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Assessment of antioxidant activities of an endemic species from Tunisia: *Rhanterium sueaveolens* Desf related to its phenolic composition

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ABSTRACT

In the present study, the phenolic composition and the antioxidant activities of aqueous methanol extracts from the flowers, stems and roots of *Rhanterium suaveolens* were investigated to assess its potential as a source of promoting bioactive constituents. The identification and the quantification of phenolic compounds were assessed by HPLC-ESI-MS. A total of 24 metabolites including phenolic acids and flavonoids were identified and reported for the first time in *R. suaveolens* species. Chlorogenic acid and its derivates; 3.4-di-O-caffeoyquinic and 4.5- di-O-caffeoyquinic acids were identified as the major compounds in the flowers and the stems extracts. They were predominant in the roots extract but with lower amounts. On the other hand, quercetin-3-O-galactoside was found to be the major flavonoids in the studied extracts. Furthermore, results showed that the flower extracts displayed the most effective DPPH scavenger ($EC_{50} = 0.331 \pm 0.009$ mg/ml) and ferric reducing power (EC1 = 0.408 ± 0.006 mg/ml). These findings support the use of *R. suaveolens* as a promising source of alternative bioactive ingredients for both industrial and medicinal uses.

1. Introduction

Due to the increase of consumer's interest for natural ingredients as well as the limitation use of synthetic chemicals due to their harmful effects, the search for natural alternatives was proved necessary. In this purpose many studies have been conducted to identify new alternative and natural sources with natural antioxidant compounds. Among the emerging source of natural ingredients, phenolic compounds have shown a big interest as agents with several healthy-promoting activities such as antimutagenic, antitumorogenic and anticarcinogenic, properties (Musa and Gasmelseed, 2012) and they present one of the most interesting groups of bioactive compound used as natural dietary antioxidants and that are widely present in vegetables, fruits and herbs (Li et al., 2017).

In Tunisia a wide range of medicinal plants are used in the traditional medicine for medicinal purpose. Despite their strong potential biological activities, many of these plants have not been yet investigated for their chemical composition as well as their biological capacities (Ben Masnsour et al., 2017). For example *Rhanterium suaveolens* Desf (*Asteraceae)*, is an endemic species from North Africa (Quezel and Santa, 1963; Wiklund, 1986) growing in arid regions of Tunisia and commonly known in Tunisia as « Arfadj ». In folk medicine, some of the *Rhanterium* species are used as an antidiuretic. They are also used by the local population in the cheese production (Hamia et al., 2013). The genus *Rhanterium* (Wiklund, 1986) belongs to the *Asteraceae* family. It is distributed over western North Africa, the Arabian Peninsula, Iraq and Iran. Several species have been reported, among which *R. adpressum* and *R. epapposum* (Kala et al., 2009; Bouheroum et al., 2007; Yaghmai and Kolbadipour, 1987).

Only a few studies have been carried out on the investigation of the secondary metabolites of *Rhanterium* species (Bouheroum et al., 2007; Kala et al., 2009; Hamia et al., 2013). Extracts of *this* species have been shown to pocess interesting biological properties such as antioxidant (Boussoussa et al., 2014; Bouaziz et al., 2009) and anticlynesterase (Chemsa et al., 2016) activities. In addition, a literature survey on the phytochemical profiles of this genus reveals that it presents a variety of bioactive molecules such as β-Eudesmol, Stigmasterol and 16-b-Hydroxylupeolyl-3-hexadecanoate identified from the chloroform extracts of

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R. adpressum aerial parts (Bouheroum et al., 2007). However, few detailed studies on the phytochemical profile of *R. suaveolens* have been reported (Bouheroum et al., 2007; Kala et al., 2009; Hamia et al., 2013). In previous studies the investigation of the phenolic composition of this plant revealed the presence of antimicrobial polyacetyleneic alcohols (Oueslati et al., 2004), ranthenone glucoside, 9-hydroxylinaloyl glucoside, scopolin, fraxetin, scopoletin, sitosterol-3β-O-[6-palmitoyl-β-D-glucopyranoside] (Oueslati et al., 2007) and ceramides (Oueslati et al., 2005).

To the best of our knowledge, this is the first report on the phytochemical profile of Tunisian *R. suaveolens*. Studies conducted on this species have for interest the prospection for new bioactive molecules with antioxidants activities. In this context, the present study was designed to screen the phenolic composition of different plant parts of *R. suaveolens*. Aqueous methanol was used to the extraction of the bioactive compounds from the plant material. The antioxidant activities (DPPH and FRAP), total phenolics and flavonoids were determined. Furthermore the main phenolic compounds of *R. suaveolens* species were identified using HPLC-ESI-MS.

2. Materials and methods

2.1. Plant material and sample preparation

Rhanterium suaveolens Desf. samples were collected from Tataouin (south of Tunisia), during the flowering period. A local botanist at the Institute of Arid zone research (IRA) in Medenine (Tunisia) Institute authenticated the harvested plant and a voucher specimen was deposited at the Herbarium of the Laboratory of Pastoral Ecology. The plant samples were separated into different parts: flowers, roots and stems. All parts were rinsed with distilled water and then allowed to air dry at room temperature. Finally the dried parts were grinded to fine powders which were stored in air-tight container.

In the present study, the extraction process was carried out using the ultrasonic assisted extraction method. The choice of this method was related to the reduction of the use of solvent, the energy and the extraction time (Falleh et al., 2019). In brief, 5 g of plant dried powder were added to 50 mL of extractive solvent (70% methanol) in graduated flask and were sonicated in an ultrasound bath **(Elmasonic S 30 H/2.75L)** at 35 °C for 30 min, working at 50 kHz of frequency. The crude extract was filtered using a Whatman n°5 filter paper and then was collected in an Erlenmeyer flask for rotary evaporation. The obtained extracts were concentrated using a rotary evaporator ((Büchi, Rungis, France) in a vacuo at 40 °C. Then, the resulting concentrates were lyophilized in a freeze-dryer (Christ, Osterode am Harz, Germany) and finally stored in dark glass at 4 °C until analysis. The yields of aqueous methanol extracts were 10.2%, 5.8% and 1.4% (w/w) for flowers, stems and roots respectively.

2.2. Phenolic composition analysis by HPLC-ESI-MS

Stock solutions with concentrations (m/v) of 5 mg/ml were prepared by dissolving each plant dried extract (DE) in methanol 70%. Before injection into the HPLC-ESI-MS. All samples were filtered through a 0.45 µm pore size nylon filter.

The analysis of phenolic compounds in the *R. suaveolens* extracts was performed on a Shimadzu Ultra Fast Liquid system (Shimadzu prominence UFLC XR, Japon) consisting on an auto-sampler, a column oven, a binary pump and a quadripole 2020 detector system. The chromatographic separation was performed using a Discovery BIO Wide Pore C_{18} column (250 mm × 4.6 mm, 5 µm) at 40 °C. The mobile phase contained 0.1% (v/v) TFA in distilled water (solvent A) and 0.1% (v/v) TFA in methanol (solvent B). The analysis was performed using a linear gradient elution program from 10% to 100% of solvent B with a flow rate of 0.5 mL/min. The binary gradient elution was described as follow: 0–14 min, 10% B; 14–27 min, 20% B; 27–37 min 55% B; 37–45 min, 100% B; 45–50 min 10% B. For peak identification *R. suaveolens* samples, a Shimadzu 2020 (Japon) Quadrupole mass spectrometer equipped with a positive/negative ESI source was used as a detector. An interface voltage of 1.2 V under a nebulising gas flow rate of 1.5 L/min, a heat block temperature of 400 °C, a desolvation line temperature of 280 °C, a drying gas flow of 15.00 L/min and a spray voltage of −3.5 V were utilized. The other analytical conditions were the same as used for HPLC analysis. Quantitative determination was carried out using calibration curves of different standards.

2.3. TPC and TFC analysis

The total phenolic content (TPC) was carried out using Folin–Ciocalteu reagent according Dewanto et al. (2002). TPC was expressed as mg of gallic acid equivalents/g of dried extract (mg GAE/g DE). The total flavonoid content (TFC) was determined using the modified assay described by Dewanto et al. (2002). The TFC value was expressed as mg catechin equivalents/g of dried extract (mg CE/g DE). All samples were analyzed in triplicates.

2.4. Determination of antioxidant capacities

The antioxidant activity of the *R. suaveolens* extracts was determined using DPPH as a free radical according Brand-Williams et al. (1995). Antiradical activity was defined as the massic concentration of plant extract necessary to decrease the initial DPPH° concentration by 50% (Efficient Concentration = EC_{50} (µg/ml)). Antioxidant activity was determined by using catechine, ascorbic acid, gallic acid (GA), trolox and BHT as positive control. The FRAP assay (Ferric Reducing Antioxidant Power assay) was performed on plant extracts and standards according the procedure of Benzie and Strain (1996). The results are expressed as Equivalent Concentration 1 or (EC_1), the concentration of extract having a ferric-TPTZ reducing ability equivalent to that of 1 mmol/L $FeSO_4$–$7H_2O$, as described previously (Pulido et al., 2000). All experiments were performed in triplicate.

2.5. Statistical analysis

The results were reported as mean ± standard deviation (SD) of three independent experiments. Statistical analysis was performed using Minitab 18 Statistical Software (Minitab Inc., U.S.A.). For evaluating differences in the studied extracts one-way analysis of variance (ANOVA) with Tukey's was used ($p < 0.05$).

3. Results and discussion

3.1. Method linearity

The linear range of the standard studied solutions was evaluated. Linearity was observed in the calibration curves of studied standards at concentrations ranging from 0.5 to 20 mg/L the obtained regression coefficients (R^2) were higher than 0.99. Fig. 1 presented the calibration curves of quinic acid, 1-3-di-O-caffeyoquinic acid, hyperoside and epicatechin.

3.2. Qualitative and quantitative characterization of phenolic extracts by LC-ESI-MS

Phenolic composition analysis of the *R. suaveolens* extracts has been carried out using LC-MS at a negative mode and the LC–ESI-TIC profiles are shown in Fig. 2. The phenolic compounds were identified by comparing their MS spectral data and retention times with those of reference compounds. For the MS detection, the selection of negative ionization mode was based on its efficiency (Brahim et al., 2017).

In total, 24 phenolic compounds including phenolic acids and flavonoids were identified and were listed in Table 1 according to their

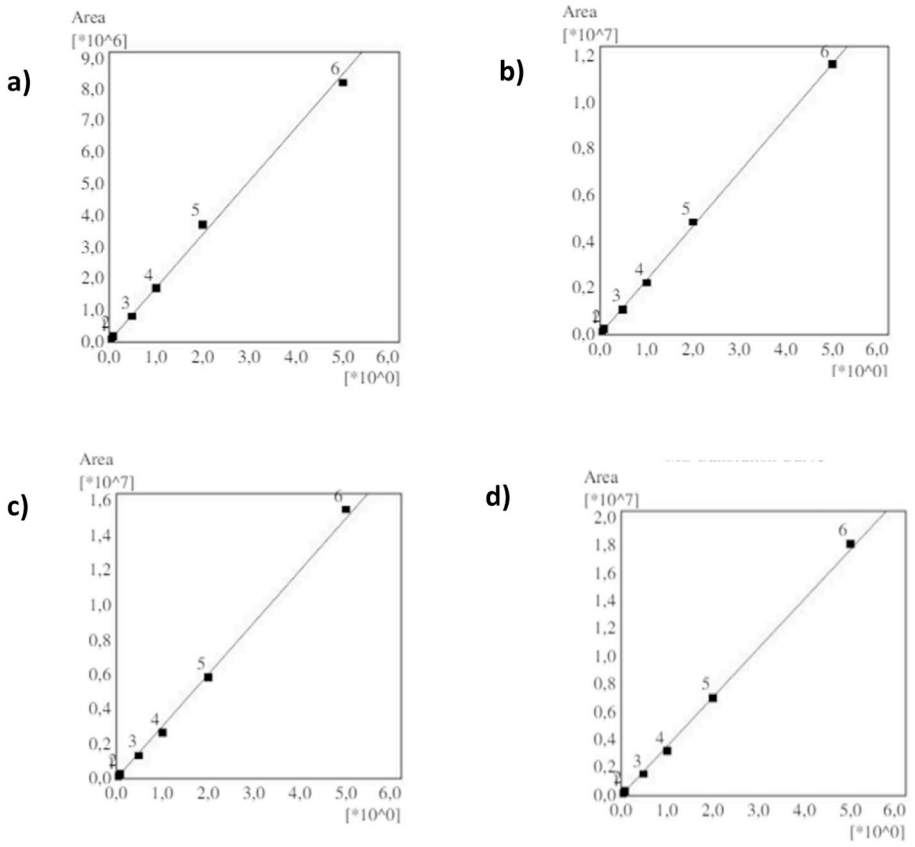

Fig. 1. Calibration curves of (a) Quinic acid, (b) 1-3-di-O-Caffeioquinic acid (c) Hyperoside and (d) Epichatechin.

elution order in LC–ESI-MS total ion current (TIC) chromatograms. The chemical profile of different extracts was found to be dominated by phenolic acids including chlorogenic acid derivates and hydroxycinnamic esters family. Moreover, the identified compounds were reported for the first time in this species however some of them have already been described in other genus from the *Asteraceae* family (Bohm and Stuessy, 2001). Indeed, Bourgou et al. (2017) demonstrated the richness of *Artemisia herba-alba* species from the *Asteraceae* family in Di-O-caffeoylquinic acids and diverse other phenolic acids.

Quantification of phenolic compounds from *R. suaveolens* flowers, stems and roots extracts was ensured using calibration curves of analytical standards. Respective amount of each identified compound is reported in Table 2. The amount of all quantified phenolic compound was determined for each extract. It was defined as the total individual phenolic content (TIPC). The stems extract presented the highest TIPC (530.73 ± 0.27 mg/g DE), followed by the flowers extract (433.10 ± 0.18 mg/g DE) which was slightly lower than that observed for the stems and finally the roots extract (170.13 ± 0.16 mg/g DE). The results indicated that phenolic acids were among the most abundant polyphenols detected in all plant parts (approximately 94.68%, 96.45% and 94.80% of TIPC for the flowers, the stems and the roots, respectively). Regarding individual compounds, the most abundant molecules in the *R. suaveolens* extracts were 4.5-di-O-caffeoylquinic acid, 3.4-di-O-caffeoylquinic acid, chlorogenic acid, 4-O-caffeoylquinic acid, quinic acid and finally quercetin-3-O-galactoside. To the best of our knowledge, this is the first study conducted on the quantification of phenolics presented in different part of this plant or in other species of *Rhanterium*.

As shown in Table 2, a high content of chlorogenic acid and its derivatives was observed in all parts of *R. suaveolens with* chlorogenic, cryptochlorogenic and 3.4-di-O-caffeoylquinic acid being predominant in the flowers while the main compound in the stems was 4.5-di-O-caffeoylquinic acid which was almost equal to 2.5 times of that found in the flowers (357.24 ± 1.10 against 125.74 ± 0.80 mg/g^{-1} DE) respectively. All these compounds were also found in lower proportions in the roots. High quantities of chlorogenic acid and hydroxycinnamic esters family in the aerial parts were reported to be well correlated with plant abiotic stress tolerance under several conditions such as extreme light and temperatures, which correspond to *R. suaveolens* growth conditions (Grace and Logan, 2000).

Many studies in the literature showed that chlorogenic acid has many health-promoting properties such as anti inflammatory (Liang and Kitts, 2016), antihypertensive (Zhao et al., 2012), antidiabetic, antilipidemic (Meng et al., 2013) and anti-oxidant activities (Liang and Kitts, 2016). It has been also reported for its antimicrobial activity against a wide range of microoganisms that makes it an excellent candidate for the formulation of functional and dietary foods (Santana-Gálvez et al., 2017).

In addition, Kang et al. (2016), demonstrated that caffeoylquinic acid derivatives including Di-caffeoylquinic acids suppress cancer cell proliferation by inducing apoptosis. Likewise, According Han et al. (2008), Di-O-caffeoylquinic acids demonstrated potent antioxidant capacity and have been reported as the main antioxidant characterizing many species of *Artemisia* from the *Asteraceae* family.

Furthermore quinic acid was identified in all studied extracts of *R. suaveolens.* The high amount of this compound was observed in the stems extract (11.19 ± 0.10 mg/g DE) and was slightly less in the flower ones (10.66 ± 1.01 mg/g DE). According to the literature, quinic acid has been reported for its antioxidant (Pero et al., 2009) and hepatoprotective (Xiang et al., 2001) properties. Moreover, it can also be used to combat prostate cancer (Inbathamizh and Padmini, 2013). *R suaveolens* extracts contain also minor amounts of *caffeic*, trans-ferulic, protocatechuic, *p*-coumaric, gallic, syringic, rosmarinic and trans-cinnamnic acids. However, gallic and rosmarinic acids were not present in the root extracts.

Recently,Othman et al., 2018 reported that extracts of *R. suaveolens*

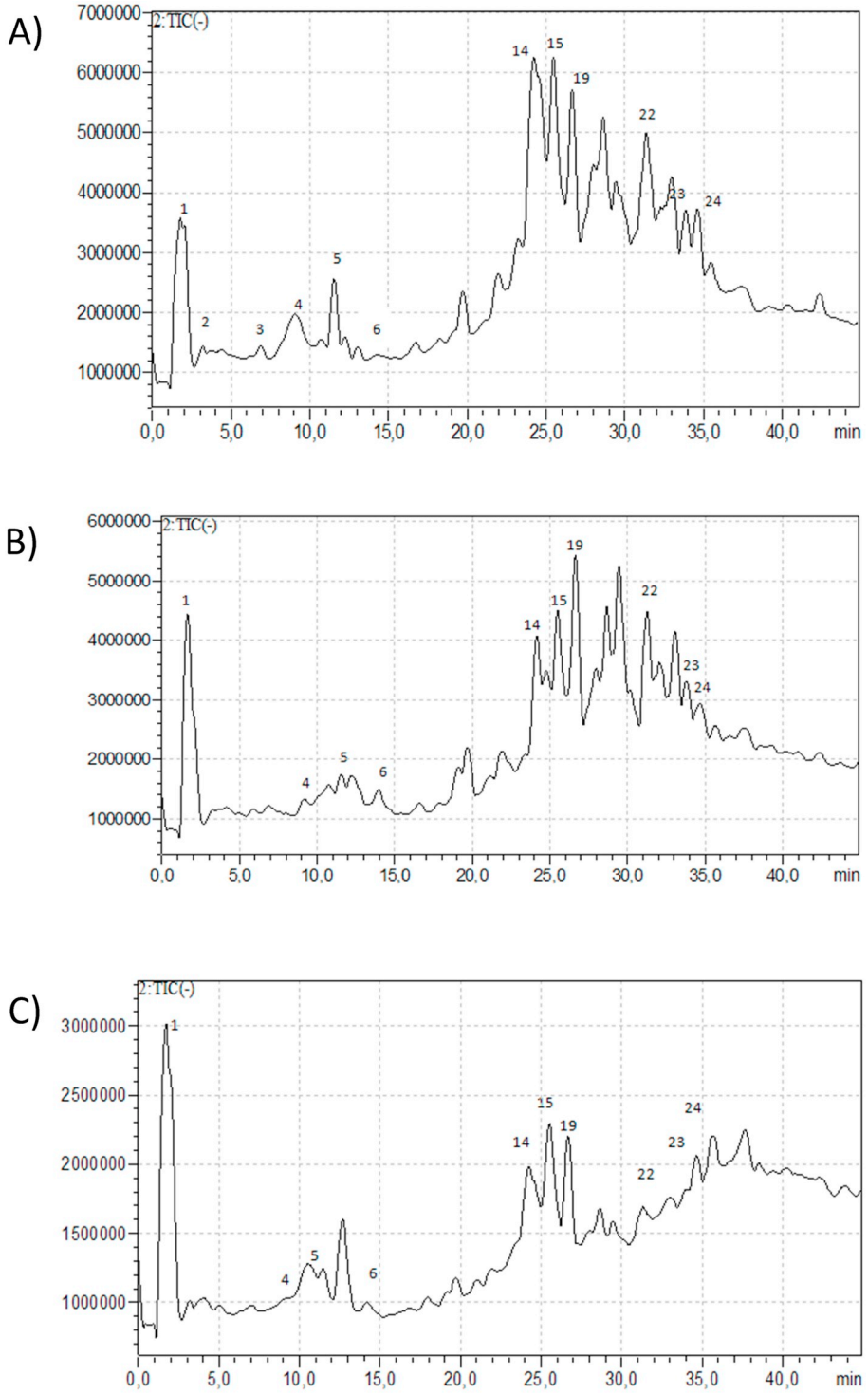

Fig. 2. LC–ESI-MS total ion current (TIC) profiles of *Rhanterium suaveolens* flowers extracts (A) stems extracts (B) and roots extracts (C).

and other species growing in Tunisian desert were shown to have a significant protective effect against heat stress induced in HSP47 cells and heat shock-induced stress in human neuroblastoma SH-SY5Y cells. This recent finding can be related to the presence of phenolic compounds such as chlorogenic acid trans-cinnamic acid and caffeoylquinic acid in these species (Bouaziz et al., 2009; Khlifi et al., 2013; Meot-Duros and Magne, 2009; Chao et al., 2013).

In addition, different types of flavonoids were detected in the *R. suaveolens* extracts. The identified flavonoids included epicatechin, rutin, quercetin, luteolin-7-o-glucoside, hyperoside, quercetrin, apigenin, naringin, luteolin and kaempferol. These phenolic compounds have already been described in other genus from the *Asteraceae* family (Bohm and Stuessy, 2001). Regarding the flavonol group, two types of quercetin derivates were identified; quercitin-3-O-galactoside and quercetrin. Quercitin-3-O-galactoside, also called hypeoside was identified in all studied extracts with an important amount. However, Quercitrin was not identified in the stems extract. In both of the flowers and the stems, approximately equal amounts of quercitin-3-O-galactoside were obtained (16.67 ± 0.10 and 16.48 ± 0.20 mg/g DE). This value was slightly less important in the

Table 1
Phenolic compounds detected in methanolic extracts by LC-ESI-MS from flowers, stems and roots of *Rhanterium suaveolens* Desf. in negative mode.

No	t_R[a](min)	[M-H]$^-$ m/z	Assigned identification[b]	Molecular formula	Flowers	Stems	Roots
1	2.059	191.00	Quinic acid	$C_7H_{12}O_6$	✓	✓	✓
2	3.928	169.00	Gallic acid	$C_7H_6O_5$	✓	✓	✓
3	6.939	153.00	Protocatechuic acid	$C_7H_6O_4$	✓	✓	✓
4	9.071	353.00	Chlorogenic acid	$C_{16}H_{18}O_9$	✓	✓	✓
5	11.554	353.00	4-O-caffeoylquinic acid	$C_{16}H_{18}O_9$	✓	✓	✓
6	14.456	179.00	Caffeic acid	$C_9H_8O_4$	✓	✓	✓
7	15.976	289.00	Epicatechin	$C_{15}H_{14}O_6$	✓	✓	✓
8	16.015	197.00	Syringic acid	$C_9H_{10}O_5$	✓	✓	✓
9	16.915	515.00	1.3-di-O-caffeoyquinic acid	$C_{25}H_{24}O_{12}$	✓	✓	✓
10	20.866	163.00	p-coumaric acid	$C_9H_8O_3$	✓	✓	✓
11	23.018	193.00	Trans-ferulic acid	$C_{10}H_{10}O_4$	✓	✓	✓
12	23.673	609.00	Rutin	$C_{27}H_{30}O_{16}$	✓	✓	✓
13	24.402	447.00	Luteolin-7-o-glucoside	$C_{21}H_{20}O_{11}$	✓	✓	✓
14	24.416	463.00	quercetin-3-o-galactoside	$C_{21}H_{20}O_3$	✓	✓	✓
15	24.695	515.00	3.4-di-O-caffeoylquinic acid	$C_{25}H_{24}O_{12}$	✓	✓	✓
16	25.793	579.00	Naringin	$C_{27}H_{32}O_{14}$	✓	✓	✓
17	26.241	359.00	Rosmarinic acid	$C_{18}H_{16}O_8$	✓	✓	✓
18	26.364	447.00	Quercetrin	$C_{21}H_{20}O_{11}$	✓	✓	✓
19	26.607	515.00	4.5-di-O-caffeoylquinic acid	$C_{25}H_{24}O_{12}$	✓	✓	✓
20	31.724	301.00	Quercetin	$C_{15}H_{10}O_7$	✓	✓	✓
21	31.730	285.00	Kaempferol	$C_{15}H_{10}O_6$	✓	✓	✓
22	31.923	147.00	Trans cinnamnic acid	$C_9H_8O_2$	✓	✓	✓
23	34.263	269.00	Apigenin	$C_{15}H_{10}O_5$	✓	✓	✓
24	34.694	285.00	luteolin	$C_{15}H_{10}O_6$	✓	✓	✓

[a] Retention time.
[b] Confirmed with analytical standards.

roots (7.16 ± 0.50 mg/g DE).

In fact flavonoids compounds have been reported for their potential effect in the treatment of many diseases (Benabderrahim et al., 2019).

Quercitin-3-O-galactoside is a flavonol glycoside occurring naturally in plants that has a broad spectrum of pharmacological and antioxidant properties. It has been reported for its anti-inflammatory, anti-diabetic, anti-cancer, anti-fungal and antioxidant activities (Raza et al., 2017). Data concerning *Rhanterium suaveolens* phenolics is scarce in the literature. Apigenin, a previously described phenolic in this species could be detected in very small proportion; astragalin (a kaempferol glycoside) was not detected, but its aglycone form was; while other previously identified phenolics such as xanthomicrol, 6-hydroxylutéoline 7,3′, 4′–trimethyl-ether, para-hydroxy benzoic acid, vanillic acid (Benaïssa, 2011), or scopoletin and fraxetin (Oueslati et al., 2007) could not be detected, suggesting they were either not extracted or extracted in quantities that were below the detection limit.

3.3. Total phenolic and flavonoid contents

Table 3 presents the TPC and TFC values. As shown, the aqueous methanol extracts from different parts of *R. suaveolens* presented variable phytochemical contents. The flower extract exhibited the highest TPC values (45.5 ± 1.0 mg GAE/g DE) followed by the stems extract (30.9 ± 0.9 mg GAE/g DE) and finally the roots extract (5.2 ± 0.3 mg GAE/g DE). On the other hand, TFC values were much lower than those of TPC. Moreover, they were following the same order as that observed for TPC (12.1 ± 0.2, 7.7 ± 0.1 and 1.9 ± 0.1 mg CE/g DE, for flowers, stems and roots extracts, respectively). The highest content of TPC recorded for the flowers and the stems are in accordance with the results regarding the quantification of phenolics using LC-MS which were obtained earlier in our study. Previously published data concerning *R. adpressum* flower methanolic extracts reported values ranging from 5.55 ± 0.18 to 22.8 ± 0.53 mg GAE/g DE, depending on seasonal variations (Boussoussa et al., 2014; Chemsa et al., 2016). Moreover, when comparing the TPC and TFC with those obtained by other authors (Al Harbi and El-Ashmawy, 2015), the values we obtained for *R. suaveolens* methanolic extract are up to 5–7 fold higher; the ultrasound assisted extraction (UAE) might account for this result since a simple infusion/filtration process was used by all other authors. Generally recovery of TPC is better in UAE as compared to conventional extraction. Indeed, the strong extraction efficiency of the UAE may be attributed to the high intensity and the frequency sound waves involved in the processing (Dhanani et al., 2013).

3.4. Antioxidant activity

The antioxidant potential of *R. suaveolens* extracts was assessed *in vitro*, using the DPPH and the FRAP assays. IC_{50} and EC_1 values of the different studied extracts are shown in Table 4. It can be seen that in both tests, the flower extract exhibited the most interesting EC_{50} and EC_1 values (333 ± 9 μg/mL and 408 ± 6 μg/mL respectively), followed by the stem and the root extracts. These results rank in the same order than the TPC and TFC values. This ranking suggests that antioxidant activity is probably correlated to the phenolic compounds content as well as their chemical structure (Bouaziz et al., 2004).

In general, the antioxidant activity of phenolics is attributed to their redox properties which allow them to act as reducing agents (Falleh et al., 2011). In addition, the synergistic effect between phytochemicals in the extract provides an antioxidant potential dependent on both the concentration, the structure and the interaction among these molecules (Heber et al., 2006).

When comparing with the antioxidant capacity of antioxidant standards (ascorbic acid, BHT and Trolox) or purified phenolics (namely gallic acid and catechin), it was found that the flower extract has a EC_{50} value about only 2–3 times lower than these and the EC_1 obtained for BHT was similar to that obtained with the stem extract (Table 4). According to these results, *R. suaveolens* aqueous methanol extracts exhibited moderate scavenging activity and reducing power. This is consistent with previously reported data concerning *R. suaveolens* ethyl acetate, butanol and methanol extracts (Amrani et al., 2014; Bouaziz et al., 2009).

Thus, further investigation must be developed in order to improve the extraction of phytochemicals from plants using green solvents such as ethanol and water instead of other organic ones (Falleh et al., 2012). In addition, in order to have an important recovery in natural antioxidants, the extraction method should be firstly optimized. Extracts that are rich in antioxidants must be then investigated as a promising source of natural ingredients for cosmetics, pharmaceutical and food industries

Table 2
Quantification of phenolic compounds in the studied extracts of *Rhanterium suaveolens* Desf.

N°	Assigned identification	Flowers	Stems	Roots
1	Quinic acid	10.66 ± 1.01^{a}	11.19 ± 0.10^{a}	5.77 ± 0.40^{b}
2	Gallic acid	0.15 ± 0.00^{a}	0.16 ± 0.02^{a}	–
3	Protocatechuic acid	0.46 ± 0.02^{a}	0.52 ± 0.01^{a}	0.01 ± 0.00^{b}
4	Chlorogenic acid	71.13 ± 2.01^{a}	29.46 ± 0.20^{b}	14.28 ± 0.20^{c}
5	4-O-caffeoylquinic acid	18.39 ± 1.00^{a}	11.71 ± 0.70^{b}	4.59 ± 0.10^{c}
6	Caffeic acid	1.11 ± 0.02^{a}	0.55 ± 0.01^{b}	0.38 ± 0.03^{b}
7	Epicatechin	0.03 ± 0.01^{a}	0.23 ± 0.01^{a}	–
8	Syringic acid	0.55 ± 0.01^{a}	0.25 ± 0.02^{a}	0.203 ± 0.03^{a}
9	1.3-di-O-caffeoyquinic acid	2.53 ± 0.30^{a}	0.54 ± 0.03^{b}	1.13 ± 0.10^{b}
10	p-coumaric acid	0.10 ± 0.00^{a}	0.18 ± 0.02^{a}	0.28 ± 0.1^{a}
11	Trans-ferulic acid	0.92 ± 0.01^{a}	0.81 ± 0.10^{a}	0.536 ± 0.02^{a}
12	Rutin	0.28 ± 0.02^{b}	1.91 ± 0.30^{a}	0.10 ± 0.01^{b}
13	Luteolin-7-o-glucoside	–	–	0.16 ± 0.02
14	quercetin-3-o-galactoside	16.67 ± 0.10^{a}	16.48 ± 0.20^{a}	7.16 ± 0.50^{b}
15	3.4-di-O-caffeoylquinic acid	268.33 ± 1.00^{a}	99.08 ± 1.0^{b}	88.42 ± 2.01^{b}
16	Naringin	0.22 ± 0.04^{a}	0.96 ± 0.10^{a}	0.24 ± 0.01^{a}
17	Rosmarinic acid	0.08 ± 0.00^{a}	0.18 ± 0.20^{a}	0.10 ± 0.01^{a}
18	Quercetrin	4.37 ± 0.30^{a}	–	0.88 ± 0.10^{b}
19	4.5-di-O-caffeoylquinic acid	125.74 ± 0.80^{b}	357.24 ± 1.10^{a}	45.72 ± 0.40^{c}
20	Quercetin	0.27 ± 0.01^{a}	0.02 ± 0.0^{a}	0.04 ± 0.00^{a}
21	Kaempferol	–	0.10 ± 0.2^{a}	0.04 ± 0.00^{a}
22	Trans cinnamnic acid	0.02 ± 0.0^{a}	0.01 ± 0.0^{a}	–
23	Apigenin	0.003 ± 0.0^{a}	0.02 ± 0.0^{a}	0.01 ± 0.00^{a}
24	luteolin	0.02 ± 0.01^{a}	0.04 ± 0.01^{a}	0.02 ± 0.00a
TIPC*		422.10 ± 0.27^{b}	530.73 ± 0.18^{a}	161.30 ± 0.16^{c}

The mean values followed by the same superscripts within a row do not differ, according to the Student's t-test ($p < 0.05$). *Total Identified Polyphenol content. Results are expressed in mg/g DE; $n = 3$.

Table 3
Total bioactive compounds in the flowers, stems and roots methanolic extracts of *Rhanterium suaveolens*.

	Total phenolics (mg GAE/g DE)	Total flavonoids (mg CE/g DE)
Flowers	45.5 ± 1.0^{a}	12.1 ± 0.2^{a}
Stems	30.9 ± 0.9^{b}	7.7 ± 0.1^{b}
Roots	23.0 ± 0.3^{c}	1.9 ± 0.1^{c}

Different letters in the same column indicate a significant difference in the studied extracts ($p < 0.05$).
Values are expressed as means ± standard error (n = 3).

(Li et al., 2006).

4. Conclusion

This is the first paper on the phytochemical profile of the endemic species *Rhanterium suaveolens* growing in Tunisia. The present investigation procured some information concerning the medicinal value of this species. In particular, The LC-MS analysis revealed the identification and the quantification of twenty four compounds in the different plant parts with the dominance of chlorogenic acid family which has interesting antioxidant activities. These findings confirmed the interesting value *of R. suaveolens* and suggest its use as a potential source of bioactive substances used as ingredients for pharmaceutical and cosmetic industry.

Table 4
Antioxidant capacity of *Rhanterium suaveolens* flowers, stems and roots methanolic extracts.

	DPPH EC_{50} (μg/mL)	FRAP EC_1 (μg/mL)
Flowers	333.0 ± 10.00^{c}	480.00 ± 10.00^{d}
Stems	450.00 ± 20.00^{b}	570.00 ± 20.00^{b}
Roots	618.00 ± 9.00^{a}	634.00 ± 25.00^{a}
Ascorbic acid	152.09 ± 0.01^{d}	N.D.
BHT	118.38 ± 0.01^{g}	538.00 ± 0.01^{c}
Trolox	150.43 ± 0.01^{e}	N.D.
GAE	107.50 ± 0.01^{h}	116.33 ± 0.01^{e}
Catechin	121.75 ± 0.01^{f}	45.05 ± 0.01^{f}

No effect at maximum concentration tested ($2\ mg\ mL^{-1}$); BHT: Butylated hydroxytoluene: GAE; Gallic Acid. N.D; Not Determined. Column with different letter were significantly different ($p < 0.05$). Values are expressed as means ± standard error (n = 3).

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101355.

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