

RESEARCH ARTICLE

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In vitro digestion, antioxidant and antiacetylcholinesterase activities of two species of *Ruta*: *Ruta chalepensis* and *Ruta montana*

Ayda Khadhri^a, Intidhar Bouali^b, Samia Belkhir^a, Rabhi Mokded^c, Samira Smiti^a, Pedro Falé^d, Maria Eduarda M. Araújo^d and Maria Luisa M. Serralheiro^d

^aFaculty of Sciences, Unity of Research of Vegetal Ecology, University of El-Manar II, Tunis, Tunisia; ^bFaculty of Sciences, Unity of biochemistry of lipids and proteins, University of El-Manar II, Tunis, Tunisia; ^cLaboratory of Extremophile Plants, Centre of Biotechnology of Borj Cedria, Hammam-Lif, Tunisia; ^dFaculty of Sciences, Centre of Chemistry and Biochemistry, University of Lisbon, Lisboa, Portugal

ABSTRACT

Context: *Ruta* genus (Rutaceae) is abundantly used and described in the most ancient systematic records of medical practice of the Mediterranean world. In Tunisia, this genus is represented by two medicinal and aromatic shrubs: *Ruta chalepensis* L. and *Ruta montana* L.

Objective: This study investigates the antioxidant and acetylcholinesterase inhibition (AChE) activities before and after *in vitro* gastrointestinal metabolism of leaf decoction of *R. chalepensis* and *R. montana*.

Materials and methods: We study, *in vitro*, the effect of the gastrointestinal juices gastric (1.75 mL) or pancreatic (2.5 mL) juices, on the biological activity by the measurement of the antioxidant activity and AChE inhibition during 4 h of decoction extract obtained from the leaves of the two species of *Ruta*.

Results: The results showed that the ability to inhibit the AChE enzyme was similar; being the greatest inhibitory activity exhibited by the ethanol extract (IC₅₀ = 12 ± 1.1 µg/mL) obtained from leaves of *R. chalepensis*.

Conclusion: In conclusion, we showed that there was no appreciable degradation and that the activity was kept constant after gastric and pancreatic juice digestion.

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Introduction

The genus *Ruta* (Rutaceae) encompasses more than 1800 species, mainly found in tropical and temperate regions with major centres of diversity in Southern Africa and Australia. Members of this genus have been used since antiquity in traditional medicine (Pollio et al. 2008) and credited with a long list of medicinal uses, including hypoglycaemic, abortive, antirheumatic, intestinal and hepatic diseases, male sterility, antihelminthic, antiepileptic and antipyretic (Di Stasi et al. 1994).

In Tunisia, this genus, known by the name of 'El Fijel', presents two species: *Ruta chalepensis* L. and *Ruta montana* L. (Pottier-Alapetite 1979). *Ruta chalepensis* is used in decoction as a medicinal remedy to treat cultural diseases known as 'empacho' (sort of indigestion), 'mal de ojo' (evil eye) and for 'spiritual cleansings'. Sometimes, it is used in amulets to keep the evil spirits away. The plant is often used for such varied conditions as hysteria, epilepsy, vertigo, colic, intestinal worms, poisonings, headache, anxiety and eye problems. The leaves of this plant infused with vinegar are given to children for the treatment of convulsion and other nervous disorders. An aqueous decoction of the leaves is used for the treatment of fever in Africa (Mansour et al. 1990).

Phytochemical screening of *Ruta* species showed the presence of alkaloids, flavonoids, coumarins, tannins, volatile oil, glycosides, sterols and triterpenes as possible active constituents (Mansour et al. 1990; El Guiche et al. 2015). In fact, flavonoids, glycosides, and tannins are considered potent inhibitors of pro-inflammatory signalling molecules (Iauk et al. 2004), which may explain in part

the bioactivities observed. The infusions/decoctions of the two species (*R. montana* and *R. chalepensis*) are widely used in folk medicine. They are used as a tonic, a febrifuge and a treatment of malaria as well as inflammatory, antioxidant and microbial processes (Khlifi et al. 2013; Kacem et al. 2014).

In the folk medicine of many countries, there are numerous plants used in fertility regulation. For example, *R. chalepensis* is used as an anti-fertility agent in Turkish and Chinese cultures. Anti-fertility activity was observed in some of the crude extracts and in some coumarins (Ulubelen et al. 1994).

Alzheimer's disease (AD), which is a progressive neurodegenerative disorder in the brain, is one of the major health problems and presents a high mortality rate, in industrialized countries, because of ageing populations. Presently, a number of treatments are used against AD as well as to counter the effect of oxidative stress. These include the use of acetylcholinesterase inhibitors (AChEIs) and high levels of antioxidants (Howes & Houghton 2003). Some adverse effects such as hepatotoxicity, gastrointestinal disturbances, nausea, vomiting, diarrhoea and dizziness have been reported with the use of most AChEIs. In some countries of the Mediterranean area, like Algeria, Cyprus or Israel, infusions of *R. chalepensis* leaves are taken to treat mental disorders (Pollio et al. 2008), which reinforces the interest in the evaluation of the activity of these infusions against AChE. Furthermore, rue is used in many countries of that area as a digestive and it is known that inhibitors of AChE are also used to increase gastrointestinal motility (Jarvie et al. 2008).

CONTACT Ayda Khadhri  khadriayda@yahoo.fr  University of El-Manar II, Faculty of Sciences, Unity of Research of Vegetal Ecology, Campus Academia, 2092 Tunis, Tunisia

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In addition, the complexity of the efficiency of a herbal drug could be further changed due to the biotransformation occurring after oral administration, i.e., bioconversion elicited by low gastric pH, the presence of digestive enzymes and the microorganisms forming the intestinal flora. It is now clear that their ultimate antioxidant potential and indeed their resulting potential bioactivity *in vivo*, is dependent on the absorption, metabolism, distribution and excretion of these compounds within the body after ingestion and the reducing properties of the resulting metabolites. In this context, the present work is focused on two species of *Ruta*: *R. montana* and *R. chalepensis* collected from Tunisian region, concerning the digestibility of the active components of the plant decoction and ethanolic extracts and their therapeutic potential as antioxidant and acetylcholinesterase (AChE) inhibitors.

The stability of extracts under gastrointestinal conditions was evaluated by HPLC before and after the submission to an *in vitro* digestion with artificial gastric and pancreatic juices. Furthermore, the antioxidant and antiAChE activities were monitored throughout the digestive process by the measurement of antioxidant activity and AChE inhibition. As to our knowledge, this is the first report on the digestibility of the active compounds from *R. chalepensis* and *R. montana*.

Materials and methods

Plant material

Leaves of *R. chalepensis* and *R. montana* were collected respectively in the South (33°53'14.82"N, 09°47'28.71"E; El Hamma) in the North of Tunisia (36°54'26.33"N, 09°25'55.13"E; Joumine) in August 2010 (Table 1). The identification of the plant was confirmed according to the 'Flore de la Tunisie' (Cuénod et al. 1954). Voucher specimens were deposited at the herbarium of the Faculty of Science of Bizerte, Tunisia.

Chemicals

All chemicals were of analytical grade. AChE type VI-S, from electric eel 349 U/mg solid, 411 U/mg protein, 5,5-dithiobis[2-nitrobenzoic acid] (DTNB), acetylthiocholine iodide (AChI), DPPH (2,2-di (4-*tert*-octylphenyl-1-picrylhydrazyl) and pancreatin were obtained from Sigma (Barcelona, Spain). Acetic acid was obtained from Fluka. Methanol and acetonitrile, both HPLC grade, were obtained from Merck (Darmstadt, Germany).

Phytochemical study

The phytochemical examination is necessary to identify the major families of compounds existing in both species.

Table 1. GPS coordinates and climatic characteristics (temperature, humidity and precipitations) of the sites where *R. montana* and *R. chalepensis* were harvested in August 2010.

Plant locality	<i>R. montana</i> Joumine (Governorate: Bizert)	<i>R. chalepensis</i> El Hamma (Governorate: Gabes)
GPS	36°54'26.33"N, 09°25'55.13"E	33°53'14.82"N, 09°47'28.71"E
Altitude (m)	247	61
Bioclimatic stage	sub-humid	Semi-arid
Average temperature (°C)	26.5	28.18
Humidity (%)	59.0	74.0
Precipitations (mm)	00.0	00.0

We characterized the presence of these secondary metabolites by using the techniques described in the works of Karumi et al. (2004).

The alkaloids have been characterized from Burchard reagent. Six millilitres of each solution was evaporated to dryness. The residue is taken again by 6 mL of alcohol with 60°. The addition of two drops of Burchard's reagent on alcoholic solution resulted in a reddish-brown precipitate and indicates a positive reaction.

For the detection of flavonoids, 2 mL of plant extracts were treated with a few drops of 37% HCl, and 0.5 g of magnesium turnings Mg²⁺. The positive test is marked by the appearance of pink or red colour, which characterizes flavonoids.

The reaction of ferric chloride (FeCl₃) was used to characterize the tannins. To 2 mL of the extracts two to three drops of 1% FeCl₃ solution were added. A positive test is indicated by the appearance of a blue-black colour (gallic tannins) or blue-green colour (tannins cathechics).

Extract preparation

Preparation of organic extracts

Ethanol extracts were obtained by Soxhlet extraction of 20 g of shoot for 6 h in about 250 mL of ethanol solvent. The extracts were concentrated to dryness and the residues were stored at 4 °C.

Decoction extract

Shoot powder (20 g) was boiled for 20 min in water (100 mL). The obtained decoctions were filtered, then freeze-dried and lyophilized.

Determination of total phenolic compounds

The total phenolic compounds content was determined by colorimetric assays using the Prussian blue assay according to the procedure illustrated by Price and Butler (1977); gallic acid was used as a standard. Briefly, 300 µL of ferric ammonium sulphate [FeNH₄(SO₄)₂, (HCl)] was added to a solution containing 100 µL extract, with a known concentration and approximate to 1 mg/mL, and 5 mL of distilled water. The solution was mixed and, after 20 min, 300 µL of potassium ferricyanide [K₃Fe(CN)₆] was added. The mixture was left to incubate for 28 min with intermittent shaking, and the absorbance was measured at 720 nm. The total phenolic content was calculated by a standard gallic acid graph, and the results expressed in mg of gallic acid equivalents per g of extract dry weight. The assay was performed in triplicate for each extract.

Absorbance = 413.25 gallic acid + 0.0243.

Antioxidant activity

The antioxidant activity was evaluated by the DPPH assay and by the iron (III) to iron (II) reduction test.

DPPH free radical-scavenging activity

The method described previously (Tepe et al. 2005) was used. The extract (50 µL) was added to 5 mL of a 0.002% methanolic solution of 2,2'-diphenylpicrylhydrazyl (DPPH). The mixture was left at room temperature for 30 min and then the

absorbance was measured at 517 nm against the corresponding blank.

$$E\% = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100,$$

where A_{blank} is the absorbance of the control reaction (a reaction with all the reagents except the sample extract), and A_{sample} is the absorbance of the sample extract.

Tests were carried out in triplicates and the extract concentration providing 50% extinction of the DPPH radical (EC_{50}) was obtained by plotting extinction percentage *versus* extract solution concentration.

Iron(III) to iron(II) reduction

The iron(III) reduction capacity of the extracts obtained with the cultivated plant at experimental plot was assessed spectrophotometrically (Dastmalchi et al. 2008) with a small modification. In brief, 1 mL of the dissolved extract with a known concentration, and approximately 1 mg/mL was mixed with 2.5 mL phosphate buffer (0.2 mol/L, pH = 6.6) and 2.5 mL of a 10 g/L potassium hexacyanoferrate [$K_3 Fe(CN)_6$] solution. After 30 min at 50 °C, 2.5 mL of a 100 g/L aqueous TCA solution was added and the mixture was stirred with a glass rod. Finally, a 2.5 mL aliquot was mixed with 2.5 mL ultra-pure water and 0.5 mL of a 1 g/L $FeCl_3$ solution and the absorbance was recorded at 700 nm. The iron (III) reduction capacity was calculated by a standard ascorbic acid graph:

$$\text{Absorbance} = 10.684 \text{ ascorbic acid (mg)} + 0.165$$

Results are presented as ascorbic acid equivalents (AscAE; mmol ascorbic acid/g sample). The assay was performed in triplicate for each extract.

Acetylcholinesterase inhibition

AChE enzymatic activity was measured using an adaptation of Ellman's method as is described by Ingkaninan et al. (2003); 325 μ L of 50 mM Tris-HCl buffer pH = 8, 100 μ L of sample (plant dried extract, dissolved in water or an aliquot coming from the enzymatic digestions) and 25 μ L AChE solution containing 0.26 U/mL were mixed in a spectrophotometer cuvette and left to incubate for 15 min at 25 °C. Subsequently, 75 μ L of a solution of AChI (0.023 mg/mL) and 475 μ L of 3 mM DTNB were added. The absorbance was read at 405 nm during the first 5 min of the reaction and the initial velocity was calculated. A control reaction was carried out using water, which was considered to possess 100% activity.

$I (\%) = 100 - [(V_{\text{sample}}/V_{\text{control}}) \times 100]$, where I is the percent inhibition of AChE, V_{sample} is the initial velocity of the extract containing reaction and V_{control} is the initial velocity of the control reaction. Tests were carried out in triplicate and a blank with Tris-HCl buffer instead of enzyme solution was used. Extract concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against extract solution concentrations.

In vitro metabolism by the synthetic gastric and pancreatic juices

The assays were adapted from Yamamoto et al. (1999). A known juice volume (1.75 mL of gastric juice or 2.5 mL of pancreatic juice) was added to an equal volume of extract solution (4 mg/

mL). The mixture was left to incubate at 37 °C for 4 h. Samples (650 μ L) were taken hourly, added to equal volume of ice-cold methanol and analyzed by HPLC. Samples (200 μ L) were taken at the same interval, centrifuged for 5 min at 5000 g and the supernatant was analyzed for antioxidant activity. Samples (500 μ L) were taken at the same interval, centrifuged for 5 min at 5000 g and the supernatant was analyzed for AChE activity, against a blank with water instead of plant extract. The synthetic gastric juice consisted of 320 mg of pepsin, 200 mg NaCl, pH 1.2 (with HCl). The pancreatic juice consisted of 250 mg of pancreatin in 10 mL of potassium-phosphate buffer 50 mM, pH 8. Assays were done in triplicates.

Antiacetylcholinesterase and antioxidant activities of the digested extracts

Aliquots were withdrawn at the beginning of the experiment and after every hour. Each sample was centrifuged at 5000 g to discard the bottom phase containing the enzyme, pepsin or pancreatin. The upper phase was used for the determination of AChE inhibition activity as well as antioxidant activity according to the procedures described previously.

HPLC analysis

The HPLC analysis was carried using a Liquid Chromatograph FinniganTM Surveyor Plus Modular LC System, Thermo-Finnigan (Waltham, MA) equipped with a Purospher STAR RP-18 column, from Merck (Darmstadt, Germany) and Xcalibur software. The extracts were analyzed by HPLC, injecting 25 μ L with an autoinjector, and using a linear gradient composed of solution A (0.05% trifluoroacetic acid), solution B (acetonitrile) and solution C (methanol) as follows: 0 min, 90% A, 10% B; 50 min 5% A, 80% B, 15% C. HPLC analysis was used to monitor changes in extract composition during *in vitro* digestion.

Statistical analysis

All results are presented as mean of five replicates $\pm$ standard deviation and the software used was Microsoft Excel 2003.

Results and discussion

Phytochemical tests

Phytochemical tests consist of detecting various families of compounds existing in the studied part of the plant by qualitative reactions of characterization. These reactions are based on phenomena of precipitation or colouring by reagents specific to each family of compounds. The results of phytochemical tests on the two studied plant are given in Table 2. In the two species of *Ruta*, the research of alkaloids, flavonoids and tannins compounds was positive.

Table 2. Results of the reactions characteristics using different chemical groups in two species of *R. chalepensis* and *R. montana*.

Classes searched	<i>R. chalepensis</i>	<i>R. montana</i>
Alkaloids	+++	+++
Flavonoids	++	++
Gallic tannins	++	++
Condensed tannins	++	++

Table 3. Total phenol contents, antioxidant activity and AChE inhibition activity of *R. chalepensis* and *R. montana* extracts.

	Total phenol contents		Antioxidant activity				AChE inhibition activity	
	(mg Eq Cat/100g extract) ^a		DPPH test (EC ₅₀ µg/ml) ^a		Ferric reducing test (mmol ascorbic acid/g of extract) ^a		AChE (IC ₅₀ µg/ml) ^a	
	<i>R. chalepensis</i>	<i>R. montana</i>	<i>R. chalepensis</i>	<i>R. montana</i>	<i>R. chalepensis</i>	<i>R. montana</i>	<i>R. chalepensis</i>	<i>R. montana</i>
Decoction of leaves	3.90 ± 0.3	10.20 ± 0.9	2.26 ± 0	1.54 ± 0.15	37.54 ± 0.12	25.95 ± 1.22	250 ± 3.6	57 ± 1.6
Decoction of stem	6.08 ± 0.37	5.84 ± 0.2	3.63 ± 0	3.64 ± 0.09	31.20 ± 1.85	18.48 ± 1.04	144 ± 2.5	343 ± 2.8
Ethanol extract of leaves	2.73 ± 0.5	5.00 ± 0.4	4.35 ± 0.18	1.47 ± 0.1	23.74 ± 0.20	25.51 ± 1.15	12 ± 1.1	76 ± 1.6
Ethanol extract of stem	5.10 ± 0.98	6.13 ± 0.85	4.26 ± 0.09	2.66 ± 0.13	18.01 ± 0.59	16.48 ± 0.28	86 ± 1.3	52 ± 0.4

The values reported are relative to weight of dried extract per ml of tested solution.

^aAverages ± standard deviation were obtained from three different experiments.

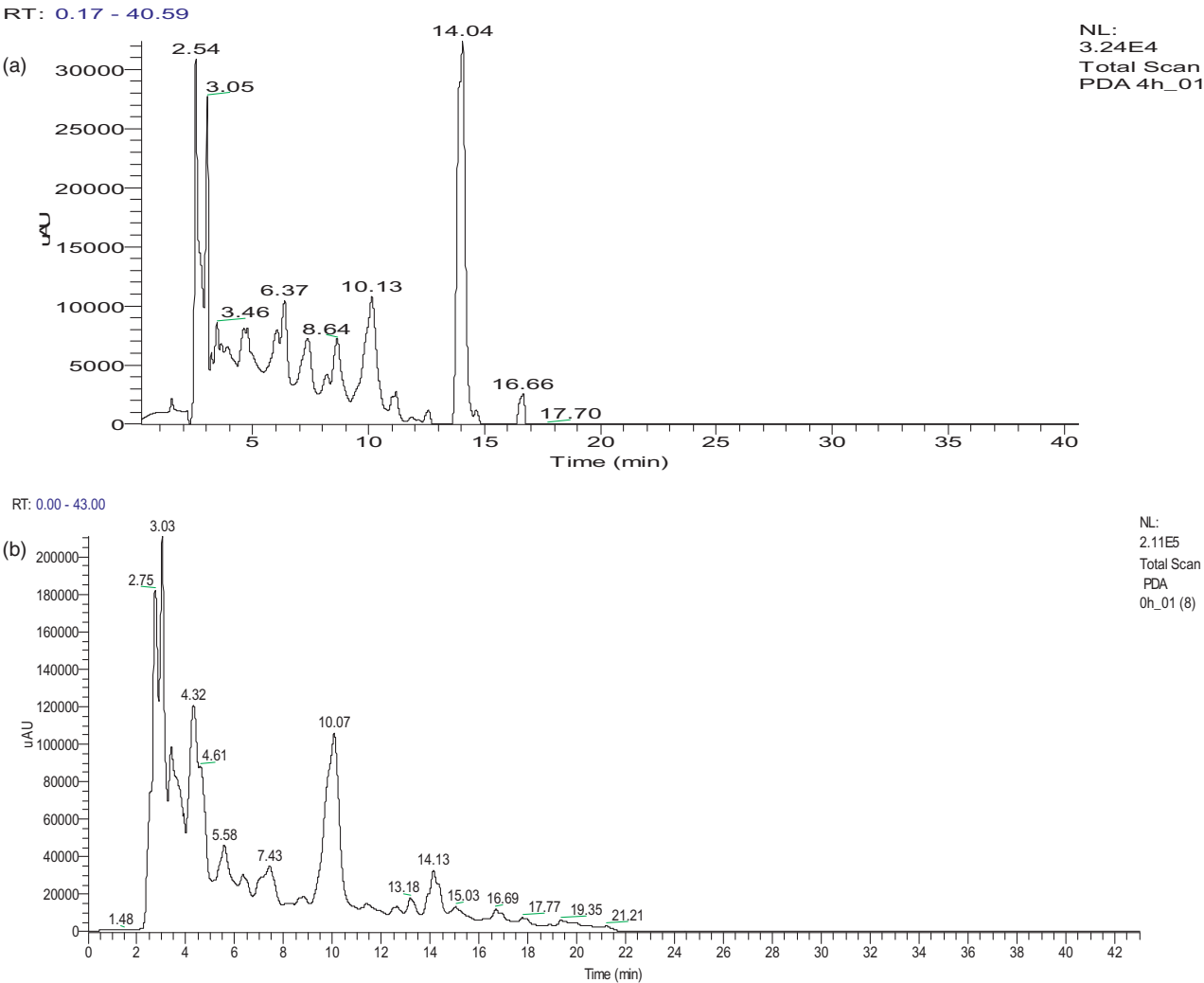


Figure 1. HPLC chromatograms of the decoction of *R. chalepensis* leaves with (a) gastric and (b) pancreatic juices.

Total phenolic content

The total phenol content was determined in decoction and ethanol extracts obtained from *R. chalepensis* and *R. monatana* stems and leaves, by the modification of Prussian blue assay of Price and Butler (1977). Results were expressed in mg catechin equivalents per 100 g dry weight of extract (Eq Cat/100 g DW), Table 3.

All tested extracts contained appreciable amounts of phenolics. The amount of total phenolics was determined dependent on the solvents used for extraction and ranged from 2.73 ± 0.5 mg Eq Cat/100 g DW (*R. chalepensis* ethanol extract) to 10.20 ± 0.9 mg Eq Cat/100 g DW (*R. montana* leaf decoction).

In *R. chalepensis*, the comparison between decoction and ethanolic extracts were organ-dependent. Stem decoction was characterized by higher polyphenol content (6.08 ± 0.37 mg Eq Cat/100 g DW) as compared to leaf decoction and ethanol extract (3.90 ± 0.3 and 2.73 ± 0.5 mg Eq Cat/100 g DW, respectively).

The highest values were obtained in *R. montana* leaf decoction (10.20 ± 0.9 mg Eq Cat/100 g DW). Ethanol extract of *R. montana* leaves was also rich in phenolic compounds showing content of 5.00 ± 0.4 mg Eq Cat/100 g DW. These results showed similar amounts of total phenolic compounds between Tunisian, Algerian and Moroccan *R. montana* extracts (Djeridane et al.

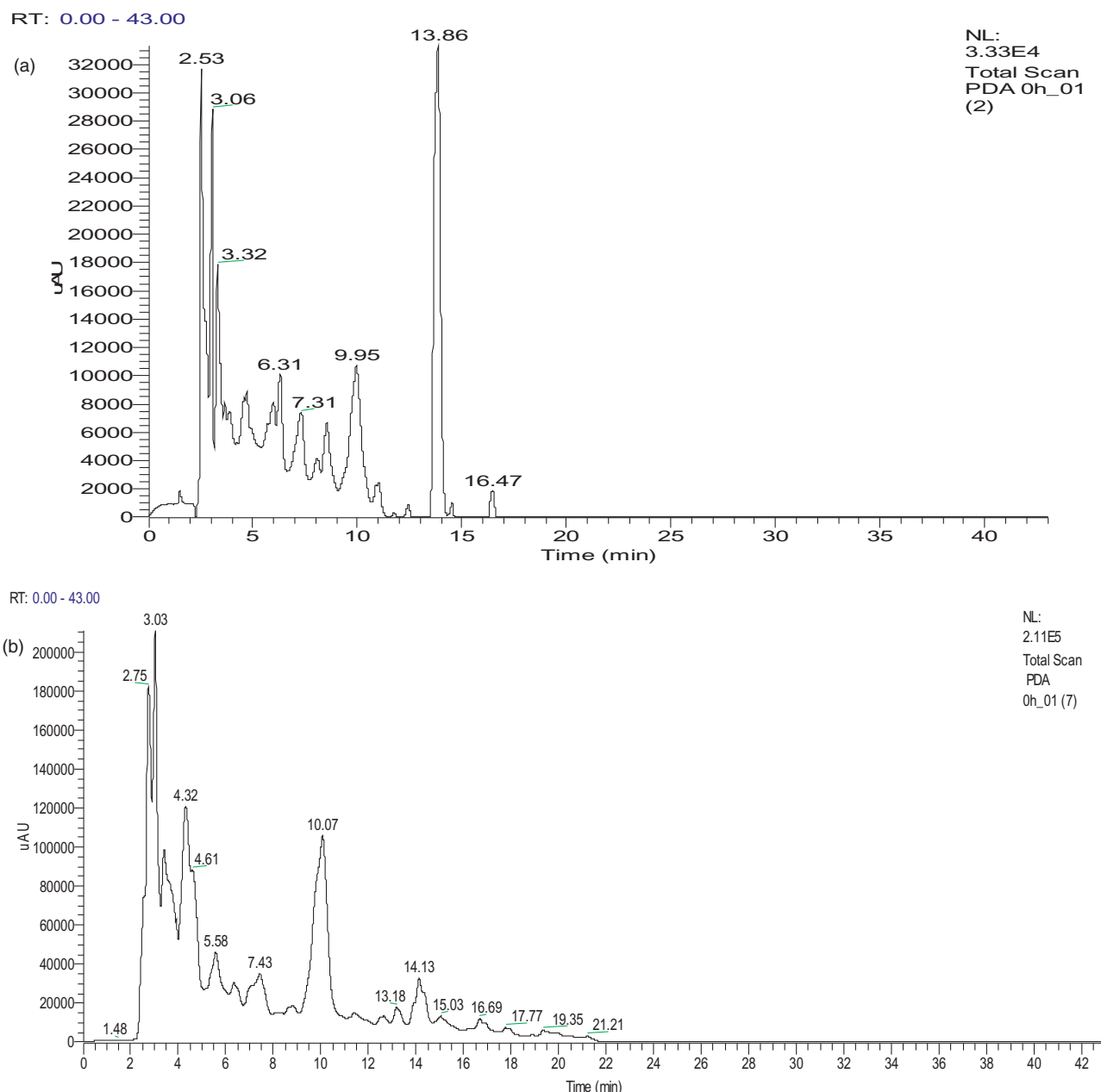


Figure 2. HPLC chromatograms of the decoction of *R. montana* with (a) gastric and (b) pancreatic juices.

2006; El Guiche et al., 2015). In addition, leaves and stems of this species were enriched in polyphenols than those of several medicinal plants (Khadri et al. 2010).

When analyzed by HPLC-DAD, the extracts (Figures 1(a) and 2(b)) did not indicate the presence of caffeic acid derivatives. The spectra exhibited by the compounds were of flavonoid type. Most of the polyphenols present in the water extracts were flavonoid derivatives or small phenolic acids.

Antioxidant activity

DPPH free radical-scavenging activity

The free radical scavenging activity was determined by the DPPH test. This test aims at measuring the capacity of the extracts to scavenge the stable radical 2,2-diphenyl-1-picryl

hydrazil (DPPH) formed in solution, by donation of hydrogen atom or an electron (Tepe et al. 2005). If the extracts have the capacity to scavenge the DPPH free radical, the initial blue/purple solution will change to a yellow colour due to the formation of diphenylpicrylhydrazine. This reaction was used as a measure of the ability of the extracts to scavenge any free radical.

The results from the radical scavenging assays for all extracts are presented in Table 3 as EC_{50} (μg dry weight extract/mL). Since EC_{50} means the concentration of the extract that is able to scavenge (extinguish) half of the DPPH free radical present in test solution, the lower is this value, the higher is the antioxidant activity of the extract.

The best results were obtained with ethanol extract from the leaves of *R. montana* ($EC_{50}=1.47 \pm 0.1 \mu\text{g/mL}$), leaf decoction from the same species ($EC_{50}=1.54 \pm 0.15 \mu\text{g/mL}$), and that of *R. chalepensis* ($EC_{50}=2.26 \pm 0 \mu\text{g/mL}$).

Table 4. Antioxidant and antiAChE activity of the decoction of *R. chalepensis* and *R. montana* leaves after *in vitro* gastrointestinal digestion.

Time (h)	DPPH (%)				AChE (%)			
	Gastric juice		Pancreatic juice		Gastric juice		Pancreatic juice	
	<i>R. chalepensis</i>	<i>R. montana</i>	<i>R. chalepensis</i>	<i>R. montana</i>	<i>R. chalepensis</i>	<i>R. montana</i>	<i>R. chalepensis</i>	<i>R. montana</i>
0	100.0 ± 1.46	100.0 ± 2.95	100.0 ± 1.74	100.0 ± 0.49	100.0 ± 2.74	100.0 ± 3.20	100.0 ± 2.75	100.0 ± 5.5
1	–	86.48 ± 1.86	–	91.33 ± 0.32	–	98.7 ± 2.99	–	96.1 ± 6.38
2	–	85.52 ± 1.18	–	88.34 ± 2.96	–	95.2 ± 2.1	–	94.3 ± 6.1
3	–	82.05 ± 2.11	–	85.06 ± 1.42	–	88.7 ± 1.7	–	91.8 ± 2.7
4	91.58 ± 1.85	80.12 ± 1.57	89.68 ± 0.25	80.14 ± 5.65	87.75 ± 1.37	85.9 ± 3.5	82.7 ± 3.38	89.2 ± 5.49

The obtained values are near to the antioxidant activity of gallic acid, a known standard, ($EC_{50} = 0.95 \pm 0.04 \mu\text{g/mL}$). Also, the values obtained are better than the antioxidant activity of butyl hydroxytoluene (BHT), a known standard, previously determined: $IC_{50} = 15.7 \mu\text{g/mL}$ (Mata et al. 2007).

These *Ruta* extracts have higher antioxidant activity than those of *R. graveolans* that showed an EC_{50} of $459 \mu\text{g/mL}$ (Ramos et al. 2003).

Antioxidant activity evaluated by Fe(III) to Fe(II) reduction test

Fe(III) to Fe(II) reduction test measures the reducing power of a compound or an extract, which means its ability to donate an electron. Hence, in order to assess the electron-donating power of *R. montana* and *R. chalepensis* extracts, their ability to reduce Fe(III) was investigated.

The results of Fe(III) reduction are presented in Table 3 as ascorbic acid equivalent (AscE) values, the higher the AscE value the greater the electron-donating power of the sample. The results suggest that the plant extracts under investigation have the ability to donate electrons, thus they can scavenge free radicals. Similarly to DPPH test results, the highest activities were obtained with *R. montana* leaf decoction ($25.95 \pm 1.22 \text{ mmol}$ ascorbic acid/g extract) and ethanol extract ($25.51 \pm 1.15 \text{ mmol}$ ascorbic acid/g extract).

Determination of acetylcholinesterase inhibitory activity

The inhibition of AChE, the key enzyme in the breakdown of acetylcholine, is considered as one of the treatment strategies against several neurological disorders such as AD, senile dementia, ataxia, myasthenia gravis and constipation (Mukherjee et al. 2007; Orhan et al. 2006). Plants have been traditionally used to enhance cognitive function and to alleviate other symptoms associated nowadays with Alzheimer's disease (Howes & Houghton 2003).

AChE inhibition was determined using an adaptation of the method described in the literature (Ferreira et al. 2006). All extracts were analyzed for their AChE inhibition activities (Table 3). The greatest inhibitory activity was exhibited by leaf ethanol extract of *R. chalepensis* ($IC_{50} = 12 \pm 1.1 \mu\text{g/mL}$). Wszelaki et al. (2010) found that hexane extract of *R. graveolens* exhibited moderate AChE inhibitory activity with an IC_{50} of 34 mg/mL . Also, Adsersen et al. (2006) found AChE inhibitory activity of the methanolic extract of *R. graveolens* (100 mg/mL), which caused 39% inhibition. Despite the large use of *R. chalepensis* and *R. montana* along the Mediterranean basin well as the fact that *R. chalepensis* is the most cultivated rue in the Atlantic coast of Iberian Peninsula, Latin America, California and Texas and the only rue used by Latin healers in New York (San Miguel

2003), no reports about the activities of the extracts of these two *Ruta* species were published.

In vitro metabolism of the extract by the gastric and pancreatic juices. Antioxidant and AChE activities

An understanding of the processes involved in the absorption and distribution of herbal tea polyphenol is essential for the determination of their potential bioactivities *in vivo* and their overall significance in disease prevention. Usually, *Ruta* species are administered orally, after which the ingredients are exposed to gastric juice and digestive and bacterial enzymes in the gastrointestinal tract.

The digestion of *R. chalepensis* and *R. montana* decoctions was carried out for 4 h under stomach acidic conditions (pepsin, pH 1). Although the digestion in the stomach may take 60–110 min, it was decided to continue the process to see if the bioconversion increased with time. Aliquots were incubated for 4 h and analyzed by HPLC (Figures 1 and 2). None of the constituents was degraded under the stomach acidic conditions, even after 4 h of digestion.

The antioxidant and AChE activities of *R. chalepensis* and *R. montana* decoctions were evaluated during the 4 h of the *in vitro* gastric process; the results are shown in Table 4. After the gastric digestion, the inhibition capacity of the extract showed only a small decline that is not statistically significant at the 95% level. The same result was noticed for the antioxidant activity, while further incubation with pepsin did not bring any further significant changes of the activity.

Pancreatic juice contains pancreatin, a mixture of hydrolytic enzymes, (amylase, lipase and protease), at basic pH, which was used to simulate the physical and chemical conditions that *R. chalepensis* and *R. montana* decoctions would be subjected to in the small intestine (Yamamoto et al. 1999). The effect of the buffer solution at pH 8 on compound degradation, without pancreatin, was also evaluated. The experiment was also carried out for 4 h. Results are presented in Table 4.

It is known that AChE inhibitors stimulate gastrointestinal motility (Jarvie et al. 2008). The results shown here point out that the AChE inhibitory activity of the decoction in the stomach is not reduced by the gastric juice, and that similar results were obtained with the pancreatic juice. The observed decreases of 10 and 20% of the activity could be due to interference with the test reagents, which are corroborated by HPLC analysis. Our results are in accordance with those reported for *Lavandula viridis* L'Hér (Lamiaceae) extracts (Costa et al. 2014) and *Erica australis* L. (Ericaceae) extracts (Dias et al. 2015).

This situation is similar to *Plectranthus barbatus* Andr. (Lamiaceae) water extract that was previously investigated during 4 h of *in vitro* gastric process (Porfírio et al. 2010). The results thus obtained showed that after the gastric digestion, the

inhibition capacity of the extract exhibited a small decline that was not statistically significant at the 95% level.

Conclusion

All extracts presented a high antioxidant activity and exhibited inhibitory properties of the AChE enzyme. This study is also the first report of the bioconversion processes of decoctions of *R. chalepensis* and *R. montana* using an *in vitro* model of the gastro-intestinal tract. Antioxidant activity is maintained even after these extracts were subjected to the action of synthetic gastric and pancreatic juices, which is a very good indication that this activity is probably maintained when these species are used in phytotherapy. AChE inhibition activity is not affected by synthetic gastric juice, although this activity was reduced to about the half of the initial value by synthetic pancreatic juice. As a final conclusion, it can be stated that these species have phytotherapeutic potential since their antioxidant and AChE inhibition activity are not destroyed if they are administered orally.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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