Kirkuk University Journal for Agricultural Sciences
ISSN:2958-6585
https://kujas.uokirkuk.edu.iq

RESEARCH ARTICLE https://doi.org. 10.58928/ku26.17126

Phytochemical Constituents, Antioxidant, and Antibacterial Activities of Gapla (Bongardia Chrysogonum) Grown Wildly in Qaradagh-Sulaymaniyah

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Received:19/09/2025 **Revised: 09/10/2025** **Accepted: 08/01/2026** **Published: 29/03/2026**

ABSTRACT

This study investigated the wildly grown (gapla) *Bongardia chrysogonum* collected from Qaradagh district, Sulaymaniyah governorate, during March–April 2024, aiming to quantify the phytochemical contents and assess antioxidant and antibacterial activities in different parts (tuber bark, tubers, stems, leaves, flower buds, and flowers). Tuber bark contained the highest phenols (164.9 mg/g). Leaves recorded the highest flavonoids (27 mg/g) and glycosides (20 mg/g), while flower buds showed the highest alkaloids (23.3 mg/g). HPLC analysis of phenolic compounds revealed distinct distributions: flower buds were rich in gallic, chlorogenic and caffeic acids with (124.9, 92.6 and 98.7 µg/g), respectively. Leaves accumulated rutin, quercetin, kaempferol and ferulic acid with (124.9, 117.9, 80.9 and 87.4 µg/g), respectively. Stems contained naringenin (80.65 µg/g) and hydroxybenzoic acid (60.9 µg/g), while tubers showed a peak in apigenin (76.9 µg/g). Alkaloids profiling detected scopolamine, quinine, caffeine, and xanthine in tubers and flowers only. Tubers contained the highest levels (147.9, 116.9, 74.6, and 98 µg/g), respectively compared to flowers (74.6, 88.5, 58.9, and 0 µg/g). Nutritionally, flower buds contained the highest carbohydrates (14.5%), nitrogen (6.3%), and phosphorus (0.88%), whereas leaves had the highest potassium (3%). All parts exhibited antioxidant activity; flower buds and tuber barks were the most effective (IC_{50} = 90.2 µg/mL and 94.1 µg/mL), stronger than vitamin C (133 µg/mL). Antibacterial assays showed tuber bark most active against gram-positive bacteria at 5 mg/mL, while leaves (10-40 mg/mL) inhibited gram-negative strains. In conclusion, this wild plant is rich in bioactive compounds, demonstrating strong nutritional, antioxidant, and antibacterial potential, and represents a promising natural resource.

Keywords: Bongardia chrysogonum, Phytochemical analysis, Antioxidant activity, Antibacterial activity, HPLC for alkaloids and phenolic compounds.

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INTRODUCTION

Medicinal plants have played a central role in traditional healthcare systems across human history. Their use as therapeutic agents dates back centuries and continues to be relevant, particularly in rural and developing regions where access to modern medicine remains limited [1]. Among these, *Bongardia chrysogonum* is a perennial herbaceous plant belonging to the Berberidaceae family. It is characterized by tuberous roots measuring 4 to 8 cm in diameter, upright flowering stems reaching 20 to 70 cm in height, and distinctive leaves composed of multiple pairs of opposite lateral leaflets, ending with a larger terminal leaflet. Its bisexual, bright yellow flowers appear in loose, branched clusters at the top of slender stems [2].

Geographically, *B. chrysogonum* is widely distributed across parts of southern Europe and western and central Asia, including Greece, Cyprus, Turkey, Iran, Afghanistan, and the northwestern regions of India [3]. In the Arab world, the plant is commonly known as "Uruf-el-Deek" [4], while in the Kurdistan Region of Iraq, it is referred to as "Geple", "Kaprooka" or "Tarshauka" [3, 5]. The people of Iraqi Kurdistan are unique in using flower buds of plants compared to other countries that widely use tubers. These unopened flower buds are used in preparing various types of food, they are mixed with eggs to make omelets or prepared with yogurt to create different kinds of salads [5].

Beyond its local dietary uses, *B. chrysogonum* is known in various cultures for its medicinal properties. Its tubers are commonly prepared as aqueous infusions and traditionally used in the treatment of urinary tract disorders, prostate enlargement [6, 7], diabetes [8], epilepsy, and cancer [9]. Recent scientific observations have suggested that extracts

from its roots may exhibit notable antioxidant effects, enhancing its potential as a natural therapeutic agent [10]. Phytochemical investigations have identified a diverse range of secondary metabolites within the tubers of *B. chrysogonum*, including various alkaloids such as bongardamine, bongardine, N-acetyl bongardine, lupanine, isoquinoline derivatives, in addition to several phenolic compounds and saponins. These constituents are believed to contribute to the plant's observed pharmacological effects [11-13]. Given the increasing global concern regarding antimicrobial resistance and the urgent need to explore alternative natural remedies, further investigation into the bioactive potential of traditionally used medicinal plants has become essential. Although *B. chrysogonum* has been cited in ethnobotanical literature, comprehensive scientific evaluation remains limited. Therefore, the present study aims to provide a detailed analysis of *B. chrysogonum* collected from the Qaradagh region in Sulaymaniyah governorate, Iraq. This research focuses on quantifying the bioactive compounds present in different plant parts using high-performance liquid chromatography (HPLC), and evaluating their antioxidant and antibacterial activities against selected antibiotic-resistant bacterial strains.

Materials and Methods

Plant collection and identification

Plants were randomly collected as follows: Plant parts, including tubers, stems, leaves, flower buds, and flowers, were collected from late March to early April 2024 with 5 replications each replicate had 6 experimental units (Fig.1), from the Qaradagh area, Sulaymaniyah governorate, Kurdistan Region, Iraq (Northeastern Iraq) (Fig. 2). The plant was identified by the Iraqi National Herbarium.

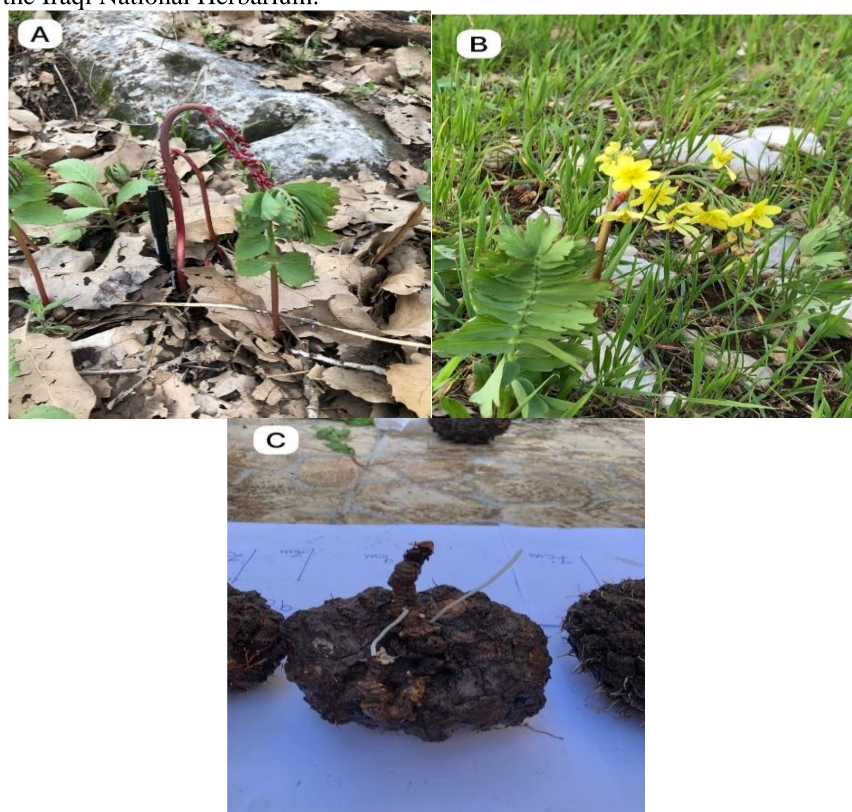

Figure 1. The plant parts of *B. Chrysogonum*: A. flower bud and stem, B. flower and leaves, C. tubers.

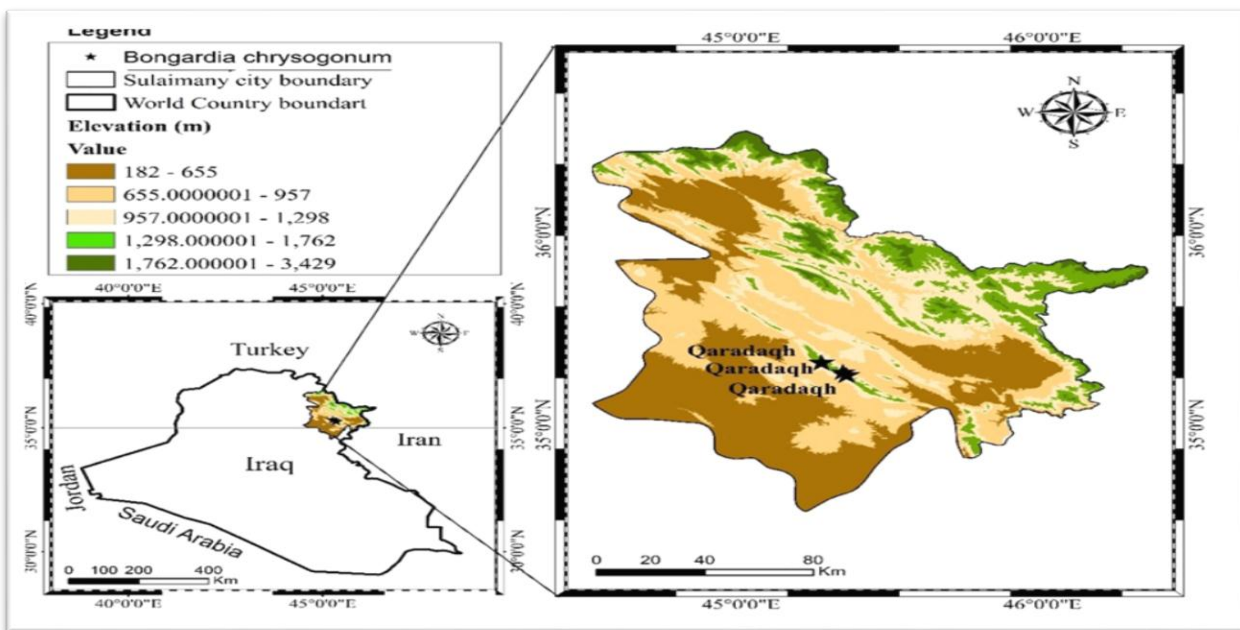

Figure 2. Map of the distribution of collection site of the *B. chrysogonum.*

Table 1. Shows monthly averages of minimum and maximum temperatures, as well as rainfall during January to June 2024 of the study location (Qaradagh).

Month	Air temperature °c		Rainfall (mm)
	Max	Min	
January	17.5	12.1	235.1
February	16.2	10	255.5
March	19	12	233.4
April	29.8	19.8	65
May	33.5	18.8	171
June	37.9	32.6	1

* The data were obtained from the meteorological station in Sulaimani.

Regarding soil characteristics, Table 2 depicts some physical and chemical characteristics of the soil taken from the study locations.

Table 2. Physical and chemical characteristics of the soil of locations under study.

Property	Units	Value
Soil depth	cm	0-30
Sand	g kg^{-1} of soil	189
Silt	g kg^{-1} of soil	437
Clay	g kg^{-1} of soil	374

Soil texture	-	Silty clay loam
Electrical Conductivity (EC)	dS m^{-1}	0.42
Soil pH	-	7.11
Organic Matters	g kg^{-1} of soil	47.12
Carbonate Minerals	g kg^{-1} of soil	211
Available (N) nitrogen	mg kg^{-1} of soil	40.67
Available (K) potassium	mg kg^{-1} of soil	188
Available (P) phosphorus	mg kg^{-1} of soil	29.74

*Soil analysis was carried out in a central laboratory for soil, water and plant analysis in the College of Agricultural Engineering Sciences / University of Baghdad.

Plant materials preparation and extraction

After collection, samples were prepared at the University of Sulaimani (Research Laboratory), washed thoroughly with running water, then separated into tubers, tuber barks, stems, leaves, and flowers. Each part was air-dried at 25 ± 2 °C in the shade with good ventilation, ground to a fine powder, and stored in sealed containers kept in a refrigerator at 4 °C for further analysis.

For extraction, 25 g of each powdered part were mixed with 250 mL of 80% ethanol and stirred for 10 min. The mixtures were incubated in a 37 °C water bath for 2 h with occasional stirring, then kept in the dark for 22 h. Filtrates were obtained using Whatman No.1 filter paper, then the extract was divided into two equal parts, one of them stored in the freezer at -8 °C for photochemical study , and the other one put in a glass to let it drying under a fan for three days, after drying stored in freezer at -8 °C for microbiological study [14].

Phytochemical analyses

Determination of total phenols

Total phenolic content was assayed in the ethanolic extracted with Folin-Ciocalteu reagent as described by [15] with some modifications. 20 µL of extract was mixed with 4 mL of 10% Folin-Ciocalteu reagent and permitted to react for (5 min) at room temperature. Then, 2 mL of 20% sodium carbonate solution was added and left for (60 min) at room temperature. In regard to blanks, the same procedure above was repeated except without the extract. Measurement was carried out by measuring with a spectrophotometer at 765 nm. Total phenolic content was expressed in milligrams of gallic acid equivalent (GAE) per gram of plant powder.

Determination of total flavonoids

The total flavonoid content was determined using a colorimetric method based on aluminum chloride, as described in references [16]. In this method, approximately 40 µL of the extract was added to 1.5 mL of 80% ethanol, followed by the addition of 0.1 mL of 10% aluminum chloride solution, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was then allowed to stand for at least 30 minutes at room temperature. A blank sample was prepared using the same reagents without the addition of the plant extract. The absorbance of the mixture was measured at 415 nm using a UV-Vis spectrophotometer. Quercetin was used to construct the calibration curve, and the results were expressed as milligrams of quercetin equivalent (QE) per gram of dry sample.

Determination of total alkaloids

The total alkaloid content was estimated by using Bromocresol Green (BCG) colorimetric assay, 1 mL of the extract was combined with 5 mL of 2N HCL and then filtered. From the filtrate, 1 mL was placed into a separating funnel, to which 10 mL of chloroform was added. The mixture was shaken well, and the organic layer was removed. The remaining solution was neutralized with 0.1 N NaOH. Subsequently, 5 mL of Bromocresol Green (BCG) reagent and 5 mL of phosphate buffer (pH 4.7) were added. The absorbance of the resulting solution was recorded at 470 nm using a UV-Vis spectrophotometer. Alkaloid concentration was quantified using a standard calibration curve, and the results were expressed as milligrams of atropine equivalent per gram of plant powder [17].

Determination of total glycosides

For the determination of glycoside content in the extract, 1 mL of the extract was mixed with 1 mL of freshly prepared Baljet's reagent: [1% picric acid (95 mL) + 10% NaOH (5 mL)]. An hour later, 20 mL of distilled water was added to dilute the solution. Then the absorbance was read at a wavelength of 495 nm using a UV-Vis spectrophotometer. The total glycoside content was approximated on the basis of a standard calibration curve prepared using securidaside and expressed as milligrams of securidaside equivalent per gram of plant powder [16, 18].

Determination of total carbohydrates

The carbohydrate percentage was determined following the phenol–sulfuric acid method [19]. For each plant part, 0.2 g of dry weight was placed in a test tube and extracted with 8 mL of 80% ethanol in a water bath at 60 °C for 30 min. The mixture was centrifuged for 15 min, and the supernatant was collected. This process was repeated three times, after which the pooled extracts were adjusted to a final volume of 25 mL with perchloric acid ($HClO_4$,1N). Subsequently, 1 mL of the extract was mixed with 1 mL of 5% phenol and 1 mL of concentrated sulfuric acid (97%), producing a brown coloration. A blank was prepared using the same procedure without plant material. Absorbance was recorded at 490 nm using a spectrophotometer, and carbohydrate concentrations were determined from the glucose standard curve. The carbohydrate percentage was calculated as:

Carbohydrates% = Concentration × dilution / Sample weight ×100

Determination of total nitrogen, phosphorus, and potassium

The digestion solution was prepared by mixing concentrated sulfuric acid (H_2SO_4) and perchloric acid ($HClO_4$) in a ratio of 4:9. One gram of powdered plant material was placed in a 100 mL glass flask, and 15 mL of the digestion solution was added. The mixture was gently heated on an electric hotplate, gradually increasing the temperature until reddish-brown nitrogen dioxide (NO_2) fumes appeared and the solution began to boil. Heating continued until the volume was reduced to 3–5 mL, taking care not to allow complete dryness. The appearance of a clear, colorless solution indicated that digestion was complete. After cooling, 20 mL of distilled water was added, and the final volume was adjusted to 100 mL with deionized water. The solution was then filtered prior to analysis.

- **Nitrogen (N)** content was determined using the Micro-Kjeldahl method.
- **Phosphorus (P)** was measured by the Molybdenum Blue method at 490 nm.
- **Potassium (K)** was quantified using Atomic Absorption Spectroscopy (AAS) [20].

Extraction and analysis of phenolic compounds using HPLC

Three grams of finely powdered plant material were extracted with 60 mL of a methanol–water mixture (40:60, v/v) and allowed to macerate for 24 hours. The mixture was then filtered, and the filtrate was concentrated under reduced pressure at 40 °C to a final volume of 5 mL. The concentrated extract was subjected to alkaline hydrolysis by adding 5 mL of 2N sodium hydroxide (NaOH) and incubating for 30 minutes. The pH was subsequently adjusted to 7.0 using 2N hydrochloric acid (HCl). Phenolic acids were extracted via liquid-liquid extraction with ethyl acetate (20 mL, three times). The combined organic phases were evaporated under vacuum, and the residue was reconstituted in 7 mL of pure methanol. A 10 µL aliquot of this solution was injected into the HPLC system for analysis. High-performance liquid chromatography (HPLC) was performed using a C18-ODS column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase consisted of 95% acetonitrile (solvent A) and 0.01% trifluoroacetic acid (solvent B), delivered at a flow rate of 1 mL/min. The gradient program was set as follows: 60% A from 0 to 5 minutes, 70% A from 5 to 7 minutes, and 40% A from 7 to 15 minutes, followed by a return to the initial conditions. Detection was carried out at 278 nm using a UV-Visible detector [21].

Extraction and analysis of alkaloid compounds using HPLC

For alkaloid analysis, 10 mg of dried sample was mixed with 50 mL of ethanol containing 25% aqueous ammonia at a ratio of 20:1. The mixture was incubated at room temperature for 30 minutes and then centrifuged. The supernatant was evaporated at 45 °C, and the residue was extracted twice with 0.7 mL of 0.1N hydrochloric acid (HCl). The combined acidic extracts were filtered through a 0.45 µm Millipore membrane. The pH of the filtrate was adjusted to 9.8 using 0.4 mL of sodium carbonate (Na_2CO_3), followed by two successive extractions with 3.0 mL of chloroform. The chloroform fractions were pooled, evaporated to dryness, and the residue was re-dissolved in 100 mL of 50% aqueous methanol. High-performance liquid chromatography (HPLC) was performed using a SYKAM system equipped with a C18–ODS column (25 cm × 4.6 mm). The mobile phase consisted of methanol and distilled water (80:20, v/v), delivered at a flow rate of 1.0 mL/min. Detection of alkaloids was carried out at 254 nm using a UV detector [22].

Antioxidant activity

The antioxidant activity was evaluated using the DPPH assay [23]. Solutions of ascorbic acid and the plant extract were prepared in 95% ethanol at concentrations of 30, 60, 120, 250, and 500 µg/mL. From each solution, 50 µL was added to 1950 µL of DPPH solution (6×10^{-5} M). The mixtures were shaken vigorously and left to stand in the dark at room temperature for 30 minutes. For the control, 50 µL of 95% methanol was combined with 1950 µL of the DPPH solution. After incubation, the absorbances of all samples were measured at 515 nm. The percentage of antioxidant activity was calculated using the formula:

Antioxidant activity (%) = (Absorbance of control - Absorbance of the sample) / (Absorbance of control) × 100.

The IC_{50} value (the concentration needed to reduce 50% of DPPH radicals) was then determined from the relationship between concentration and percentage inhibition.

Antibacterial activity

Five Multidrug-Resistant bacteria, including, gram-positive species (*Staphylococcus haemolyticus* and *Staphylococcus aureus*) and gram-negative species (*Proteus mirabilis, Klebsiella pneumoniae,* and *Escherichia coli*) were employed in the antimicrobial study, the samples were from urine infection. The isolates were identified by VITEK2 compact system at the microbiology lab of the Sulaimani Teaching Hospital.

Agar well diffusion assay

Agar well diffusion assay was conducted to analyze the antimicrobial activity of *B. chrysogonum* extracts, according to a procedure obtained from [24]. Each bacterial strain was grown overnight in nutrient broth at 37 °C. The bacterial suspension was adjusted to 1.5×10^8 CFU/mL, which is optically equivalent to 0.5 McFarland standard. Thirty (30 mL) of nutrient agar were poured in to Petri dishes and allowed to solidify at room temperature. A sterile cotton swab was used to evenly spread the freshly made bacterial inoculum throughout the whole agar surface. Wells were created using sterile gel puncture tool, and 100 µL of dry plant extract in concentration 20 mg/mL was then added to the well. After 1 hour of standing at room temperature, the plates were incubated overnight (24 hours) at 37 °C. The zones of inhibition were measured in millimetres (mm).

Minimum inhibitory concentration (MIC) & minimum bactericidal concentration (MBC)

The Minimum Inhibitory Concentration (MIC) of *B. chrysogonum* extracts was determined using the microdilution technique [25]. The MIC is defined as the lowest concentration of an antimicrobial agent that inhibits visible bacterial growth after overnight incubation. After dissolving the extract in 1% (v/v) dimethyl sulfoxide (DMSO), serial doubling dilutions of the extract were made in distilled water at concentrations varying between 1.25 mg/mL to 40 mg/mL. To find the MIC, the fresh overnight bacterial culture was adjusted to 0.5 McFarland [26]. In Eppendorf tubes, 250 µL of bacterial culture was mixed with 250 µL of extract at each concentration (1.25, 2.5, 5, 10, 20, and 40 mg/mL). Blank: 250 µL nutrient broth + 250 µL extract (per concentration). Furthermore, a positive control was administered using 250 µL of bacterial culture combined with 250 µL of nutrients broth, and a negative control was 500 µL of nutrient broth. Tubes were incubated at 37 °C for 24 hours, after which MIC values were determined by visual inspection of turbidity (indicating bacterial growth). Five replicates were tested per concentration. Additionally, 5 µL of each Eppendorf was sub-cultured in nutrient agar plates to determine the minimum bactericidal concentration (MBC). The plates were then incubated for 24 hours at 37 °C. MIC determinations were repeated at least twice for each bacterium and each tested agent in different times.

Statistical analysis

Statistical analysis was performed using the XLSTAT (Version: 2019.2.2) statistical software tool, and a Complete Randomized Design (CRD) was carried out for five replications of the phytochemical investigation using a single factor (plant parts).

Results and Discussions

Total phenols, flavonoids, alkaloids, and glycosides

The data presented in (Fig. 3) demonstrate clear differences in the distribution of phytochemicals across the various plant parts. Tuber barks were found to be the richest source of phenolic compounds, reaching (164.9 mg/g), whereas flower buds and leaves contained moderately high levels (98.17 and 90.48 mg/g, respectively). The tubers also showed a noticeable amount (66.85 mg/g), while the stem exhibited the lowest phenolic concentration at (32.72 mg/g). This distribution indicates a strong relationship between the high concentrations of phenolics and the defensive function of different plant parts. The role of phenolic compounds is well recognized in protecting plants against oxidative damage, environmental stress, microbial infection, and herbivores. Their high abundance in tuber barks, which serve as

essential structures for storage and regeneration, is consistent with the findings of [27] in their study on *Xylia xylocarpa*. Similarly, Witzell and Martín (2008) reported that phenolics in tree bark play a dual role as antioxidants and antifungal agents[28]. The quantitative phytochemical analysis performed by Gezici and Şekeroğlu (2022) reported that the ethanolic extracts of *B. chrysogonum* parts (leaves, stems, tubers) contain significant levels of polyphenols, specifically, the leaves extract) [4].

Flavonoid content was most abundant in the leaves (27 mg/g), followed by the flowers (24.63 mg/g) and flower buds (20 mg/g). The stem also contained a moderate level of flavonoids (15.54 mg/g). The lowest concentrations were detected in the tubers (5.93 mg/g) and tuber barks (4.56 mg/g), with significant difference between these two parts. The findings revealed that leaves contained the highest levels of flavonoids, likely due to their direct exposure to ultraviolet (UV) radiation and their essential role in photosynthesis. Elevated flavonoid concentrations help protect leaves from UV damage and oxidative stress [29]. This agrees with the observations of [30] in *Moringa oleifera*, where leaves were identified as the richest source of flavonoids. Likewise, [31] reported that *Clerodendrum trichotomum* leaves are abundant in flavonoids and phenolic glycosides, while Tungmunnithum *et al.* (2018) highlighted the protective role of flavonoids as a natural barrier against UV radiation [32]. The earlier research on *B. chrysogonum* conducted in Turkey indicated a significant leaf flavonoid concentration of (240.74 mg QE/g), which strongly corroborates our findings. This agreement reinforces the notion that the species is an abundant source of bioactive flavonoids. Additionally, the consistent distribution pattern, with the highest concentration in leaves, a moderate level in stems, and the lowest in roots, further supports our results.

For alkaloids, flower buds recorded the highest accumulation (23.3 mg/g), which was significantly greater than in the other plant organs. Flowers (20.5 mg/g) and leaves (18.5 mg/g) also showed relatively high amounts, while the stem contained a lower level (5.8 mg/g). The tubers (3.5 mg/g) and their bark (4.4 mg/g) contained the lowest levels without a significant difference between them. The results indicated that flower buds and flowers contained the highest concentrations of total alkaloids, which is reasonable since these are the plant's reproductive organs that require protection against microbial infection and herbivores. Similar findings were reported [33] in *Zilla spinosa* and by [31] in *Clerodendrum trichotomum*. [34] also confirmed the presence of alkaloids in the flowers of *Nyctanthus*.

Regarding glycosides, the results revealed that the leaves contained the highest amount (20 mg/g), showing a significant difference compared to other plant parts. This was followed by the flowers (13 mg/g), which did not differ significantly from the flower buds (12.4 mg/g). Moderate levels were found in the tuber bark (2.6 mg/g) and tubers (2.3 mg/g), while the lowest value was recorded in the stem (1.1 mg/g). The high concentration of glycosides in leaves can be attributed to their role as the primary site of photosynthesis, where many secondary metabolites are synthesized from carbohydrate-derived pathways. In addition, glycosides act as defensive compounds against herbivores, pathogens, and environmental stress, particularly UV radiation and oxidative damage, to which leaves are highly exposed [35, 36]. Similar results were observed in the leaves of *Moringa oleifera*, which are rich in glycosides and flavonoids [30] and in *Clerodendrum trichotomum*, where leaves contained high levels of phenolic glycosides [31]. These findings are consistent with the general view that secondary metabolites, including glycosides, tend to accumulate in metabolically active and environmentally exposed tissues such as leaves [32, 37, 38].

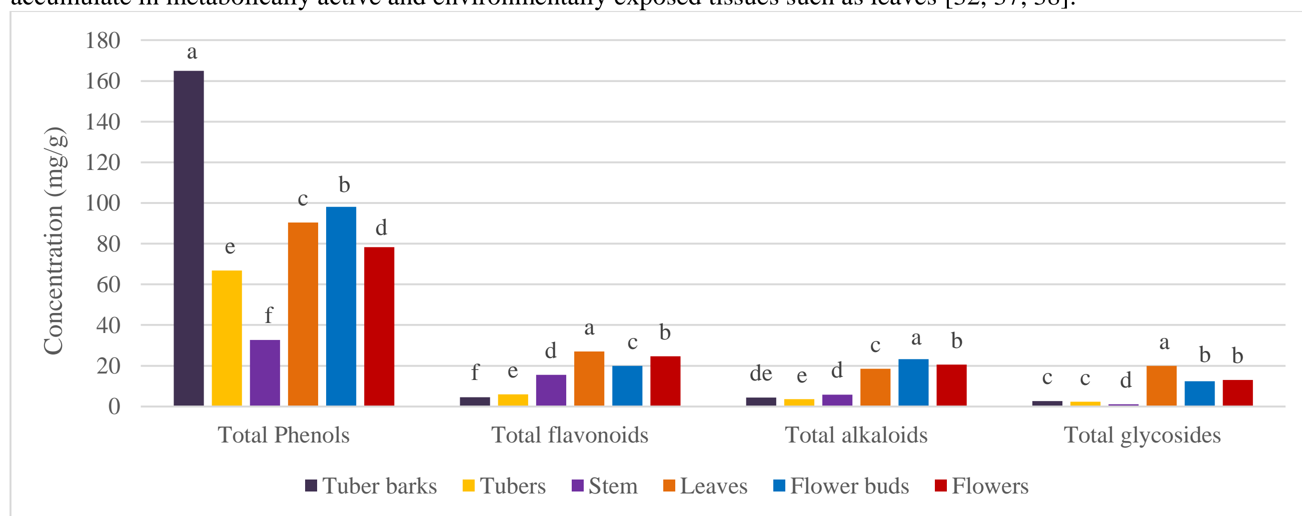

Figure 3. The total contents of phenols, flavonoids, glycosides, and alkaloids in the extracts of different plant parts of *B. chrysogonum* are presented. Different letters indicate significant differences among means according to Duncan's Multiple Range Test ($P \leq 0.05$).

Total carbohydrates, nitrogen, phosphorus, and potassium

Figure 4 demonstrates noticeable differences among the plant parts in their levels of total carbohydrates and the macronutrients N, P, and K. Carbohydrates were most abundant in the flower buds (14.5%), followed by the flowers (13.6%) and stems (13.2%), while the tuber bark contained the lowest proportion (3.3%).

Carbohydrate levels are higher in flower buds and flowers than in other plant parts due to their rapid growth and high energy demands for flowering and gamete production. These carbohydrates are temporarily accumulated in the form of sucrose and fructose, supplied from other parts [39]. Tubers mainly store starch as a long-term reserve, which may not appear clearly in rapid assays [37]. Leaves continuously produce carbohydrates but export much of it to developing organs, reducing their relative accumulation [40]. This reflects the source sink relationship, where carbohydrates are redistributed according to the metabolic needs of active tissues [41].

Nitrogen and phosphorus showed a parallel pattern, reaching their highest values in the flower buds (6.3% and 0.88%, respectively) and decreasing progressively in the leaves and flowers, with the lowest amounts again recorded in the tuber bark. The higher concentrations of nitrogen (N) and phosphorus (P) observed in flower buds and flowers can be explained by their role as highly active reproductive organs. Nitrogen is essential for the synthesis of amino acids, proteins, and enzymes that support rapid cell division and growth [42]. Phosphorus, on the other hand, is required for nucleic acids and plays a key role in energy production through ATP, which fuels metabolic and developmental processes in these tissues. In addition, the translocation of nutrients from storage organs such as tubers or from leaves to the developing reproductive structures further enhances the accumulation of N and P in buds and flowers during the flowering stage [39].

Potassium, however, exhibited a distinct distribution. The leaves accumulated the greatest percentage (3.0%), followed by the flower buds (2.87%), whereas flowers and stems, displayed nearly equal levels. The minimum potassium concentration was observed in the tuber bark (1.05%). The high concentration of potassium in leaves can be explained by its vital role in these active vegetative tissues. It is a key osmotic regulator that controls water movement and the opening and closing of stomata, which explains its accumulation in leaves [39]. It also contributes to the activation of many enzymes associated with photosynthesis and metabolism, including carbon fixation and protein synthesis [42].

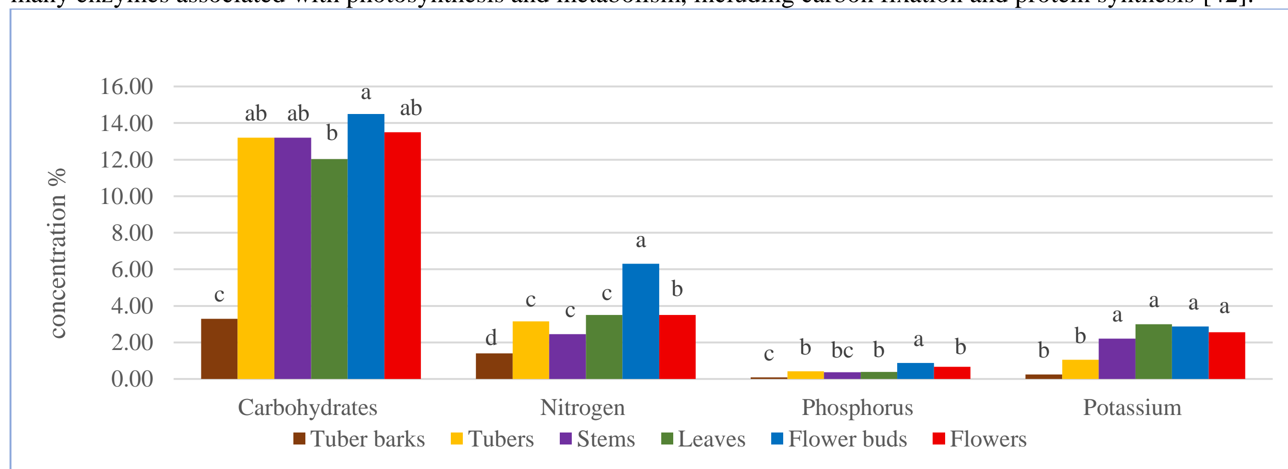

Figure 4. The total carbohydrates, Nitrogen, phosphorus, and potassium in the extracts of different plant parts of *B. chrysogonum* are presented. Different letters indicate significant differences among means according to Duncan's Multiple Range Test ($P \leq 0.05$).

HPLC analysis of phenolic compounds

HPLC analysis revealed a highly variable and diversified profile of phenolic compounds among the different plant parts of *B. chrysogonum* (Table 3). The compound accumulation was highly tissue-specific. Gallic acid was the most prevalent phenolic acid in all the examined parts, and its content was highly significant in the reproductive tissues: flower buds (124.9 µg/g) and flowers (118.8 µg/g). Similarly, quercetin was found at high levels in the above-ground components, and leaves had the maximum level (117.9 µg/g), higher than flower buds (114.7 µg/g) and flowers (98.8 µg/g). Rutin was also predominant in leaves (124.9 µg/g), and it exhibited much higher levels significantly than a group of other examined components. Kaempferol was detected in all components except the reproductive organs, and it was maximally concentrated in leaves and tubers. Naringenin was detected in all components except tubers and leaves, and it was concentrated maximally in the stem. Caffeic and chlorogenic acids were detected solely in the above-ground parts, the flower buds having the highest concentrations for both (98.7 µg/g and 92.6 µg/g, respectively). Other compounds had a more restricted occurrence. Apigenin was exclusively detected in the underground components, at higher levels in the tubers than in the tuber barks. Ferulic acid showed a significantly higher

concentration in the leaves (87.4) compared with the flower (80.2) and flower buds (79.2), whereas hydrobenzoic acid was detected exclusively in the stems (60.9 µg/g). The exclusive compartmentalization of phenolic compounds thus leans toward distinctive biological roles for each part of the plant. The compartmentalized and distinctive phenolic profile in *B. chrysogonum* is not random but reflects the specialized physiological and ecological function of plant parts. The far higher concentration of gallic acid, quercetin, and rutin in the aerial parts, particularly in leaves and reproductive organs (flower buds and flowers), is a common defense mechanism in plants. These organs are most sensitive to abiotic stressors such as UV radiation, herbivory, and pathogen infection [43]. The presence of apigenin specifically in the below-ground parts (tubers and tuber barks) may be due to its role in protecting against soil-borne pathogens or in regulating rhizosphere interactions because certain flavonoids were known to influence microbial communities in the soil [44]. Furthermore, organ-specific accumulations of ferulic acid (in leaves alone) and hydrobenzoic acid (in stems alone) suggest that there is a rigorous control of their biosynthetic pathways, perhaps based on the structural and functional demands of each organ. Ferulic acid, for instance, is a key monomer of lignin biosynthesis and plays a role in cell wall stiffness and integrity [45].

Table 3. HPLC analysis of *B. chrysogonum* parts for phenolic compounds. Different letters indicate significant differences among means according to Duncan's Multiple Range Test ($P \leq 0.05$)

Compounds	Tuber barks µg/g	Tubers µg/g	Stems µg/g	Leaves µg/g	Flower buds µg/g	Flowers µg/g
Gallic acid	80.7 c	77.9 d	70.6 e	65.4 f	124.9 a	118.8 b
Rutin	88.7 b	82.6 c	-	124.9 a	80.6 c	74.8 d
Quercetin	-	-	79.8 d	117.9 a	114.7 b	98.8 c
Kaempferol	73.6 c	80.8 a	77.4 b	80.9 a	-	-
Naringenin	70.9 c	-	80.65 a	-	77.9 b	70.8 c
Caffeic acid	-	-	-	92.5 b	98.7 a	90.8 b
Chlorogenic acid	-	-	-	-	92.6 a	81.4 b
Apigenin	71.6 b	76.9 a	-	-	-	-
Ferulic acid	-	-	-	87.4 a	79.2 c	80.2 b
Hydrobenzoic acid	-	-	60.9	-	-	-

HPLC analysis of alkaloid compounds

HPLC analysis confirmed the presence of four alkaloid compounds in the tubers of *B. chrysogonum*; scopolamine, quinine, caffeine, and xanthine were restricted to the tubers and flowers only, with the highest concentrations detected in the tubers (147.9, 116.9, 74.6, and 98 µg/g, respectively) (Fig.5). These findings are consistent with previous phytochemical reports on this plant, which determined the alkaloid compounds present in its tubers [46].

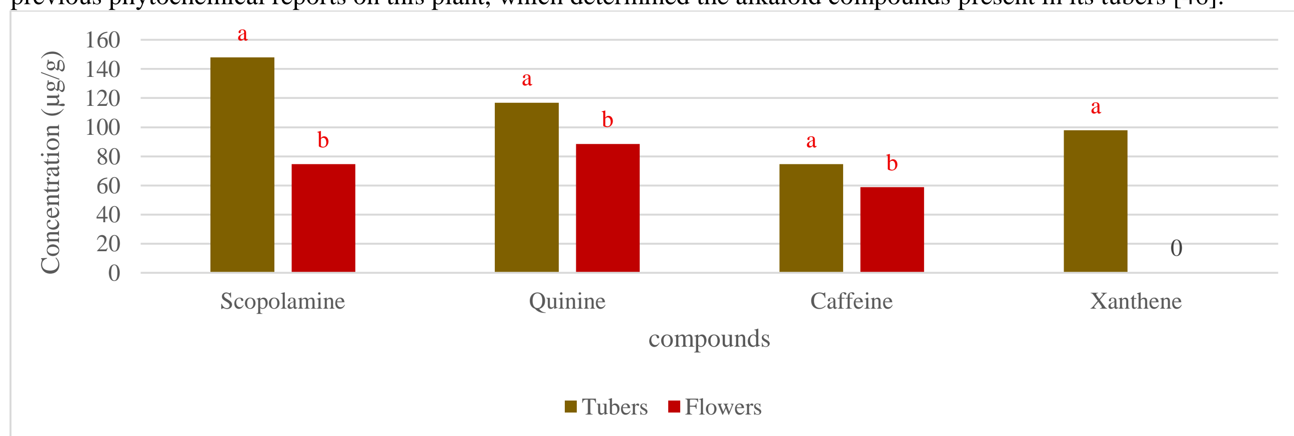

Figure 5. HPLC analysis of *B. chrysogonum* parts for alkaloid compounds.

Antioxidant activity

The DPPH assay revealed a concentration-increased enhancement of antioxidant activity of all plant parts, which were all more than the activity of the vitamin C standard (Fig. 6). The flower buds were especially high on efficacy individually at concentrations of 30, 60, 120, and 250 µg/mL. Further, the leaves presented high activity at all tested concentrations, especially at 250 µg/mL (69.80%), equivalent to that of flower buds (70.25%). However, at the highest concentration examined (500 µg/mL), the efficacy of the tuber bark was the highest with activity of 84.55%, very close to that of the flower buds which had 79.08%. In short, flower buds, tuber bark, and leaves expressed maximum activity for antioxidants, followed by flowers and tubers, with stems showing lowest activity. The flowers, leaves, and bark of the tubers of this plant also contain high amounts of secondary metabolites like phenolic compounds, flavonoids, alkaloids, and glycosides, which are accountable for their great antioxidant ability. Phenolic compounds, and flavonoids specifically, are recognized for their ability to neutralize free radicals, chelate transition metals, and avert oxidative insults. Alkaloids also raise antioxidant potential by transferring electrons or hydrogen atoms to scavenge reactive oxidative species (ROS) and by increasing antioxidant defense enzymes. These collaborative actions diminish cell damage by oxidative stress and lipid peroxidation. In summary, these secondary metabolites are important for cell protection and redox balancing of biological systems [47, 48].

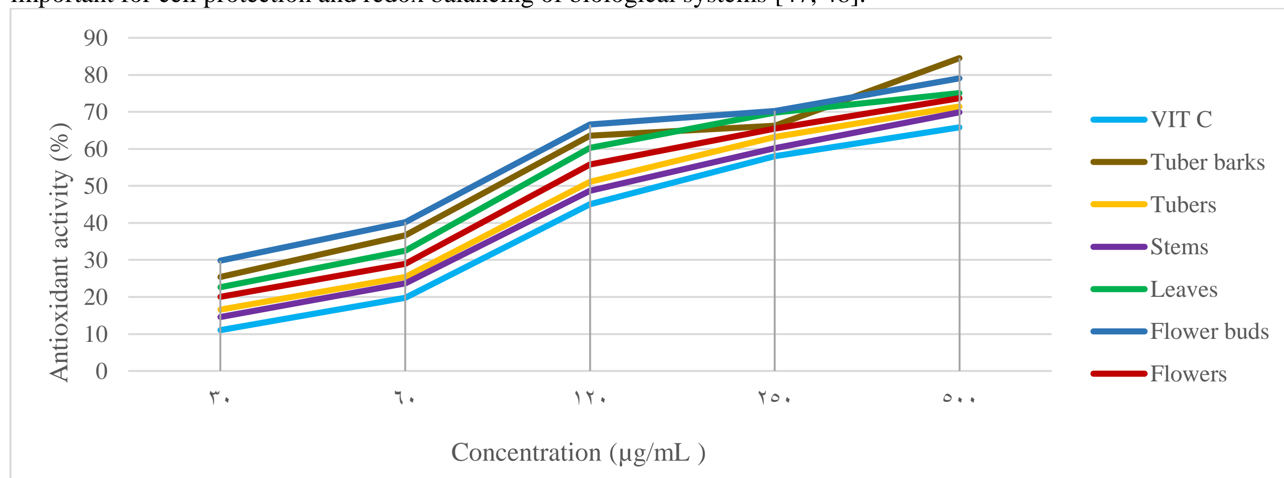

Figure 6. Antioxidant activity (%) of extracts from different parts of *B. chrysogonum*.

IC_{50} determination

The IC_{50} values shown in (Fig. 7) indicate variation among the different plant parts in their antioxidant capacity. Lower IC_{50} values reflect stronger antioxidant activity, and the results demonstrated that all plant parts exhibited greater antioxidant potential than vitamin C. The strongest activity was observed in the flower buds (90.2 µg/mL), which was significantly higher than all other plant parts, followed by the tuber barks (94.1 µg/mL) and the leaves (99.6 µg/mL), while vitamin C showed a higher IC_{50} value (133 µg/mL). Both the flower bud and tuber bark exhibited the highest antioxidant activity compared to other plant parts and even vitamin C, which is attributed to their high content of phenolic compounds and flavonoids capable of scavenging free radicals [48]. The low IC_{50} values in these parts indicate the strong antioxidant potential of their extracts, as lower concentrations are sufficient to achieve effects comparable to or greater than vitamin C. Our results are consistent with those [4, 49].

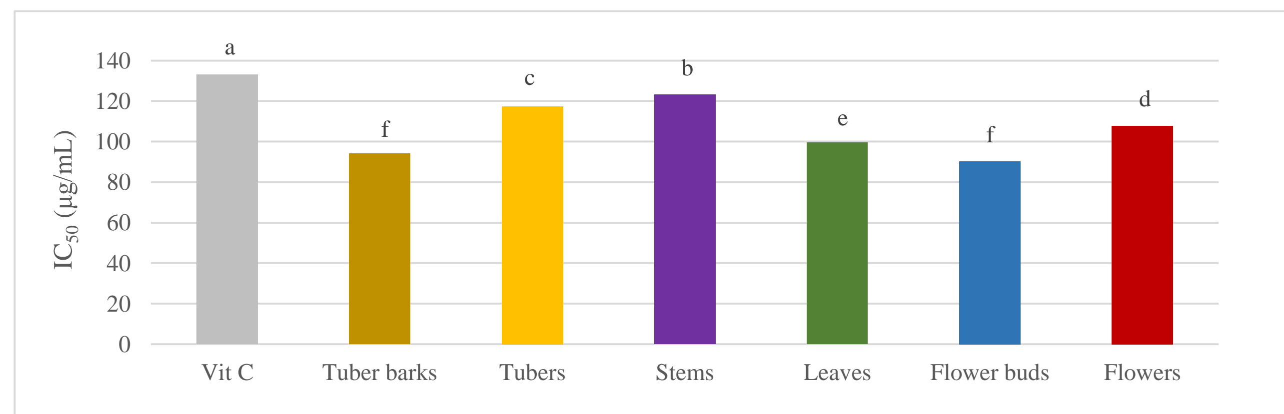

Figure 7. Antioxidant capacities of the extracts regarding IC_{50} (µg/mL) values. Different letters indicate significant differences among means according to Duncan's Multiple Range Test ($P \leq 0.05$)

Antibacterial activity

The antibacterial activity of the plant ethanolic extract of concentration (20 mg/mL) against gram-positive (*S. haemolyticus* and *S. aureus*) and gram-negative (*E. coli, P. mirabilis,* and *K. pneumoniae*) was determined by the presence of inhibition zones. The antibacterial activity was observed just in tuber barks against gram-positive bacteria, *S. aureus* (15 mm), *S. haemolyticus* (12.66 mm). In our tests, however, none of the examined plant extracts exhibited antibacterial efficacy against gram-negative bacteria (Table 4).

Table 4. Bacteria inhibition zone (mm) of *B. chrysogonum* extracts against bacterial strains: *S. aureus, S. haemolyticus, E. coli, K. pneumoniae*, and *P. mirabilis*.

Plant part	*Staphylococcus aureus* mm	*Staphylococcus haemolyticus* mm	*Escherichia coli* mm	*Proteus mirabilis* mm	*Klebsiella pneumonia* mm
Tuber barks	15	12.66	0	0	0
Tubers	0	0	0	0	0
Stems	0	0	0	0	0
Leaves	0	0	0	0	0
Flower buds	0	0	0	0	0
Flowers	0	0	0	0	0

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *B. chrysogonum*

Based on previous section results, the part of the plant which had the broadest inhibition zone (IZ) were subjected to more investigation to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for gram-positive bacteria. The tubers barks extract of *B. chrysogonum* showed a significant inhibitory effect against the gram-positive bacteria *S. aureus* and *S. haemolyticus* with a MIC and an MBC value of 5 mg/mL (Tables 5 and 6).

Table 5. MIC and MBC of *S. aureus* using tuber barks.

Concentration	Tuber barks extract concentration mg/mL					
	40	20	10	5	2.5	1.25
MIC	-	-	-	-	+	+
MBC	-	-	-	-	+	+

+ is refers to bacterial growth

-refers to an effect that is bacteriostatic or bactericidal.

Table 6. MIC and MBC of *S. haemolyticus* using tuber barks.

Concentration	Tuber barks extract concentration mg/mL					
	40	20	10	5	2.5	1.25
MIC	-	-	-	-	+	+
MBC	-	-	-	-	+	+

+ is refers to bacterial growth

-refers to an effect that is bacteriostatic or bactericidal.

Because the agar well diffusion test did not have inhibitory zones, all parts of plant were screened for gram-negative bacteria using broth microdilution MIC and MBC. After doing MIC and MBC for gram-negative bacteria. The leaves extract was the only one with activity at different concentration (1.25 to 40 mg/mL) (Tables 7, 8, and 9). The leaves extract showed inhibitory effect against *P. mirabilis* with an MIC and an MBC value 10 mg/mL. Whereas

in *E. coli*, the bacteria exhausted at a higher MIC and MBC value of 20 mg/mL. However, the last bacteria *K. pneumoniae* recorded MIC value 20 mg/mL and MBC value 40 mg/mL.

Table 7. MIC and MBC of *P. mirabilis* using leaves.

Concentration	Leaves extract concentration mg/mL					
	40	20	10	5	2.5	1.25
MIC	-	-	-	+	+	+
MBC	-	-	-	+	+	+

+ is refers to bacterial growth.
-refers to an effect that is bacteriostatic or bactericidal.

Table 8. MIC and MBC of *E. coli* using leaves.

Concentration	Leaves extract concentration mg/mL					
	40	20	10	5	2.5	1.25
MIC	-	-	+	+	+	+
MBC	-	-	+	+	+	+

+ is refers to bacterial growth.
-refers to an effect that is bacteriostatic or bactericidal.

Table 9. MIC and MBC of *K. pneumoniae* using leaves extract.

Concentration	Leaves extract concentration mg/mL					
	40	20	10	5	2.5	1.25
MIC	-	-	+	+	+	+
MBC	-	+	+	+	+	+

+ is refers to bacterial growth.
-refers to an effect that is bacteriostatic or bactericidal.

The plant extract's reported antibacterial activity points to the presence of bioactive substances that can stop bacteria from growing. Secondary metabolites such as phenolics, flavonoids, and alkaloids, which are known to damage bacterial cell membranes, obstruct enzyme activity, or prevent the formation of nucleic acids are responsible for the efficacy [50]. This study indicates that *B. chrysogounm* phytochemicals have antibacterial potential since they inhibit the development of both gram-positive and gram-negative bacteria. The significant antibacterial activity observed in the tuber bark and leaves is likely due to their high phenolic content (164.9 mg/g in tuber bark) and the presence of flavonoids and glycosides in the leaves (27 and 20 mg/mL, respectively). Abuhamdah *et al.* (2017) similarly evaluated the antimicrobial properties of *B. chrysogonum* tuber extract using both agars well diffusion and microdilution techniques. In the agar diffusion technique, both ethanolic and aqueous extracts exhibited antibacterial activity, giving inhibition zones that ranged from 0.3 mm to 4.08 mm against *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Quantitative analysis using the microdilution method showed minimum inhibitory concentration (MIC) values ranging from 600-950 μg/mL for the aqueous extract and 620-950 μg/mL for the ethanolic extract. Additionally, the minimum bactericidal concentration (MBC) values were slightly higher, ranging from 790–900 μg/mL for the aqueous extract and 800-980 μg/mL for the ethanolic extract [51]. As is in agreement with us, past research confirms that Gram-negative bacteria generally require higher amounts of antimicrobial agents for successful inhibition, further emphasizing their increased inherent resistance compared to Gram-positive bacteria. Such strength is largely attributed to the protective outer membrane of Gram-negative organisms, acting as a barrier to permeability, complemented by their efficient efflux pump mechanisms actively extruding harmful substances, e.g., antibiotics, out of the cell [52].In contrast, *B. chrysogonum*

ethanol extract of tubers did not exert any detectable antibacterial effect on any of the bacterial strains examined in this study [49]. The findings presented no inhibition for all examined bacteria both gram-positive (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633) and gram-negative (*Enterobacter aerogenes* ATCC 13048 and *Escherichia coli* ATCC 35218) at concentrations starting from 4.9 µg/mL to 2500 µg/mL. That is, even at the highest dose tested (2500 µg/mL), the extract was unable to inhibit apparent bacterial growth under the experimental conditions. In a separate investigation, Assaf *et al.* (2013) tested the antimicrobial potential of *B. chrysogonum* tuber ethanol extract at 5 mg/mL using the agar dilution method. Surprisingly, the extract showed no inhibitory effects against common pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Propionibacterium acnes*, suggesting limited antimicrobial activity under these experimental conditions [53].

Conclusion

Bongardia chrysogonum exhibits strong antioxidant and antibacterial activities, predominantly in the flower buds, leaves, and tuber bark, due to their high contents of phenolic compounds, flavonoids, and alkaloids. These observations confirm its traditional usage in herbal medicine and suggest it as a promising natural source of drugs. Since this species has been subjected to low-level studies on the international level, the current study represents the first study conducted within Iraq. These outputs therefore provide a critical addition to the field and firmly establish *Bongardia chrysogonum* as a promising candidate for future phytochemical and pharmacological studies.

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المكونات الكيميائية النباتية وانشطة المضاد للأكسدة وللبكتيريا لنبات الكابلا (*Bongardia chrysogonum*)النامي بريًا في قرداغ – السليمانية

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قسم البستنة، كلية علوم الهندسة الزراعية، جامعة السليمانية، السليمانية، العراق.

الخلاصة

تم اجراء هذه الدراسة على نبات ***Bongardia chrysogonum*** النامي بريا والذي جُمِع من منطقة قرداغ في محافظة السليمانية خلال شهري آذار–نيسان 2024، بهدف تقدير المحتوى الكمي للمركبات الكيميائية النباتية وتقييم انشطة المضاد للأكسدة والبكتيريا في أجزائه المختلفة (قلف الدرنات، الدرنات، السيقان الأوراق، البراعم الزهرية، والأزهار). أظهر قلف الدرنات أعلى محتوى من الفينولات الكلية (164.9 ملغم/غم). كما سجلت الأوراق أعلى محتوى من الفلافونيدات (27 ملغم/غم) والجلايكوسيدات (2 ملغم/غم)، بينما احتوت البراعم الزهرية على أعلى تركيز من القلويدات (23.3 ملغم/غم) .أوضحت تحاليل ***HPLC*** تباين التوزيع بين الأجزاء النباتية؛ إذ كانت البراعم الزهرية غنية بأحماض الغاليك، الكلور وجينك والكافيك معطيا (124.9، 92.6 و98.7 ميكروغرام/غم)، على التوالي. أما الأوراق فقد تميزت بتراكم الروتين، الكور سيتين، الكامب فيرول، وحمض الفيريولك معطيا (124.9، 117.9، 80.9 و87.4 ميكروغرام/غم)، على التوالي. واحتوت السيقان على النارنجين (80.65 ميكروغرام/غم) وحمض الهايدروبنزويك (60.9 ميكروغرام/غم)، بينما أظهرت الدرنات تركيزًا مرتفعًا من الأبيجينين (76.9 ميكروغرام/غم)**.** أما بالنسبة للقلويدات فقد تم الكشف عن أربعة مركبات هي الكينين، الكافايين، الزانثين، والسكوبولامين، وجدت في الدرنات والأزهار فقط. سجلت الدرنات أعلى تراكيز (116.9، 74.6، 98، 147.9 ميكروغرام/غم) مقارنة بالأزهار (88.5، 58.9، 0، 74.66 ميكروغرام/غم)**.** من الناحية التغذوية، احتوت البراعم الزهرية على أعلى نسب من الكربوهيدرات (***14.5%***)، النتروجين (***6.3%***)، والفسفور (***0.88%***)، بينما سجلت الأوراق أعلى تركيز للبوتاسيوم (***3%***)**.** أظهرت جميع الأجزاء النباتية نشاطًا مضادًا للأكسدة، وكانت الفعالية الأعلى في البراعم الزهرية وقلف الدرنات اذ كان ***IC50***لهما 90.2 و94.1 ميكروغرام/مل متفوقة على فيتامين ***C*** (133 ميكروغرام/مل). كما أظهر قلف الدرنات فعالية قوية ضد البكتيريا موجبة الغرام بتركيز 5 ملغم/مل، بينما أبدت الأوراق (10–40 ملغم/مل) فعالية ضد السلالات سالبة الغرام .تُشير النتائج إلى أن هذا النبات البري يُعد غنيًا بالمركبات الفعالة ذات القيمة الغذائية والطبية، ويمثل مصدرًا واعدًا مضادًا للأكسدة والبكتيريا.

الكلمات المفتاحية: *Bongardia chrysogonum*، التحليل الكيميائي النباتي، النشاط المضاد للأكسدة، النشاط المضاد للبكتيريا، HPLC للقلويدات والمركبات الفينولية.