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Original Research Article

Antioxidant properties of various extracts from selected wild Moroccan aromatic and medicinal species

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ABSTRACT

Antioxidant properties of various extracts from different wild Moroccan aromatic and medicinal plants (*Achillea ageratum* L., *Salvia officinalis* L., *Thymus saturooides* Cosson., *Mentha suaveolens* subsp. *timija* (Briq.) Harley and *Cotula cinerea* Del.) were examined by applying DPPH free radical-scavenging activity, β -carotene/linoleic acid bleaching and reducing power assays. Total phenol and flavonoid contents of the extracts were also determined. Antioxidant activity was compared with that of synthetic antioxidants including butylated hydroxytoluene (BHT) and quercetin. Overall, the highest antioxidant properties were shown by the hot water and methanolic extracts obtained from all species studied. The hot water extract of *M. suaveolens* subsp. *timija* (Briq.) presented the best contribution in DPPH and reducing power assays (IC_{50} = 14.04 \pm 0.14 and 17.36 \pm 0.1 μ g/mL, respectively) which was significantly associated with its high phenolic content (134.49 \pm 2.14 μ g GAE/mg extract). However, in β -carotene-linoleic acid bleaching assay, the hot water extract of *S. officinalis* L. was the most effective with an IC_{50} value of 14.49 \pm 0.40 μ g/mL. These findings may provide new information on the considerable potential of the five Moroccan medicinal and aromatic plants as rich a source of natural antioxidant substances. Therefore, they could be suitable for using as antioxidative additives in the food and pharmaceutical industries.

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1. Introduction

Formation of free radicals may play an important role in the origin of life and biological evolution. In fact, oxidation is essential to many living organisms for the production of energy to fuel biological processes (McCord, 2000). However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cardiovascular problems, and diabetes as well as in degenerative processes associated with ageing. In order to protect human beings from oxidative stress damage, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) have widespread use as food additives in many countries (Xiu-Qin et al., 2009). Recently, many reports reveal that these compounds may be implicated in many health risks, including

cancer and carcinogenesis (Prior, 2004; Sivasothy et al., 2011). Hence, there is a tendency towards the use of natural antioxidants of plant origin to replace these synthetic antioxidants. In fact, the evaluation of natural products containing antioxidants for reducing oxidative stress damages and to prevent the rancidity and oxidation of lipids has increased considerably in the food industries (Sarikurku et al., 2010). Within these natural resources, extracts from aromatic and medicinal plants constitute one of the main sources of new antioxidant agents and healthcare products, which can be used for different applications, particularly as food additives. The antioxidant capacity of these plants extracts is generally attributed to the presence of many secondary plant metabolites (Sacchetti et al., 2005; Issa et al., 2006; Mohammadhosseini, 2017). Among them, the phenolic compounds, a group of low and medium molecular weight that can embrace a great diversity

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of substances (Gouveia and Castilho, 2011). These substances are biosynthesized by plants to prevent pathogen attack, biotic or abiotic stress. Furthermore, the phenolic compounds including flavonoids, phenolic acid and tannin are a major group of phytochemicals which exhibited strong antioxidant potency (Pietta, 2000). This activity can be related to a number of different mechanisms, such as free radical-scavenging, hydrogen-donation, singlet oxygen quenching, metal ion-chelation, and acting as substrates for radicals such as superoxide and hydroxyl (Tepe et al., 2007).

In this paper, we investigated for the first time the antioxidant properties of some polar extracts obtained from five Moroccan medicinal and aromatic plants. According to our recent ethno-pharmacological investigations, these herbs are widely used as flavorings and food additives. Among the studied plants, *Mentha suaveolens* subsp. *timija* (Briq.) is a strict endemic perennial species. Mint *timija* products have been used in Moroccan folk medicine as a powder or infusion for the treatment of coughs, antispasmodic and as an excellent carminative (Bellakhdar, 2006). As an aromatic plant, mint *timija* is considered as an important flavouring agent, commonly used in herbal tea for its tonic and stimulant properties. The second plant, *Thymus satureioides* Coss. is a North African species typical of arid habitats used in the Moroccan folk medicine in the form of infusions and decoctions to treat whooping cough, bronchitis and rheumatism (Bellakhdar, 2006) and generally for its anti-inflammatory properties after topical or oral administration. As medicinal plant, it has been reported that *T. satureioides* Coss. possesses numerous pharmacological and biological properties including microbiocidal and antioxidant activities (Alaoui Jamali et al., 2012; El Bouzidi et al., 2013; Kasrati

et al., 2014). The third species, *Achillea ageratum* L. is a Mediterranean medicinal shrub very appreciated for its therapeutic properties. In south-western Morocco, particularly in the Demnate region, flowers are largely used as powder mixed with honey to treat stomach and gastro-intestinal disorders (Bellakhdar, 2006). Many pharmacological studies supported the cytostatic, anti-inflammatory, analgesic and antipyretic activities of *A. ageratum* L. extracts (El Bouzidi et al., 2011; Mohammadhosseini et al., 2017). The fourth plant, *Cotula cinerea* Del. (synonym=*Brocchia cinerea* (Del.) Vis) is used in Moroccan folk medicine as a stomachic and antiseptic and against gastro-intestinal problems (Bellakhdar, 2006). It has been reported that *C. cinerea* Del. extracts possess some pharmacological properties, analgesic, anticandidal and antibacterial activities (Markouk et al., 1999; Bensizerara et al., 2013; El Bouzidi et al., 2013). Finally, *Salvia officinalis* L. is a perennial woody sub-shrub native to the Mediterranean. It is cultivated in several countries mainly to obtain dried leaves to be used as raw material in pharmaceutical, perfumery, and food industries. In folk medicine of Morocco, the aerial parts of sage were particularly recognized since earliest times for their cardiovascular, anticancer (Loizzo et al., 2007), anti-inflammatory (Baricevic et al., 2001), antioxidant (Fellah et al., 2006), allelopathic (Bouajaj et al., 2013), antileishmanial (Et-Touys et al., 2016) and antimicrobial properties (Hayouni et al., 2008).

2. Experimental

2.1. Preparation of extracts

During the period of April-May 2014, the aerial



Thymus satureioides Coss.



Mentha suaveolens subsp. *timija* (Briq.) Harley.



Cotula cinerea Del.



Salvia officinalis L.



Achillea ageratum L.

Fig. 1. Aerial parts of the five Moroccan aromatic and medicinal plants.

Table 1

Localization (site and geographical coordinates) of the five aromatic and medicinal species studied.

Species	Species code	Harvesting place	Voucher specimen	Latitude/Longitude
<i>M. suaveolens</i> subsp. <i>timija</i>	MS	Ourika valley	MST05	31°23'N - 7°42'W
<i>S. officinalis</i>	SO	Ourika valley	SO046	31°20'N - 7°45'W
<i>A. ageratum</i>	AA	Demnate	ACHAG045	31°43'N - 06°58'W
<i>T. satureioides</i>	TS	Idni	TS067	30°54'N - 08°17'W
<i>C. cinerea</i>	CC	Zagora	COTCIN03	30°20'N - 05°52'W

parts of five wild medicinal species (*A. ageratum* L., *S. officinalis* L., *T. satureioides* Coss., *M. suaveolens* subsp. *timija* (Briq.) Harley and *C. cinerea* Del.) (Fig. 1) were harvested from different locations in south and south-west of Morocco (Table 1). The identification was done by one of the authors (A. Abbad) and voucher specimens were deposited at the Laboratory of Phytochemistry and Pharmacology of Aromatic and Medicinal Plants, Faculty of Science Semlalia, Marrakech, Morocco. The collected materials were air-dried at room temperature (≈ 25 °C) in the shade. The extracts were obtained by maceration of dry plant material (20 g) in boiling water (100 mL) for 3 h (hot water extract) and in cold water (100 mL) for 2 days at room temperature (cold water extract) and in methanol (100 mL) for 2 days at room temperature (methanolic extract). The resulting extracts were filtered by using Whatman filter paper (No. 1) and then concentrated in vacuum at 40 °C using a Büchi® rotary evaporator Model R-200.

2.2. Antioxidant activity

2.2.1. DPPH free radical-scavenging activity

The antioxidant activity of plant extracts was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Sahin et al., 2004). When this radical encounters a proton-donating substrate, such as an antioxidant, it changes colour from purple to yellow, and a decrease in absorbance results. Fifty microlitres of various concentrations of the samples (extracts, quercetin and BHT) in methanol were added to 2 mL of a 60 μ M methanolic solution of DPPH. Absorbance measurements were read at 517 nm, after 20 min of incubation in the dark at room temperature. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control. Butylated hydroxytoluene (BHT) and quercetin were used as positive controls. The percentage inhibition of the DPPH radical was calculated according to the following equation (Eqn. 1):

$$\% \text{ Inhibition} = (A_b - A_s / A_b) \times 100 \quad (\text{Eqn. 1})$$

Where, A_b is the absorption of the blank sample and A_s is the absorption of the tested extracts. The sample

concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. The test was carried out in triplicate and IC_{50} values were reported as means \pm SD.

2.2.2. Reducing power determination

Reductive ability was investigated by the Fe^{+3} to Fe^{+2} transformations in the presence of extracts, using the method of Oyaizu (1986). The different extracts were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1.0%). The mixture was then incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. Finally, the upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. The concentration of extract providing 0.5 of absorbance (IC_{50}) was calculated by plotting absorbance at 700 nm against the corresponding extract concentration. BHT and quercetin were used as reference compounds. The test was carried out in triplicate and EC_{50} values were reported as means \pm SD.

2.2.3. β -Carotene/linoleic acid bleaching assay

The β -carotene/linoleic acid test evaluates the inhibitory effect of a compound or a mixture on the oxidation of β -carotene in the presence of molecular oxygen (O_2) (Bamoniri et al., 2010). Assay of the remained β -carotene gives an estimation of the antioxidant potential of the sample. The method described by Miraliakbari and Shahidi (2008) was used. A mixture of β -carotene and linoleic acid was prepared by adding together of 0.5 mg β -carotene in 1 mL chloroform (HPLC grade), 25 μ L linoleic acid and 200 mg Tween 40. The chloroform was then completely evaporated under vacuum and 100 mL of oxygenated distilled water was subsequently added to the residue and mixed to form a clear yellowish emulsion. 350 μ L of various concentrations of the sample (extracts, quercetin and BHT) were added to 2.5 mL of the above emulsion in test tubes and mixed. The test tubes were incubated in a water bath at 50 °C for 2 h together with a negative

control (blank) contained the same volume of methanol instead of the sample. The absorbance values were measured at 470 nm. Antioxidant activities (inhibition percentages, I%) of the samples were calculated using the following equation (Eqn. 2):

$$I\% = \left(\frac{A_{\beta\text{-carotene after 2 h assay}}}{A_{\text{initial } \beta\text{-carotene}}} \right) \times 100 \quad (\text{Eqn. 2})$$

Where $A_{\beta\text{-carotene after 2 h assay}}$ is the absorbance values of β -carotene after 2 h assay remaining in the samples and $A_{\text{initial } \beta\text{-carotene}}$ is the absorbance value of β -carotene at the beginning of the experiment. The test was carried out in triplicate and IC_{50} values were reported as means \pm SD of triplicates.

2.3. Determination of total phenolic content

Total phenolic content of the extracts was determined by the Folin-Ciocalteu micro-method (Arabshahi-Delouee and Urooj, 2007; Ali et al., 2014; Zengin et al., 2014). A 20 μ L aliquot of extracts solution was mixed with 1.16 mL distilled water and 100 μ L of Folin-Ciocalteu reagent, followed by the addition of 300 μ L of Na_2CO_3 solution (20%). Subsequently, the mixture was incubated in a shaking incubator at 40 $^{\circ}C$ for 30 min and its absorbance at 760 nm was measured. Gallic

acid was used as a standard for calibration curve. Total phenolic content expressed as gallic acid equivalent (GAE) was calculated using the following linear equation (Eqn. 3) based on the calibration curve:

$$A = 0.094C - 0.025 \quad (R^2 = 0.997) \quad (\text{Eqn. 3})$$

Where A is the absorbance and C is the concentration (μ g GAE /mg extract).

3. Results and Discussion

3.1. Antioxidant activity

In a first approach, the extract yields obtained from each plant were determined and the results are depicted in Table 2. The yields obtained present the following increasing order of occurrence: methanol extract, cold water extract and hot water extract.

Owing to the complex reactive facets of phytochemicals, the antioxidant activities of plant extracts cannot be evaluated only by a single method. Two or more methods should always be employed in order to evaluate the total antioxidative effects (Schlesier et al., 2002). There are several methods for determining antioxidant function, each of which acting

Table 2

Yield of the methanol, hot and cold water extracts obtained for each plant studied.

Extracts	MS ^a	SO	AA	TS	CC
Methanol	5.7 \pm 0.02	7.15 \pm 0.15	8.6 \pm 0.21	12.25 \pm 0.05	7.25 \pm 0.07
Hot water	14.1 \pm 0.8	27.8 \pm 0.3	17.2 \pm 0.13	12.7 \pm 0.67	19.5 \pm 1.4
Cold water	12.5 \pm 1.2	7 \pm 0.75	16.7 \pm 1.02	11.6 \pm 0.34	9.5 \pm 0.23

Details concerning the codes of the species are given in Table 1.

Table 3

Antioxidant activity of the different extracts obtained from the five species studied.

Species	Extracts	MS ^a	DPPH	Reducing power	β -Carotene bleaching
			(IC_{50} , μ g.mL ⁻¹)	(IC_{50} , μ g.mL ⁻¹)	(IC_{50} , μ g.mL ⁻¹)
	Hot water		14.04 \pm 0.14 ^b	17.36 \pm 0.1	27.37 \pm 1.27
	Cold water		55.83 \pm 0.91	79.14 \pm 0.93	70.66 \pm 1.39
	Methanol		19.51 \pm 0.04	24.77 \pm 0.54	64.92 \pm 0.90
	Hot water	SO	18.79 \pm 0.19	24.17 \pm 0.2	14.49 \pm 0.40
	Cold water		62.03 \pm 0.89	85.56 \pm 1.08	80.13 \pm 1.12
	Methanol		24.69 \pm 0.39	25.51 \pm 0.30	20.89 \pm 0.67
	Hot water	AA	19.67 \pm 0.47	26.31 \pm 0.11	14.86 \pm 0.6
	Cold water		42.17 \pm 0.09	70.81 \pm 0.22	70.48 \pm 1.7
	Methanol		21.91 \pm 0.17	34.8 \pm 0.29	30.32 \pm 0.9
	Hot water	TS	15.99 \pm 0.47	20.33 \pm 0.19	14.69 \pm 0.69
	Cold water		53.42 \pm 1.17	64.32 \pm 0.52	50.20 \pm 0.33
	Methanol		30.24 \pm 0.19	30.48 \pm 0.52	86.38 \pm 0.85
	Hot water	CC	48.31 \pm 0.98	84.37 \pm 0.68	74.16 \pm 2.45
	Cold water		161.19 \pm 0.77	135.17 \pm 1.78	233.74 \pm 1.09
	Methanol		58.51 \pm 1.22	48.53 \pm 0.76	169.14 \pm 1.01
Synthetic antioxidant	Quercetin	-----	1.07 \pm 0.01	2.29 \pm 0.1	0.95 \pm 0.02
	BHT	-----	4.21 \pm 0.08	7.09 \pm 0.1	4.30 \pm 0.33

^aValues represent means \pm standard deviations for triplicate experiments; ^bDetails concerning the codes of the species are given in Table 1.

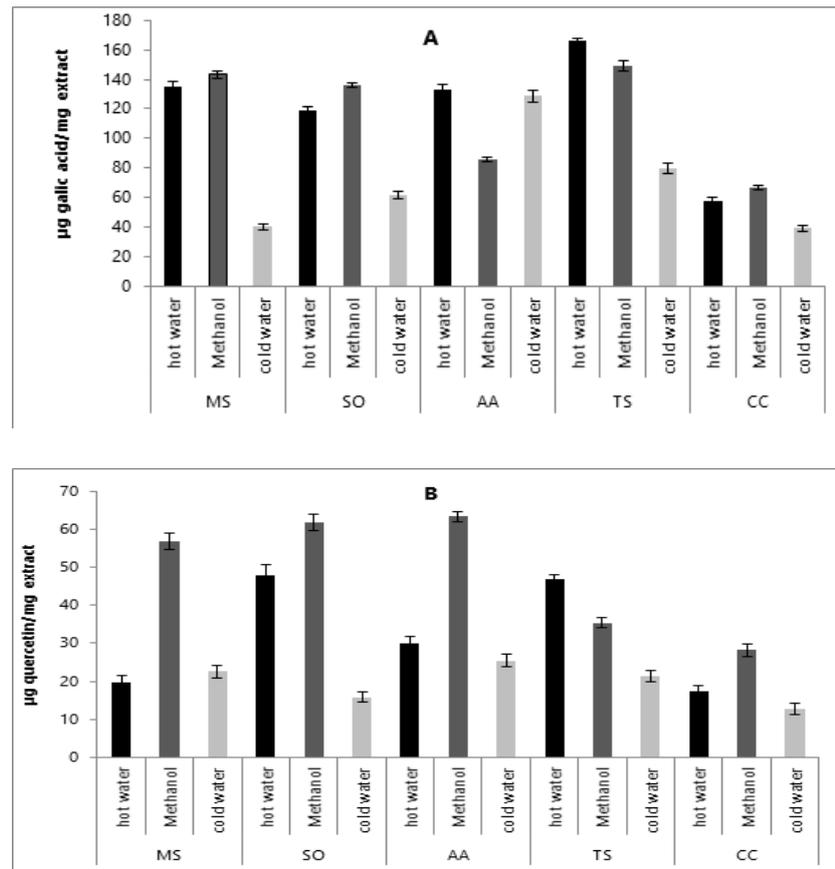


Fig. 2. Amount of total phenols (A) and flavonoids (B) of the studied plant extracts. The bars represent the average and the deviation standard.

by different mechanisms (Huang et al., 2005). For this reason, the antioxidant activity of *A. ageratum* L., *S. officinalis* L., *T. satureioides* Coss., *M. suaveolens* subsp. *timija* (Briq.) Harley and *C. cinerea* L. extracts were assessed by three complementary *in vitro* antioxidant assays: *i*) the DPPH assay, evaluating the H-donating or radical scavenging ability of the extracts using the stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) as a reagent, *ii*) an assay estimating the ferric-reducing capacity of the extracts and *iii*) the β -carotene-linoleic acid bleaching assay. The concentrations that led to 50% inhibition or effectiveness (IC_{50}) are given in Table 3. The lower IC_{50} values reflected better protective action.

Generally, in the three assays, all the extracts revealed interesting antioxidant properties. This activity is comparable to the positive controls, i.e. BHT and quercetin. Regarding the IC_{50} values, both hot water and methanolic extracts of all species displayed the best antioxidant activity in all assays compared with the cold water extracts. This finding is in parallel with previous published results which showed that methanolic and hot water extracts are more antioxidants than cold water extracts (Gonçalves et al., 2013; Skotti et al., 2014). Interestingly, the hot water and methanolic extracts obtained from *M. suaveolens* subsp. *timija* (Briq.) Harley presented the highest potency in DPPH and reducing power tests (IC_{50} values varied between

14.04 \pm 0.14 μ g/mL to 24.77 \pm 0.54 μ g/mL). This activity may be attributed to the higher phenolic content (134.49 \pm 4.14 and 143.49 \pm 2.51 μ g GAE/mg extract, respectively). However, the weakest radical scavenging and reducing power activities were registered by *C. cinerea* Del. cold water extract (IC_{50} =161.19 \pm 0.77 and 135.17 \pm 1.78 μ g/mL) which may be explained by the lower phenolic (38.96 \pm 1.74 μ g GAE/mg extract) and flavonoid (12.65 \pm 0.45 μ g QE/mg extract) contents. The results obtained for the hot water and methanolic extracts of *M. suaveolens* subsp. *timija* (Briq.) Harley are in agreement with what has been reported in previous studies of *Mentha* species extracts (Kamkar et al., 2010; Belfeki et al., 2016; Benabdallah et al., 2016; Boussouf et al., 2017). In fact, literature review shows the presence of prevalent phenolics in mint family such as rosmarinic acid, luteolin and caffeic acid (Padmini et al., 2010). These compounds are commonly soluble in water and organic solvents such as methanol and the presence of them in the hot water and methanolic extracts of *M. suaveolens* subsp. *timija* (Briq.) Harley may be the main cause of its strong antioxidant activity.

In the case of β -carotene-linoleic acid bleaching assay, antioxidant ability of the hot water and methanolic extracts of *S. officinalis* L. were superior in all the samples tested with an IC_{50} value of 14.49 \pm 0.40 μ g/mL and 23.89 \pm 0.67 μ g/mL, respectively. This activity



is apparently related to the important phenolic and flavonoid contents (118.96 ± 1.43 and 136.21 ± 1.24 μg GAE/mg extract; 47.95 ± 1.58 and 61.72 ± 2.22 μg QE/mg extract respectively). Nevertheless, *C. cinerea* again for the cold water extract gave the lowest contribution in this test ($\text{IC}_{50} = 233.74 \pm 1.09$ $\mu\text{g}/\text{mL}$). According to literature survey, it was found out that some polar extracts from *Salvia* species studied for their antioxidant effect contain several antioxidant compounds (Şenol et al., 2010; Kocak et al., 2016; Alimpić et al., 2017; Toplan et al., 2017). In addition, *S. officinalis* L. contains a range of different phenolic components, especially flavonoids, such as apigenin and luteolin glycosides, phenolic acids such as caffeic acid, carnosic acid and rosmarinic acid (Jang et al., 2005; Imanshahidi and Hosseinzadeh, 2006). For the three tests applied, hot water and methanolic extracts of *T. satureoides* Coss., and *A. ageratum* L. expressed in general intermediate activity. This activity may be linked to the presence of many phenolic and flavonoid compounds such as rosmarinic acid, luteolin-7-glycoside, hesperetin, hispidulin and ciresileol (Vieira et al., 1997; Ramchoun et al., 2012).

3.2. Phenolic and flavonoid contents

Polyphenolic compounds are the main class of secondary metabolites responsible of the antioxidant potency of medicinal and aromatic plants. Therefore, in the present study, the total phenolic and flavonoid contents of the studied plants extracts were calculated, and the obtained results are given in Fig. 2. The data clearly indicated that the hot water and methanolic extracts of all species showed the highest contents of phenols and flavonoids. The phenolic content values varied from 57.22 ± 2.42 to 165.82 ± 2.25 μg GAE/mg extract, while the amounts of total flavonoid were ranged from 17.35 ± 1.50 to 63.32 ± 1.36 μg QE/mg extract. However, the lowest amounts of these compounds were obtained by the cold water extracts.

In general, in all the samples examined, higher levels of total phenolic and flavonoid contents were obtained when extracts were prepared by hot water and methanol, compared to those obtained with cold water. Similar results have been previously reported indicating that the extraction procedure appears to affect total phenolic and flavonoid content and consequently the antioxidant activity of each plant (Sultana et al., 2009; Addai et al., 2013; Pereira et al., 2017). Furthermore, other previous work showed the efficiency of hot water and organic solvent such as methanol in the extraction of the antioxidant compounds (Kamatou et al., 2010; Skotti et al., 2014; Teixeira et al., 2013). According to these authors, extraction at high temperature and using organic solvents induced cell disruption and improved penetration of solvent to the plant tissue, and consequently breaking the vegetal cells and release the cell contents into the extraction medium (Toma et al., 2001).

4. Concluding remarks

The data presented in this study are the first information on the antioxidant properties of the hot water, cold water and methanolic extracts obtained from five Moroccan medicinal and aromatic plants (*A. ageratum* L., *S. officinalis* L., *T. satureoides* Coss., *M. suaveolens* subsp. *timija* (Briq.) Harley and *C. cinerea* Del.). The results obtained showed that all the plants extracts exhibited interesting antioxidant activity. Generally, the hot water and methanolic extracts especially those of *M. suaveolens* subsp. *timija* (Briq.) Harley and *S. officinalis* L. showed the strongest antioxidant potency and the highest phenolic and flavonoid contents compared with the cold water extracts. These results provide additional information on the economic value of Moroccan plant extracts as an effective alternative antioxidant agent for the preservation of processed food and in pharmaceutical industries. However, further investigations are necessary to evaluate the toxicity level of these Moroccan plant extracts, in order to develop an optimized dose to maintain the safety and the shelf-life of the product.

Conflict of interest

The authors declare that there is no conflict of interest.

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