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Original Article

Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian *Ruta chalepensis*

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ABSTRACT

The antioxidant and antibacterial activities of phenolic compounds from cultivated and wild Tunisian *Ruta chalepensis* L. leaves, stems, and flowers were assessed. The leaves and the flowers exhibited high but similar total polyphenol, flavonoid, and tannin content. Moreover, two organs showed strong, although not significantly different, total antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl scavenging ability, and reducing power. Investigation of the phenolic composition showed that vanillic acid and coumarin were the major compounds in the two organs, with higher percentages in the cultivated organs than in the spontaneous organs. Furthermore, *R. chalepensis* extracts showed marked antibacterial properties against human pathogen strains, and the activity was organ- and origin-dependent. Spontaneous stems had the strongest activity against *Pseudomonas aeruginosa*. From these results, it was concluded that domestication of *Ruta* did not significantly affect its chemical composition and consequently the possibility of using *R. chalpensis* organs as a potential source of natural antioxidants and as an antimicrobial agent in the food industry.

1. Introduction

Plants constitute a valuable source of natural antioxidants such as vitamins, phenolic compounds, and flavonoids [1]. Because of their potential carcinogenicity, the utilization of synthetic antioxidants is progressively restricted in the food industry. This trend is concomitant with an increasing interest in the identification and valorization of natural antioxidants of plant origin. Moreover, microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety. Concern over pathogenic and spoilage

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microorganisms in foods is increasing because of the increase in outbreaks of food-borne disease. There is growing interest in using natural antibacterial compounds such as plant extracts of herbs and spices for the preservation of foods because these extracts possess a characteristic flavor and sometimes show antioxidant activity and antimicrobial activity [2].

Ruta is a genus of Rutaceae family and features primarily shrubby plants that are native to the Mediterranean region and usually grow on rocky slopes. The three most diffused species are *Ruta chalepensis* L., *Ruta graveolens* L., and *Ruta montana* L. [3]. It is used for culinary purposes to flavor foods and as an aperitif [4]. This plant is commonly used as a traditional medicinal plant. It is protective against various disorders such as rheumatism, fever, mental disorders, dropsy, neuralgia, menstrual problems, convulsions, and other bleeding and nervous disorders [3,5,6]. With regard to the phytochemical composition of *Ruta chalepensis* extracts, Aguilar-Santamaria and Tortoriello [5] have reported that its leaves and young stems contain alkaloids, flavonoids, phenols, amino acids, furocoumarins, and saponins. However, data on the bioactivities of *R. chalepensis* are scarce. Almog et al [7] found that the ethanol extract of the plant reduced the oxidative stress in experimental animal models by modulating the activities of various antioxidant enzymes. Moreover, Fakhfakh et al [8] reported interesting *in vitro* antioxidant activities of the essential oil and solvent extracts of wild *R. chalepensis*. Studies on the antimicrobial activity of *R. chalepensis* are limited; however, Al-Bakri and Afifi [9] found that dimethyl sulfoxide extracts of *R. chalepensis* possess promising antibacterial activity. In Tunisia, there are two *R. chalepensis* provenances: the cultivated *R. chalepensis* (i.e., in garden houses) and the spontaneous *R. chalepensis* (i.e., wild, usually growing in mountains). Several researchers suggested the relevance of identifying the metabolic differences between wild and cultivated specimens to assess their quality during the domestication process [10]. In some instances, the wild forms of plants may demonstrate different phytonutrient profiles from the cultivated ones. Several studies have stated that most wild species preserve higher biological capacity and richer secondary metabolites, compared with the cultivated species [11,12]. According to previous literature reports illustrating the importance of *Ruta* as a medicinal plant, and because of its availability in Tunisia, in this work we aimed to evaluate the phenolic composition and the antioxidant and antibacterial properties of the methanolic extracts of Tunisian *R. chalepensis* as function of the plant parts (i.e., leaves, stems, and flowers) and provenance (i.e., cultivated and wild *Ruta*). In addition, the objective of this study was to establish differences between the two origins and to evaluate which is the most interesting. The results of this work highlighted the possibility of using *R. chalepensis* organs as a potential source of natural antioxidants and as an antimicrobial agent in the food industry.

2. Materials and methods

2.1. Plant material

Cultivated *R. chalepensis* originated from an orchard in the Center of Tunisia from El Ala (Central Tunisia: latitude 35°36′5782″ (N), longitude 9°33′34″ (E), altitude 151.80 km). Spontaneous *Ruta chalepensis* was collected from the mountain Traza (El Ala). The two provenances were collected in May 2012 at the flowering stage, and the different organs (i.e., leaves, stems, and flowers) were air-dried at room temperature. They were then ground with a blade-carbide grinding (Type A:10; IKA-WERK).

2.2. Preparation of extracts

Extracts were obtained by stirring 1 g of dry *R. chalepensis* powder of the organs with 10 mL of pure methanol for 30 minutes at 120 rpm using a magnetic stirrer plate. The extracts were then maintained for 24 hours at 4°C, filtered through Whatman No. 4 filter paper, and evaporated under vacuum to dryness. They were subsequently stored at 4°C, until analyzed.

2.3. The determination of the total phenolic content

The total phenolic content was assayed using Folin–Ciocalteu reagent, following Singleton's method, which was slightly modified by Dewanto et al [13]. An aliquot (0.125 mL) of a suitable diluted methanolic extract (0.25 mg/mL) was added to 0.5 mL of deionized water and 0.125 mL of the Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 minutes before adding 1.25 mL of 7% sodium carbonate (Na_2CO_3) solution. The solution was then adjusted with deionized water to a final volume of 3 mL and mixed thoroughly. After incubation for 90 minutes at 23°C, the absorbance of this solution versus a prepared blank was read at 760 nm. The total phenolic content of the organ extracts (i.e., three replicates per treatment) was expressed as milligrams gallic acid equivalent (GAE) per gram of dry weight (mg GAE/g DW) through a calibration curve with gallic acid. The calibration curve range was 50–400 mg/mL ($R^2 = 0.99$).

2.4. Determination of the total flavonoid content

The total flavonoid content was measured by a colorimetric assay, based on the method described by Dewanto et al [13]. An aliquot (250 μL) of the appropriately diluted plant extract was mixed with 75 μL sodium nitrite ($NaNO_2$; 5%). After 6 minutes, we added 150 μL of 10% aluminum chloride. Five minutes later, 500 μL of sodium hydroxide (NaOH; 1M) was added to the mixture. The mixture was finally adjusted to 2.5 mL with distilled water. The absorbance of the mixture versus a prepared blank was read at 510 nm. The total flavonoid content of flowers (i.e., three replicates per treatment) was expressed as mg catechin equivalent (CE)/g DW through the calibration curve with catechin. The calibration curve range was 50–500 mg/mL.

2.5. The determination of the condensed tannin content

The condensed tannins (i.e., proanthocyanidins) were analyzed by the method of Sun et al [14]. An aliquot of 50 μL of the extract appropriately diluted, was added to 3 mL of 4% methanol vanillin solution and 1.5 mL of sulfuric acid (H_2SO_4). After 15 minutes, the absorbance was measured at 500 nm. The condensed tannin content of the extracts was expressed

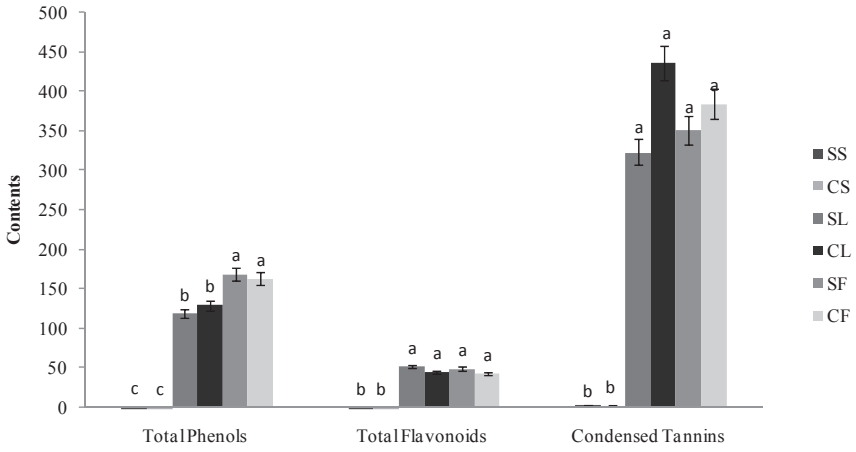


Figure 1 – The total phenol (mg GAE/g DW), total flavonoid (mg CE/g DW) and condensed tannin (mg CE/g DW) contents in spontaneous and cultivated parts (i.e., stems, leaves, and flowers) from Tunisian *Ruta chalepensis*. The values are presented as the mean of three replicates ± the standard deviation. The data marked with different letters share significance at $p < 0.05$ (based on the Duncan test). CE ¼ catechin equivalent; CF ¼ cultivated flowers; CL ¼ cultivated leaves. CS: cultivated stems; DW: dry weight; GAE: gallic acid equivalent; SF: spontaneous flowers; SL: spontaneous leaves; SS: spontaneous stems.

as mg CE/g DW through the calibration curve with catechin. The calibration curve range was 50–600 mg/mL.

2.6. *Identification of phenolic compounds using reverse-phase high-performance liquid chromatography*

The obtained methanolic extracts was filtered through a 0.45 μm membrane filter and injected into a high-performance liquid chromatography (HPLC) system. The phenolic compounds were analyzed by using a reverse-phase-HPLC system (RP-HPLC; Agilent Technologies 1100 Series; Agilent Technologies, Santa Clara, California, USA) that was coupled with an ultraviolet/visible spectrum multiwavelength detector. The separation was performed on a 250 mm × 4.6 mm, 4 μm Hypersil ODS C18 reversed-phase column (Thermo Fisher Scientific Inc., Waltham, MA, USA) at ambient temperature. The mobile phase consisted of acetonitrile (Solvent A) and water with 0.2% sulfuric acid (Solvent B). The flow rate was maintained at 0.5 mL/min. The gradient program was as follows: 15% A/85% B for 0–12 minutes; 40% A/60% B for 12–14 minutes; 60% A/40% B for 14–18 minutes; 80% A/20% B for 18–20 minutes; 90% A/10% B for 20–24 minutes, and 100% A 24–28 minutes.

2.7. *Evaluation of the total antioxidant capacity*

The assay is based on the reduction of molybdenum (VI) [Mo (VI)] to Mo (V) by the extract and subsequent formation of a

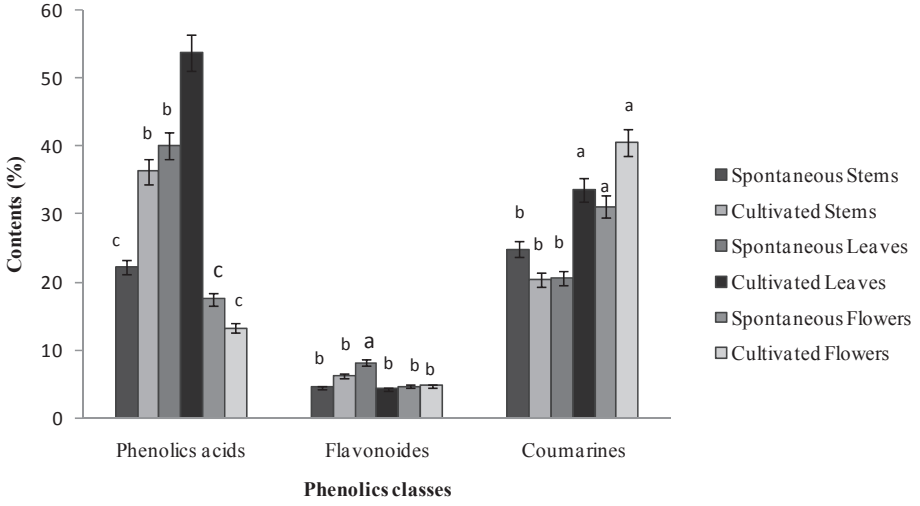


Figure. 2 – Percentage of chemical classes of phenolic compounds in extracts of the spontaneous and cultivated parts (i.e., stems, leaves, and flowers) from Tunisian *Ruta chalepensis*. The values are presented the mean of three replicates ± the standard deviation. The data marked with different letters share significance at $p < 0.05$ (based on the Duncan test).

Table 1 – The percentages of phenolic compound of different Tunisian *Ruta chalepensis* parts.

	SS	CS	SL	CL	SF	CF
Protocatechuic acid	—	7.27 ± 0.75^{a}	0.63 ± 0.73^{c}	2.57 ± 0.35^{b}	0.88 ± 0.06^{c}	3.46 ± 0.53^{b}
Gallic acid	3.4 ± 0.77^{a}	4.27 ± 0.01^{a}	—	2.1 ± 0.03^{b}	0.82 ± 1.31^{c}	1.79 ± 0.11^{b}
3,4 Dihydroxybenzoic acid	0.49 ± 0.85^{a}	0.19 ± 0.12^{b}	0.12 ± 0.99^{b}	0.06 ± 0.02^{c}	0.14 ± 1.02^{b}	—
Chlorogenic acid	5.41 ± 0.01^{a}	0.41 ± 0.32^{b}	0.71 ± 0.12^{b}	0.54 ± 0.56^{b}	—	0.5 ± 0.26^{b}
Catechin hydrate	1.09 ± 1.13^{c}	1.09 ± 0.01^{c}	5.23 ± 0.57^{a}	2.84 ± 0.11^{b}	0.09 ± 0.99^{d}	0.11 ± 0.45^{d}
Caffeic acid	1.52 ± 0.16^{b}	2.01 ± 0.02^{b}	0.5 ± 1.14^{c}	4.08 ± 0.36^{a}	0.52 ± 0.53^{c}	0.49 ± 0.28^{c}
Syringic acid	1.56 ± 0.02^{b}	3.76 ± 0.05^{a}	2.54 ± 0.20^{b}	4.25 ± 0.25^{a}	2.01 ± 0.64^{b}	0.95 ± 0.98^{c}
Vanillic acid	5.21 ± 0.15^{d}	14.25 ± 0.02^{c}	32.95 ± 1.23^{b}	38.97 ± 1.27^{a}	4.99 ± 0.08^{d}	3.57 ± 0.84^{d}
Naringin	0.8 ± 0.98^{c}	3.23 ± 0.65^{a}	1.68 ± 1.04^{b}	0.98 ± 0.04^{c}	0.43 ± 0.94^{c}	0.22 ± 0.63^{d}
Ferulic acid	1.69 ± 0.25^{a}	0.72 ± 0.65^{b}	0.26 ± 0.9^{b}	0.4 ± 0.21^{b}	—	—
trans-Hydroxycinnamic acid	1.16 ± 0.30^{a}	0.74 ± 0.3^{b}	0.25 ± 1.92^{b}	0.12 ± 0.02^{b}	0.16 ± 0.71^{b}	0.46 ± 0.01^{b}
Luteolin	0.67 ± 1.12^{b}	—	0.34 ± 0.12^{b}	—	1.73 ± 0.43^{a}	1.47 ± 0.17^{a}
Kaempferol	—	0.83 ± 1.15^{a}	—	0.1 ± 0.05^{b}	—	—
Naphtoresorcinol	0.63 ± 0.35^{a}	—	0.19 ± 0.15^{b}	—	0.22 ± 0.27^{b}	0.22 ± 0.44^{b}
Apigenin	0.29 ± 0.65^{b}	0.9 ± 0.06^{a}	0.26 ± 0.4^{b}	0.25 ± 0.91^{b}	0.14 ± 0.16^{b}	0.87 ± 0.02^{a}
Flavone	1.07 ± 0.01^{b}	0.21 ± 1.07^{c}	0.48 ± 0.62^{c}	0.13 ± 0.52^{c}	2.08 ± 0.04^{a}	1.93 ± 0.55^{a}
Coumarin	24.88 ± 0.85^{c}	20.38 ± 0.08^{d}	20.57 ± 0.11^{d}	33.53 ± 0.07^{b}	31.09 ± 0.31^{b}	40.54 ± 0.39^{a}
Carnosic acid	0.37 ± 0.75^{c}	0.71 ± 0.11^{c}	1.34 ± 0.01^{b}	—	5.88 ± 0.22^{a}	1.18 ± 0.22^{b}
2,5 Dihydroxybenzoic acid	1.4 ± 0.32^{a}	1.78 ± 0.15^{a}	0.69 ± 0.02^{b}	0.58 ± 0.65^{b}	1.91 ± 0.72^{a}	0.43 ± 0.19^{b}
Salicylic acid	—	0.17 ± 0.09^{b}	—	—	0.21 ± 0.29^{b}	0.43 ± 1.05^{a}
TPI	51.64	62.92	68.74	91.5	53.3	58.62

CF = cultivated *Ruta* flowers; CL = cultivated *Ruta* leaves; CS = cultivated *Ruta* stems; SF = spontaneous *Ruta* flowers; SL = spontaneous *Ruta* leaves; SS = spontaneous *Ruta* stems; TPI = represent the total phenols identified. Values with different superscripts (a–d) are significantly different at $p < 0.05$ (means of three replicates).

green phosphate/Mo (V) complex at acid pH. An aliquot of the sample extract was combined with 1 mL of a reagent solution (0.6M sulfuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate). The mixture was incubated in a thermal block at 95°C for 90 minutes. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank [15]. The antioxidant capacity was expressed as mg GAE/g DW. All samples were analyzed in three replications.

2.8. *Scavenging ability on the 2,2-diphenyl-1-picrylhydrazyl radical*

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability of the organ extracts was measured by the method of Hanato et al [16]. One milliliter of the extract at known concentrations was added to 0.25 mL of 0.2 mmol/L DPPH methanolic solution. The mixture was shaken vigorously, and then allowed to stand at room temperature for 30 minutes in the dark. The absorbance was then measured at 517 nm. The antiradical activity was expressed as IC_{50} (μg/mL), which is the concentration required to cause a 50% inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

in which A_0 is the absorbance of the control at 30 minutes, and A_1 is the absorbance of the sample at 30 minutes.

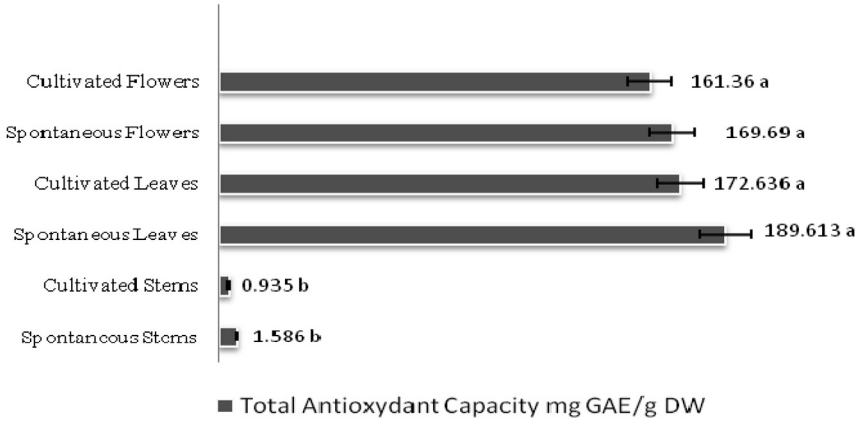
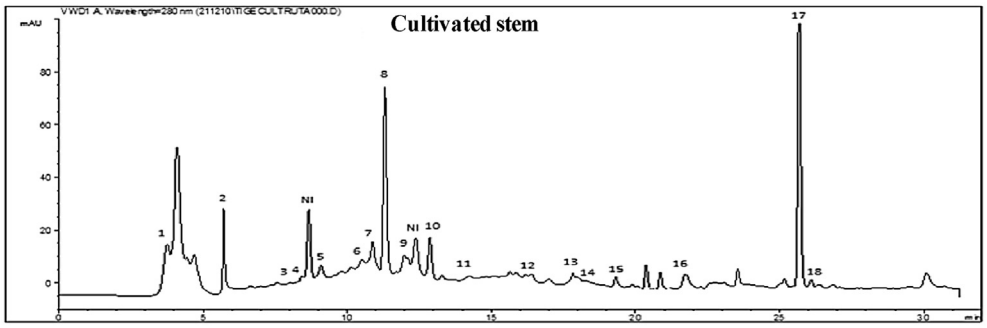


Figure 3 – The total antioxidant capacity in spontaneous and cultivated parts (i.e., stems, leaves, and flowers) from Tunisian *Ruta chalepensis*. The values are presented as the mean of three replicates ± the standard deviation. The data marked with different letters share significance at $p < 0.05$ (based on the Duncan test).

A



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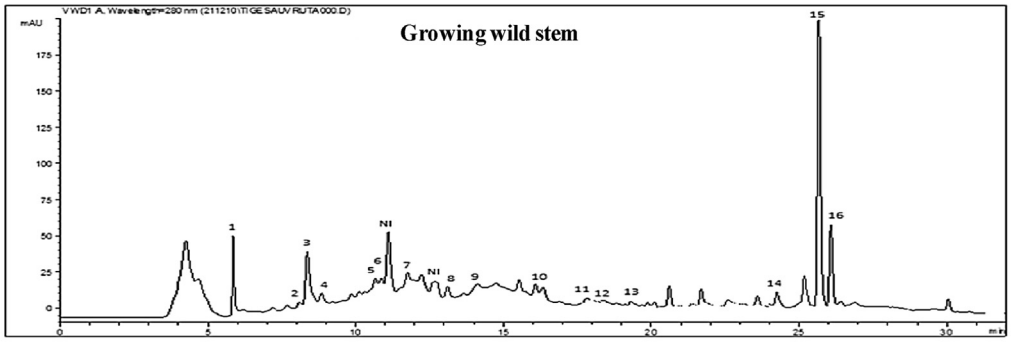
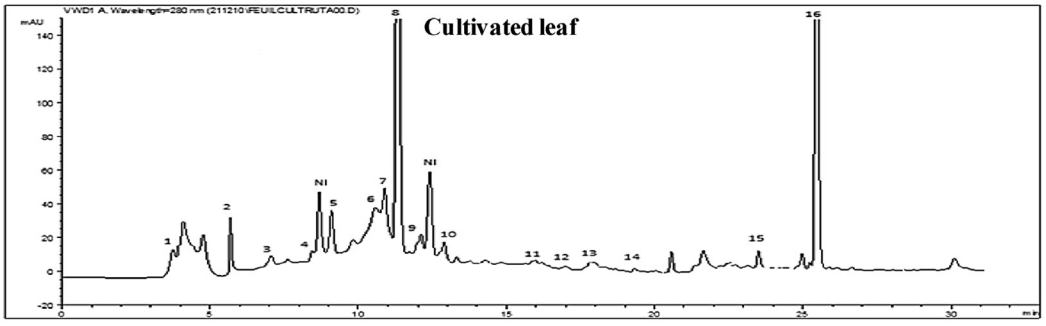


1. Gallic acid; 2. 3,4 dihydroxybenzoic acid; 3. Chlorogenic acid; 4.Catechin; 5.Cafeic acid; 6. Syringic acid; 7. 2,5-dihydroxybenzoic acid; 8. Naringin acid; 9. Ferulic acid; 10. trans hydroxycinnamic acid; 11. Luteolin; 12. Naphtoresorcinol; 13. Apigenin; 14. Flavone 15. Coumarine; 16. Carnosic acid

B



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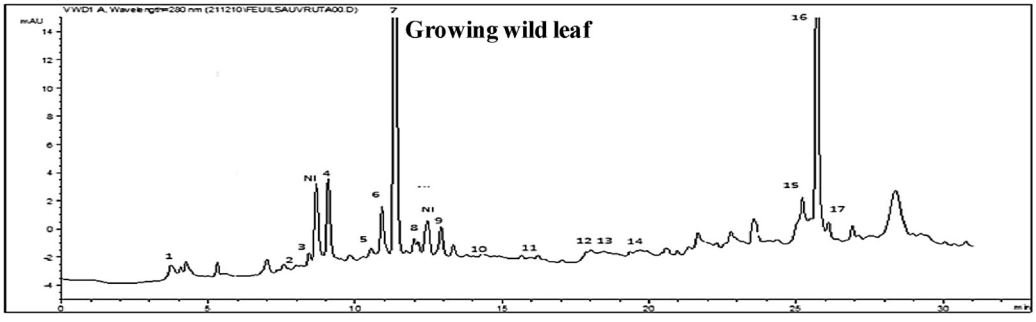

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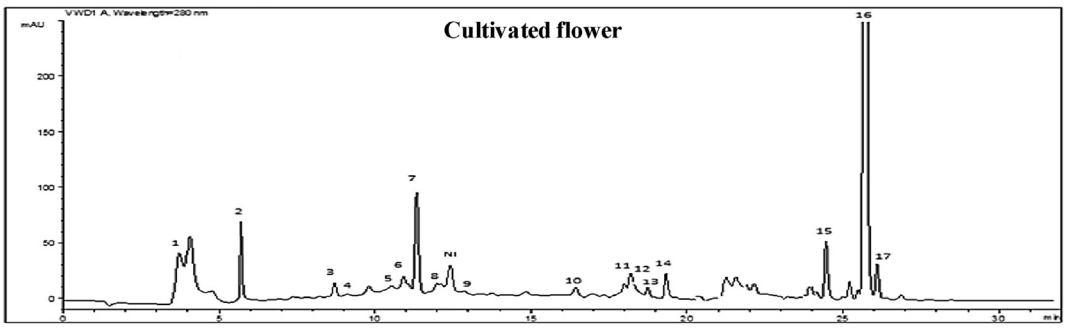
C



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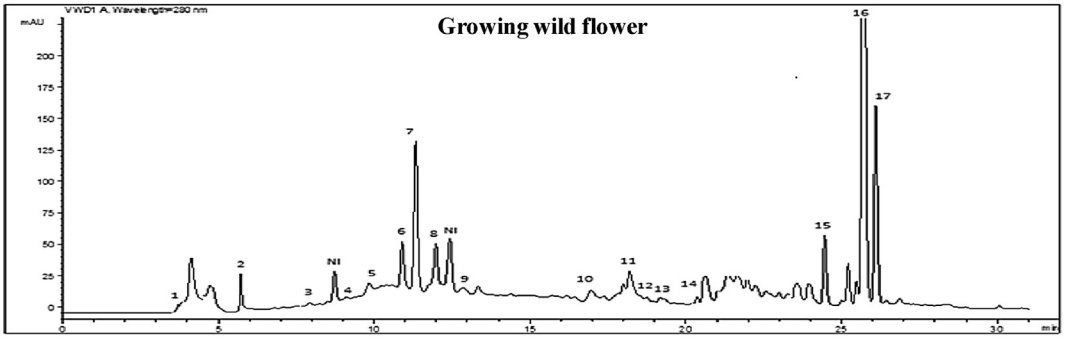

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Figure 4 – (*continued*).

Butylated hydroxytoluene (BHT) was used as a positive control. The samples were analyzed in triplicate.

2.9. *Iron-reducing power*

The reducing power was assessed using the method of Oyaizu [17]. An aliquot of 1 mL was mixed with 2.5 mL of a 0.2M sodium phosphate buffer (pH = 6.6) and 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$), and incubated in a water bath at 50°C for 20 minutes. Thereafter, 2.5 mL of 10% trichloroacetic acid were added to the mixture, which was centrifuged at 650 g for 10 minutes. The supernatant (2.5 mL) was then mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride solution. The intensity of the blue-green color was measured at 700 nm. The EC_{50} value (mg/mL)—the extract concentration at which the absorbance was 0.5 for the reducing power—was calculated from the graph of absorbance at 700 nm against the extract concentration. Ascorbic acid was used as the positive control.

2.10. *Screening of the antibacterial activity*

The antibacterial activity of the extracts (leaves, stems, and flowers) was assessed by the agar disk diffusion assay [18] against three human pathogenic bacteria: Gram positive bacteria, which included *Staphylococcus aureus* (ATCC 25923) (Collection National Institut of Neurologie, Tunisia), and Gram negative bacteria, which included *Escherichia coli* (ATCC 35218), and *Pseudomonas aeruginosa* (ATCC 27853). The bacterial strains were first grown on Muller Hinton medium at 37°C for 24 hours before seeding onto the nutrient agar. One or several colonies of the indicator bacteria were transferred into active pharmaceutical ingredient (API) suspension medium (BioMérieux, Hazelwood, MO, USA) and adjusted to the 0.5 McFarland turbidity standard with a Densimat (BioMérieux). A sterile filter disc, 6 mm in diameter (Whatman paper No. 3), was placed on the infusion agar seeded with bacteria and 50 μL of several extract concentrations were dropped onto each paper disc, representing 5 mg per disc. The treated Petri dishes were incubated at 37°C for 24 hours. Antibacterial

Figure 4 – A. Reverse-phase high-performance liquid chromatography (RP-HPLC) chromatographic profiles of the phenolic compounds in cultivated and wild-grown *Ruta chalepensis* stem extract monitored at 280 nm. B. The RP-HPLC chromatographic profiles of the phenolic compounds of cultivated and wild-grown *R. chalepensis* leaf extract monitored at 280 nm. C. The RP-HPLC chromatographic profiles of the phenolic compounds of cultivated and wild-grown *R. chalepensis* flower extract monitored at 280 nm.

activity was assessed by measuring the zone of growth inhibition surrounding the discs. Standard discs of gentamycin (10 UI) served as positive antibiotic controls according to CASFM 2005 guidelines.

3. Results and discussion

3.1. Total polyphenol, flavonoid, and condensed tannin content

The results in Figure 1 indicated that the total phenolic content was more organ- than provenance-dependent and varied 0.2–168.91 mg of GAE/g DW. Of the two provenances, flowers were the richest organ for polyphenols. The levels were high but not significantly different between the provenances. In fact, the values were 168.91 mg CE/g DW and 163.26 mg CE/g DW for the wild and cultivated *Ruta*, respectively. This phenomenon was also noted for the leaves, which presented a high but not significantly different content of polyphenols at 129.69 mg CE/g DW and 119.2 mg CE/g DW for the wild and cultivated *Ruta*, respectively. By contrast, the stems of the two *Ruta* exhibited weak content. The two *Ruta* provenances exhibited no significant differences in the flavonoid and tannin content of their leaves and flowers s (Figure 1). In fact, the flavonoid content was high and approximately 50 mg CE/g DW. Moreover, for the two *Ruta* provenances, the leaves and flowers were particularly rich in condensed tannin (Figure 1) with the highest content in the cultivated *Ruta* leaves at 435.89 mg EC/g DW. The content was approximately 350 mg EC/g DW in the cultivated flowers and in the spontaneous leaves and flowers. For the total polyphenols, the stems of the two provenances showed weak flavonoid and tannin content. For the leaves, the polyphenol and flavonoid content in our experiment were close to the content reported by Fakhfakh et al [8] in the Tunisian wild *Ruta*. However, compared with other *Ruta* species, the Tunisian *R. chalepensis* seems to be a rich source of phenolics. In fact, a lower content of polyphenols was reported in *R. graveolens* shoots (37 mg GAE/g DW [19]) and leaves (4.3 mg GAE/g DW, [20]), and in *R. montana* extracts (3.13 mg GAE/g DW, [21]). Independent of the provenance, the variation of polyphenol, flavonoid, and tannin concentrations in our experiment between *R. chalepensis* organs suggests that phenolic production in the plant is primarily allocated to the reproductive organ (i.e., the flowers) and secondarily to the leaves. Moreover, our results demonstrated that the wild and cultivated *Ruta* exhibited similar phenolic contents. This finding is in accordance with Vogel et al [22]. Our results indicate that *Ruta* is suitable for cultivation and that its cultivation under a protected environment does not alter the production of secondary metabolites such as polyphenols, flavonoids, and condensed tannins.

3.2. Identification of phenolic compounds using RP-HPLC

Twenty phenolic compounds were tentatively identified by HPLC analysis in the different parts of the two *Ruta* provenances (Figure 4) and included 12 phenolic acids with three hydroxycinnamic acids (i.e., caffeic, *trans*-hydroxycinnamic, and ferulic acid), and nine hydroxybenzoic acids (i.e., protocatechuic acid, 3,4-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, gallic acid, chlorogenic acid, syringic acid, carnosic acid, salicylic acid, and vanillic acid). In addition, seven flavonoids were identified: apigenin, catechin hydrate, flavone, luteolin, naringin, kaempferol, and naphthoresorcinol. The phenolic acids and coumarin represented the major classes of phenols in the two *Ruta* provenances organs, whereas the flavonoids were weakly represented with percentages that did not exceed 10% of the total phenols (Figure 2). A comparison of the two provenances revealed that stems and leaves of the cultivated *Ruta* exhibited significantly higher phenolic acid levels than the wild *Ruta*. Moreover, higher coumarin levels were encountered in the leaves and the flowers of cultivated *Ruta,* compared with the wild *Ruta*. The stems of cultivated *Ruta* and the leaves of cultivated and spontaneous *Ruta* were characterized by the predominance of vanillic acid (Table 1). Moreover, the extracts of spontaneous and cultivated *Ruta* stems and flowers were particularly rich in coumarin (Table 1). In accordance with our results, Al-Said et al [23] showed that the extract of the aerial parts of *R. chalepensis* contain a high quantity of coumarins, flavonoids, and tannins. On the other hand, our results suggested that coumarin can confer an important value to *Ruta* flowers and stems, which may therefore be an alternative commercial source of natural coumarins, whereas *Ruta* leaves can be a natural source of vanillic acid. Analysis of the phenolic composition indicated that kaempferol was detected only in cultivated *Ruta* stems and leaves, and naphthoresorcinol and luteolin were detected only in the stems and leaves of spontaneous *Ruta* (Table 1). The variation of phenolic compounds between the two *Ruta* provenances could be attributed to the effect of climate and location [24]. On the other hand, variations were observed between different *Ruta* parts; for example, ferulic acid was detected in stems and leaves, but absent in flowers; salicylic acid was detected in the stems and flowers, but absent in the leaves. The variations could be related to morphological differentiation occurring during the phenological cycle.

3.3. Evaluation of total antioxidant capacity and scavenging ability on DPPH radical and iron-reducing power

Several methods are commonly used to measure the antioxidant capacity of extracts. Each method results in the generation of or uses a different radical that is directly involved in the oxidative process through a variety of mechanisms. No single assay can represent the total antioxidant capacity, and for this reason three different and complementary assays were used to evaluate the extract antioxidant activities: total antioxidant capacity, DPPH free radical scavenging, and the reducing power.

As shown in Figure 3, the total antioxidant capacity of *R. chalepensis* was high in the leaves and flowers of the two provenances with significantly lower levels in stems: 189.61 mg GAE/g DW and 172.63 61 mg GAE/g DW for spontaneous and cultivated *Ruta* leaves, respectively, and 169.6 mg GAE/g DW and 161.36 mg GAE/g DW for spontaneous and cultivated *Ruta* flowers, respectively. For the total antioxidant activity, spontaneous and cultivated *Ruta* leaf and flower extracts both had higher scavenging activity than the stem

Table 2 – The antioxidant activities of the methanolic extract from different Tunisian *Ruta chalepensis* parts.

	Reducing power (EC_{50} mg/mL)	DPPH (IC_{50} μg/mL)
Spontaneous stems	1.96 ± 5.492^{a}	79.47 ± 0.192^{b}
Cultivated stems	2.05 ± 1.786^{a}	80.77 ± 0.150^{a}
Spontaneous leaves	0.90 ± 6.527^{c}	30.69 ± 0.041^{c}
Cultivated leaves	1.71 ± 6.008^{b}	28.89 ± 0.171^{c}
Spontaneous flowers	1.10 ± 1.372^{c}	23.73 ± 0.1^{c}
Cultivated flowers	0.92 ± 0.113^{c}	28.48 ± 0.06^{c}
BHT	—	25 ± 0.2^{c}
Ascorbic acid	$40.10^{-3} \pm 0.13^{d}$	—

EC_{50} = the effective concentration at which the absorbance is 0.5 for reducing power; IC_{50} = the concentration at which DPPH radicals are scavenged by 50%.
The values are presented as the mean ± the standard deviation ($n = 3$). Mean values with different letters within a row are significantly different ($p < 0.05$).

extracts (Table 2). This radical scavenging ability was high with IC_{50} values ranging 23.73–30.69 μg/mL. Furthermore, the scavenging ability of the spontaneous flower extract (23.73 μg/mL) was higher than that of BHT (25μg/mL).

In the reducing power assay (Table 2), spontaneous *Ruta* leaf and cultivated *Ruta* leaf and flower extracts demonstrated the highest reducing power (0.90 mg/mL, 0.92 mg/mL, and 1.10 mg/mL, respectively), followed by the stem extracts (1.96 mg/mL and 2.05 mg/mL for the spontaneous and cultivated provenances, respectively). These results demonstrated that *R. chalepensis* extracts exhibited a variable activity to reduce iron as a function of the plant part and provenances studied.

3.4. Correlations

The results of the total phenols (TP), total flavonoids (TF), total condensed tannins (CT) analysis and the different antioxidant assays used in the present investigation were compared and correlated with each other. Correlations between the results of different assays are shown in Table 3. Significant positive correlations (R^2 = 0.66–0.97) were observed between the total phenolic content and values for total antioxidant and DPPH scavenging activities of the leaves and flowers of the two *Ruta* origins. Moreover, total phenolics were highly correlated with the reducing power in the spontaneous flowers (R^2 = 0.96) and cultivated flowers (R^2 = 0.99), indicating the significant contribution of phenolics to these antioxidant assays. Furthermore, the strong correlations (R^2 = 0.95–0.99) between the total antioxidant activity and DPPH scavenging activity and condensed tannin content of the spontaneous and cultivated *Ruta* leaves suggest that the high antioxidant capacities of these organs may in large part result in the contribution of these compounds. For the spontaneous flowers, the free radical scavenging activity was closely correlated with the flavonoid content (R^2 = 0.99).

Investigation of correlations between the percentage of coumarin, the main phenolic compound in the *Ruta* organs, and the different antioxidant assays indicate that samples of both spontaneous and cultivated *Ruta* leaves and flowers showed a linear correlation between the content of coumarin and total antioxidant activity, DPPH scavenging ability, and reducing power (R^2 = 0.76–0.99). These results indicate that coumarin is the major contributor to the antioxidant activities of extracts from the leaves and the flowers of the two *Ruta* origins. Thus, antioxidant activity of extracts from different parts of *R. chalepensis* may be primarily explained by their phenolic composition. Coumarin and vanillic acid were the major components identified in *R. chalepensis* and are expected to quench DPPH radicals.

3.5. Screening for antibacterial activity

The emergence of multidrug resistance in human and animal pathogenic bacteria and undesirable adverse effects of certain antibiotics have triggered immense interest in the search for new antimicrobial drugs of plant origin. In the present study, methanolic extracts of different *R. chalepensis* parts have been tested against drug-resistant bacteria and pathogenic yeast, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The antimicrobial activity of the extracts and their potency were quantitatively assessed by the presence or absence of an inhibition zone and zone diameter, respectively, as presented in Table 4. The results presented in Table 4 show that the extracts from the wild and cultivated *Ruta* organs exhibit marked antibacterial activities. Spontaneous *Ruta* stem extract were particularly effective against *S. aureus* and *P. aeruginosa* with diameters of inhibition of 16.3 mm and 17.7 mm, which was close to that of the standard antibiotic gentamycin. Moreover, cultivated *Ruta* stem extract exhibited the highest antibacterial activity against *Escherichia coli*.

On the other hand, the efficiency of these extracts against the bacteria could in part be because of their phenolic composition. In fact, several studies attributed the inhibitory effect of plant extracts against bacterial pathogens to their

Table 3 – The correlation between antioxidant assays and total phenol, flavonoid, and tannin contents.

	SS			CS			SL			CL			SF			CF		
	TAC	IC_{50}	CE_{50}	TAC	IC_{50}	CE_{50}	TAC	IC_{50}	CE_{50}	TAC	IC_{50}	CE_{50}	TAC	IC_{50}	CE_{50}	TAC	IC_{50}	CE_{50}
TP	0.99	0.54	0.32	0.75	0.87	0.53	0.80	0.77	0.61	0.97	0.82	0.43	0.97	0.98	0.96	0.87	0.66	0.99
TF	0.02	0.69	0.96	0.82	0.92	0.62	0.80	0.77	0.62	0.63	0.77	0.48	0.99	0.96	0.98	0.78	0.75	0.96
CT	0.62	0.81	0.10	0.25	0.97	0.1	0.99	0.99	0.97	0.95	0.99	0.43	0.67	0.75	0.69	0.30	0.25	0.89

CF = Cultivated flower; CL = Cultivated leaf; CS = Cultivated stem; CT = condensed tannins; EC_{50} = Reducing power; IC_{50} = 2,2-diphenyl-1-picrylhydrazyl scavenging activity; SF = Spontaneous flower; SL = Spontaneous leaf; SS = Spontaneous stem; TAC = Total antioxidant capacity; TF = total flavonoids; TP = total phenols.

Table 4 – Antibacterial activity of the extract from Tunisian *Ruta chalepensis* parts against human pathogenic bacteria.

	Inhibition zone diameter					
	Gram positive bacteria		Gram negative bacteria			
	Staphylococcus aureus		*Pseudomonas aeruginosa*		*Escherichia coli*	
Spontaneous stems	16.3 ± 0.6^{b}	++	17.7 ± 0.4^{a}	++	13.3 ± 1.1^{c}	+
Cultivated stems	14.7 ± 1.2^{b}	++	7.7 ± 0.2^{c}	–	17.3 ± 0.3^{a}	+
Spontaneous leaves	15.3 ± 1.2^{b}	++	16.7 ± 0.6^{a}	++	14.3 ± 0.4^{c}	++
Cultivated leaves	12.3 ± 1.5^{b}	+	9.7 ± 1.3^{c}	+	16.3 ± 0.6^{a}	++
Spontaneous flowers	15 ± 0.6^{b}	++	16.3 ± 1.1^{a}	++	15.7 ± 0.9^{b}	++
Cultivated flowers	16 ± 1^{a}	++	15 ± 0.5^{b}	++	13 ± 1.2^{c}	+
Gentamycin	24–28.5	+++	15.5–22.5	+++	22–26.1	+++

"–" = Ø < 8 mm; "+" = 9 mm < Ø < 14 mm; "++" = 15 mm < Ø < 19 mm; "+++" = Ø < 20 mm.
The data are presented as the mean ± the standard deviation.
The values are presented as the mean of three replicates ± the standard deviation. The data marked with different letters share significance at $p < 0.05$ (based on the Duncan test).

phenolic composition [25,26]. The inhibitory effect of phenolic compounds could be explained by adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation [27]. Moreover, comparison of the antibacterial ability between cultivated and spontaneous *Ruta* organs revealed that the activity was strain- and origin-dependent. Spontaneous and cultivated *Ruta* organs possess similar phenolic and flavonoid and tannin contents; therefore, the differences in the antibacterial activity could be because of the presence of specific antibacterial compounds, in addition to the phenolic compounds.

4. Conclusion

Our results indicated that the wild and cultivated *R. chalepensis* organs had similar contents of polyphenols, flavonoids, and tannins. Moreover, the organs were rich on vanillic acid and coumarin with the highest levels in the cultivated leaves and flowers, respectively. The results indicated that the leaves and flowers of the cultivated and wild-grown *Ruta* possess strong antioxidant and antibacterial activities with no significant differences. The cultivation of *Ruta* is easy; therefore, the leaves and flowers of this plant could be a prospective source of natural bioactive molecules that could replace synthetic antioxidants and serve as a potential source of antibacterial agents in the food industry.

Conflicts of interest

All contributing authors have no conflicts of interest to declare.

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