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Extracts of *M. pulegium* (L.) and *M. spicata* (L.): Effect of Extraction Conditions on Phenolics and Flavonoids Contents and Their Antioxidant Power

Nadia Zekri [a,b]*, Hannou Zerkani [b], Hanane Elazzouzi [a,b], Touria Zair [b] & Mohammed Alaoui El Belghiti [a]

[a]*Laboratory of Spectroscopy, molecular modeling, materials and nanomaterials, Water and Environment – Department of Chemistry, University Med V- Faculty of Sciences.4- Avenue Ibn Battouta. B.P.1014 RP, Rabat, Morocco.*

[b]*Laboratory of Chemistry of Bioactive Molecules and Environment, Department of Chemistry, University Moulay Ismail, Faculty of Sciences, BP 11201. Zitoune, Meknès, Morocco.*

Abstract

The correlation between the contents of polyphenols and flavonoïds and the degree of antioxidant activity of various extracts from *M. pulegium* (L.) and *M. spicata* (L.), obtained by different solvents and methods, was investigated. The crude extracts were prepared by mixing areal parts in powder with methanol/water solution. They were subjected later to liquid-liquid extraction via solvents with progressive polarity (chloroform, ethyl acetate and *n*-butanol) by maceration and soxhlet techniques. The total phenol and flavonoïds contents from crude extracts and their fractions were determined by using Folin-Ciocalteu and $AlCl_3$ assays respectively. The antioxidant activity of extracts was evaluated by DPPH˙ (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging test. This activity was measured by inhibition concentration 50% (IC_{50}) values. Generally, higher extract yields were obtained by the soxhlet extraction technique; the crude extracts recorded the best yields for *M. pulegium* by soxhlet (26.37%) and maceration (13%) while for *M. spicata*, the aqueous extract by soxhlet (34.9%) and crude one by maceration (9.4%) showed the higher yields. The higher phenolic and flavonoïds contents were found in crude extracts by maceration for both mints whereas by soxhlet, the ethyl acetate and/or n-butanol extracts demonstrated the strongest contents. These extracts, rich in flavonoïds, showed a positive correlation since they have exhibited better antioxidant activity compared to ascorbic acid as the antioxidant reference (IC_{50}= 0.051 mg/ml).

Keywords: *Mentha*, polyphenols, flavonoïds, extraction, DPPH˙ test.

1. Introduction

Polyphenols are among important bioactive compounds in plants that gained great attention of scientific community due to their beneficial effects on human health [1]. There are many studies on antioxidant and other biological effects of polyphenols which exert the prevention of diverse pathologies particularly diabetes, cancers and cardiovascular diseases [2]. Likewise, recent epidemiological researches highly recommend consumption of diets rich in plant polyphenols in order to prevent the development of such diseases [3].

The polyphenols extracted from plant materials are considered as a source of bioelements used mainly for the preparation of food ingredients and pharmaceutical products. The extraction of phenolic compounds is performed frequently by organic solvents as methanol and/or with different proportions of water [4]. This solvent is considered as the best for the extraction of polyphenols from Lamiaceae family [5,6]. However, differences in the structure of phenolic compounds determine their solubility in solvents of different polarity. Therefore, type of extraction solvent as well as the extraction methods may have significant impact on the yield of extraction and the content of polyphenols from plants material [7].

The ability of polyphenols to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations provides them the property as antioxidants [8]. The optimization of the conditions

*Corresponding author e-mail: nadia1zekr@yahoo.fr.; (Nadia Zekri).
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for extracting the content of phenolic compounds and the antioxidant activities of certain plants was the subject of some reports but others have reported that the optimal procedure is generally different according to the plant matrices [7,9;10].

In the present study, *M. pulegium* (L.) and *M. spicata* (L.) were selected as the most common herbs consumed for culinary and therapeutic purposes. They are among the most promising sources for the recovery of polyphenols that could be added as antioxidants to foods, food supplements, or cosmetics [11,12,13].

The objectives of the present work were to determine the effect of extraction conditions on the contents of polyphenols and flavonoïds occurring in the extracts of *M. pulegium* L. and *M. spicata* L. and on the degree of their power to scavenge the free radical DPPH•(1,1-Diphenyl-2-picrylhydrazyl).

2. Materials and methods

2.1. ***Plant material***

The areal part (leaves and flowers) of *M. pulegium* (L.) and *M. spicata* (L.) were collected from Azrou region in Moroccan Middle-Atlas (Latitude: 33° 25′ 59″; Longitude: 5° 13′ 01″; Altitude: 1278m). The climate is semi-humid with strong continental influence with an annual average temperature of 20°C. The dried leaves and flowers were pulverized and then used for preparation of various extracts.

2.2. ***Preparation of extracts from leaves and flowers of M. pulegium (L.) and M. spicata (L.) by maceration and soxhlet***

For solid liquid extraction of total phenols and flavonoïds in the solvents, 30 g of ground material from a dry pulverized sample was macerated in aqueous methanol solution 100 ml (80/20%) (v/v) at room temperature every 48 hours (3 replicates). After filtration and vacuum concentration, the aqueous phase was subjected to successive extractions (splitting) of liquid-liquid using organic solvents with increasing polarity (chloroform, ethyl acetate and *n*-butanol).

By soxhlet, the mixture of methanol/water 100 ml (80/20%) (v/v) was added to the plant material (30 g) already dried and ground then refluxed for three hours. The hydromethanolic extract (crude extract) was filtered then evaporated by a rotary evaporator. Thereafter, the same protocol of maceration was followed for the polyphenols fractionation.

The determination of total phenols was conducted according to the method adapted by Singleton and Rossi using the Folin-Ciocalteu reagent [14]. While the flavonoïds content in the samples was evaluated by the aluminum trichloride ($AlCl_3$) method adapted by Djeridane et al. [15].

2.3. ***Determination of polyphenols content (PPC)***

The amount of phenolic total in the extracts of *M. pulegium* (L.) and *M. spicata* (L.) leaves and flowers was determined by the method described by Dehpour et al. [16] with slight modification. They used the Folin-Ciocalteau method to determine the polyphenols content of a plant extract.

Different concentrations: 0.08, 0.04, 0.16, 0.32, 0.48, 0.6, 0.96 and 1.28 µg/ml, were prepared, in volumetric flasks, for each solution a volume of 1.5ml of Folin-Ciocalteu (10%). The mixture was stirred and allowed to stand for 6 minutes before the addition of 1.5 ml of Na_2CO_3 solution (7.5%). The solutions were, adjusted with distilled water to reach a final volume of 100 ml, shaken immediately and kept in the dark for 2h at room temperature.

The absorbance of each solution was determined at 765 nm with a spectrophotometer Shimadzu UV-MINI 1240. The quantitative analysis of total phenols in our extracts was carried out by adapting the same procedure used for the preparation of the curve calibration, replacing gallic acid with a volume of extract to an appropriate concentration. The total polyphenols concentrations of each extract was calculated from the regression equation of the calibration range established with gallic acid ($y = 0.095x + 0.003$).

The results, expressed in milligrams of gallic acid equivalent/ gram of dry matter (GAE mg/g plant), were used to provide estimates on total polyphenols contained in the leaves and flowers of *M. pulegium* (L.) and *M. spicata* (L.). The total phenol content is calculated according to the following formula:

$$T = (C \times V / m_{\text{dry material}}) \times D$$

T: Total Phenolics Content
C: Concentration evaluated according to the calibration curve
V: Volume of overall Extract
m: Mass of the extract (dried material)
D: Dilution Factor

2.4. ***Determination of flavonoïds content***

The quantification of flavonoïds was carried out by a colorimetric method adapted by Djeridane et al. [15]. From the methanolic solution of Quercetin, different concentrations: 5, 10, 15, 20, 25 and 30 µg/ml were prepared in volumetric flasks (50 ml) by

adding to each solution 20 ml of distilled water. After 5 min, 100 µl of aluminum trichloride ($AlCl_3$) at 10% (w/v) was added. The solutions were adjusted to 50 ml with methanol, shaken immediately and then kept in the dark for 30 minutes at room temperature. The absorbance of each concentration was determined by a spectrophotometer at 333nm as mentioned previously for the determination of total phenolic content. Quantitative analysis of flavonoïds in our extracts was conducted by adapting the same procedure used for the preparation of the calibration curve, replacing the quercetin by a volume of the extract until an appropriate concentration.

The flavonoïds concentrations of each extract were calculated from the regression equation of the calibration range established with quercetin (y = 0.073x – 0.081).

2.5. ***Evaluation of antioxidant activity of M. pulegium and M. spicata extracts by DPPH• (1,1-Diphenyl-2-picrylhydrazyl) test***

The experiment was performed by the spectrophotometer at 515 nm. The solution of DPPH• at 6 10^{-5}M was obtained by dissolving 2,4 mg of the powder in 100 ml of ethanol while the samples were prepared by dissolving in ethanol at 1,6 mg /ml [17].

The test was carried out by mixing 2,8 ml of the prepared solution DPPH• with 200 µl of the crude, ethyl acetate and *n*-butanol extracts or standard antioxidant (ascorbic acid) at different concentrations (0 to 200 µg/ml). After 30 minutes of incubation in the dark at room temperature, the absorbance is read at 515 nm against a blank control containing only ethanol. The positive control contains DPPH• solution without the extract. The obtained values are then converted into percentages of inhibition using the following equation:

$$AA\% = ((A_{control} - A_{sample}) / A_{control}) \times 100$$

AA% : Percentage of antioxidant activity

$A_{control}$: Absorbance of the solution containing only radical DPPH• solution

A_{sample}: Absorbance of the sample solution to be tested in the presence of DPPH•

The values of IC_{50} of different extracts (concentration corresponding to the loss of 50% of free radicals activity) was determined graphically from the 3rd degree polynomial trend curves.

3. Results and discussion

3.1. ***Yield of extraction***

The yields of different extracts obtained from pennyroyal and spearmint have been summarized in Table (1)

- Extraction yield by soxhlet (Figure 1): It emerges through the observation of extraction yields, that the hydromethanolic extract *M. pulegium* gives the best extraction yield (26.37%) for while the highest yield for *M. spicata* (L.) was recorded by the aqueous extract (34.9%) followed by the crude extract (23.07%). On the other hand, chloroform gives the lowest yield (0.8%) for *M. pulegium* (L.) as well as the ethyl acetate extract (1.47%) for *M. spicata* (L.).

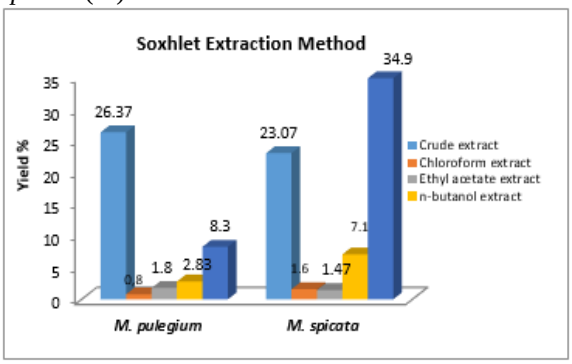

Figure 1: Yields of various extracts obtained by soxhlet method

- Extraction yield by maceration (Figure 2): the results of the yields given by maceration show that the best yield was also obtained by the crude extract followed by the aqueous extract for *M. Pulegium* (13 - 3.8%) and *M. spicata* L. (9.4 - 4.9%) respectively while the chloroform extracts of both mints' extracts showed the lowest yields.

Table. 1: Yields values of different extracts of both mints

Extract	*M. pulegium:* yield %		*M. spicata:* yield %	
	Maceration	Soxhlet	Maceration	Soxhlet
Crude extract	13	26.37	9.4	23.07
Chloroform extract	0.36	0.8	0.33	1.6
Ethyl acetate extract	1.7	1.8	0.87	1.47
***n*-butanol extract**	1.26	2.83	2.2	7.1
Aqueous extract	3.8	8.3	4.9	34.9

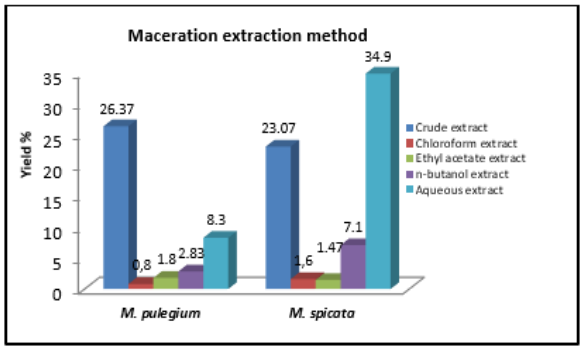

Figure 2: Yields of various extracts obtained by maceration method

Table 2: Polyphenol contents of various extracts from Mentha species

Extract	*M. spicata* (mg GAE / g DM)		*M. pulegium (* mg GAE/ g DM)	
	Maceration	**Soxhlet**	**Maceration**	**Soxhlet**
Crude extract	5.98	15.44	7.23	14.24
Chloroform extract	1.03	2.43	3.38	7.22
Ethyl acetate extract	0.52	16.11	9.70	9.66
***n*-butanol extract**	4.38	7.11	4.88	11.88
Aqueous extract	4.17	1.55	3.67	1.36

- Extraction yield by mint species: the extracts of pennyroyal presented highest yields compared to those from spearmint. The crude extracts, from maceration and soxhlet, gave the strongest yields (13 - 26.37%) followed by aqueous extracts (3.8 - 8.3%) and *n*-butanol (1.26 - 2.83%) respectively.

These results are almost similar to those cited by Bencheikh et al. [18] and Khennouf et al. [19], the crude and aqueous extracts from Algerian pennyroyal, obtained by maceration, also recorded the best yields (14.4 - 13.87%) respectively. However, the extracts of spearmint by maceration generally have the lowest yields compared to those of *M. pulegium* L.; as for the aqueous extract of the same mint, obtained by soxhlet, contains higher yield (34.9%) than all obtained extracts, followed by the crude (23.07%) and butanol (7, 1%) extracts. Likewise, Barchan et al. [20] found similar results in which the aqueous extract of *M. spicata* (L.), from Northern Morocco, got stronger yield (29.4%) than that of *M. pulegium* (6.42 %).

The extraction yields obtained therefore varied depending on the nature of solvent, the extraction technique and on the tested species. So, the best extraction yields are recorded by soxhlet and particularly for *M. pulegium* (L.).

The common use of the extraction by solvents, for preparing plant extracts, was due to their ease, effectiveness and wide applicability. Other works demonstrated that the extraction yield depended also on the extraction time, the temperature, the sample/solvent ratio and the chemical composition and physical characteristics of the samples [21]. Tay et al. [22] reported that the tested concentrations of the ethanol used for the extraction of polyphenols, and the sample/solvent ratio had a significant effect on yields. Likewise, Mata et al. [23] found that the aqueous extracts of some *Mentha* species from Portugal were richer in polyphenols than ethanolic extracts. Stankovic et al. [5] have also found that methanol was the best solvent followed by water and ethyl acetate. Moreover, Barchan et al. [20] concluded that the best yields are recorded from aqueous and methanolic extracts.

Previous studies have shown that methanol was the best solvent for extraction of phenolic substances from Lamiaceae species. Sharififar et al. [6] found that methanol gave higher yield than water, petroleum ether and chloroform. Likewise, methanol extracted more polyphenols than acetone, chloroform and petroleum ether, from some Lamiaceae species studied by Çakir et al. [24]. Indeed, methanol and ethanol and its mixtures with water gave the highest yields. Moreover, ethyl acetate and acetone have been also used in the extraction of plant polyphenols. However, the use of water and ethanol remain better because of their low toxicity and high extraction efficiency but some antioxidants with the low solubility such as carotenoids can give too low yields [25].

3.2. Content of total phenolics

The results of the colorimetric analysis by Folin-Ciocalteu reagent, the contents of total phenolic compounds of studied pennyroyal and spearmint extracts are presented in Table (2). They showed that the crude extracts generally had the highest contents of total phenols, whether by maceration or soxhlet and for both mints tested. Second was the ethyl acetate extract from *M. spicata* L. (16.11 mg GAE/ g DM) and *n*-butanol extract of *M. pulegium* (11.88 mg GAE/ g DM). By maceration, the ethyl acetate fraction of *M. pulegium* which showed the most interesting polyphenols content (9.70 mg EAG/ g DM) followed by the crude fraction (7.23 mg GAE/ g DM). These results are confirmed by Khennouf et al. [19], they found that the polyphenols content in the different fractions of the Algerian pennyroyal decreased as follows: the ethyl acetate fraction (191.99 ± 0.016 µg GAE/ g Extract)> the crude fraction (183.45 ± 0.125 µg GAE/ g Extract)> the chloroform fraction (119.73 ± 0.036 µg GAE/ g Extract)> the aqueous fraction (88.84 ± 0.112 µg GAE/ g Extract).

The fractions obtained by the soxhlet technique recorded the highest levels of phenolics compared to those from maceration for all tested mints. The most important values in the different fractions were

recorded for *M. pulegium* followed by *M. spicata* (L.) respectively (Figure 3&4). On the other hand, the crude extracts and the polar fractions exhibited high polyphenols content. Senevirathne et al. [26] studied the antioxidant potential of the different fractions of the methanolic extract from *Ecklonia cava* species and reported that among the organic fractions, the ethyl acetate fraction had the highest level of total phenols. The chloroform fraction and the methanolic extract also showed a high content of phenolic compounds. However, our results have shown that the chloroform extracts of both species contained the low total polyphenols contents.

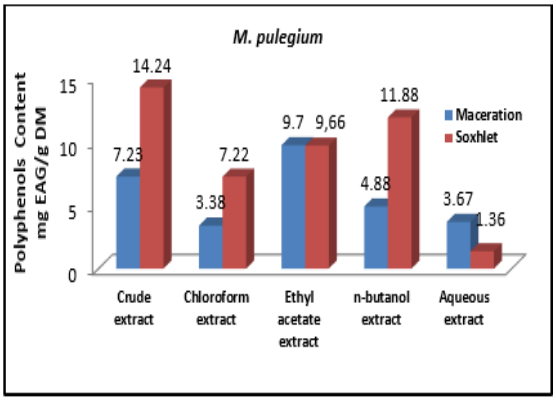

Figure 3: Polyphenols contents of extracts of *M. pulegium* (L.)

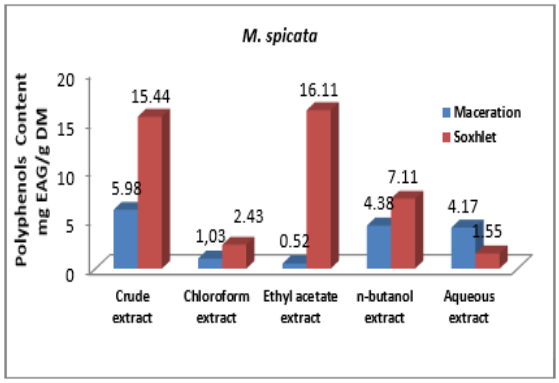

Figure 4: Polyphenols contents of extracts of *M. spicata* (L.)

The variability of the polyphenols contents in these species is probably due to the phenolic composition of the extracts [27], the biotic (species, organ and physiological stage) and abiotic conditions: the nature of the soil and the type of bioclimate and also the bioclimatic stages where these plants grow [28].

The assessment of the polyphenols content in mints, from different regions of the world, has been the subject of previous researches. Bencheikh et al. [18] found also that the ethyl acetate extract of *M. pulegium* (L.) which recorded the highest polyphenols content (191.99 µg EAG/ g Extract) followed by the methanolic (183.45 µg EAG/ g Extract) and chloroformic (119.99 µg EAG/ g Extract) extracts. Similarly, Khaled-Khodja et al. [29] deduced that the methanolic extract of *M. pulegium* was the richest in polyphenols among four studied plants (72.84 mg EAG/ g Extract). The polyphenols content of the methanolic extract of Greek pennyroyal reached a value, close to ours, about of 13.4 ± 0.2 mg EAG/ g DM [30]. Inversely, Stagos et al. [31] found that the aqueous extract of Greek pennyroyal was richer in polyphenols (188 mg EAG/ g DM) than the methanolic extract (138 mg EAG/ g DM). However, our results did not concord with those concluded by Derakhshani et al. [32], in their study on some Lamiaceae from Iran; they found that the methanolic extract of spearmint had higher polyphenols content (22.43 ± 1.13 mg EAG/ g DM) than that of pennyroyal (15.95 ± 0.52 mg EAG/ g DM). The content of total phenolic compounds in the aqueous extract of Indian spearmint was found to be around 25.62 ± 3.14 mg EAG/ g wet weight of sample. Dorman et al. [33] reported total phenol content in the range of 128 - 230 mg EAG/ g of extract from different *Mentha* species.

In fact, the obtained polyphenols contents indicated that they therefore depended on the used solvent, the extraction technique and the tested species. In addition to these factors, there are the water/solvent ratio, the sample/solvent ratio, the number and the extraction conditions [7,34,35].

According to obtained data, the used solvents extracted different types of phenolic compounds. Thus, the more polar fractions should contain a greater amount of hydrophilic phenols while the chloroform extracts, which manifested low polyphenols content, may include the low molecular weight hydrophobic phenolic compounds. On the other hand, the crude extracts should have been rich in phenolic compounds of both types [36].

3.3. Content of flavonoids

According to the absorbance values of various extracts, compared to the standard solution of quercetin equivalent (QE), the results of the colorimetric analysis of total flavonoïds are given in Table (3). For *M. pulegium*, the content of flavonoïds would have varied from 0 to 9.91 mg QE/ g DM by Maceration and from 1.14 to 14.85 mg QE/ g DM by Soxhlet. This content recorded values between 0 and 7.53 mg QE/ g DM by maceration and between 1.37 and 20.53 mg EQ/ g DM by soxhlet for *M. spicata* (L.). On the other hand, the contents obtained by soxhlet were higher for both mints than those obtained by maceration. For the same extraction

Table 3. The flavonoïds contents of the different fractions of *M. pulegium* and *M. spicata*

Extract	*M. spicata* (L.) (mg EQ/ g DM)		*M. pulegium* (L.) (mg EQ/ g DM)	
	Maceration	Soxhlet	Maceration	Soxhlet
Crude extract	3.54	9.1	3.21	14.12
Chloroform extract	0	11.61	0	1.37
Ethyl acetate extract	0.01	14.85	7.53	20.53
n-butanol extract	9.91	5.01	4.63	12.50
Aqueous extract	6.31	1.14	2.65	6.15

method, the extracts of *M. pulegium* generally recorded the stronger values than those of *M. Spicata* ones.

The high flavonoïds contents were observed in the extracts obtained by polar solvents (ethyl acetate and *n*-butanol) for both tested plants by maceration and by soxhlet (Figures 5&6). However, the chloroform extract of *M. spicata* L. has reached a significant content by the soxhlet technique of the order of 11.61 mg EQ/ g DM. Similary, Meziti et al. [37] and Hossain et al. [38] found comparable results in which the chloroform extract contained more polyphenols and flavonoïds than other tested extracts. Inversely, the chloroform extracts resulting from maceration for *M. spicata* and *M. pulegium* were very lacking in flavonoïds. While the ethyl acetate extracts of *M. pulegium* by maceration and soxhlet (7.53 - 20.53 mg EQ/ g DM) respectively recorded the highest values compared to other extracts. Similar results were reported by Bencheikh et al [18] whose the ethyl acetate extract from Algerian pennyroyal was the richest in flavonoïds than other extracts with a content about 110.03 ± 0.023 µg EQ/g Extract.

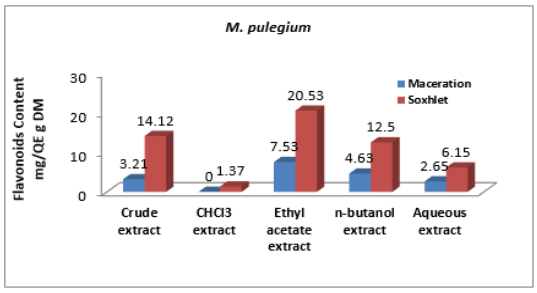

Figure 5. Flavonoïds Contents of extracts of *M. pulegium* (L.)

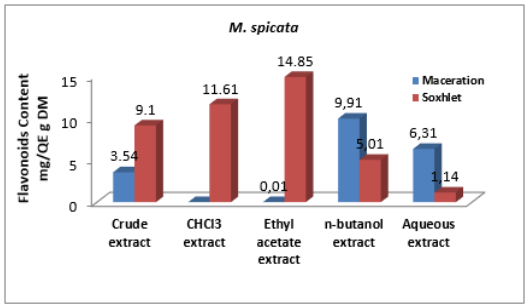

Figure 6. Flavonoïds Contents of extracts of *M. spicata* (L.)

The flavonoïds contents, like those of the polyphenols, also depended on the extraction technique, the tested species and the used solvent. This is consistent with results found in numerous works [25;39;40]. As we have already pointed out, pennyroyal had generally lower flavonoïds contents than those of *M. spicata* in contrast to the yield values and those of total polyphenols.

Many works have been interested to the extraction of plants by different solvents and in the determination of polyphenols and flavonoïds contents by various techniques but those concerning mints are not numerous. In this regard, the used solvents for the extraction of polyphenols and flavonoïds from different species of mint, which have been the subject of previous research, were often methanol, water or ethanol. The methanolic extract of *M. pulegium* (L.) from Algeria has reached approximately 13.82 mg EC/g of flavonoïds extract [29]. Moreover, the flavonoïds content of the aqueous extract of *M. spicata* (L.) from India was 13.5 ± 1.38 mg EC/g extract [41].

It is noted that the flavonoïds contents in some extracts from both studied mints were higher than that of polyphenols. This could be explained by the fact that not all phenolic compounds could be estimated by single extraction or by a single method due to the complexity of the compounds. While the majority of flavonoïds are phenolic compounds, which means that they contained at least one unique phenolic group.

Regarding the obtained data of total phenolic compounds contents, the Folin-Ciocalteu procedure may not give a complete image of the quality or quantity of the phenolic constituents in the extracts [42]. Despite its great sensitivity, the Folin-Ciocalteu method can present interference problems. In fact, the Folin-Ciocalteu reagent can react with non-phenolic constituents [43]. Similarly, Talbi et al. [44] found that the flavonoïds content was higher than that of polyphenols in methanolic and aqueous extracts of *Nigella sativa* as well as for the aqueous, hydroethanolic and ethanolic extracts of *Cucumero psisedulis* and *Garcinia kola* studied by Pélagie et al. [45]. Furthermore, Settaraksa et al. [46] showed that the curry paste produced higher flavonoïds content 81.62 ± 0.03 mg EC/100 g than the polyphenols (34.02 ± 0.03 mg GAE/ g). Additionally, the hydromethanolic and chloroform extracts of *Leonorus cardiaca* (L.) had interesting flavonoïds contents 50.21 ± 0.65 and 27.25 ± 0.670 mg Hyperoside equivalent/ g while the polyphenols have recorded contents in the order of 42.95 ± 3.55 and 4.90 ± 0.98 mg GAE/ g respectively [47]. Likewise, the aqueous and ethanolic extracts

from 44 Australian species showed higher flavonoïds contents compared to those of polyphenols [48].

The obtained data indicated that the flavonoïds in the extracts from both mints were much more polar than apolar and the high yields are especially obtained with the crude and aqueous extracts. Thus, the apolar Flavonoïds were less present because the low yields and the low flavonoïds contents are recorded by chloroform extracts. As well, the addition of chloroform caused the separation of flavonoïds into glycosylated fractions and aglycones. So, the glycolysed flavonoïds were more abundant than aglycone flavonoïds since the chloroform extracts, in which they are soluble, have given the lowest yields.

The variation in yields and in the polyphenols and flavonoïds contents is due to the effect of many factors, the main ones being: climatic and environmental factors such as light, precipitation, topography, season and the type of soil, the harvest period, the genetic heritage such as the concentration of polyphenols that varies from a species to another, and finally the extraction method [49].

3.4. ***Antioxidant activity of M. pulegium and M. spicata extracts***

The DPPH• (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging assay is a convenient and fast technique to evaluate antioxidative activity. This test aims to measure the capacity of the extracts to trap the stable DPPH• radical formed in solution by donation of a hydrogen atom or an electron [50]. The antioxidant activity of different extracts and ascorbic acid (standard reference) was determined by visible UV spectrometry by following the reduction of DPPH•, translated by its change from purple to the yellow color, measurable at 515 nm.

The antioxidant power was characterized by the parameter IC_{50}. The values of the inhibitory concentration at 50% (IC_{50}) of ascorbic acid (Figure 7) and *M. pulegium* and *M. spicata* (L.) extracts (Figures 8-13) were obtained from 3rd degree polynomial trend curves. So, the calculated IC_{50} values revealed that the different extracts showed antiradical activity. The lower the IC_{50} value, the higher the antioxidant activity.

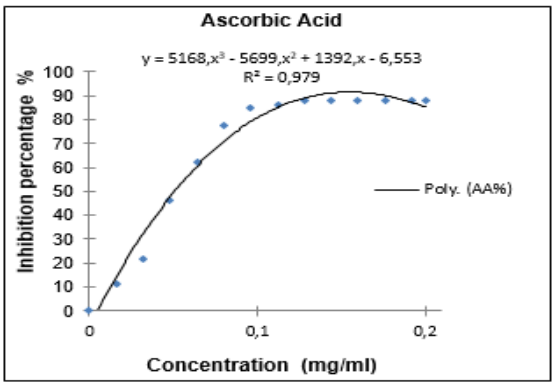

Figure 7. Percentage of DPPH• inhibition according to concentrations of Ascorbic Acid

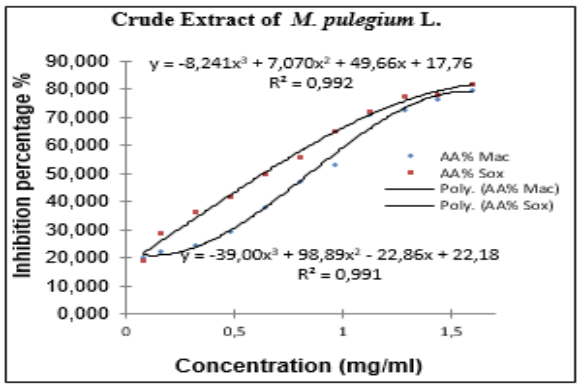

Figure 8. Percentage of inhibition of DPPH• by crude extract of *M. pulegium* L.

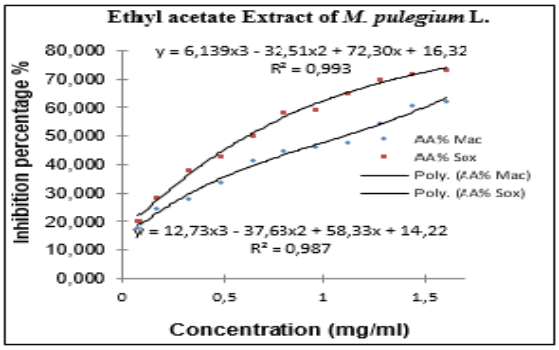

Figure 9. Percentage of inhibition of DPPH• by ethyl acetate extract of *M. pulegium* L.

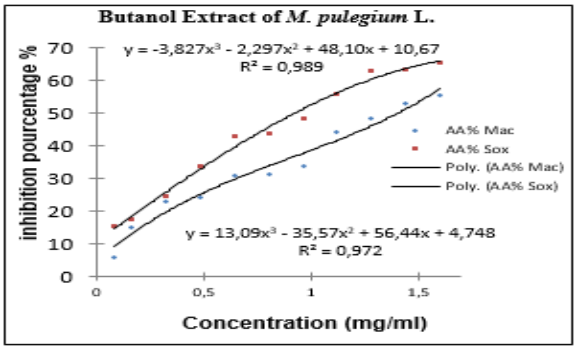

Figure 10. Percentage of inhibition of DPPH• by *n*-butanol extract of *M. pulegium* L.

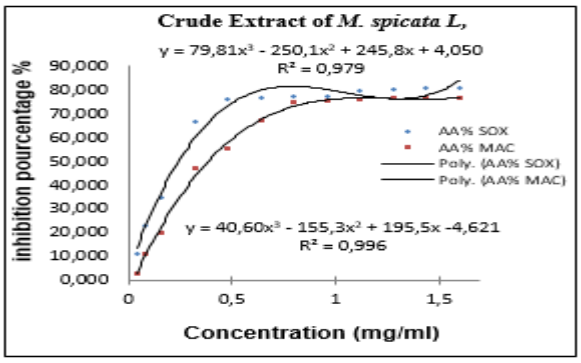

Figure 11. Percentage of inhibition of DPPH• by crude extract of *M. spicata* L.

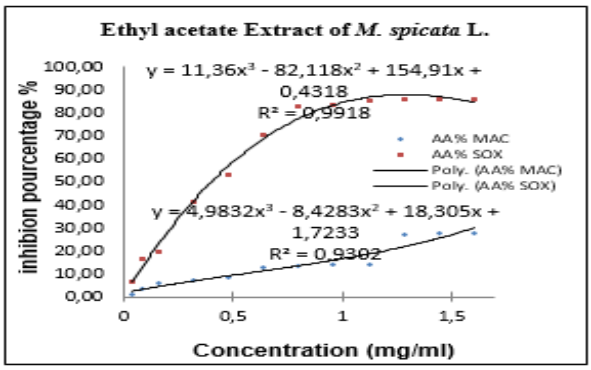

Figure 12. Percentage of inhibition of DPPH• by ethyl acetate extract of *M. spicata* L.

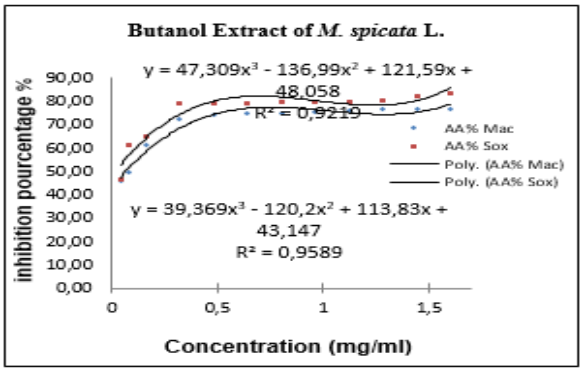

Figure 13. Percentage of inhibition of DPPH• by *n*-butanol extract of *M. spicata* L.

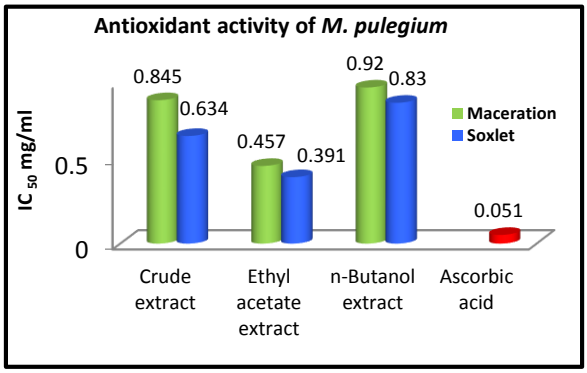

Figure 14: IC_{50} values of different extracts of *M. pulegium*

3.4.1. *Evaluation of antioxidant power of M. pulegium* (L.) extracts

According to the values of the IC_{50}, the extracts of *M. pulegium* (L.) showed the ability to reduce the free radical DPPH˙ (Table 4). They exhibited considerable antioxidant capacity compared to that of the standard (IC_{50} of ascorbic acid = 0.051 mg/ml).

Table 5. IC_{50} values of DPPH by different M. pulegium extracts

Extract	CI_{50} (mg/ml)	
	Maceration	Soxhlet
Crude extract	0.845	0.634
Ethyl acetate extract	0.457	0.391
***n*-butanol extract**	0.92	0.83

We noted that the extracts obtained by the soxhlet technique have greater antioxidant activity than those prepared by maceration so the ethyl acetate extract, by both extraction techniques, seemed the most active compared to other extracts with an IC_{50} in order of 0.457 mg/ml and 0.391 mg/ml by maceration and soxhlet respectively (Figure 14). Bencheikh et al. [18] also found similar results for which the ethyl acetate extract from the Algerian pennyroyal has higher antioxidant activity (IC_{50}= 0.017 µg/ml) than the crude, chloroform and aqueous extracts.

Other works had also shown that pennyroyal extracts had strong ability to act as antioxidants. The methanolic extract of pennyroyal from Algeria showed strong antiradical power (IC_{50} = 0.051 ± 0.001 mg/ml) compared to BHT standard (IC_{50} = 0.041 ± 0.001 mg/ml) [29]. In a study of Kamkar et al.[51], the IC_{50} of the aqueous extract of pennyroyal was 5.5 ± 0.3

µg/ml as for the methanolic extract was 6.1 ± 0.1 µg/ml comparable to that of BHT (4.9 ± 0.2 µg / ml). This activity is considered to be higher than that reported by Nickavar et al. [52] on the ethanolic extract (17.92 µg/ml) and Mata et al. [23] on ethanol (24.9 µg / ml) and aqueous extract (8.9 µg/ml) of *M. pulegium* L.

The antiradical activity was correlated with nature of used solvents. So, ethanol and water extracts showed very good radical scavenging activities [23] (Mata et al., 2007). The best results have been obtained with the aqueous extract of pennyroyal (IC_{50} = 8.9 ± 0.2 µg / m1). This value is lower than that of BHT (15.7 ± 0.2 µg / m1). The aqueous extract of pennyroyal was more active than that of ethanol. Likewise, the hot water extract from Portuguese pennyroyal showed high antiradical activity (EC_{50} = 16.3 ± 0.4 g/ml) followed by the ethanolic and cold water extract [53]. The methanolic extract of pennyroyal from Iran, evaluated by Derakhshani et al. [32], had significant antioxidant efficiency and that extracted from flowers are highly active than that from leaves: 2.94 ± 0.05 and 3.35 ± 0.08 mmol Fe/ 100 g fresh weight. However, Stagos et al. [31] found that the aqueous and methanolic extracts of pennyroyal from Greece had moderate antiradical activity compared to other tested Lamiaceae species; the IC_{50} values obtained are around 26 ± 0.6 µg/m1 and 28 ± 0.1 µg/m1 respectively. Vladimir-Knezevic et al. [54] also found that the antiradical activity of the pennyroyal ethanolic extract from Croatia was moderate compared to the other Lamiaceae tested (IC_{50} = 24.27 ± 0.21 µg / ml and to the IC_{50} of Trolox (reference standard) (1.99 ± 0.03µg / ml). The same level of activity was observed for the methanolic extract of Tunisian pennyroyal, but with a higher IC_{50} value reached 48 µg/ ml [55].

3.4.2. Evaluation of the antioxidant power of extracts of M. spicata L.

The results of antiradical power of *M. spicata* extracts had significant antiradical activity. The values found of IC_{50} of the extracts are comparable to that of ascorbic acid (Figure 15).

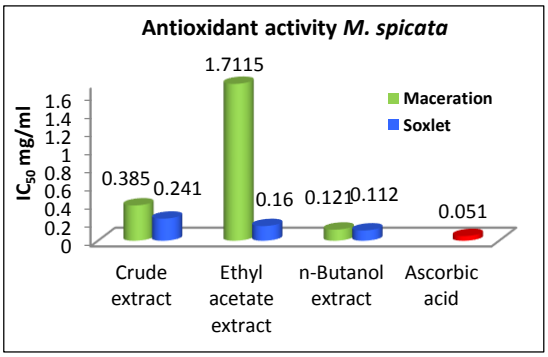

Figure 15: IC_{50} values of different extracts of *M. spicata* (L.)

The relative data indicated that the *n*-butanol fractions recorded the lowest IC_{50}values: 0.112 mg/ml by maceration and 0.121 mg/ml by soxhlet (Table 5). As for the fraction of ethyl acetate obtained by soxhlet, the IC_{50} was equal to 0.160 mg/ml while that obtained by maceration recorded the lowest antioxidant activity (IC_{50}= 1.712 mg/ml). Contrarily, the ethyl acetate fraction of *M. spicata* (L.) from India showed stronger antioxidant activity (95%) than that of hexanic (18%) and chloroform (22%) fractions [56].

Studies, carried out to determine the antiradical activity of *M. spicata* (L.), have shown that this species had remarkable antioxidant potential, while others have found that its activity was displayed to be moderate or low. Mata et al.[23] (2007) reported that the aqueous extract was found to be significantly so active (IC_{50}= 5.7 ± 0.4 µg/ml) compared to BHT (Butylhydroxytoluene) standard (15.7 µg/ml). In addition, Barchan et al. [20] found that the activity of the aqueous extract of spearmint from northern Morocco reached 90.32% approximately equal to that of antioxidant BHT (90.61%) and higher than that of the methanolic extract (89,38%). Naidu et al. [57] and Kanatt et al. [40] also reported that the methanolic extract of spearmint from Malaysia and India had significant antiradical activity with an IC_{50} around of 25.2 µg/ml (IC_{50} of ascorbic acid= 18 µg/ml) and 25.8 µg/ml (BHT IC_{50} = 10.1 µg/ml) respectively. However, Moldovan et al. [58] found that the ethanolic extract was moderately active (IC_{50}= 151.05 ± 1.95 µg/ml) compared to the Trolox standard (12 ± 0.54 µg/ml). Similarly, the ethanolic extract of Iranian spearmint recorded the lowest trapping activity of the DPPH• (IC_{50} = 87.89 µg / ml) among the five tested mints [

4. Discussion

In the present study, the Lamiaceae selected species showed a significant variation in the content of phenolic compounds and in the antioxidant potential and this is in agreement with previous studies on the antioxidant properties of certain Lamiaceae plants [52;59;60].

Our results on the antioxidant activity of mint extracts indicated that each species reacted differently towards the free radical DPPH• according to the used solvents and extraction methods. The highest antiradical activity was recorded by the ethyl acetate fraction of *M. pulegium* (L.) (Table 6) and *n*-butanol fraction of *M. spicata* (L.) (Table 7). Furthermore, according to the yields of different extracts and to the contents of phenolic compounds, it seems that the soxhlet extraction technique was the most effective

compared to the maceration. The same result was obtained by Bimakr et al. [61], the yields and flavonoïds contents soxhlet extraction were higher than those extracted by the supercritical carbon dioxide extraction method.

It is difficult to compare the antioxidant activity of these two mints due to the interaction of several parameters: the effect of solvent characteristics, the extraction technique, the tested mint part and the species itself. However, data from the literature on the antioxidant activity of *Mentha* species are often difficult to compare due to differences in methodology [59]. Some of our results were generally similar to those obtained previously about *M. pulegium* or *M. spicata*.

The highest inhibition percentages were observed for *M. spicata* (86.22%) followed by *M. pulegium* (81.57%) compared to that of ascorbic acid (90%). Likewise, the aqueous extract of *M. spicata* (L.) was found to be more active than that of *M. pulegium* (L.) [23]. However, some works performed by Ahmad et al. [3, Derakhshani et al. [32], Moldovan et al. [58], Nickavar et al. [52] and Mata et al. [23] have reported that *M. pulegium* with different extracts have shown greater antioxidant potential than those from *M. spicata*.

Note that the highest levels of polyphenols and flavonoïds in the extracts have been observed for those which have shown significant antiradical activity: the ethyl acetate extract of *M. pulegium* (L.) and that of *n*-butanol extract from *M. spicata* L. Consequently, this activity could be attributed to the abundance of phenolic compounds in these extracts.

A positive correlation has been found between the polyphenols content and the degree of antioxidant activity. The correlation coefficients (r^2) obtained are: 0.877 for *M. pulegium* and 0.815 for *M. spicata* extracts by maceration. Apart from those with high activity, the fractions extracted by soxhlet showed some heterogeneities between the polyphenols content and the antiradical activity (r^2<0.3). This heterogeneity may be due to the nature of phenolic compounds which contain different antioxidant capacity [56] or to other compounds which are not phenolic and which are partly responsible for this activity [52].

Many researchers have reported a positive correlation between the free radical scavenging activity and the content of phenolic compounds [60]. Romero-Jimenez et al. [61] indicated that the level of antioxidant activity was strongly associated with the content of phenolic compounds in the extracts. Furthermore, Barchan et al. [20] also found that the antioxidant activity is well correlated with the phenolic content (r^2 = 0.88 and 0.66 for *M. spicata* and *M. pulegium* respectively). Likewise, Mata et al. [23] deduced that the antioxidant potential of mints depended greatly on the presence of phenolic compounds.

5. Conclusions

This study was performed to assess and compare the antioxidant efficiency of different extracts from *M. pulegium* (L.) and *M. spicata* (L.) according to the contents of polyphenols and flavonoïds present in these extracts.

The obtained contents of polyphenols and flavonoïds seem to be depended on solvent types, extraction method and tested species. Thus, the highest contents were observed with polar solvents and by soxhlet method. As well, the extracts from *M. spicata* were generally more active than those of *M. pulegium*.

The results indicated that the polyphenols and flavonoïds contents were positively and significantly correlated with the antioxidant activity. So, the extracts, presented the strongest contents, showed important antioxidant potential comparable to that of ascorbic acid. Consequently, the extracts of these mints could be a source of useful antioxidants in the food and pharmaceutical fields.

Table 6. Polyphenols and flavonoïds contents and IC_{50} values of *M. pulegium* (L.) extracts

***M. pulegium* (L.)**	Maceration (mg/ml)			Soxhlet (mg/ml)		
	IC_{50}	**PPC***	**FC****	**IC_{50}**	**PPC**	**FC**
Crude extract	0.845	7.23	3.21	0.634	14.24	14.12
Ethyl acetate extract	0.457	9.70	7.53	0.391	9.66	20.53
***n*-butanol extract**	0.92	4.88	4.63	0.83	11.88	12.50

Table 7. Polyphenols and flavonoïds contents and IC_{50} values of *M. spicata* (L.) extracts

***M. spicata* (L.)**	Maceration (mg/ml)			Soxhlet (mg/ml)		
	IC_{50}	**PPC**	**FC**	**IC_{50}**	**PPC**	**FC**
Crude extract	0.385	5.98	3.54	0.241	15.44	9.1
Ethyl acetate extract	1.712	0.52	0.01	0.160	16.11	14.85
***n*-butanol extract**	0.121	4.38	9.91	0.112	7.11	5.01

***PPC: polyphenols content**
**** FC: Flavonoids content**

6. Conflicts of interest

There are no conflicts to declare.

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