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Algerian mint species: high performance thin layer chromatography quantitative determination of rosmarinic acid and *in vitro* inhibitory effects on linoleic acid peroxidation

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PEER REVIEW

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Comments

This study showed that Algerian mint species are rich in phenolic compounds, and their antioxidant property was demonstrated. Therefore, these traditionally used species could be exploited for further studies.

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ABSTRACT

Objective: To determine the quantitative parameters of rosmarinic acid in Algerian mints, *Mentha spicata* L. (*M. spicata*), *Mentha pulegium* L. and *Mentha rotundifolia* (L.) Huds by high performance thin layer chromatography (HPTLC)–densitometric method and screen the effects of these plant extracts on linoleic acid peroxidation.

Methods: The analyses were performed on HPTLC silica gel 60 F254 plates with chloroform: acetone: formic acid (75:16.5:8.5, v/v) as the mobile phase. Rosmarinic acid was determined in UV at 365 nm and fluorescence at λ_{exc} 325 nm with a 550 nm filter, respectively. The effects of plants extracts on linoleic acid peroxidation were measured by an indirect *in vitro* colorimetric method.

Results: Chromatographic resolution permitted reliable quantification in both measurement modes and calibration curves were linear in a range of 150–1000 ng/spot. *M. spicata* was found to contain significantly higher concentrations of rosmarinic acid. The densitometric quantification allowed the analysis of many samples in a short time with reasonable precision (total precision for *Mentha* spp extracts, 5.1% and 5.8% for UV and fluorescence detection, respectively). The HPTLC data, allied to assays of linoleic acid peroxidation prevention, suggested the potential of *M. spicata* (52% Trolox® equivalents) as a natural source for inhibitors of lipid peroxidation.

Conclusions: Densitometry can be used for routine determination and quality control of rosmarinic acid in herbal and formulations containing *Mentha* species.

KEYWORDS

Densitometry, High performance thin layer chromatography, Lipid peroxidation, *Mentha* sp, Rosmarinic acid

1. Introduction

The genus *Mentha* which comprises 20 species distributed all over the world is among the major genera belonging to Lamiaceae family^[1]. *Mentha* species are traditionally used in foods and cosmetics as well^[2].

The *Mentha* genus is considered to be a rich source of

secondary metabolites including monoterpenes–based essential oils, simple phenols, flavonoids, phenolic acids^[3–5] and higher terpenoids, including diterpenes (phytol) and tetraterpenes (α and β carotene)^[6]. The most common and popular mints for cultivation are the species pennyroyal [*Mentha pulegium* L. (*M. pulegium*)], spearmint [*Mentha spicata* L. (*M. spicata*)] and (more recently) apple mint

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(*Mentha suaveolens* Ehrh.)^[1].

M. spicata L., commonly known in the Algerian systems of medicine as “Naana”, has many culinary and medicinal uses in Maghreb. It is popularly consumed in the form of tea and added to several preparations as a flavor enhancer; the dry or fresh leaf of spearmint is added specially during the brewing of tea. Biliary disorders, menstrual cramps, stomach pain, constipation, gingivitis and odontalgias are treated with the decoction of spearmint leaves. The leaves are also a poultice used to relieve rheumatism and combat fever^[7]. *M. pulegium* L., known as “Feliou” in Algeria, is used for the treatment of flatulent dyspepsia and intestinal colic due to its carminative and antispasmodic properties^[8]. *Mentha rotundifolia* (*M. rotundifolia*), “Timija” in Algeria, is also widely used in Maghreb, primarily for external use. The crushed leaves decoction treats furunculosis and abscesses. It would reduce fever in friction and eliminate dental pains as a mouthwash^[7].

Although many compounds have been isolated from different species of the genus *Mentha*, notably *Mentha piperita*, the only few reports available on the chemical composition of the species growing in the Algeria concentrated on essential oils^[4]. Phenolic compounds are, however, quite interesting due to the great variety of biological properties displayed by most of them^[9]. An extensive investigation on Lamiaceae family, mainly qualitative data^[10], can be afforded, and the acid phenol rosmarinic acid (Figure 1) commonly occurs in many species of this family. Rosmarinic acid is considered as an excellent chemotaxonomic marker^[11]. Rosmarinic acid attracts major current interest for its antibacterial, antioxidant, anti-carcinogenic, anti-inflammatory, anti-viral, immunosuppressant, hepato- and neuro-protective activities^[12]. It is also investigated for the effect on autoimmune arthritis, heart disease, suppression of autoimmune rejection in human skin transplant patients, as well as for its multipurpose activities against reverse transcriptase, integrase and RNase H in HIV infections^[13]. Moreover, rosmarinic acid has recently been reported to inhibit the hemorrhagic effect of snake venoms^[14], and for its positive effects on Alzheimer’s disease^[15].

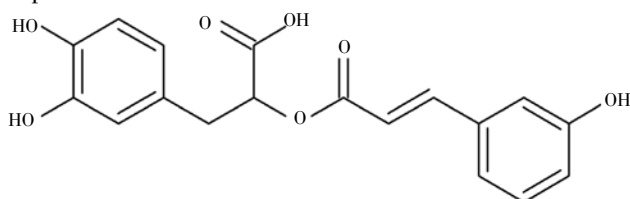


Figure 1. Chemical structure of α -O-caffeoyl-3,4-dihydroxyphenyl-lactic acid ester (rosmarinic acid).

High performance thin layer chromatography (HPTLC) has emerged as one of the most efficient tools in the last two decades for the separation and quantification of secondary metabolites, especially for the evaluation of botanical materials^[16,17]. In comparison with high performance liquid chromatography (HPLC), HPTLC presents a series of advantage as (i) the methods usually do not require extensive clean-up procedures of crude plant extracts, even for quantitative analysis^[17]; (ii) numerous samples can be run

in a single analysis thereby dramatically reducing analysis time^[10], and (iii) the totality of the sample is visualized on the chromatoplate, whereas compounds stuck on a HPLC column can be missed; this is a major advantage in detecting unknown contaminants. The main drawbacks of HPTLC resides in the linearity range (typically only about 1 log) and the precision of analytical determinations; although many papers claim high precisions, the relative standard deviations achievable in practice typically range 2% to 5% (total precision, *i.e.* including the between-plates component) a precision that is usually sufficient for the standardization of phytodrugs^[18,19].

Owing to the significant nutritional, medicinal and commercial value of Algerian *Mentha* species, the present work aims to develop a simple, rapid and effective quantitative method for the determination of their rosmarinic acid content. Moreover, in view of the potential use of mints as a source of natural antioxidants and as possible functional food to prevent the consequences of oxidative stress, the antioxidant capacity of mint extracts was evaluated by an *in vitro* method based on the inhibition of linoleic acid peroxidation. To the best of our knowledge, this activity was determined for the first time on *M. rotundifolia* extract and the three species together. A correlation between the antioxidant activity and rosmarinic acid content was assessed.

2. Materials and methods

2.1. Herbal material

The leaves and herbarium samples of *M. spicata*, *M. pulegium* and *M. rotundifolia* were collected from the region of Bejaia, Algeria from June to August 2009. *M. spicata* was harvested in Tichy (Latitude: 36°40' N, Longitude: 5°10' E), *M. pulegium* in Chemini (Latitude: 36°36' N, Longitude: 4°36' E) and *M. rotundifolia* in Smaoun (Latitude: 36°36' N, Longitude: 4°49' E). The samples were authenticated by Professor J. Lejoly in the Laboratory of Systematical Botany and Phytosociology, Free University of Brussels, Belgium. Voucher specimens were deposited in the Herbarium of the National Botanical Garden of Meise (Belgium) referring BR 0000006946227 to *M. spicata*, BR 0000006946043 to *M. pulegium*, and BR 000000 6946197 to *M. rotundifolia*. The collected plant material was dried at room temperature under shade and powdered to 250 mesh.

2.2. Chemicals

The reference standard rosmarinic acid (purity 99%, w/w) was purchased from Carl Roth (Karlsruhe, Germany). HPTLC plates (20 cm×10 cm, 0.2 mm thickness, silica gel 60 F₂₅₄) and solvents were purchased from Merck (Darmstadt, Germany). All other reagents were purchased from Aldrich.

2.3. Samples and standards preparation

Powdered samples (0.4 g) were extracted with 15 mL of 50%

aqueous ethanol for 10 min in an ultrasonic bath (Eurosonic 44). The extracts were filtered through Whatman filter paper No. 1 which was rinsed and made up to 20 mL in volumetric flasks^[20]. Stock solutions of rosmarinic acid (1 mg/10 mL) were prepared in 50% aqueous ethanol and diluted to give 1 mL solutions in the appropriate concentrations range (15 to 100 µg/mL).

2.4. HPTLC conditions

Duplicate standards and triplicate samples solutions (10 µL) were applied using an ATS4 automated thin layer chromatography (TLC) sampler (Camag, Switzerland) in 7 mm bands at 10 mm from the bottom, 15 mm from the sides and with 8.5 mm space between. Plates were developed to a height of about 7 cm from the starting line with chloroform: acetone: formic acid (75:16.5:8.5, v/v) in a saturated vertical chamber. After development, the plates were dried at 110 °C for 20 min (Camag TLC Plate Heater III) and spots were visualized under UV light (254 and 366 nm) (Figure 2). Quantitative evaluation of the plates was performed in reflectance/absorbance mode at 365 nm and in fluorescence mode with excitation at 325 nm using a Camag TLC Scanner III (filter K400 at 550 nm; slit width, 4 mm×0.3 mm; scanning speed, 1 mm/s; data resolution, 50 µm/step; WinCats 1.3.2). The peak areas and absorption spectra were recorded. The amounts of rosmarinic acid in the sample were calculated in the two detection modes using the respective calibration curves.

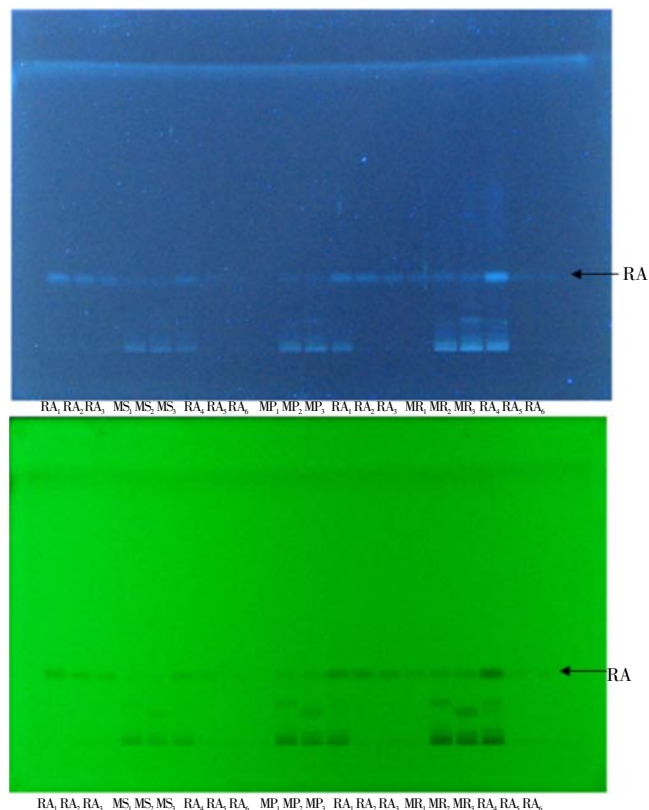


Figure 2. HPTLC fingerprints of mint extracts and rosmarinic acid under UV₃₆₆ nm and UV₂₅₄ nm.

Mobile phase: chloroform: acetone: formic acid (75:16.5:8.5, v/v); RA: Rosmarinic acid; MS: *M. spicata* leaves extract; MP: *M. pulegium* leaves extract; MR: *M. rotundifolia* leaves extract.

2.5. Inhibition of linoleic acid peroxidation

The inhibition of the Fenton reaction–induced linoleic acid peroxidation was measured by an indirect *in vitro* colorimetric method based on the hydroperoxides– and hemoglobin–catalyzed oxidation of benzoyl leucomethylene blue into methylene blue, which has a strong absorption band at about 660 nm. The reaction mixture contained 125 µL phosphate buffer (0.4 mol/L, pH 6.75, air–saturated), 25 µL of extract solutions (serial dilutions 1/2 in methanol; initial concentration, 10 mg/mL), 10 µL of hydrogen peroxide (16 mmol/L), 10 µL of ferrous sulfate (16 mmol/L)–ethylene diamine tetraacetic acid Na₂H₂ (15 mmol/L) and 80 µL of linoleic acid (8 mmol/L in borate 50 mmol/L, nitrogen–saturated, pH 9). After 10 min at room temperature in the dark, 75 µL of each reaction well were transferred to another plate containing 150 µL of a benzoyl leucomethylene blue–hemoglobin solution (5 mg benzoyl leucomethylene blue, in 8 mL dimethyl formamide, added to 80 mL phosphate buffer (0.2 mol/L, pH 5), containing 1.4 g Triton X–100 and 5.6 mg of hemoglobin; diluted to 100 mL with phosphate buffer; to be prepared extemporaneously, strictly observing the order of reagents addition) per well. After 30 min at room temperature in the dark, the absorbances were measured with a Labsystems iEMS reader/dispenser MF (Labsystems, Finland) at 660 nm. All the experimental results obtained from two separate experiments in triplicate were fitted to a parametric function in Systat 7.1 (Systat Software). The IC₅₀ (the concentrations of plant extracts that inhibit 50% of methylene blue formation) were computed. Results were expressed as Trolox® equivalent antioxidant capacity, calculated as IC₅₀ Trolox®/IC₅₀ plant extract^[21].

2.6. Statistical analyses

The precision data were analyzed by a Two–way ANOVA with repetition, considering 2 random effects, sample (or concentration level) and plate. The within–plate and total (between–plates) variations for the whole analytical procedure were computed^[19].

Assay data are expressed as mean±SD and compared by One–way ANOVA with *post hoc* tests (Bonferoni correction). The differences between individual means were deemed to be significant at $P \leq 0.05$. All analyses were performed by using the Statistica v 5.1 software.

3. Results

An active marker compound, rosmarinic acid, was quantified by a HPTLC densitometric method in three *Mentha* species (*M. spicata*, *M. pulegium*, and *M. rotundifolia*). The mobile phase was selected after studying and testing numerous variants reported in the literature. The mobile phase proposed by Wagner and Bladt was retained^[16]. It was found that rosmarinic acid was well resolved from other components of the sample extract at R_f 0.43 in the solvent system chloroform: acetone: formic acid (75:16.5:8.5). In order to confirm the identity of the bands for rosmarinic acid in

the samples extracts, these were co–chromatographed with authentic standard of rosmarinic acid and UV spectra were visually compared, indicating the specificity of the method (Figure 3).

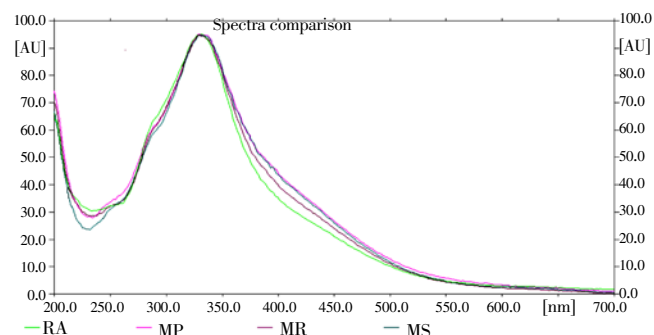


Figure 3. Comparison of UV spectra of rosmarinic acid in standards and extracts between 200 and 700 nm.

Spectra recorded on HPTLC plates after separation in the conditions as in Figure 2; RA: Rosmarinic acid; MP: *M. pulegium* leaves extract; MR: *M. rotundifolia* leaves extract; MS: *M. spicata* leaves extract.

Given the typically low linearity range of the densitometric methods, it is suggested that calibration curves should be prepared to cover the possible range of concentrations in the samples. An excellent linearity was achieved in the concentration ranges of 150–1000 ng/spot for rosmarinic acid (typically $R^2=0.996$ in absorbance mode, $R^2=0.997$ in fluorescence mode, concentrations in duplicate). Table 1 details the components of the variance for the precision data. For the standards, the precision was influenced by both investigated factors, level and chromatographic plate. Concentration levels can be clearly distinguished but measured densities depend on the chromatographic run. This difference justifies the need for complete calibration curves on each developed plate. The total precisions for the

analysis of rosmarinic acid in *Mentha* spp extracts were 5.1% and 5.8% for UV and fluorescence detection, respectively.

The results of densitometric determinations of rosmarinic acid in the three *Mentha* species (Table 2) shown that *M. spicata* leaves contained the highest amounts of studied phenol from the yielded comparable data of UV and fluorescence, which points to an absence of significant interferences in measurements. Figure 4 presents the chromatographic profiles obtained for the extracts of *M. spicata*, *M. pulegium* and *M. rotundifolia* in UV and fluorescence. Rosmarinic acid was obviously dominant in the leaves of *M. spicata*, while there were no significant differences in rosmarinic acid content between *M. pulegium* and *M. rotundifolia* (Table 2).

Table 2

Rosmarinic acid content in *M. spicata*, *M. pulegium* and *M. rotundifolia*.

| Sample | Absorbance mode | | | Fluorescence mode | | |
|------------------------|-------------------|------|---------|-------------------|------|---------|
| | % w/w | SD | RSD (%) | % w/w | SD | RSD (%) |
| <i>M. spicata</i> | 3.63 ^a | 0.10 | 2.8 | 3.30 ^a | 0.09 | 2.7 |
| <i>M. pulegium</i> | 1.04 ^b | 0.05 | 4.8 | 1.06 ^b | 0.02 | 1.9 |
| <i>M. rotundifolia</i> | 0.96 ^b | 0.03 | 3.1 | 0.77 ^b | 0.04 | 5.2 |

^{a,b}: Means followed by the same small letter did not share significant differences at $P \leq 0.05$; SD: Standard deviation; RSD: Relative standard deviation.

Lipid peroxidation is the oxidative degradation of polyunsaturated fatty acids and involves free radicals (superoxide and hydroxyle) and singlet oxygen produced in biological systems[22]. It's an early event in the deterioration of food quality during processing and/or storage[23]. Free radicals scavenging and singlet oxygen quenching are generally the accepted mechanism for antioxidants inhibiting lipid oxidation [22,24].

After peroxidation of linoleic acid by a Fenton reaction, the resulting hydroperoxides were reacted with N–benzoyl leucomethylene blue in the presence of hemoglobin to yield

Table 1

Precision study: two–way ANOVA with repetition; 2 random factors (sample and plate).

| Source of variation | | Degrees of freedom (df) | Sum of squares | Mean square | Variance ratio (F) | P^c | RSD% (within–plate) | RSD% (total) |
|---|----------------------------|-------------------------|----------------|-------------|--------------------|--------------------------|---------------------|--------------|
| Standards UV ^a | Plate | 1 | 1606084 | 1606084 | 23.14 | 0.009 ^{**} | 4.9 | 13.0 |
| | Level | 4 | 35387987 | 8846997 | 127.49 | 1.81×10 ^{−4***} | | |
| | Plate×Level (Interaction) | 4 | 277584 | 69396 | 2.81 | 0.085 | | |
| | Residual (error) | 10 | 247325 | 24732 | | | | |
| | Total | 19 | 37518980 | | | | | |
| Standards fluorescence ^a | Plate | 1 | 534514 | 534514 | 9.06 | 0.040 [*] | 3.3 | 7.1 |
| | Level | 4 | 94471245 | 23617811 | 400.60 | 1.86×10 ^{−5***} | | |
| | Plate×Level (Interaction) | 4 | 235826 | 58956 | 4.66 | 0.022 [*] | | |
| | Residual (error) | 10 | 126544 | 12654 | | | | |
| | Total | 19 | 95368129 | | | | | |
| <i>Mentha</i> samples UV ^b | Plate | 1 | 5233 | 5233 | 0.03 | 88.700 | 5.1 | 5.1 |
| | Level | 2 | 3474246 | 1737123 | 8.57 | 10.400 | | |
| | Plate×Sample (Interaction) | 2 | 405382 | 202691 | 14.82 | 0.057 | | |
| | Residual (error) | 12 | 164160 | 13680 | | | | |
| | Total | 17 | 4049021 | | | | | |
| <i>Mentha</i> samples fluorescence ^b | Plate | 1 | 24546 | 24546 | 0.08 | 80.100 | 5.8 | 5.8 |
| | Sample | 2 | 7369378 | 3684689 | 12.39 | 7.500 | | |
| | Plate×Sample (Interaction) | 2 | 594789 | 297394 | 18.51 | 0.022 [*] | | |
| | Residual (error) | 12 | 192851 | 16071 | | | | |
| | Total | 17 | 8181564 | | | | | |

^a: 5 different levels have been analyzed in duplicate on 2 different plates; ^b: 3 samples have been analyzed in triplicate on 2 different plates; ^c: Non significant, $P > 0.05$; ^{*}: Significant, $0.01 < P < 0.05$; ^{**}: Highly significant, $P < 0.001$; ^{***}: Very highly significant; RSD: Relative standard deviation.

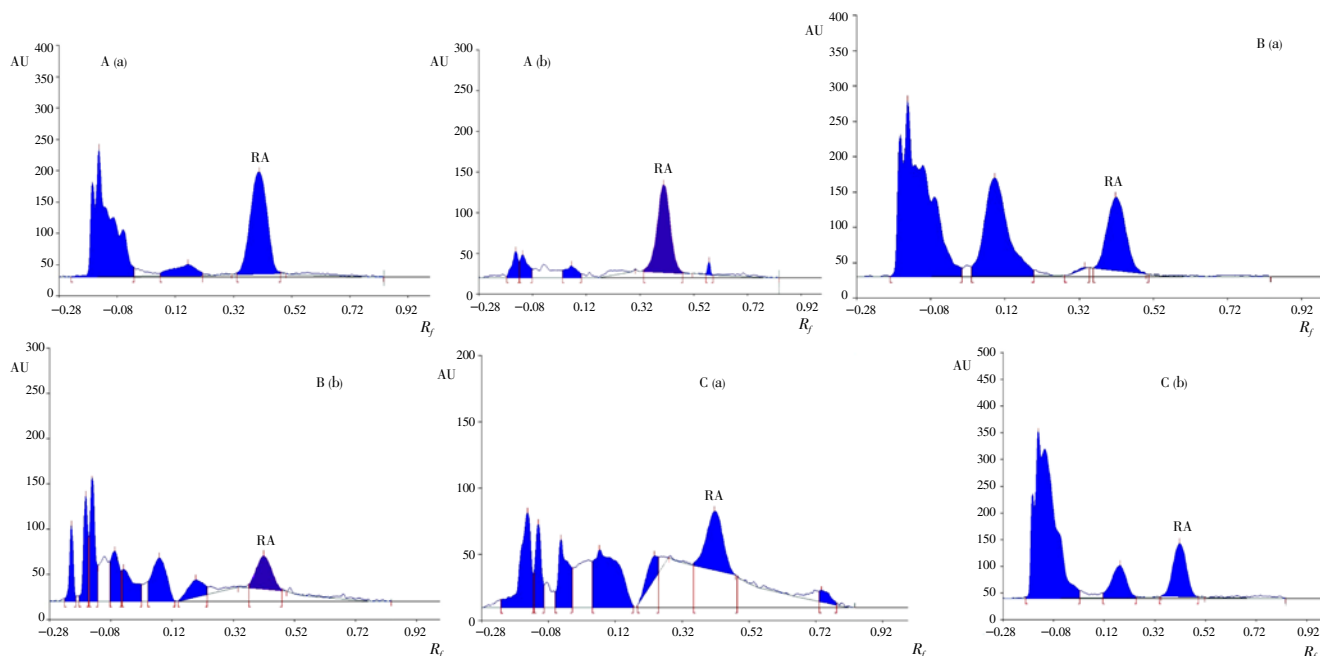


Figure 4. HPTLC profiles of *M. spicata* (A), *M. pulegium* (B) and *M. rotundifolia* (C) extracts in UV and fluorescence. a: Scan at 365 nm in UV; b: Scan at λ_{exc} 325 nm in fluorescence; Chromatographic conditions as in Figure 2.

methylene blue, which has a strong absorption band at about 660 nm. Hydroperoxides oxidize leucomethylene blue to methylene blue was schematized by Auerbach *et al*[25].

The presence of antioxidants, susceptible to inhibit at any of these steps, results in a decreased methylene blue production[21]. This inhibition could be caused (i) by the absence of ferryl–perferryl complex formation; (ii) by scavenging the hydroxyl radical or the superoxide radicals; (iii) by changing the Fe^{3+}/Fe^{2+} ratio; (iv) by reducing the rate of conversion of ferrous to ferric; or (v) by chelating the iron itself[26].

Curve fitting allowed to compute IC_{50} values for the extracts of the three plant species (Table 3); these IC_{50} s indicated the ability of the plant extracts to inhibit lipid peroxidation and were expressed as Trolox® equivalents.

Table 3

Inhibition of linoleic acid peroxydation assay by mint extracts compound.

| Samples | $IC_{50} \pm SD^a$ ($\mu g/mL$) | Trolox® equivalent ^{a,b} |
|------------------------|-----------------------------------|-----------------------------------|
| <i>M. spicata</i> | 6.8 ± 0.4 | 0.52 |
| <i>M. pulegium</i> | 10.0 ± 0.5 | 0.34 |
| <i>M. rotundifolia</i> | 26.0 ± 3.0 | 0.14 |
| Trolox®1 | 3.5 ± 0.3 | – |
| Trolox®2 | 3.8 ± 0.3 | – |

^a: Curve fitted from two experiments in duplicate; ^b: Computed from Trolox® IC_{50} measured along each experiment.

These three mints extracts inhibited lipid peroxidation with IC_{50} values ranging between 6 and 26 $\mu g/mL$ (0.51–0.14 Trolox® equivalents).

4. Discussion

The amount of rosmarinic acid in aromatic herbs was determined essentially using HPLC method. Previous HPLC analyses of spearmint (*M. spicata*) clones grown in a normal,

controlled environment revealed that rosmarinic acid was the primary phenolic compound present. The rosmarinic acid content ranged from 55% to 85% of the total phenolics profile with the remainder of the phenolics was m–coumaric, p–coumaric, caffeic and ferulic acids[13]. Kivilompolo and Hyotylainen measured, using two–dimensional liquid chromatography with mass detection, an amount of 0.56% w/w (dry weight) of rosmarinic acid in spearmint leaves[27].

Dorman *et al.* and Kosar *et al.* analyzed aqueous extracts of *M. spicata* by HPLC, and identified rosmarinic acid as the major component with a concentration of 0.46% (w/w of extract)[3,28]. Based on non–specific spectrophotometric methods, Wang *et al.* reported a rosmarinic acid content of 0.71%–1.43% w/w for *M. spicata*[29]; Fletcher *et al.* reported a *M. spicata* clone (HMS–21) with enhanced rosmarinic acid levels of up to 6% w/w[13].

So far, to the best of our knowledge, there have been no attempts to determine the amounts of rosmarinic acid in *M. pulegium* and *M. rotundifolia*.

As mentioned here above, rosmarinic acid is a molecule of interest, owing to its multiple biological activities and high content, especially in *M. spicata*, and probably participates to some of the activities alleged in traditional medicine.

In the test of the lipid peroxidation, *M. spicata* proved to be the most potent. This is in line with previous results that exposed its role as a potent inhibitor of lipid peroxidation[30]. This activity is most probably associated with the high amount of rosmarinic acid in the extracts of this plant. Fadel *et al.* showed the high efficiency of rosmarinic acid in preventing lipid peroxidation[31].

Soobrattee *et al.* have shown that the order of effectiveness of the hydroxycinnamic acids in inhibiting low density lipoprotein oxidation was: rosmarinic acid>chlorogenic acid>caffeic acid>ferulic acid>coumaric acid[32]. Yang *et al.* reported the interaction of caffeic acid and rosmarinic acid with respect to the hepatic glutathione levels, lipid

peroxidation and other antioxidant enzyme activities involved in hepatic protection against oxidative stress using *in vitro* human hepatocyte HepG2 and *in vivo* rat liver^[24]. According to del Bano *et al.*^[22], the protective effects of hydroxycinnamic acids correlated with the number of hydroxyl substituents. Rosmarinic acid, in comparison with caffeic acid and chlorogenic acid, presented two dihydroxyphenyl propanoic acid moieties. The presence of two catechol structures conjugated with a carboxylic acid group increased the antioxidant activity in aqueous or aqueous alcoholic media^[22].

Fadel *et al.* have shown that a small amount of rosmarinic acid was able to spontaneously insert inside membranes^[31]. This fraction of rosmarinic acid could be extremely efficient to prevent lipid peroxidation. They proposed two hypotheses to explain these antioxidant effects: (i) rosmarinic acid molecules in the bulk phase could stop the propagation of the free radical before they reach the lipids; and/or (ii) rosmarinic acid molecules associated with the lipid membrane could block the propagation of free radicals within the bilayer (either by modifying the membrane fluidity or by direct free radicals scavenging)^[31].

The data obtained herein suggest, for the studied *Mentha* species, a direct correlation between total antioxidant capacity expressed as Trolox[®] equivalent and rosmarinic acid content ($R^2=0.863$), indicating that rosmarinic acid considerably contributes to this biological activity.

These results are in line with previous studies^[13], which demonstrated that many phenolic acids, including rosmarinic acid and related polyphenols, significantly contribute to the antioxidant capacity of many fruits and vegetables. The present study opens the eventuality to explore the possible use of *M. spicata* L. (spearmint), which contains a considerable amount of rosmarinic acid, as a preventive and therapeutic approach in disorders related to oxidative stress but also as an antioxidant for agri–food storage.

Rosmarinic acid, a major contributor to mints numerous health–promoting properties, has been successfully quantified in different mint extracts by the proposed HPTLC–photodensitometric method. This simple and rapid analytical method has been for the first time applied to the quantification of this major bioactive compound in three mints, *M. spicata*, *M. pulegium* and *M. rotundifolia*.

There is a significant quantitative variation in rosmarinic acid according to the species. *M. spicata* was particularly rich in this phenolic acid, and could be used as a cost–effective, readily exploitable industrial resource for rosmarinic acid production.

The developed analytical method permits an easy determination of the compound. Moreover, its validation for application in quantification of *Mentha* species extracts and formulations is worthy to pursue as the method could allow a fast and efficient quality control.

The *in vitro* capacity to inhibit linoleic acid peroxidation could be correlated with the content of the major phenolic compound, rosmarinic acid. The rosmarinic acid content and inhibition of lipid peroxidation of mints proved to be high, especially in *M. spicata*. This later plant could be considered as a possible functional food, which makes it a promising

candidate for more detailed *in vitro* and *in vivo* studies.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

We thank Pr. J. Lejoly for his identification of plants studied and all members of the Pharmacognosy, Bromatology and Human Nutrition laboratory from ULB for their help, especially M. Faes and O. Vaillant for maintaining good conditions of work in the laboratory.

Comments

Background

The mint species are very important and used in traditionally medicinal in Maghreb, particularly in Algeria. So, it is important to use certain methods such as HPTLC for the quantitative determination of rosmarinic acid, an important secondary metabolite, which has some properties.

Research frontiers

This study is HPTLC quantitative determination of rosmarinic acid and *in vitro* inhibitory effects on linoleic acid peroxidation.

Related reports

The phytochemical analyses were performed on HPTLC silica gel 60 F₂₅₄ plates with chloroform: acetone: formic acid (75:16.5:8.5, v/v) as the mobile phase. Rosmarinic acid was determined in UV at 365 nm and fluorescence at λ_{exc} 325 nm with a 550 nm filter, respectively. The effects of plants extracts on linoleic acid peroxidation were measured by an indirect *in vitro* colorimetric method.

Innovations and breakthroughs

The study demonstrated that *M. spicata* was particularly rich in this phenolic acid, and could be used as a cost–effective, readily exploitable industrial resource for rosmarinic acid production. Therefore, the developed analytical method permits an easy determination of the compound; its validation for application to quantification in *Mentha* species, extracts and formulations is worthy to pursue as the method could allow a fast and efficient quality control.

Applications

According to certain studies, *Mentha* species of Algeria have some significant nutritional, medicinal and commercial values. Moreover, in view of the potential use of mints, it could use as a source of natural antioxidants and as a possible functional food to prevent the consequences of oxidative stress.

Peer review

This study showed that Algeria mint species were rich in

phenolic compounds, and their antioxidant property was demonstrated. Therefore, these traditionally used species could be exploited for further studies.

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