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Research Article

Polyphenols and antioxidant properties of extracts from *Mentha pulegium* L. and *Matricaria chamomilla* L.

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ABSTRACT: **Introduction:** *Mentha pulegium* L. and *Matricaria chamomilla* L. are used in the Algerian folk medicine. This study was carried out to determine the antioxidant activity of these plants and their polyphenols contents. **Methods:** The antioxidant capacity of the plant extracts was determined by the scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and β-carotene/linoleic acid model system. Total phenolic content in these extracts were determined using Folin–Ciocalteu's reagent. **Results:** The methanolic extract (MeE) of *Mentha pulegium* L. showed the highest yield of extraction (14.4%). Whereas, the aqueous extract (AqE) of *Matricaria chamomilla* L. had the highest yield (18.56%) from total extracted plant material. Moreover, the ethyl acetate extract of *Mentha pulegium* L. contains high amount of total polyphenols; tannins and flavonoids (191.99 µg gallic acid equivalent/g dry extract; 265.33 µg tannic acid equivalent/g dry extract; 110.37 µg quercetin equivalent/g dry extract; 151.11 µg rutin equivalent/g dry extract) respectively. This extract possessed high antioxidant activity (IC_{50} = 0.017 mg/ml) in the DPPH assay whilst the chloroformic extract (ChE) is more efficient in the β carotene/linoleic acid assay with (61.07%). The ChE of *M. chamomilla* contains the higher value of flavonoids (197.43 µg quercetin equivalent/g) and total polyphenols were abundant in MeE. The ethyl acetate extract of this plant had the highest antioxidant activity (IC_{50} = 0.0111 mg/ml) in the DPPH assay, while chloroformic extract shows appreciable inhibition value (37.15%) in the β carotene/linoleic acid assay. **Conclusion:** These results provide useful information about the utilization of these plants as natural antioxidants in food and in folk medicine.

KEYWORDS: Antioxidants, polyphenols, *Mentha pulegium*, pennyroyal, *Matricaria chamomilla*, chamomile

INTRODUCTION

Reactive oxygen species and their involvement in some human diseases have attracted growing interest over the last few decades. Recently, there has been increased interest in naturally occurring antioxidants which can be used to protect the human beings against the damage from the oxidative stress.[1] Many plants contain natural antioxidants which counteract the endogenous production of free radicals and other oxidizing species.[2] The antioxidants mainly come from plants including phenolic compounds (flavonoids, phenolic acids, alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids.[1] Medicinal plants are good sources of these compounds.

Pennyroyal (*Mentha pulegium*) is an aromatic perennial and is common wild or garden plant. It is a spontaneous species in the whole of Europe, west of Asia and the north of Africa.[3] This plant is a good digestive tonic, it stimulates digestive juices, relieves flatulence and colic. It is also a good remedy for headaches and for minor respiratory infections and a powerful stimulant to the uterine muscle encouraging menstruation.[4] It can be used externally to relieve rheumatic conditions including gout.[3] However, additional research is necessary to evaluate the practical values of therapeutic application.

Chamomile (*Matricaria chamomilla*) was known for centuries and is well established in therapy. In traditional popular medicine, it is used in the form of tea, for gastric and

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intestinal painful diseases like diarrhea and the distensions.[5] It is also used for the inflammatory, gastric and intestinal diseases.[3] In external use, chamomile is applied in the form of compress against badly healed wounds, like a bath for hemorrhoids and genital diseases, as rinsing of the mouth reached of ignitions of the oral cavity and the pharynx, like vapor inhaled for the treatment of the acne, the nasal flow and like baths for babies in order to soften the skin.[3] Current natural medicine also uses it as anti-inflammatory drug and as a disinfectant.[4] Currently, chamomile is used to treat all the disorders where the spasm occupies a significant place, such as in the case of painful digestive spasms and for dysmenorrhoea.[6] *Mentha pulegium* L. was described as a potential source of phenolic compounds.[7] *M. chamomilla* contains three different classes of secondary metabolites: sesquiterpenes, coumarins, and flavonoids. The major components of the essential oil are (-)-α-bisabolol and α-farnesene. This plant also has high levels of polyphenolic compounds such as coumarins and flavonoids. The coumarins are herniarin, umbelliferone, and esculetin. The major flavonoid components are apigenin, luteolin, and quercetin. Thus, chamomile is one of the richest sources of antioxidants.[5] The present study was carried out to determine the antioxidant activity of *Mentha pulegium* L. and *Matricaria chamomilla* L. extracts and their polyphenols contents.

MATERIALS AND METHODS

Plant material

Mentha pulegium L. leaves were collected in September 2011 and the flowers of *Matricaria chamomilla* L. were collected at the end of May 2011, in Algiers, Algeria. Plants were identified by Pr. Laouer Hocine from the Faculty of natural and life Sciences. Department of Ecology and Vegetal Biology, University Ferhat Abbass, Sétif, Algeria. The leaves and the flowers were separated from the other parts and dried at room temperature. The plant samples were air dried in shadow and finely powdered in a rotating knife grinder. The powder was sieved through a 1 mm mesh to remove large fragments. Each plant powder was then used for the extraction procedure.

Polyphenols extraction procedures

100 g of *Matricaria chamomilla* L. flowers or the leaves of *Mentha pulegium* L. are macerated in 1 liter 85% methanol and the mixture was subjected to agitation for three days at 4°C. The suspension was filtered through a Buchner funnel and concentrated under reduced pressure on a rotary evaporator to give an initial crude extract (CE). Fractionation is carried out using solvents with increasing polarity.[8] The aqueous solution was initially mixed with the hexane to eliminate lipids. The extraction is repeated several times until the solvent (hexane) becomes transparent. The residual aqueous phase was subjected to further extraction using chloroform, and finally by ethyl acetate, following the same protocol as for the first extraction by hexane. This series of extractions makes it possible to obtain different fractions; the crude extract (CE), a chloroform fraction (CHE), an ethyl acetate fraction (ACE) and an aqueous fraction (AQE). These fractions were subjected to a freeze-drying and were preserved at –20°C until use.

Determination of total polyphenols

In order to measure phenolic compounds in plant extracts/fractions (crude extract, chloroform, ethyl acetate and aqueous fractions), we used the Folin–Ciocalteu assay.[9] The reagent of Folin–Ciocalteu consists of a mixture of acid phosphotungstic and phosphomolybdic acid. During oxidation, it is reduced to a mixture of blue oxide. The color produced is proportional to the amount of polyphenols present in the extract.[10]

An aliquot of 50 µL from each extract was mixed with 250 µl Folin-Ciocalteu reagent (diluted 10 times) and 250 µl of sodium carbonate (7.5%). After 90 min incubation, the absorption was measured at 765 nm. The results were expressed results in mg of gallic acid per g (GEA) of dry weight of plant.

Determination of flavonoids

The total flavonoids in plant extracts were determined using the aluminium trichloride ($AlCl_3$) method.[11] Briefly, 1 ml of each extract/fraction (suitable dilutions in methanol or distilled water) was added to 1 ml of $AlCl_3$ solution (2% in methanol). After 10 minutes of incubation, the absorbance is measured at 430 nm and the flavonoids content was expressed in mg per g of quercetin equivalent (QE).

Determination of tannins

The test of haemoglobin precipitation by tannins compounds was used.[12] Briefly, a volume of each plant extract was mixed with an equal volume of heamolysed sheep blood (absorbance = 1.6). After 10 minutes incubation, this solution was centrifuged for 20 minutes and the absorbance of the supernatant was measured at 576 nm against the blank. Different concentrations of tannic acid were also mixed with an equal volume of heamolysed blood and the absorbance was measured in the same manner. The effectiveness of the precipitation of the solutions tested is expressed as µg tannic acid equivalent/g extract.

DPPH radical scavenging activity of plant extract

The antioxidant capacity of the extracts/fractions, expressed as the donation of an electron or a hydrogen atom to radical free 2,2'-diphenyl-1-picrylhydrazyl (DPPH), was measured by a spectrophotometric method.[13] Aliquots (50 µl) of various concentrations of the extracts/fractions were added to 5 ml DPPH solution (0.004%). After 30 minutes incubation in the darkness, the absorbance was read at 517 nm. The positive control is represented by the BHT. The antioxidant activity, which expresses the capacities to trap the free radical, is estimated by the percentage of discoloration of the DPPH solution in methanol (Inhibition% or I%) according to the formula:

$$\text{Inhibition\%}\ (IC_{50}) = (ABS_{control} - ABS_{test}) \times 100 / ABS_{control}.$$

Where $ABS_{control}$: Absorbance of solution without extract; ABS_{test}: Absorbance of the sample. IC_{50} value is defined as the concentration of the substrate which causes the loss of 50% of the activity of the DPPH. The values of IC_{50} were calculated by the linear regression where the X-coordinate is represented by the concentration of the compounds tested and ordered by (I%) the percentage of inhibition.[14]

β-carotene/linoleic acid assay

The antioxidant capacity of the extracts/fractions was given by measuring the inhibition of the oxidative decomposition of β-carotene (discolouration) by the products of oxidation of the linoleic acid.[15] An emulsion of linoleic acid/β-carotene was prepared by dissolving 0.5 mg of β-carotene in 1 ml of chloroform, 25 µl of the linoleic acid and 200 mg of Tween 40. Chloroform was completely evaporated with the rotary evaporator, and then 100 ml of distilled water saturated with oxygen was added. The resulting emulsion was agitated vigorously and 350 µl of the extracts/fractions or reference antioxidant (BHT) (2 mg/ml) was added to 2.5 ml of emulsion.

The kinetics of discoloration of the emulsion in the presence and the absence of antioxidant is measured at 490 nm at intervals over 48 hours (1, 2, 3, 4, 6, 24, and 48 hrs) of incubation at ambient temperature and in the darkness.

The percentage of inhibition of the extracts was measured as follows: $AA\% = ABS_{test} / ABS_{BHT} \times 100$. ABS_{test}: Absorbance in the presence of the extract; ABS_{BHT}: Absorbance in the presence of positive control BHT.

Statistical analysis

Experimental results are expressed as the mean ± standard deviation (SD) of triple determinations. The data were analyzed by one-way analysis of variance (ANOVA). Tests of significant differences were determined by Tukey multiple range tests at $p < 0.05$.

RESULTS AND DISCUSSION

Total polyphenols, flavonoids and tannins in *Mentha pulegium* L extracts

Total phenolics content in *Mentha pulegium* extract/fractions were in the following order: AcE (191.99 ± 0.016 µg GAE/g of extract) > MeE (183.45 ± 0,125 µg GAE/g of extract) > CHE (119.73 ± 0.036 µg GAE/g of extract) > AqE (88.84 ± 0.112 µg GAE/g of extract) (Table 1). AcE contained the highest amount of tannins (265.33 ± 0.030 µg TAE/gE), followed by ChE (209 ± 0.017 µg TAE/gE), MeE (149.33 ± 0.0046 µg TAE/gE) and the AqE (137.22 ± 0.029 µg TAE/gE).

Total flavonoids were in the following order: AcE (110.37 ± 0.023 µg QE/gE) > MeE (59.87 ± 0.005 µg QE/gE) > ChE (19.50 ± 0.013 µg QE/gE) > AqE (1.19 ± 0.004 µg QE/gE) (Table 1). Our study is in agreement with previous studies which also reported *Mentha pulegium* to contain polyphenols and flavonoids. These constituents may account for the high antioxidant activity observed for the polar extracts of these aromatic herbs.[16,17]

Total polyphenols, flavonoids and tannins in *Matricaria chamomilla* L. extracts

The total flavonoid contents of different *Matricaria chamomilla* L. extract/fractions are reported in Table 2. The fractions contain flavonoids in the following order: ChE (197.43 ± 0.033 µg QE/gE) > AcE (173.33 ± 0.007 µg QE/gE) > ME (35.16 ± 0.028 µg QE/gE) > AqE (27.65 ± 0.007 µg QE/gE). In comparison to the flavonoids components, polyphenols in the extracts were in the following order: ME (299.14 ± 0.102 µg GAE/g of extract), AcE (2079.65 ± 0.048 µg GAE/g of extract), AqE (146.97 ± 0.046 µg GAE/g of extract) and ChE (104.53 ± 0.033 µg GAE/g of extract). Table 2 shows also the relative contents of tannins in these extracts.

Table 1: Total polyphenols, flavonoids and tannins in *Mentha pulegium* L. extracts

Extracts	Flavonoids[a]	Polyphenols[b]	Tannins[c]
Methanolic	59.87 ± 0.01	183.45 ± 0.13	149.33 ± 0.00
Chloroform	19.50 ± 0.01	119.73 ± 0.034	209.00 ± 0.02
Ethyl acetate	110.37 ± 0.02	191.99 ± 0.02	265.33 ± 0.03
Aqueous	1.19 ± 0.00	88.84 ± 0.11	137.22 ± 0.03

[a]µg Quercetin equivalent per gram of extract. [b]µg Gallic acid equivalent per gram extract. [c]µg Tannic acid equivalent per gram extract. The values present the mean of three measurements ± SD.

Table 2: Total polyphenols, flavonoids and tannins in *Matricaria chamomilla* L. extracts

Extracts	Flavonoids[a]	Polyphenols[b]	Tannins[c]
Methanolic	35.16 ± 0.028	299.14 ± 0.102	145.55 ± 0.067
Chloroform	197.43 ± 0.033	104.53 ± 0.033	245.11 ± 0.039
Ethyl acetate	173.33 ± 0.007	2079.65 ± 0.048	201.66 ± 0.165
Aqueous	27.65 ± 0.007	146.97 ± 0.046	132.22 ± 0.023

[a]µg Quercetin equivalent per gram extract. [b]µg Gallic acid equivalent per gram of extract. [c]µg Tannic acid equivalent per gram extract. The values present the mean of three measurements ± SD.

Flavonoids represent the major fraction of water-soluble components in chamomile. Chamomile flavonoids were recognized to be spasmolytic and antiphlogistic and are therefore of great interest.[3] Apigenin was the first flavone to be isolated from chamomile.[3] DPPH scavenging activity of extracts of *Mentha pulegium* L.

DPPH scavenging activity of extracts of *Mentha pulegium*

Antioxidant activity profiles show that extracts/fractions of *Mentha pulegium* had dose-dependent antioxidant activities and the IC_{50} of each extract was determined (Figure 1). All extracts and standards (BHT, gallic acid, quercetin, rutin) depleted the initial DPPH concentration by 50% within 30 min. A lower IC_{50} value indicates a higher free radical scavenging activity. The free radical scavenging activities of the extract/fractions of *Mentha pulegium* were in this order: ethyl acetate > methanolic > chloroform > aqueous. The ethyl acetate extract (which contained most tannin; Table 1) had the highest free radical scavenging activity. All extracts had higher IC_{50} values compared to the gallic acid, quercetin, rutin and BHT controls. When compared to the BHT, rutin, gallic acid and quercetin controls, the ethyl acetate fraction and the methanolic extract did not show any significant differences ($P > 0.05$), Whilst the chloroformic extract and aqueous extract were significantly different ($P < 0.05$). The effect of MeE and AcE extracts is very probably attributed to their high phenolic compounds and flavonoids.[4]

In the present study, the increase in DPPH radical-scavenging activity by aqueous (IC_{50}: 5.5 lg/ml) and methanolic extract (IC_{50}: 6.1 lg/ml) extracts of *M. pulegium* was higher than those previously reported for an ethanol extract (17.92 lg/ml)[18] and for ethanol (24.9 lg/ml) and water extract (8.9 lg/ml).[19] This difference could be due to the nature of extraction and the origin of the plant material which can affect polyphenolic contents in the plant.

DPPH scavenging activity of extracts of *Matricaria chamomilla*

Figure 2 shows that the ethyl acetate extract of *Matricaria chamomilla* had the highest radical scavenging activity ($IC_{50} = 0.01 \pm 0.0009$ mg/ml) followed by aqueous extract, methanolic extract and chloroformic extract. It was noted that the scavenging effect of the extract/fractions was inferior compared to the levels of standards: BHT, gallic acid, quercetin and rutin. BHT was significantly more potent than the chloroformic extract ($P < 0.05$). The results of DPPH radical scavenging activities showed that chamomile ethyl acetate fraction exhibited the greatest free radical scavenging activity. The antioxidant effect of plant extract is likely related to the amount of polyphenols present.[20–22] The antioxidant effect of an extract may also differ depending on the quality of

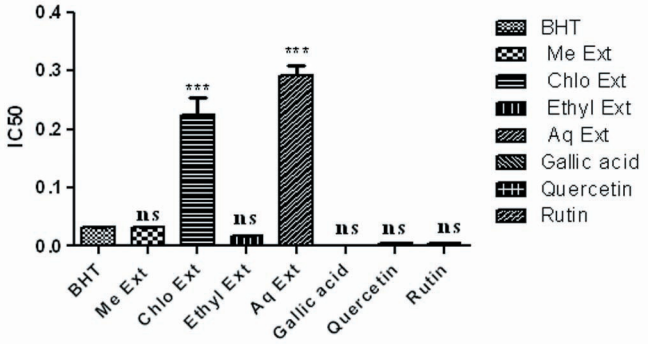

Figure 1. IC_{50} (mg/ml) values of *Mentha pulegium* L. extracts for DPPH free radical scavenging activity. Results represent mean ± SD (n = 3). Lower IC_{50} value indicates higher antioxidant activity. Me Ext: methanolic extract, Chlo Ext: chloroform extract, Ethyl Ext: ethyl acetate extract, Aq Ext: aqueous extract. ns: no significant difference from BHT value, ***($p < 0,001$) compared to BHT as standard.

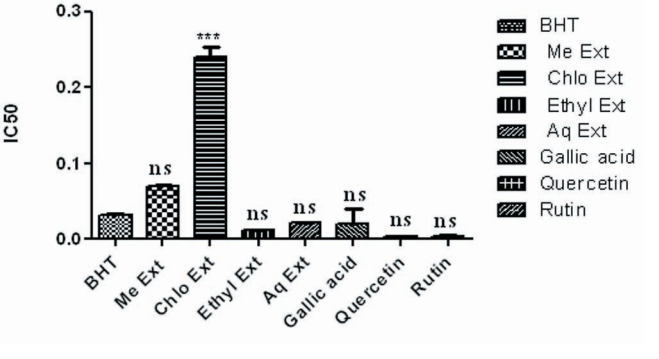

Figure 2. IC_{50} (mg/ml) values of *Matricaria chamomilla* L. extracts for DPPH free radical scavenging activity. Results represent mean ± SD (n = 3). Lower IC_{50} value indicates higher antioxidant activity. Me Ext: methanolic extract, Chlo Ext: chloroform extract, Ethyl Ext: ethyl acetate extract, Aq Ext: aqueous extract. ns: no significant difference from BHT value, ***($p < 0,001$) compared to BHT as standard.

polyphenols and flavonoids and on other factors including the presence of metallic ions in test solution.[23] The mechanism of the reaction between the antioxidants and DPPH depends on the structural conformation of the antioxidant.[24] Some compounds react rapidly with the DPPH, with the reducing the number of DPPH equal to that of the hydroxyl groups present in the the antioxidant compound.[25,26]

Antioxidant activity of *Mentha pulegium* and *Matricaria chamomilla* extracts

The antioxidant activities of the extracts/fractions were determined by the β-carotene/linoleic acid system assay (Figures 3 and 4). The antioxidant activity of samples was reflected in their ability to inhibit the bleaching of β-carotene. In this assay, the ethyl acetate extract of

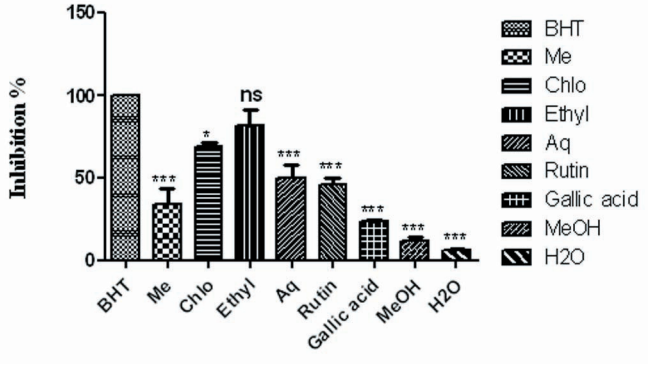

Figure 3. Inhibition percentage of divers extracts of *Mentha pulegium* in linoleic acid/β-carotene assay after 24 h. Results represent the means ± SD (n = 3). High inhibition percentage indicates higher antioxidant activity. Me: methanolic extract, Chlo: chloroform extract, Ethyl: ethyl acetate extract, Aq: aqueous extract. ** ($p < 0,001$) compared to BHT as standard.

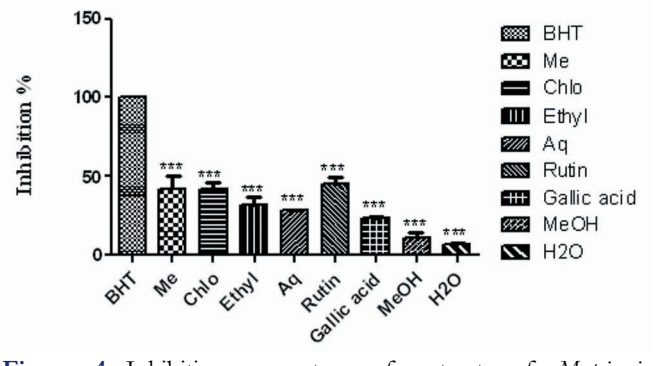

Figure 4. Inhibition percentage of extracts of *Matricaria chamomilla* L in β-carotene/linoleic acid assay after 24 h (using Me OH, H_2O, BHT as standards). Results represent the means ± SD (n = 3). High inhibition percentage indicates higher antioxidant activity. MeOH: methanolic extract, Chlo: chloroform extract, Ethyl: ethyl acetate extract, Aq: aqueous extract. ** ($p < 0,001$) compared to BHT as standard.

M. pulegium possessed better antioxidant activity than the other fractions/extract and the rutin and gallic acid controls, but did not reach that of BHT control. The other fractions/extracts were also effective in inhibiting lipid peroxidation in the following order: chloroform extract > aqueous extract > methanolic extract (Figure 3).

The results obtained from extracts of *Matricaria chamomilla* flowers were all significantly different ($P < 0.05$) (Figure 4). The percentage inhibition varies between 25.59 ± 0.002% and 37.04 ± 0.074%. All were lower than the BHT control (100% inhibition). Both chloroform and ethyl acetate extracts of *M. pulegium* effectively inhibited the linoleic acid oxidation by as much as 60.38% and 91.67%, respectively. In this respect, it was reported that BHT was more potent than the water and ethanol extract of this plant.[19] A linear correlation between antioxidant activity and phenolic contents of the plant extracts is shown in the present study as reported previously plant extracts.[26,27] Flavonoids are able to scavenge hydroxyl radicals, superoxide anions and lipid peroxyl radicals.[28,29] Moreover, a potent antioxidant activity for terpenoids in this plant has been shown.[30] The presence and synergism of different antioxidants in an extract will determine the antioxidative or the proxidative properties of a specific extract.[28,31] Several studies have shown that the antioxidant effect of natural sources is related to the presence of phenolic compounds,[32,33] the ChE of *M. pulegium* shown the highest polyphenol content and the best activity in this study.

CONCLUSION

These results provide support about the beneficial utilization of these plants as natural antioxidants in food and in folk medicine.

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