

Full Length Research Paper

Antioxidant and anti-browning activities of *Mentha suaveolens* extracts

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The inhibition of enzymatic browning and oxidation is still an important challenge in food processing. This study was designed to examine *in vitro* the inhibition of enzymatic browning in apple and the antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferrous ion chelating activity by *Mentha suaveolens* phenol, methanol and aqueous extracts. The use of various extracts in the prevention of the enzymatic browning, catalysed by polyphenol oxidases (PPO) and peroxidases (POD) showed that the phenol extract had a high effect on PPO more than synthetic inhibitor (potassium sorbate), whereas all extracts of *M. suaveolens* presented only a minor effect on POD of apple. The radical scavenging activity of various extracts of *M. suaveolens* using DPPH showed that the phenol extract had same activity as butyl hydroxyl toluene (BHT) which is a known inhibitor for its antioxidant activity. The highest ferrous ion chelating activity was found in phenol and methanol extracts. The chemical analysis of the phenol extract using the high performance liquid chromatography (HPLC) suggests that the rosmarinic acid could explain the observed inhibition. In conclusion, these data suggest that the *M. suaveolens* compounds can act as preservatives of food against oxidation and enzymatic browning.

Key words: *Mentha suaveolens*, phenolic compounds, enzymatic browning, antioxidant activity.

INTRODUCTION

Oxidation and enzymatic browning occurring during the manufacturing process, storage and processing of food cause loss of quality (Mau et al., 2004). It is a particular problem in fruits with white flesh such as apples, pears and bananas. Enzymatic browning usually starts with the

oxidation of phenols to quinones by polyphenol oxidases brown colored products (Murata et al., 1995). Although, (PPO) in the presence of oxygen. Quinones are subjected to further reactions leading to the formation of peroxidase activity (POD) is limited by the availability of electron acceptor compounds like hydrogen peroxide, it is also relevant to enzymatic browning of various fruits (Chisari et al., 2007). The oxidative deterioration of foods is responsible for the rancid odours and flavours, consequently decreasing the nutritional quality and safety of food due to the formation of secondary, potentially toxic compounds (Zainol et al., 2003). Synthetic substances, such as sulphites are widely used for preventing the enzymatic browning, but they are not always accepted because the toxicological risks which they represent (Taylor and Nordlee, 1993). Thus, an active

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Abbreviations: DPPH, 1,1-Diphenyl-2-picrylhydrazyl radical; PPO, polyphenol oxidase; POD, peroxidase; BHT, butyl hydroxyl toluene; HPLC, high-performance liquid chromatography; PVPP, polyvinylpyrrolidone; GAE, gallic acid equivalents.

field of research is currently under development to find antioxidants and non-sulfite anti-browning agents for the food industry.

Ascorbic acid is commonly used to prevent browning of fruits (Mc Evily et al., 1992). However, its effect is temporary since once it is added, it is completely oxidized and o-quinones could accumulate, leading to browning pigment formation (Rojas-Grau et al., 2006). Thus, various reducing and chelating agents such as citric acid, N-acetylcysteine and oxalic acid have been investigated to prevent browning (Son et al., 2001). Therefore, the use of plants as antioxidants in processed foods had become of increasing importance in the food industry as an alternative to synthetic antioxidants. Lamiaceae family is one of the important families used as a source of extracts with high antioxidant effect. In this family, the genus *Mentha* comprises approximately 25 to 30 species. Members of this genus are highly valued as condiments. A review of the literature reveals that the aerial parts of some members are used for herbal teas and condiments as spasmolytics, antibacterial agents, and for their analgesic and antigenotoxic properties (Basüer and Kurkcuoglu, 1999).

To the best of our knowledge, there is no major research on the antioxidant and anti-browning activities of *Mentha suaveolens*. Therefore, we suggest searching for potential alternatives able to effectively inhibit the oxidation and enzymatic browning phenomena.

MATERIALS AND METHODS

All chemicals, solvents and standards were purchased from Sigma/Aldrich (Germany). Ethanol used for extraction was of chemical grade and other reagents were of analytical grade.

Plant material

M. suaveolens was collected from the Ourika region (Marrakesh, south of Morocco) and identified by the plant Ecology Laboratory of the Faculty of Science Semlalia Marrakesh under the number 6590. Golden delicious apples fruits were purchased locally.

Preparation of plant extracts

The aqueous extract of *M. suaveolens* was obtained by mixing 10 g of aerial parts powder with 100 ml of distilled water for 8 h at room temperature with stirring. The homogenate was centrifuged at 5000 g for 15 min and the supernatant was collected. We also prepared an aqueous extract (10 g/ 100 ml) of *M. suaveolens* with 2 g of the insoluble polyvinylpyrrolidone (PVPP) to trap the phenolic compounds contained in the medium. Furthermore, the methanol extract was obtained from 30 g of the powder of *M. suaveolens* aerial parts mixed with 100 ml of methanol using Soxhlet extractor for 8 h. The phenol extract was prepared according the method of El modafar et al. (1996); 5 g of aerial parts powder of *M. suaveolens* was extracted by a water-alcohol mixture (ethanol / water) (80 / 20 V/ V) for 15 min stirring at 4°C in the dark. After centrifugation, the hydro-alcoholic extract was evaporated in a vacuum to obtain a concentrated aqueous extract. The aqueous

phase of extract was depleted by petroleum ether (1/2 V) to remove all traces of apolar compounds. The aqueous phase thus obtained was then extracted three times with ethyl acetate (V/ V). The resulting organic phase was dried by sodium sulphate anhydride (Na_2SO_4) to eliminate all traces of water. After filtration the solvent was evaporated at 60°C. The residue was taken in methanol and stored at 4°C until tested. The extracts were used for the determination of antibrowning and antioxidant activities and evaluation of total phenol content. The concentrations of each sample are expressed by mg of dry powder per ml of extractant agent.

Inhibition of enzymatic browning

Extraction of crude enzyme of apple

The mashed apple (2 g) was homogenized with 2 ml of potassium phosphate buffer at pH 6.8 for 5 min. The homogenate was centrifuged at 15.000 g for 30 min and supernatant was collected. The supernatant contains two enzymes responsible for enzymatic browning (PPO and POD).

Measurement of polyphenol oxidase activity

PPO activity was measured as per the method of Haplin and Lee (1987) with some modifications. One hundred microliter of different concentrations (2 to 10 mg/ ml) of *M. suaveolens* extracts were pre-incubated at 25°C with 10 μL of enzymatic extract and 1 ml of pyrocatechol 10 mM as a substrate. PPO activity was assayed by a spectrophotometric procedure and expressed as the increase in absorbance at 410 nm for 3 min. The percentage inhibition was calculated as $[(A_0 - A_1) / A_0] * 100$, where A_0 is the activity of the control with extractant and A_1 is the activity of the inhibited enzyme with *M. suaveolens* extracts. The potassium sorbate and ascorbic acid were used as standards.

Measurement of peroxidase activity

POD activity was assayed spectrophotometrically according to Maehly and Chance (1954) method. One hundred microliter of *M. suaveolens* extracts were pre-incubated with 2 ml of potassium phosphate buffer and 1 ml of guaiacol as substrate. The reaction was initiated by the addition of 0.5 ml of H_2O_2 0.03% and the optical density was read at 470 nm. The inhibition rate was also calculated as $[(A_0 - A_1) / A_0] * 100$, where A_0 is the activity of the sample with extractant (control) and A_1 is the activity of the inhibited enzyme with *M. suaveolens* extracts. For the standard, the extracts were replaced by potassium sorbate and ascorbic acid.

Preparation of apple purees and their treatments with various *M. suaveolens* extracts

Ten grams of apple puree were dipped in 1 ml of *M. suaveolens* extracts (10 mg/ml). Excess surface liquid was removed. Controls apple purees were dipped in methanol and water. Potassium sorbate and ascorbic acid were used as standards. Videos images of samples were captured after 8 h of storage at room temperature.

Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl assay

The method of Wang et al. (2002) was used for measuring the

DPPH radical scavenging ability of *M. suaveolens* extracts. One hundred microliters of various concentrations (0.05 to 5 mg/ml) of extracts were mixed with 900 μ L of a 0.004% methanol solution of DPPH. The mixture was then vortexed. After 30 min of incubation at 25°C, the absorbance was measured at 517 nm. The inhibition of free radical DPPH was calculated as: $I\% = (A_0 - A_1 / A_0) * 100$, where A_0 and A_1 correspond to the absorbance of the sample with extractant (control) and the sample with extract, respectively. The butyl hydroxyl toluene (BHT) was used as a standard.

Ferrous ion- chelating activity

The chelating activity of the *M. suaveolens* extracts for ferrous ions was measured following the method of Dinis et al. (1994) with minor modifications. The reaction mixture contained 0.1 ml of extracts and 0.04 ml of $FeCl_2$ (2 mM). The reaction was initiated by the addition of 0.1 ml of ferrozine (5 mM), and then the mixture was shaken vigorously and incubated at room temperature for 10 min. The absorbance of the solution was measured at 562 nm. The percentage inhibition (I %) of ferrozine- Fe^{2+} complex formation was calculated as: $I\% = (1 - A_1 / A_0) * 100$, where A_1 was the absorbance of the sample with extract and A_0 was the absorbance of the sample with extractant (control). Ascorbic acid was used as a standard.

Determination of total phenol content

The phenol content in the various extracts of *M. suaveolens* was estimated by Folin-Ciocalteu method (Singleton and Rossi, 1965) using gallic acid as standard. Ten microliters of the sample was mixed with 250 μ L of Folin-Ciocalteu reagent (diluted 3-fold with distilled water), 500 μ L of sodium bicarbonate 20% and 1.745 ml of distilled water. The solution was kept at 40°C for 30 min. The absorbance was then measured at 760 nm and the total phenols were expressed as gallic acid equivalents (mg GAE/ g dry weight).

Analysis of individual phenolic compounds by analytical high-performance liquid chromatography (HPLC)

The chromatographic analysis of the phenolic extract of *M. suaveolens* was made using HPLC apparatus Waters 600 equipped with a pump, photodiode array detector (Waters 2996), a column Altima reversed phase C18 (250 \times 4 mm, 5 μ M) under a flow rate of 1 ml/min. The mobile phase consisted of a mixture of two solvents, acetic acid-water (2:98, v/v) (solvent A) and acetonitrile (solvent B). The composition of A was increased to 30% in 10 min, then to 100% in 30 min, and then returned to the initial condition in 5 min. A 10 min equilibrium time was allowed between injections. Then, 20 μ L of the phenolic extract was injected after a filtration with a syringe filter (25 m 0.45 mm GHP, GHP Acrodisc). Spectra UV/VIS was recorded in a range of 240 to 400 nm. Moreover, the HPLC chromatograms were analyzed at three wavelengths 280, 350 and 320 nm, which correspond to absorption maxima of benzoic acid, flavonoids and hydrocinnamic derivatives. These spectral data were processed with software Millennium 32 LC.

Data processing and statistical analysis

Data were analyzed for significant differences by the test of variance (ANOVA) follow-up test. LSD was made with the SPSS 17. The level of signification was set at 5%. All measurements were performed in triplicate.

RESULTS

Effect of *M. suaveolens* extracts on apple PPO activity

The percentage of inhibition decreased in the order of ascorbic acid > phenol extract > methanol extract >= potassium sorbate > aqueous extract > aqueous extract with PVPP (Figure 1). Statistically, the methanol extract had the same inhibitory effect as potassium sorbate. The aqueous extract with PVPP presented a very minor effect on the apple PPO. At 8 mg/ml, *M. suaveolens* extracts and chemical anti-browning agents did not cause a substantial increase in inhibition, indicating maximum inhibition was achieved.

Effect of *M. suaveolens* extracts on apple POD activity

The evolution of inhibition of apple POD by *M. suaveolens* extracts was low (Figure 2). Indeed, the phenol and methanol extracts had a minor effect on POD activity; their inhibition did not exceed 20.6 and 18.7%, respectively. On the other hand, the other extracts had considerably a very low inhibition effect on POD of apple.

Effect of *M. suaveolens* extracts on apple purees

Compared with standards, the best color preservation for the purees apple was obtained with phenol and methanol extracts after 8 h of storage period, while no anti-browning effect was shown with aqueous extract with or without PVPP (Figure 3).

Determination of DPPH radical scavenging activity

DPPH free radical scavenging activity is shown in Figure 4. At first, phenol and methanol extracts had almost equal antioxidant potency and they showed significantly a superior antioxidant effect than BHT. Up to 0.5 mg/ml, the activity of phenol extract was always greater than that of the other extracts. Aqueous extract showed a relatively medium scavenging potential, while aqueous extract with PVPP showed the least DPPH scavenging activity which does not exceed 8.12%.

Determination of ferrous ion- chelating activity

Generally, there was no significant difference between the ferrous ion- chelating capacity of phenol, methanol extracts and ascorbic acid (Figure 5). At 5 mg/ml, the phenol and methanol extracts, demonstrated a best ferrous chelating efficiency, their activity exceeded 78% followed by aqueous extract (46.3%). The metal chelating

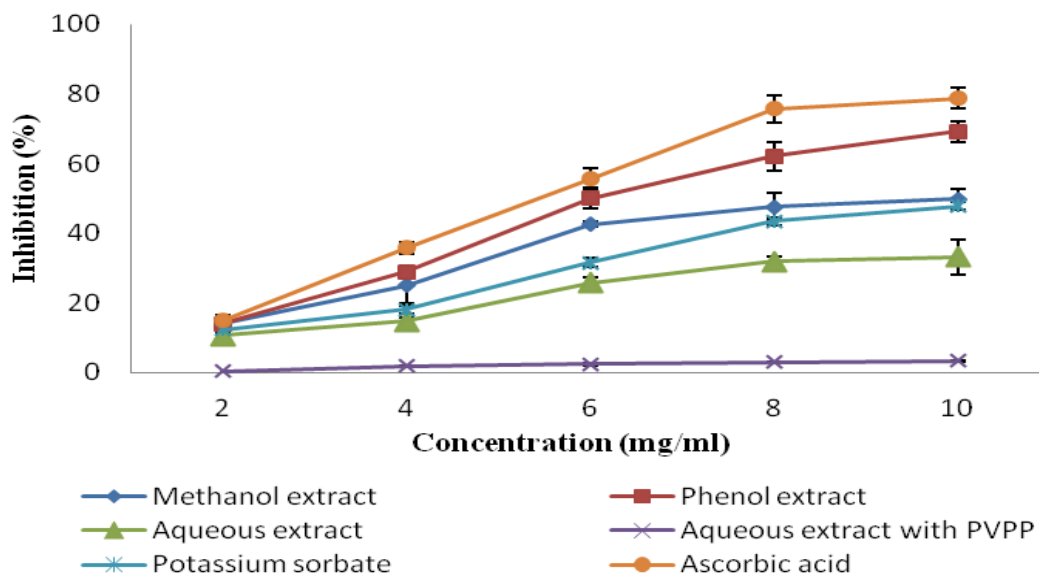


Figure 1. Effect of *Mentha suaveolens* extracts and anti-browning agents on apple PPO activity. Each measure is the mean of 3 replicates. PPO, Polyphenol oxidase.

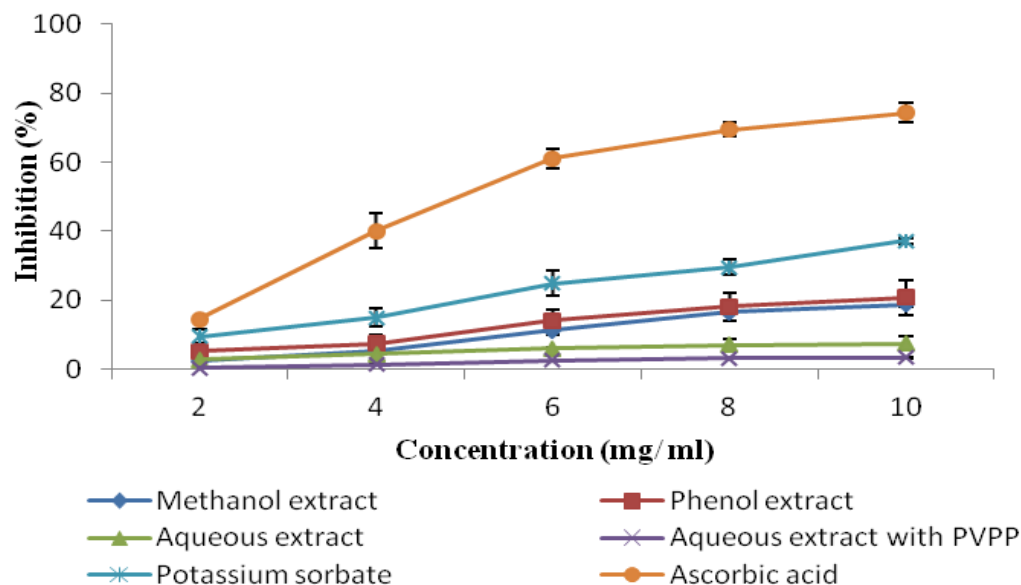


Figure 2. The inhibitory effect of *Mentha suaveolens* extracts and anti-browning agents on apple POD activity. Each measure is the mean of 3 replicates. POD, Peroxidase.

activity in aqueous extract with PVPP was inferior to 4%.

Total phenol content (TPC)

Significant differences found in TPC among different extracts of *M. suaveolens* varied from 0.25 ± 0.04 to 2.65 ± 0.03 mg gallic acid equivalents/g dry weight and followed the order of phenol extract > methanol extract > aqueous extract > aqueous extract with PVPP (Table 1).

Compositional analysis

In this study, HPLC identified seven phenolic compounds in the phenol extract including eriocitrin, gallic acid, rosmarinic acid, p- coumaric acid, ferulic acid, vanillic acid, and luteolin (Figure 6). The different compounds were identified by comparisons to the retention times and UV spectra of authentic standards, while the quantitative data were calculated from their calibration curves. Total amount was 52.9, 50.14, 1743, 46.2, 130.5, 268.1 and

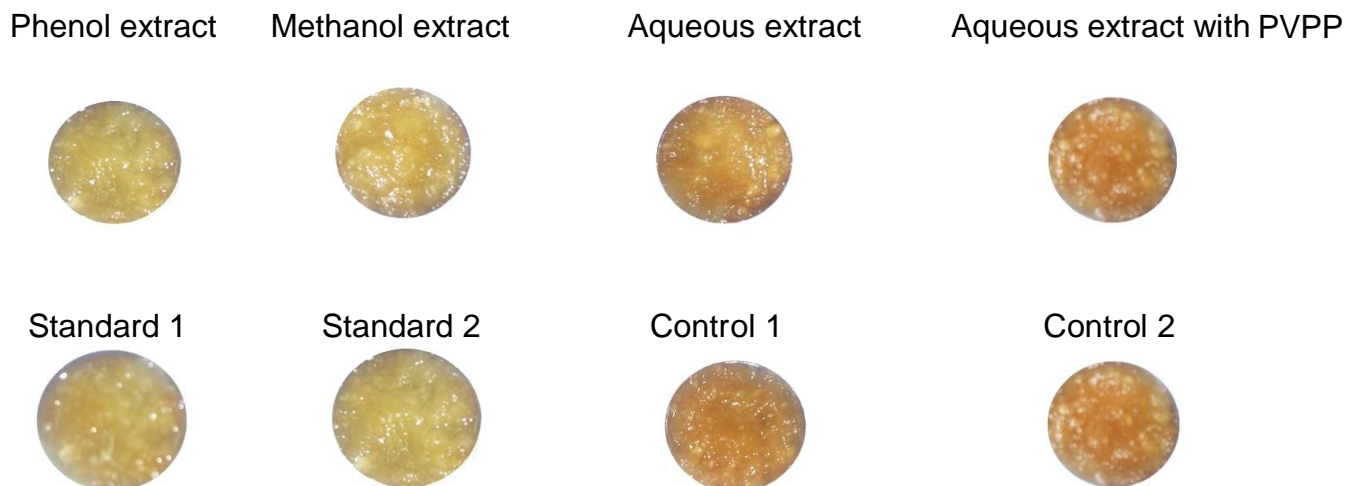


Figure 3. Video images of apple purees treated by *Mentha suaveolens* extracts. Control 1, sample dipped in water; control 2, sample dipped in methanol. Standard 1, Ascorbic acid; standard 2, potassium sorbate.

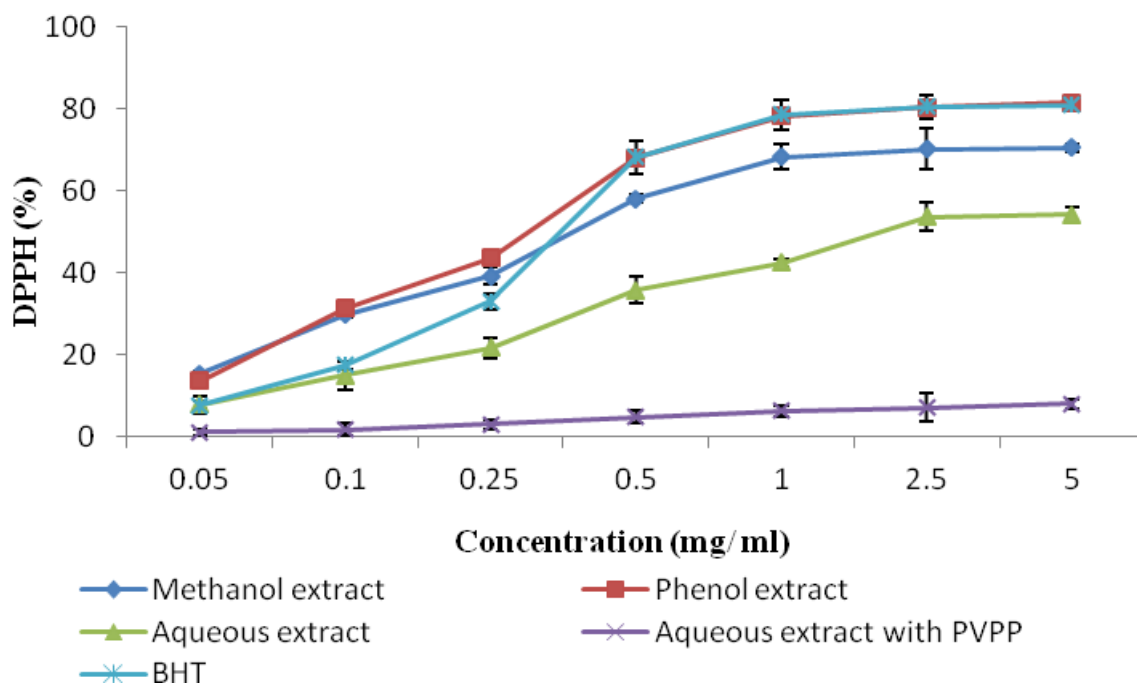


Figure 4. Antioxidant activity of *Mentha suaveolens* extracts determined by DPPH radical-scavenging activity. Each measure is the mean of 3 replicates. DPPH, 1, 1-Diphenyl-2-picrylhydrazyl radical.

56.8 μg GAE/g dry weight, respectively. Rosmarinic acid was identified as the major component.

DISCUSSION

Inhibition of enzymatic browning

The extracts of *M. suaveolens* showed a significant inhibitory effect on apple PPO. Son et al. (2000) also find

that the natural extracts such as rhubarb and pineapple, are potential inhibitors of enzymatic browning. The general mechanism of PPO inhibition was reviewed by Iyengar and McEvily (1992). The active site of the PPO comprises two copper atoms through which the enzyme interacts with its phenolic substrates and oxygen. On the other hand, chelating agents would act as a PPO inhibitor by making the active site copper unavailable since they have the ability of reacting with metal through chelation. However, the extracts did not have the same inhibitory

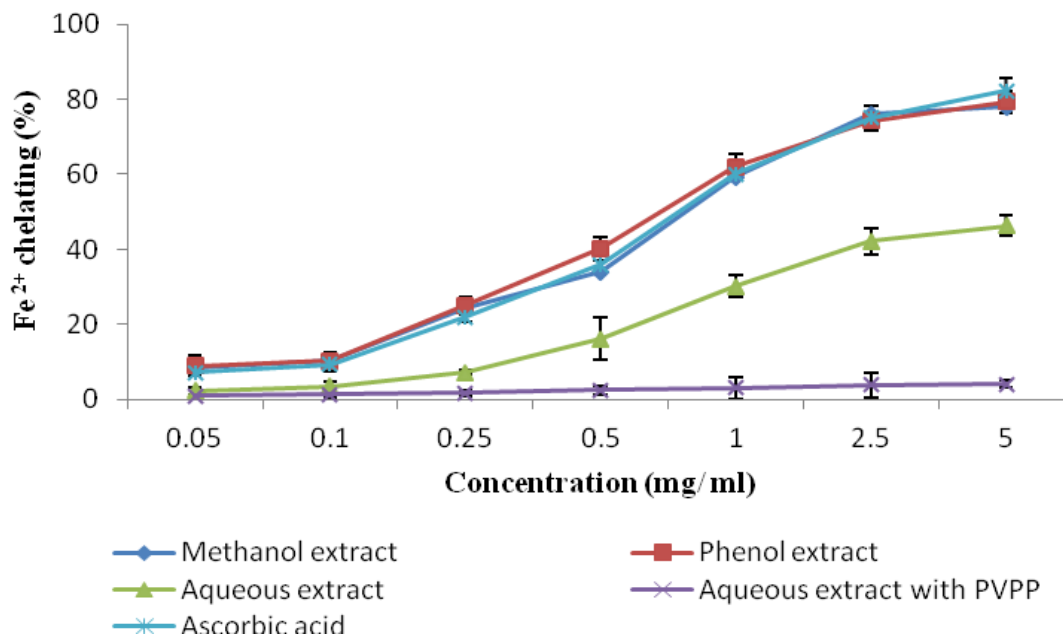


Figure 5. Ferrous ion-chelating activity of different *Mentha suaveolens* extracts. Each measure is the mean of 3 replicates.

Table 1. Quantities of phenol content in various extracts of *Mentha suaveolens* (mg equivalent gallic acid/ g dry weight).

Extract	Total phenol (mg EGA/g dry weight)
Phenol extract	2.7 ± 0.03
Methanol extract	2.6 ± 0.01
Aqueous extract	1.5 ± 0.06
Aqueous extract with PVPP	0.2 ± 0.04

PVPP, Polyvinylpyrrolidone.

effect on PPO and POD of apple, contrary to the chemical anti-browning agents (ascorbic acid and potassium sorbate) that had no great significant difference between the inhibition of PPO or POD. They are not specific towards the browning enzymes nor do not act directly on the enzyme structure, while the compounds contained in our extracts could have specificity towards inhibition of enzymes. Ferrar and Walker (1996) implied that the sensitivity to inhibitors is directly related to intrinsic characteristics of enzymes and more particularly to the accessibility of the prosthetic group of enzymes to exogenous ligands. They argued that the mechanism of inhibition depends on the substrate and the type of enzyme, and thus a single inhibitor can be characterized by several types of apparent inhibition.

The study on the apple purees confirmed our results on polyphenol oxidase and peroxidase activities. Phenol and methanol extracts had an anti-browning activity on the apple purees more apparent as aqueous extracts; this

effect may be due to phenolic compounds which are able to inhibit browning either by acting on the enzymes of oxidation, on the substrate or the reaction products. Similar observations were made by Luo et al. (1992) who assumed that the application of a crude extract of papain delay browning of apple slices preserving the texture of these walls.

Determination of antioxidant activity

Compared to other extracts, the phenol extract of *M. suaveolens* presented highest antioxidant activity. Effectively, phenolic compounds are considered as a major group of compounds that contribute to the antioxidant activities of botanical materials because of their scavenging ability on free radicals due to their hydroxyl groups (Djeridane et al., 2006). The antioxidant activity of phenolic compounds is described as being largely

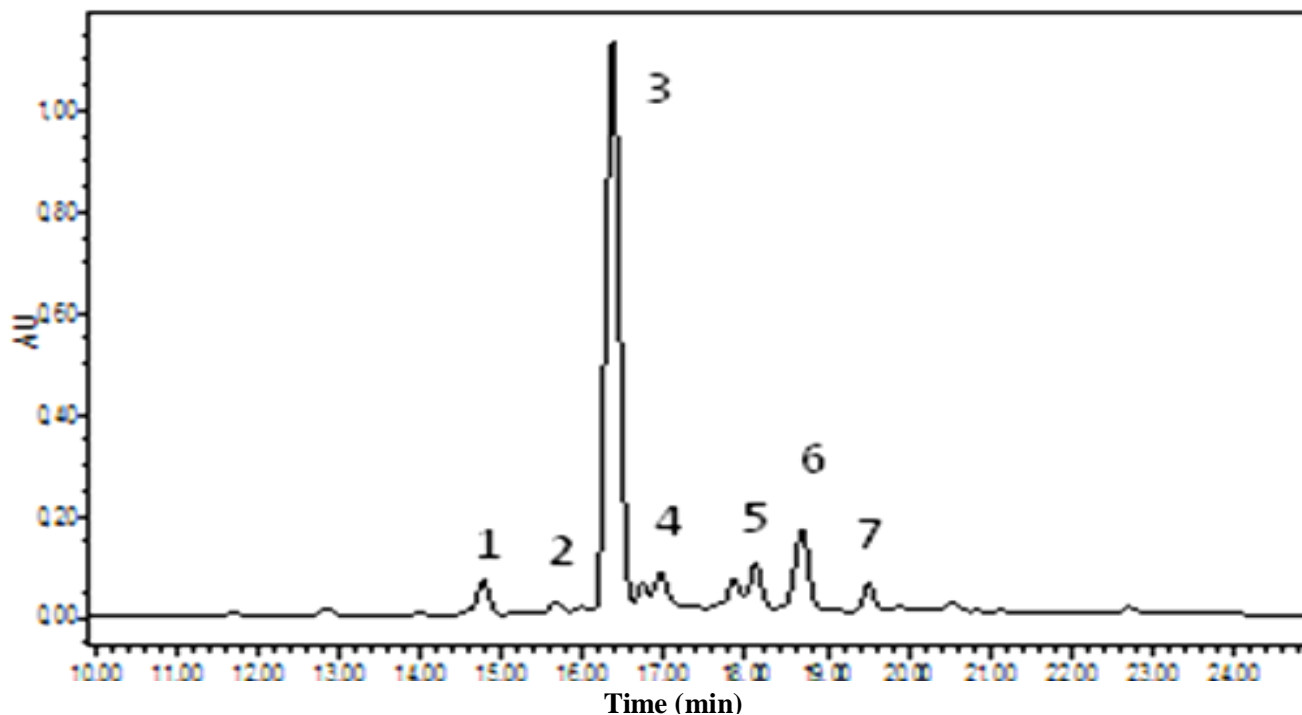


Figure 6. HPLC profile of phenol extract of *Mentha suaveolens* at 280 nm. (1) Eriocitrin; (2) gallic acid; (3) rosmarinic acid; (4) P-coumaric acid; (5) ferulic acid; (6) vanillic acid; (7) luteolin. HPLC, High-performance liquid chromatography.

influenced by the number of hydroxyl groups on the aromatic ring (Wang et al., 2003). Also, this activity is due to their ability to scavenge free radicals, donate hydrogen atoms or electrons, or chelate metal cations (Amarowicz et al., 2004). The metal chelating potency of phenolic compounds are dependent upon their unique phenolic structure (Santoso et al., 2004). In this system, the structure of the antioxidant compounds present in the *M. suaveolens* extracts is important for its capacity of donating hydrogen ions. Indeed, the structure of phenolic compounds is a key determinant of metal chelating activity, and this is referred to as structure-activity relationships. In the case of phenolic acids for example, the antioxidant activity depends on the numbers and positions of the hydroxyl groups in relation to the carboxyl functional group (Robards et al., 1999).

The aqueous extract with PVPP showed a very low antioxidant activity, which proves that the phenols trapped in this extract are generally responsible for antioxidant activity. The low activity of this extract may be due to other compounds. These results are consistent with those of Javanmardi et al. (2003), who also found that the antioxidant activity of plant extracts is not limited to phenolics. It may also come from other antioxidant secondary metabolites, such as volatile oils, carotenoids and vitamins. In contrast, Aehle et al. (2004) showed that the antioxidant efficiency of the spinach polyphenol extracts was significantly increased by the elimination of a major part of the non phenolic components.

Total phenol content and compositional analysis

From the statistical test, it could be concluded that the total phenol content of our extracts was influenced significantly by the type of solvent. Phenolic compounds were more soluble in polar organic solvents than in water. This result was, however, in contrast with studies of Waterman and Mole (1994) who indicated that more extract dissolved in low polarity solvent than in high polarity solvent. In this study, the major phenolic compound found in *M. suaveolens* was rosmarinic acid. This phenol has a high antioxidant activity. Several studies have shown that the antioxidant properties of plants from Lamiaceae family and this activity correlate with the presence of rosmarinic acid (Exarchou et al., 2002; Zheng and Wang, 2001). Rosmarinic acid inhibits the production of nitric oxide (NO) and other reactive species of oxygen and nitrogen in macrophages, thus preventing damage caused by oxidant stress or cellular aging (Sroka et al., 2005; Qiao et al., 2005).

The other minor phenolics compounds contained in the *M. suaveolens* are also known for their antioxidant activity. Indeed, Miller (1996) showed that the luteolin is a significantly more potent antioxidant than the synthetic antioxidant butylated hydroxytoluene (BHT), which is generally used in oxygen sensitive processes (Lgile et al., 1994). Gallic acid is also a good natural antioxidant. It is a strong chelating agent and forms complexes of high stability with iron (III) (Zheng and Wang, 2001).

In conclusion, the results of this study indicate that phenolic compounds present in the *M. suaveolens* could be largely responsible for the antioxidant activities. The anti-browning and antioxidants effects recorded by *M. suaveolens* phenol and methanol extracts suggest potential application in food, cosmetic and pharmaceutical industries.

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