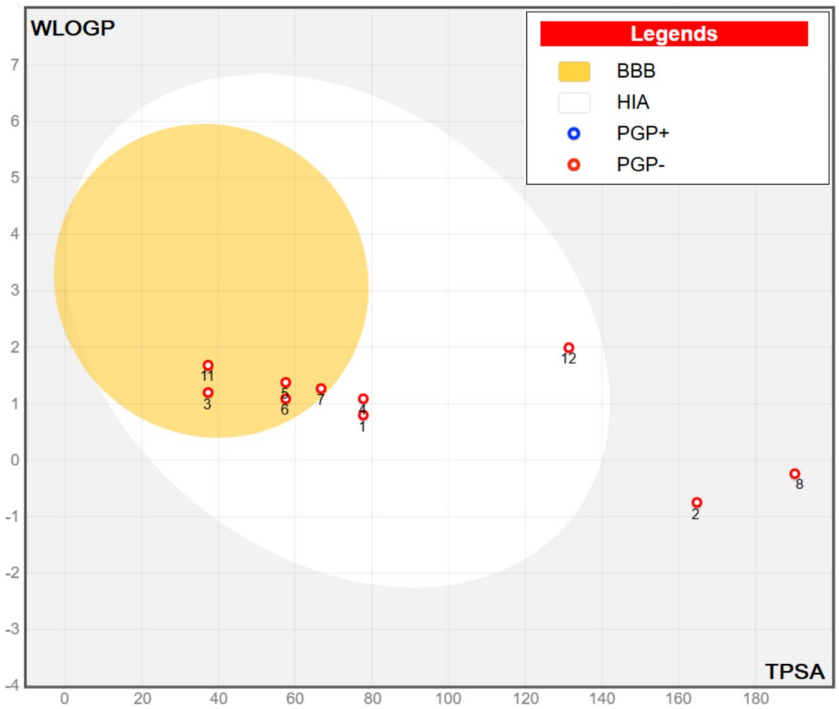

Fig. 6 Boiled-egg model of the molecules detected and quantified by LC–ESI–MS/MS analysis in the ethanolic extract of *Glebionis coronaria*. 1—Protocatechuic acid, 2—Chlorogenic acid, 3—Hydroxybenzaldehyde, 4—Caffeic acid, 5—o-Coumaric acid, 6—Salicylic acid, 7—Protocatechuic ethyl ester, 8—Hesperidin, 9—Isoquercitrin, 10—Kaempferol-3-glucoside, 11—trans-Cinnamic acid, 12—Quercetin

drug-likeness properties. The bioavailability scores of these compounds range from 0.11 (Chlorogenic acid) to 0.85 (o-coumaric acid, salicylic acid and trans-cinnamic acid). Molecules with higher bioavailability scores, such as trans-cinnamic acid, are promising for oral bioavailability, suggesting they could be administered effectively via the oral route (Ayvaz et al. 2022). Protocatechuic acid, caffeic acid, and quercetin, with moderate bioavailability scores (around 0.55), also show potential for good bioavailability under optimized conditions. Synthetic accessibility scores indicate how readily these compounds might be produced. Hydroxybenzaldeyde, with a synthetic accessibility score of 1.00, suggests ease of synthesis, whereas hesperidin, with a score of 6.34, may present challenges for scalable synthesis. Figure 6 illustrates the distribution of molecules from the ethanolic extract of *G. coronaria* according to the boiled-egg model. This model was used to assess how the molecules interact with different phases, providing insights into their potential bioactivity and partitioning behavior within the system.

Conclusion

The present study highlights the significant optimization of extraction conditions for phenolic and flavonoids compounds from *Glebionis coronaria* using ethanol and liquid–liquid fractionation with solvents of varying polarity. The n-butanol fraction exhibited the highest total phenolic and flavonoid content, alongside potent antioxidant and metal-chelating activities, underscoring its potential for therapeutic applications. Cytotoxicity assays revealed significant anticancer effects of the ethanolic extract against the CAPAN-1 cell line, while minimal toxicity was observed in the healthy L929 cell line. LC–ESI–MS/MS analysis identified several bioactive compounds, with chlorogenic acid as the major constituent, known for its strong antioxidant and anticancer properties. The pharmacokinetic and bioavailability profiles of these compounds suggest their favorable absorption and distribution, further supporting their potential as therapeutic agents. These findings offer promising prospects for the development of *Glebionis coronaria* as a source of bioactive compounds with applications in cancer therapy and other health-related fields. Future research should focus on exploring the full therapeutic potential of these compounds, including in vivo studies and their application in medicinal formulations.

Acknowledgements This work was supported and funded by the Deanship of Scientific Research at Imam Mohammad Ibn Saud Islamic University (IMSIU) (grant number IMSIU-DDRSP2501).

Author Contributions "I.D., H.B. Conceptualization; R.A., F.B. Data curation; I.D. F.B. Formal analysis; H.B. Funding acquisition; L.D. I.Y. Investigation; H.B. Methodology; H.B. Project administration; R.A., K.R. Resources; K.R. Software; H.B., S.G. Supervision; H.B. Validation; I.D. Visualization; L.D. Writing – original draft; H.B., S.G. Writing – review and editing. All authors reviewed the manuscript."

Funding Open access funding provided by Università degli Studi di Roma La Sapienza within the CRUI-CARE Agreement. This work was supported and funded by the Deanship of Scientific Research at Imam Mohammad Ibn Saud Islamic University (IMSIU) (grant number IMSIU-DDRSP2501).

Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to Participate Not applicable.

Competing interests The authors declare no competing interests.

References

Aggarwal V, Tuli HS, Thakral F, Singhal P, Aggarwal D, Srivastava S et al (2020) Molecular mechanisms of action of hesperidin in cancer: recent trends and advancements. Exp Biol Med 245:486–497

Akash MSH, Rehman K, Chen S (2014) Effects of coffee on type 2 diabetes mellitus. Nutrition 30(7–8):755–763

Al-Owaisi M, Al-Hadiwi N, Khan SA (2014) GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of Moringa peregrina (Forssk.) Fiori leaves. Asian Pac J Trop Biomed 4(12):964–970

Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA (2017) Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 6(4):42

Ameer K, Shahbaz HM, Kwon JH (2017) Green extraction methods for polyphenols from plant matrices and their byproducts: a review. Compr Rev Food Sci Food Saf 16(2):295–315

Aungst BJ (1993) Novel formulation strategies for improving oral bioavailability of drugs with poor membrane permeation or presystemic metabolism. J Pharm Sci 82(10):979–987

Aydin A, Erenler R, Yılmaz B, Tekin Ş (2016) Antiproliferative effect of Cherry laurel. J Turkish Chem Soc Sect A: Chem 3(3):217–228

Ayvaz U, Gürüler H, Khan F, Ahmed N, Whangbo T, Bobomirzaevich AA (2022) Automatic speaker recognition using mel-frequency cepstral coefficients through machine learning. Comput Mater Continua,71(3)

Belhachat D, Tidjani R, Makhlouf A, Balhachat M (2023) Phytochemical screening, composition and in vitro antioxidant activity of algerian *Chrysanthemum coronarium* (L.) extracts. South Asian J f Ex Biol 13(3)

Belmamoun AR, Ammam A, Mhamdia C, Chadli R, Baki A, Tou A, ... Belhouadjeb FA (2024) *Chrysanthemum coronarium* L: chemical composition and gastroprotective potential of methanolic leaf extract in ethanol-induced gastric ulcers in male Wistar rats. Indian J Pharm Educ Res 58(1):145–153

Benabderrahim MA, Sarikurkcu C, Elfalleh W, Ozer MS (2019) *Datura innoxia* and *Dipsacus laciniatus*: biological activity and phenolic composition. Biocatal Agric Biotechnol 19:101163

Blois MS (1958) Antioxidant determinations by the use of a stable free radical. Nature 181(4617):1199–1200

Brglez Mojzer E, Knez Hrnčič M, Škerget M, Knez Ž, Bren U (2016) Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. Molecules 21(7):901

Cacace JE, Mazza G (2003) Mass transfer process during extraction of phenolic compounds from milled berries. J Food Eng 59(4):379–389

Cancemi G, Cicero N, Allegra A, Gangemi S (2023) Effect of diet and oxidative stress in the pathogenesis of lymphoproliferative disorders. Antioxidants 12(9):1674

Chadni M, Isidore E, Diemer E, Ouguir O, Brunois F, Catteau R, Cassan L, Ioannou I (2022) Optimization of extraction conditions to improve chlorogenic acid content and antioxidant activity of extracts from forced witloof chicory roots. Foods 11(9):1217

Chuda Y, Suzuki M, Nagata T, Tsushida T (1998) Contents and cooking loss of three quinic acid derivatives from garland (Chrysanthemum coronarium L.). J Agric Food Chem 46(4):1437–1439. https://doi.org/10.1021/jf9708554

Derbak L, Bendif H, Ayad R, Bensouici C, Yildiz İ, Demirtas I, ... Boufahja F (2024) Optimization of polyphenol extraction, phenolic profile by LC-ESI-MS/MS, antioxidant, anti-enzymatic, and cytotoxic activities of *Physalis acutifolia*. Open Chem 22(1):20240040

Dzah CS, Duan Y, Zhang H, Wen C, Zhang J, Chen G, Ma H (2020) The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: a review. Food Biosci 35:100547

El Aanachi S, Gali L, Nacer SN, Bensouici C, Dari K, Aassila H (2020) Phenolic contents and in vitro investigation of the antioxidant, enzyme inhibitory, photoprotective, and antimicrobial effects of the organic extracts of *Pelargonium graveolens* growing in Morocco. Biocatal Agric Biotechnol 29:101819

Farag NF, Farag MA, Abdelrahman EH, Azzam SM, El-Kashoury ESA (2015) Metabolites profiling of *Chrysanthemum pacificum* Nakai parts using UPLC-PDA-MS coupled to chemometrics. Nat Prod Res 29(14):1342–1349

Gezici S, Sekeroglu N (2019) Current perspectives in the application of medicinal plants against cancer: novel therapeutic agents. Anticancer Agents Med Chem 19(1):101–111. https://doi.org/10.2174/1871520619666181224121004

Griffith CM, Thai AC, Larive CK (2019) Metabolite biomarkers of chlorothalonil exposure in earthworms, coelomic fluid, and coelomocytes. Sci Total Environ 681:435–443

Grigoraș CD, V^ aşcaZamfir D, Vînatoru C, Mușat B, Bratu C, Dobre O, Barcanu Tudor E. ALSE:105–10

Gupta A, Atanasov AG, Li Y, Kumar N, Bishayee A (2022) Chlorogenic acid for cancer prevention and therapy: current status on efficacy and mechanisms of action. Pharmacol Res 186:106505

Hajialyani M, Hosein Farzaei M, Echeverría J, Nabavi SM, Uriarte E, Sobarzo-Sánchez E (2019) Hesperidin as a neuroprotective agent: a review of animal and clinical evidence. Molecules 24(3):648

Hemwimon S, Pavasant P, Shotipruk A (2007) Microwave-assisted extraction of antioxidative anthraquinones from roots of *Morinda citrifolia*. Sep Purif Technol 54(1):44–50

Hosni K, Hassen I, Sebei H, Casabianca H (2013) Secondary metabolites from *Chrysanthemum coronarium* (Garland) flowerheads: chemical composition and biological activities. Ind Crops Prod 44:263–271

Ivashchenko IV (2017) Chemical composition of essential oil and antimicrobial properties of *Chrysantemum coronarium* (Asteraceae). Biosystems Diversity 25(2):119–123

Ivashchenko I, Bakalova A, Rakhmetov D, Fishchenko V (2019) Biochemical peculiarities of *Glebionis coronaria* (Asteraceae) introduced in Central Polissya of Ukraine. Plant Fungal Res, 2, 32–39. Int J Environ Sci 27(1), 45–58

Kalemba MRK, Makhuvele R, Njobeh PB (2024) Phytochemical screening, antioxidant activity of selected methanolic plant extracts and their detoxification capabilities against AFB1 toxicity. Heliyon 10(2)

Kandylis P (2022) Phytochemicals and antioxidant properties of edible flowers. Appl Sci 12(19):9937

Karan T, Erenler R (2018) Fatty acid constituents and anticancer activity of Cladophorafracta (OF Müller ex Vahl) Kützing. Trop J Pharm Res 17(10):1977–1982

Kellal R, Left DB, Azzi M, Zertoubi M (2023) Insight on the corrosion inhibition performance of *Glebionis coronaria* plant extract in various acidic mediums. J Appl Electrochem 53(4):811–832

Khan FA, Maalik A, Murtaza G (2016) Inhibitory mechanism against oxidative stress of caffeic acid. J Food Drug Anal 24(4):695–702

Köktürk M, Atalar MN, Odunkıran A, Bulut M, Alwazeer D (2022) Evaluation of the hydrogen-rich water alleviation potential on mercury toxicity in earthworms using ATR-FTIR and LC–ESI–MS/MS spectroscopy. Environ Sci Pollut Res 1–15

Lee KD, Yang MS, Ha TJ, Park KM, Park KH (2002) Isolation and identification of dihydrochrysanolide and its 1-epimer from *Chrysanthemum coronarium* L. Biosci Biotechnol Biochem 66(4):862–5

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 23(1–3):3–25

Lou SN, Hsu YS, Ho CT (2014) Flavonoid compositions and antioxidant activity of calamondin extracts prepared using different solvents. J Food Drug Anal 22(3):290–295

Mahdi SK, Abdelaal M, El-Sherbeny GA, Mashaly IA, Yahia AA, Ramadan S (2023) Phytochemical content, antioxidant activity, essential oils, and antibacterial potential of Egyptian *Phlomisfloccosa* D. Don and *Glebionis coronaria* (L.) Cass. exSpach. Catrina: Int J Environ Sci 27(1):45–58

Mhadhbi N, Dgachi S, Belgacem S, Ahmed AB, Henry N, Loiseau T, ... Naïli H (2023). Design, theoretical study, druggability, pharmacokinetics and properties evolution of a new organobromocadmate compound as prospective anticancer agent. J Mol Struct 1274:134439

Mohammed HA, Emwas AH, Khan RA (2023) Salt-tolerant plants, halophytes, as renewable natural resources for cancer prevention and treatment: roles of phenolics and flavonoids in immunomodulation and suppression of oxidative stress towards cancer management. Int J Mol Sci 24(6):5171

Müller L, Gnoyke S, Popken AM, Böhm V (2010) Antioxidant capacity and related parameters of different fruit formulations. LWT-Food Sci Technol 43(6):992–999

Naczk M, Shahidi F (2004) Extraction and analysis of phenolics in food. J Chromatogr A 1054(1–2):95–111

Nakatani N, Kayano SI, Kikuzaki H, Sumino K, Katagiri K, Mitani T (2000) Identification, quantitative determination, and antioxidative activities of chlorogenic acid isomers in prune (*Prunus domestica* L). J Agric Food Chem 48(11):5512–5516

Naveed M, Hejazi V, Abbas M, Kamboh AA, Khan GJ, Shumzaid M, ... XiaoHui Z (2018) Chlorogenic acid (CGA): a pharmacological review and call for further research. Biomed Pharmacother 97:67–74

Niedzwiecki A, Roomi MW, Kalinovsky T, Rath M (2016) Anticancer efficacy of polyphenols and their combinations. Nutrients 8(9):552

Oyaizu M (1986) Antioxidative activities of browning reaction prepared from glucosamine. Jpn J Nutr 44:307–315

Özyürek M, Güngör N, Baki S, Güçlü K, Apak R (2012) Development of a silver nanoparticle-based method for the antioxidant capacity measurement of polyphenols. Anal Chem 84(18):8052–8059

Pal D, Mitra AK (2006) MDR-and CYP3A4-mediated drug–drug interactions. J Neuroimmune Pharmacol 1:323–339

Paniwnyk L, Cai H, Albu S, Mason TJ, Cole R (2009) The enhancement and scale up of the extraction of anti-oxidants from *Rosmarinus officinalis* using ultrasound. Ultrason Sonochem 16(2):287–292

Patel K, Singh GK, Patel DK (2018) A review on pharmacological and analytical aspects of naringenin. Chin J Integr Med 24:551–560

POWO (2024) Glebionis coronaria. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:1013480-1

Quezel P, Santa S (1962) New flora of Algeria and southern desert regions

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

Rojas-González A, Figueroa-Hernández CY, González-Rios O, Suárez-Quiroz ML, González-Amaro RM, Hernández-Estrada ZJ, Rayas-Duarte P (2022) Coffee chlorogenic acids incorporation for bioactivity enhancement of foods: a review. Molecules 27(11):3400

Singh SK, Thakur K, Sharma V, Saini M, Sharma D, Vishwas S, ... Gadewar MM (2023) Exploring the multifaceted potential of chlorogenic acid: journey from nutraceutical to nanomedicine. South Afr J Bot 159:658–677

Singh R, Khanna L (2021) Glebionis coronaria (L.) Cass. exSpach (Asteraceae)-a new fabric dye with potential antibacterial properties. J Indian Chem Soc 98(11):100193

Srief M, Bani M, Mokrani EH, Mennai I, Hamdi M, Boumechhour A, ... Akkal S (2023) Evaluation of in vitro and in silico anti-Alzheimer potential of nonpolar extracts and essential oil from *Mentha piperita*. Foods 12(1):190

Sulas L, Petretto GL, Pintore G, Piluzza G (2017) Bioactive compounds and antioxidants from a Mediterranean garland harvested at two stages of maturity. Nat Prod Res 31(24):2941–2944

Szydłowska-Czerniak A, Dianoczki C, Recseg K, Karlovits G, Szłyk E (2008) Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. Talanta 76(4):899–905

Tabrez S, Priyadarshini M, Urooj M, Shakil S, Ashraf GM, Khan MS, ... Damanhouri GA (2013). Cancer chemoprevention by polyphenols and their potential application as nanomedicine. J Environ Sci Health Part C 31(1):67–98

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

Treml J, Šmejkal K (2016) Flavonoids as potent scavengers of hydroxyl radicals. Comprehensive Reviews in Food Science and Food Safety 15(4):720–738

Upadhyay RK (2014) Drug delivery systems, CNS protection, and the blood brain barrier. Biomed Res Int 2014(1):869269

Varma MV, Panchagnula R (2005) Enhanced oral paclitaxel absorption with vitamin E-TPGS: effect on solubility and permeability in vitro, in situ and in vivo. Eur J Pharm Sci 25(4–5):445–453

Wan C, Li S, Liu L, Chen C, Fan S (2017) Caffeoylquinic acids from the aerial parts of *Chrysanthemum coronarium* L. Plants 6(1):10. https://doi.org/10.3390/plants6010010

Wang F, Miao M, Xia H, Yang LG, Wang SK, Sun G (2016) J Food Nutr Res 61(1): 1265324 (1–9)

Wijaya EJ, Nathanael J, Carolan O, Adiyanto SA, Bun WB, Sahamastuti AAT (2020) Indones. J Sci Technol 44–55

Yilmaz MA (2020) Simultaneous quantitative screening of 53 phytochemicals in 33 species of medicinal and aromatic plants: a detailed, robust and comprehensive LC–MS/MS method validation. Ind Crops Prod 149:112347

Youssef HA, Ali SM, Sanad MI, Dawood DH (2023) Chemical investigation of phenolic profiles and antioxidant activity of *Chrysanthemum Morifolium*. Egypt J Chem 66(10):35–47

Zerrouki S, Mezhoud S, Yaglioglu AS, Bensouici C, Atalar MN, Demirtas I, ... Mekkiou R (2022). Antioxidant, anticancer activities, and HPLC-DAD analyses of the medicinal halophyte *Limoniastrum guyonianum* Dur. extracts. J Res Phar 26:598–608