Antioxidant activity of Leptocarpha rivularis DC flower extract and Globularia alypum L.

SUMIT SHEORAN[1], Swati arora[1], and Garima Uppal[2]

[1]Lovely Professional University
[2]Innocent Hearts Group of Institutions

February 23, 2024

Abstract

The aim of this analysis was to look into the complete phenolic and flavonoid contents, and also the antioxidant activity, of leaf extracted from Globularia alypum L. collected in the Taza region of northeast Morocco and flower derives from Leptocarpha rivularis DC. Consequently, the extracts' individual phenolics and volatiles were measured. Soxhlet extraction was used to produce organic extracts of this plant using two separate solvents, ethyl acetate and chloroform. The antioxidant ability of leaf extracts was assessed using DPPH, ABTS, and FRAP assays; antioxidant and anticancer viability was evaluated in Leptocarpha rivularis DC flower specimens employing four solvents (n-hexane (Hex), dichloromethane (DCM), and acetone) (DCM),ethyl acetate (AcOEt), and ethanol (EtOH)). The antioxidant activity (2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), ferric reducing antioxidant ability (FRAP), overall reactive antioxidant properties (TRAP), and oxygen radical absorbance capability (ORAC) of extracts were contrasted through a defined value of reduced/oxidized glutathione (GSH/GSSG). The findings affirm the value of this plant as a sources of antioxidants.

Antioxidant activity of *Leptocarpha rivularis DC flower extract,* and *Globularia alypum L.*

SUMIT SHEORAN[1*], SWATI ARORA[1*], GARIMA UPPAL[2]

Corresponding author: Sumit Sheoran, Swati Arora

Equal First Authorship: Garima Uppal, Swati Arora, Sumit Sheoran

Abstract: The aim of this analysis was to look into the complete phenolic and flavonoid contents, and also the antioxidant activity, of leaf extracted from Globularia alypum L. collected in the Taza region of northeast Morocco and flower derives from Leptocarpha rivularis DC. Consequently, the extracts' individual phenolics and volatiles were measured. Soxhlet extraction was used to produce organic extracts of this plant using two separate solvents, ethyl acetate and chloroform. The antioxidant ability of leaf extracts was assessed using DPPH, ABTS, and FRAP assays; antioxidant and anticancer viability was evaluated in Leptocarpha rivularis DC flower specimens employing four solvents (n-hexane (Hex), dichloromethane (DCM), and acetone) (DCM),ethyl acetate (AcOEt), and ethanol (EtOH)). The antioxidant activity (2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), ferric reducing antioxidant ability (FRAP), overall reactive antioxidant properties (TRAP), and oxygen radical absorbance capability (ORAC) of extracts were contrasted through a defined value of reduced/oxidized glutathione (GSH/GSSG). The findings affirm the value of this plant as a sources of antioxidants.

Keyboard: *Globularia alypum L.* , phenolic compounds, flavonoids, antioxidant activity, *Leptocarpha rivularis DC*

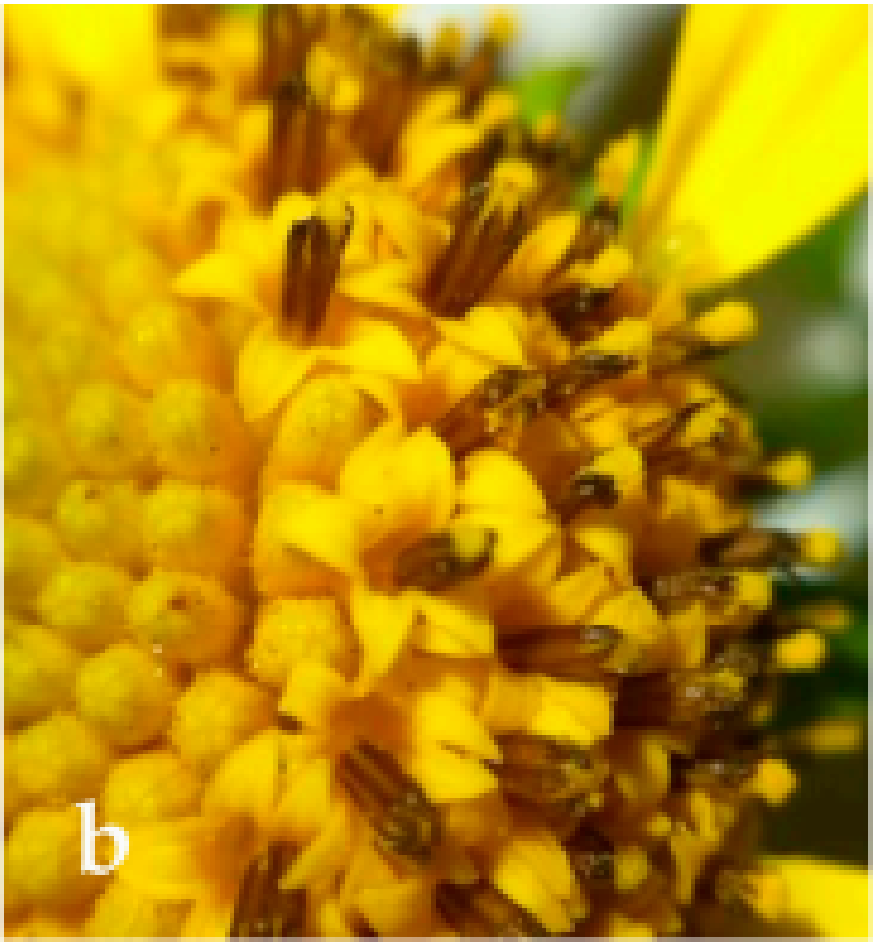
Introduction:

Medicinal plants have been used for the treatment of many diseases from very ancient time. In present, in spite of the evolution of current medicine and the efficiency of synthetic drug in remedy for different ailments, Various people take traditional treatment, due to the less side effect in comparison to synthetic treatment or drug. The plants which have medicinal properties can give rise no. of bioactive compounds, especially secondary metabolites. Due to this, different researchers, and scientist work on different plant extracts to determine their bioactivities such as anticancer, antioxidants and other bioactivities of that extracts. (1-3)

ROS works as signalling molecule for usual biologic processes; generally, the asymmetry in between manufacturing of reactive oxygen species and the biotic system ability of the normal detoxication and due to this oxidative stress occurs. These disruption in the usual cell's redox state originate toxic effects by the producing free radicals that react with cellular constituents and that originate serious cell damage (4-6).

In present, many reports shows that L. flos-cuculi hold secondary phytochemicals. (7-10).

Leptocarpha rivularis DC is from Compositae family and commonly known as "Palo negro". It is persisting for many growing seasons native to southern chile and its growns in humid and sunny soil types having orocca. two meters in height (11). Globularia alypum L. (G. A.) found all over mediterranean area, perennial plant from family plantaginaceae. In the origin reason their name is "Ain Larnab" or "Tasselgha and known for their different objectives in moroccan habitual medicine (12). Globularia alypum L. leaves used for various purposes like antidiabetic agent etc (13). In the treatment of urinary unrestraint and problems of skin, like eczemas this plant is used. According to an ethnobotany examination, which indicates that Globularia alypum L. (G. A.) is ver important plant which have medicinal properties utilized by the Algerian peoples as traditional remedies (14,15).



Flowers of L. rivularis plants grown in Araucania Region, South Chile

Globularia alypum L. Flower

2. Material & method:

2.1. General

All chemicals and solvents were obtained from Aldrich (St. Louis, MO, USA) and were used without further purification.

2.2. Plant Material

Flowers of L. rivularis DC were collected in Temuco during the flowering season (November 2019). A voucher specimen (No. Lr-11119) was stored in the Herbarium of the Natural Products Laboratory, "LPNSO," Department of Chemistry, Universidad de Playa Ancha, Valparaso, Chile. Globularia alypum L. was collected in the Taza region of Morocco during the spring season, between March and April 2018, and deposited in the Faculty's herbarium under the voucher specimen code GA-LABP01. New G. A. leaves were air dried at

room temperature in the shade for around 2 weeks. After thoroughly drying the leaves, they were powdered and placed in a glass bottle in the dark for further study.

2.3. Extraction

G. A. was processed using the Soxhlet apparatus using chloroform and ethyl acetate to obtain two extracts with distinct polarities. 50 g of dry G. A. leaves were rigorously collected with 250 mL of each solvent, and the extracted extracts were concentrated and solvent-free using a rotary evaporator under reduced pressure. At the conclusion of the extraction procedure, two crude extracts were collected and weighed to determine the extraction yield for each solvent before being placed in an airtight bottle in a refrigerator (4 C) before further study. Five grammes of plant powder were defatted three times in 50 mL of n-hexane for GC-MS examination, and the extraction was done by sonication in an ultrasound bath (130 kHz) for 45 minutes. Prior to GC-MS review, the supernatant was purified through a paper filter, dried with a rotary evaporator, and reconstituted with n-hexane after being centrifuged for 5 minutes. The dried powdered flowers of L. rivularis DC (200 g) were extracted thoroughly with 70% ethanol (300 mL) using a Soxhlet extractor at 50 C for 16 hours. The polar extract was evaporated under low pressure to produce crude ethanol extract, which was then fractionated into hexane, dichloromethane, ethyl acetate, and ethanol extracts (16). Many of the solvents used were of the chromatographic grade. In a nutshell, the extraction was carried out for 72 hours using an orbital shaker (170 rpm) at 25 C. The resulting mixture was purified into Whatman No. 1 filter paper (Sigma-Aldrich, Darmstadt, Germany), and the hexane was extracted from the filtrate under reduced pressure using a rotatory evaporator (BÜCHI, Barcelona, Spain, Rotavapor R-300). The residue was then removed sequentially and sequentially with dichloromethane, ethyl acetate, and ethanol. Finally, the yield was determined by measuring each extract. L. Until further study, rivularis DC flower extracts were held at 4 C.

2.4. Phytochemical Screening of Flower Extracts

Using normal protocols, all flower extracts were subjected to qualitative chemical tests to classify different groups of bioactive chemical constituents contained in the leaves (17).

2.4.1. Determination of Total Phenols

The total phenolic content (TPC) of the flower extracts was determined using the Folin-Ciocalteu reagent (18). Gallic acid (20–100 g/mL) was used as a reference norm for plotting the calibration curve. A amount of 0.5 mL of the flower extract (100 g/mL) was combined with 1.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with deionized water) and neutralised with 3 mL of 7.5 percent w/v sodium carbonate solution. For colour growth, the reaction mixture was kept in the dark at room temperature for 30 minutes with occasional shaking. The absorbance of the resultant blue colour was measured using a double beam UV-Vis spectrophotometer (UV Analyst-CT 8200) calibrated to 765 nm. The TPCs were estimated using the linear regression equation derived from the gallic acid standard map. The overall phenolic compound content was measured as mean standard deviation (n = 3) and expressed as mg/g gallic acid equivalent (GAE) of dry extract.

2.4.2. Estimation of Total Flavonoid Content (TFC)

The TFC in flower extracts was measured using Madaan's (19) stated method, and quercetin was used as a standard to create the calibration curve. In a nutshell, 10 mg of quercetin was dissolved in 80% ethanol and then diluted to 20, 40, 60, 80, and 100 g/mL. In a test tube, diluted regular solutions of quercetin or plant extracts (0.5 mL) of varying amounts is combined with 1.5 mL of 95 percent ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of purified water. To complete the reaction, the test tubes were incubated at room temperature for 30 minutes. The absorbance of the reaction mixture was measured against a blank using a double beam UV-Vis spectrophotometer at 415 and (G.A.)510nm. Both reagents were found in a standard blank solution, except for aluminium chloride, which was substituted with the same volume of purified water. The flavonoid concentration was determined using the linear regression equation derived from the quercetin calibration. Both of the experiments were carried out in triplicate.

2.4.3. Total Anthraquinone Content (TAC)

The recorded protocol (20,21) was used to evaluate TAC in flower extracts, and emodin was used as a norm to create the calibration curve. In short, 1 mL of 2% w/v aluminium trichloride (AlCl3) in ethanol was combined with the same amount of ethanol extract solution (1.0 mg/mL). The mixture was incubated at room temperature for 10 minutes before measuring absorbance at 486 nm against a blank sample made up of 1.0 mL of extract solution and 1.0 mL of methanol without AlCl3. The overall anthraquinone content was expressed in emodin equivalents (EE) per gramme of dry extract. Both of the experiments were carried out in triplicate.

2.5. Chromatographic Analysis

The Hex, DCM, and AcOEt extracts were diluted with chloroform before being analysed by gas chromatography (Hewlett Packard, Palo Alto, CA, USA) using the process mentioned elsewhere [22,23]. To achieve the optimum isolation using a capillary Rtx-5MS column, the operating conditions were as follows: on-column injection; injector temperature, 250 C; detector temperature, 280 C; carrier gas, He at 1.0 mL/min; oven temperature programme: 40 C rise to 260 C at 4 C /min, and then 260 C for 5 min. The mass detector ionisation used a 70 eV electron effect. The chromatograms' compounds were described by matching their mass spectra to those in the NIST/EPA/NIH Mass Spectral Library (24). When the similarity index (MATCH) and reverse similarity index (RMATCH) of a chromatographic peak is less than 850, it was considered "unknown" and discarded in this recognition process [25]. These criteria are referred to by the degree to which the target spectrum meets the regular spectrum in the NIST Library (a value of 1000 implies a great fit), as well as by a comparison of their retention index with those stated in the literature (24) for the same form of column or commercial requirements, where available. Under the same operational conditions, the retention indices were measured in relation to a homologous n-alkanes sequence (C8–C36) using the equation:

$$RI = 100 \times (n + Tr(unknown) - Tr(n)/Tr(N) - Tr(n)), \quad (1)$$

where n = the number of carbon atoms in the smaller n-alkane; N = the number of carbon atoms in the larger n alkane; and Tr = the retention time. Component relative concentrations were obtained by peak area normalization.

2.6. Antioxidant Assay

2.6.1. DPPH Radical Scavenging Assay :

The DPPH assay was carried out exactly as mentioned previously (26). In short, 0.1 mL of sample (ranging from 0 to 4 mg/L of flower extracts) was combined with 2.9 mL of freshly formulated DPPH* solution (50 M). As a control, 2.9 mL 50 M DPPH* solution with 0.1 mL ethanol was used. After 15 minutes at room temperature, the absorbance of the resulting solutions, the power, and the blank (with only the reagents) were measured. Three times, each sample was repeated. At 517 nm, the absence of DPPH* was observed spectrophotometrically. The following equation was used to measure the percentage RSC (Radical Scavenging Capacity):

$$RSC\ (\%) = 100\% \times (Acontrol - Asample)/Acontrol \quad (2)$$

From the obtained RSC (%) values the IC50 value, which represent the concentrations of the resinous exudate and the major compounds that caused 50% inhibition, was determined by linear regression analysis.

2.6.2. Ferric Reducing Antioxidant Potential (FRAP) Assay:

The FRAP assay(27) with slight modifications was used to evaluate the ferric reducing strength of plant extracts. Per day, 10 volumes of 300 mM acetate buffer, pH 3.6, is combined with 1 volume of 10 mM TPTZ in 40 mM hydrochloric acid and 1 volume of 20 mM ferric chloride to create the working FRAP reagent. On the day of planning, both solutions were used. A 100 L sample of the extracts (1.0 mg/mL) in 300 L of deionized water was combined with 3 mL of freshly prepared FRAP reagent. The reaction mixture was incubated in a water bath for 30 minutes at 37 degrees Celsius. The samples' absorbance was then measured

at 593 nm. A blank ethanol reading was also taken. The difference in absorbance between the sample and the blank was measured and used to measure the FRAP value. The FRAP values were given in millimoles of Trolox. Both measurements were taken three times.

2.6.3. Total Reactive Antioxidant Properties (TRAP) Assay:

The assay was carried out using the previously mentioned procedure (28). A 10 mM ABAP solution was combined with a 150 mM ABTS*+ solution in a 100 mM PBS solution with a pH of 7.4. For 30 minutes, the mixture was incubated at 45 degrees Celsius. A sample solution of 10 L was applied to 990 L of the resulting blue-green ABTS*+ solution. After 30 seconds at room temperature, the absorbance of TRAP solutions and ABTS*+ was measured. The samples' absorbance was then measured at 734 nm. Using a Trolox normal curve (0–120 mg/L), the overall antioxidant potential TRAP of the extracts was represented in mM Trolox equivalents (TEAC). Any calculation was repeated three times.

2.6.4. Oxygen Radical Absorbance Capacity (ORAC) Assay:

Alarcon's ORAC assay was used to assess oxygen radical scavenging ability (29). ORAC-fluorescein is a simple tool for measuring the antioxidant potential of pure compounds or complex mixtures such as herbal infusions and teas. It has previously been shown that the ORAC-index is affected by the target molecule (30). Centered on a Trolox standard curve, the ORAC values of the extracts were expressed as M of Trolox equivalent antioxidant potential (M TEAC). GA and BHT were used as positive controls. Both tests were conducted in triplicate.

2.6.5. Reduced Glutathione Assay:

GSH was determined using an enzymatic recycling technique in which it was sequentially oxidised by 5,5'-dithiobis (2-nitrobenzoic acid) and reduced by NADPH in the presence of GR according to existing protocols [31,32] and adapted to L. rivularis DC flower extracts from the various solvents studied. In brief, g dry weight (DW) of L. rivularis DC flower extracts were extracted in 5% sulphosalicylic acid, and the extent of 2-nitro5-thiobenzoic acid formation was controlled at 412 nm for GSH plus GSSG assessment. To evaluate GSSG alone, the dry extract was pretreated with 2-vinylpyridine to derivatize GSH and scavenge it.

3. Result: 3.1. Extract Yields:

The sequential soxhlet extraction process was changed to acquire various flower extracts using hexane (Hex), dichloromethane (DCM), ethyl acetate (AcOEt), and ethanol (EtOH). The largest yields were obtained for ethanol (15.52 percent by weight), followed by hexane (6.50 percent by weight), dichloromethane (5.80 percent by weight), and ethyl acetate (4.60 percent by weight).

3.2. Phytochemical Content:

Following the extraction of flower extracts, the phytochemical content (total phenolic contents, flavonoids, and anthraquinone) was calculated using colorimetric assays, as summarised in Table 1. Total anthraquinone and flavonoids were significantly higher in EtOH extracts (p 0.05) than in other extracts. Table 1 shows the total phenols (TPC) of flower extracts expressed in terms of GAE mg L1 and presented in table 1. The overall phenolic content in both the Hex and EtOH extracts differed significantly from the other extracts tested (p 0.05). TPCs is determined using the linear regression equation derived from the gallic acid standard plot: y = 0.001x + 0.021, r2 = 0.998.

Extract	Total Phenols (GAE mg·L-1)	S.D.	Total Flavonoids (QE mg·L-1)	S.D.	Total Anthraquinones (EE mg
Hex	27.8423 a	1.7642	70.9333 a	1.8986	37.8368 a
DCM	41.8810 b	1.4569	26.4295 b	0.4815	10.4092 b
AcOEt	47.8899 b	0.0449	33.8589 b	1.0233	12.9897 b
EtOH	82.6696 c	5.8292	103.6202 c	3.9844	75.2023 c

Table 1. Phytochemical contents of different flower extracts of L. rivularis. Hex: hexane; DCM: dichloromethane; AcOEt: ethyl acetate; EtOH: ethanol; GAE: gallic acid equivalent; QE: quercetin equivalents; EE: emodin equivalents; SD: standard deviation. Different letters in the same column indicate significant differences; $p < 0.05$, n = 3.**3.2.1. Total Antioxidant Activity:**

The antioxidant activity of L. rivularis flower extracts was tested in vitro using DPPH, FRAP, TRAP, and ORAC assays (see Table 2). The DPPH assay revealed that the Hex extract performed poorly (p 0.05) as opposed to the positive control (Trolox) (T). As compared to AcOEt and EtOH, DCM was the most active extract, but it was less active than T (p 0.05). According to the FRAP assay, DCM, AcOEt, and EtOH extracts had stronger antioxidant activity than positive controls (p 0.05). Under TRAP, Hex extract was the least active as compared to positive controls (gallic acid (GA) and butylhydroxytoluene (BHT), p 0.05).

Extract	DPPH (IC50 mg·mL-1)	S.D.	FRAP (TEAC mM)	S.D.	TRAP (TEAC mM)	S.D.	ORAC (TEAC
Hex	4.1863 a	0.1834	0.3141 a	0.0131	0.0074 a	0.0022	442.57 a
DCM	1.8953 b	0.0437	0.2656 a	0.0145	0.0961 b	0.0047	932.40 b
AcOEt	2.4088 c	0.0758	0.3063 a	0.0081	0.1288 b	0.0057	1878.17 c
EtOH	2.8300 c	0.0626	0.3687 b	0.0277	0.0729 b	0.0204	744.20 d
T	0.1060 d	0.0050	n.a.	n.a.	n.a.	n.a.	n.a.
GA	n.a.	n.a.	1.7200 c	0.0200	1.1300 c	0.0100	696.22 e
BHT	0.0600 d	0.0010	1.5200 d	0.0700	1.0600 c	0.0200	593.87 f

Table 2. Antioxidant activity of flower extracts of L. rivularis. Hex: hexane; DCM: dichloromethane; AcOEt: ethyl acetate; EtOH: ethanol; T: Trolox; GA: gallic acid; BHT: Butylhydroxytoluene; (2,2- diphenyl-1-picryl-hydrazyl-hydrate (DPPH), ferric reducing antioxidant potential (FRAP), total reactive antioxidant properties (TRAP) and oxygen radical absorbance capacity (ORAC)); IC50: represents the concentrations of extracts that caused the neutralization of 50% of the radical; TEAC: Trolox equivalent antioxidant capacity; SD: standard deviation. Different letters in the same column indicate significant differences; $p < 0.05$, n = 3; n.a.: not applicable.

3.2.2. Reduced Glutathione Assay

Under normal conditions, the ratio of reduced GSH to oxidised GSH (GSSG) is a measure of cellular fitness, with reduced GSH constituting up to 98 percent of cellular GSH. The findings of the reduced glutathione assay using pooled flower extracts of L. rivularis in the various solvents studied are shown in Table 3. Ethanol extracts contained substantially more GSH than the other solvents, but they also contained significantly more GSSG (4 times over AcOEt, and 10 times over n-hexane and DCM). Thus, the DCM extract had the highest GSH/GSSG ratio content—significantly higher than the other extracts—and the lowest GSH to GSSG conversion rate.

Extract	GSH	GSSG	GSH/GSSG
µmol/gDW	µmol/gDW	µmol/gDW	µmol/gDW
Hex	1.179 ± 0.909 d	16.432 ± 3.756 c	0.062 ± 0.042 b
EtOH	86.456 ± 1.755 a	267.863 ± 9.157 a	0.323 ± 0.009 b
DCM	28.185 ± 3.385 b	22.110 ± 5.684 b	1.376 ± 0.204 a
AcOEt	11.461 ± 1.924 c	50.427 ± 5.686 b	0.224 ± 0.013 b

Table 3. Glutathione pool of L. rivularis DC flower extracts. GSH: reduced glutathione; GSSG: oxidized glutathione; GSH/GSSG: ratio GSH/GSSG; gDW: grams dry weight of extract; DCM: dichloromethane; AcOEt: ethyl acetate; EtOH: ethanol. Different letters indicate significant differences ($p < 0.05$; Tukey test) ANOVA. Each value represents mean ± SE (n=3).

Antioxidant Properties (Mean IC50 Value µg/mL ± Standard Deviation)

Plant Extracts	DPPH	ABTS	Reducing Power (mg AAE/g DW)
Ethyl acetate	12.3 ± 3.83	37.0 ± 2.45	531.1 ± 1.08
Chloroform	69.8 ± 1.89	114.6 ± 0.63	473.2 ± 1.88

Table 4. Antioxidant activity of G. A. extracts.

4. **Conclusion:**

L. rivularis DC flower extracts, as well as ethyl acetate and chloroform leaves extracts of Globularia alypum, L., were tested for their antioxidant and cytotoxic activities. Only in-vitro experiments are performed on these plants; no in-vivo studies are conducted. The findings obtained are very positive, and if accompanied by "in vivo" experiments, such a species may be proposed for therapeutic purposes. This research indicates that the flowers of L. rivularis DC and Globularia alypum, L. may be potential natural antioxidants as well as other antitumour or bioactive molecules for both the pharmaceutical and food industries.

Reference:

1. Cai, Y.; Luo, Q.; Sun, M.; Corke, H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004, 74, 2157–2184.
2. Miliauskas, G.; Venskutonis, P.R.; Van Beek, T.A. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 2004, 85, 231–237.
3. Altemimi, A.; Lakhssassi, N.; Baharlouei, A.; Watson, D.G.; Lightfoot, D.A. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 2017, 6, 42.)
4. Auten, R.L.; Davis, J.M. Oxygen toxicity and reactive oxygen species: The devil is in the details. Pediatr. Res. 2009, 66, 121–127.
5. Satoh, T. Astaxanthin: Health Benefits and Toxicity in Nutraceuticals; Elsevier: Amsterdam, The Netherlands, 2016; pp. 531–539.
6. Farhan, H.; Malli, F.; Rammal, H.; Hijazi, A.; Bassal, A.; Ajouz, N.; Badran, B. Phytochemical screening and antioxidant activity of Lebanese Eryngium creticum L. Asian Pac. J. Trop. Biomed. 2012, 2, S1217–S1220
7. Bucharow, W.G.; Chirva, V.J.; Bucharowa, I.L. Triterpene glycosides from Coronaria flos-cuculi. Pharmazie 1974, 29, 540.
8. Báthori, M.; Lafont, R.; Girault, J.P.; Máthé, I. Structural diversity of ecdysteroids of Lychnis flos-cuculi. Acta Pharm. Hung. 2001, 71, 157–167.
9. Tomczyk, M. Preliminary phytochemical investigation of Lychnis flos-cuculi herbs. J. Nat. Med. 2008, 62, 473–475.
10. Costea, T.; Nencu, I.; Gîrd, C.E.; Popescu, M.L. Ragged robin (Lychnis flos cuculi L.) aerial parts—Botanical characterization, phytochemical screening and antioxidant activity. Studia Univ. Vasile Goldis Arad Seria Stiintele Vietii 2017, 27, 231–238.
11. Riedemann, P.; Aldunate, G. Flora Nativa de Valor Ornamental. Identificación y Propagación. Chile—Zona Sur, 2nd ed.; Editorial Andrés Bello: Santiago, Chile, 2011; pp. 202–203
12. Hammiche, V.; Merad, R.; Azzouz, M. Plantes Toxiques à Usage Médicinal du Pourtour Méditerranéen; Springer: Paris, France, 2013.
13. Jouad, H.; Haloui, M.; Rhiouani, H.; El Hilaly, J.; Eddouks, M. Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the North centre region of Morocco (Fez–Boulemane). J. Ethnopharmacol. 2001, 77, 175–182.
14. Boudjelal, A.; Henchiri, C.; Sari, M.; Sarri, D.; Hendel, N.; Benkhaled, A.; Ruberto, G. Herbalists and wild medicinal plants in M'Sila (North Algeria): An ethnopharmacology survey. J. Ethnopharmacol. 2013, 148, 395–402.
15. Telli, A.; Esnault, M.-A.; Khelil, A.O.E.H. An ethnopharmacological survey of plants used in traditional diabetes treatment in south-eastern Algeria (Ouargla province). J. Arid Environ. 2016, 127, 82–92

16. De Castro, M.L.; Garcıa-Ayuso, L.E. Soxhlet extraction of solid materials: An outdated technique with a promising innovative future. Anal. Chim. Acta 1998, 369, 1–10.
17. Mohammad, N.; Sajid, A.; Muhammad, Q. Preliminary phytochemical screening of flowers, leaves, bark, stem and roots of Rhododendron arboreum. Middle East J. Sci. Res. 2011, 10, 472–476.
18. Aryal, S.; Baniya, M.K.; Danekhu, K.; Kunwar, P.; Gurung, R.; Koirala, N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants 2019, 8, 96.
19. Madaan, R.; Bansal, G.; Kumar, S.; Sharma, A. Estimation of total phenols and flavonoids in extracts of Actaea spicata roots and antioxidant activity studies. Indian J. Pharm. Sci. 2011, 73, 666–669.]
20. Mellado, M.; Madrid, A.; Pena-Cortes, H.; López, R.; Jara, C.; Espinoza, L. Antioxidant activity of anthraquinones isolated from leaves of Muehlenbeckia hastulata (je sm.) johnst.(polygonaceae). J. Chil. Chem. Soc. 2013, 58, 1767–1770.
21. Kurkin, V.A.; Shmygareva, A.A.; Ryazanova, T.K.; San'kov, A.N. Quantitative Determination of Total Anthraquinone Glycosides in Cassia Syrup Preparation. Pharm. Chem. J. 2017, 50, 691–694
22. Montenegro, I.; Madrid, A.; Zaror, L.; Martínez, R.; Werner, E.; Carrasco, H.; Cuellar, M.; Palma, H. Antimicrobial activity of ethyl acetate extract and essential oil from bark of Laurelia sempervirens against multiresistant bacteria. Bol. Latinoam. Caribe Plant. Med. Aromat. 2012, 11, 306–315.
23. Canales, N.; Montenegro, I.; Párraga, M.; Olguín, Y.; Godoy, P.; Werner, E.; Madrid, A. In vitro antimicrobial activity of embothrium coccineum used as traditional medicine in patagonia against multiresistant bacteria. Molecules 2016, 21, 1441
24. NIST/EPA/NIH Mass Spectral Library with Search Program (Data Version: NIST 11, Software Version 2.0 g. Available online: http://webbook.nist.gov/chemistry/name-ser.html (accessed on 15 May 2016.
25. Santander, R.; Creixell, W.; Sánchez, E.; Tomic, G.; Silva, J.R.; Acevedo, C.A. Recognizing Age at Slaughter of Cattle from Beef Samples Using GC/MS-SPME Chromatographic Method. Food Bioprocess Technol. 2013, 6, 3345–3352
26. Leyton, M.; Mellado, M.; Jara, C.; Montenegro, I.; González, S.; Madrid, A. Free radical-scavenging activity of sequential leaf extracts of Embothrium coccineum. Open Life Sci. 2015
27. Dudonné, S.; Vitrac, X.; Coutière, P.; Woillez, M.; Mérillon, J.M. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J. Agric. Food Chem. 2009, 57, 1768–1774.
28. Romay, C.; Pascual, C.; Lissi, E.A. The reaction between ABTS radical cation and antioxidants and its use to evaluate the antioxidant status of serum samples. Braz. J. Med. Biol. Res. 1996, 29, 175–183.
29. Alarcón, E.; Campos, A.M.; Edwards, A.M.; Lissi, E.; López-Alarcón, C. Antioxidant capacity of herbal infusions and tea extracts: A comparison of ORAC-fluorescein and ORAC-pyrogallol red methodologies. Food Chem. 2008, 107, 1114–1119.
30. Lopez-Alarcon, C.; Lissi, E. A novel and simple ORAC methodology based on the interaction of Pyrogallol red with peroxyl radicals. Free Radic. Res. 2006, 40, 979–985.
31. Griffith, O.W. Determination of glutathione disulphide using glutathione reductase and 2-vinylpyridine. Anal. Biochem. 1980, 106, 207–212.
32. Lutts, S.; Lefevre, I.; Delperee, C.; Kivits, S.; Dechamps, C.; Robledo, A.; Correal, E. Heavy metal accumulation by the halophyte species Mediterranean saltbush. J. Environ. Qual. 2004, 33, 1271–1279.