DETERMINATION OF THE BIOACTIVE COMPOUNDS, ANTIOXIDANT AND ANTIFUNGAL ACTIVITIES OF DIFFERENT EXTRACTS OF *Marrubium vulgare* L.

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Abstract. The current work concerns a medicinal plant *Marrubium vulgare* L. grown in Souk Ahras region (Northeastern of Algeria). Widely used by the natives of this region for their extraordinary therapeutic properties such as: antioxidant, anti-inflammatory, antibacterial and analgesic activities. The aims of this study were to evaluate the phenolics, flavonoids and tannins composition, and antioxidant activities of hexane, dichloromethane, acetone and methanol extracts from leaves of *Marrubium vulgare* L. by using different methods. The extracts were a good source of phenolics and flavonoids. Acetonic extract from the aerial part of *M. vulgare* had the highest total phenolic and flavonoid contents with 171.45±3.38 µg GAE mg $^{-1}$ E and 78.33±0.29 µg QE mg^{-1} E, respectively. While the hexane extract had the most condensed tannins content with 90±0.01 µg CE mg $^{-1}$ E. The antioxidant activity showed the good antioxidant capacity of extracts (dichloromethane, acetone and methanol) in DPPH, ABTS, β-Carotene, CUPRAC, Phenanthroline and GOR assays. While the hexane extract showed the lowest antioxidant activity in DPPH, β-Carotene and GOR assay. Weak antioxidant activity in Reducing power was observed for all extracts with >200 µg/mL. On the other hand, these extracts showed no activity in Ferrous ions chelating assay, exceptionally the methanol extract which showed a very weak activity >800 µg/mL. The results showed that the concentrations of TPC, TFC and CTC were not significantly different ($p > 0.05$). However, a highly significant difference in all antioxidant activities was observed ($p < 0.001$) except Phenanthroline activity which presented a non-significant difference ($p > 0.05$). We have also tested the effect of the different extracts against two phytopathogenic fungi strains by the disc-diffusion method. The inhibitory effect were important for the two tested strains. The extracts of *M. vulgare* have important antifungal activity. Statistical analysis indicates no significant differences at $P > 0.05$. Based on These results, it is right to conclude that *M. vulgare* is an important source of bioactive compounds with antioxidant and antifungal properties.

Key words: *Marrubium vulgare* L.; phenolic compounds; antioxidant activity; flavonoids; antifungal activity.

INTRODUCTION

For millennia, herbs have played an important role in daily life. Many native people use medicinal plant extracts for their health care. One of the most valuable herbal families is the Lamiaceae family which includes a large variety of plants with medical and biological application [48]. The Lamiaceae plant family is one of the largest families used as a framework to determine the typical secondary metabolites [49].

M. vulgare is commonly known as "Marrioua" in Algeria, "Marrubia" in Tunisia, "Merrîwt" in Maroc and in Brazil is called "marroio". This plant is regularly used in traditional medicine to treat diverse diseases [12, 27].

The traditional use and scientific research explained the efficacy and the safety of medicinal plants [46]. Because of their accessibility, the comparison to modern medicine and modern drugs, plants become an excellent part of the health public and primary health care [46].

According to the World Health Organization (WHO), about 80 percent of the world population consumes mainly drugs that are derived from plants [7]. The Lamiaceae family incorporates many species that are used in traditional medicine. The aerial parts of *M. vulgare* (leaves and stems) are known to have antidiabetic, antispasmodic, diuretic, expectorant, antiseptic and tonic roles [31, 41].

Many phytochemical studies on different parts of *M. vulgare* have reported the presence of secondary metabolites such as lactones, alkaloids, tannins, steroids, diterpenoids and a series of phenylpropanoid esters [36]. Furthermore, bioactive compounds such as diterpenoids, phenylethanoid, glycosides and essential oils are known for their antioxidant activities [1, 23].

M. vulgare is very useful in traditional medicine for its various therapeutic properties. This plant exhibits multiple activities such as antimicrobial [40], antibacterial [20], and cytotoxic [21].

Several applications including natural therapies, pharmaceuticals, cosmetics, raw and processed food preservation and alternative medicine have developed basically by the antimicrobial activities of different plant extracts [26].

The aim of this research was to evaluate the antifungal activity and the antioxidant activities of different extracts of aerial parts of *M. vulgare* by the use of different methods supported by a quantification of total bioactive compounds, including phenolic, flavonoid and tannin.

MATERIAL AND METHODS

Plant material

The aerial parts of *M. vulgare* were collected on May 2019, during the flowering season from Souk Ahras (East region of Algeria).

Study site

The study was conducted in Souk Ahras province as shown in Figure 1. Souk Ahras is situated in the Northeastern part of Algeria (Latitude 36° 17′ 15″ (N); longitude 7° 57′ 15″ (E); Altitude 653 m above sea) [8].

Souk Ahras is characterized by sub-humid climate with annual humidity 71%, precipitation is around a

Figure 1. Geographical location of the study site [3]

350 mm/an [8]. The daily average temperatures vary according to the seasons (from 10 °C in January to 45°C in August). Average monthly temperatures are 15°C in January and 35°C in July [3].

Preparation of extracts

After harvesting, aerial parts (leaves) of the plant were separated, cleaned and dried under shade at room temperature for 10 days to preserve the molecules integrity. After drying the samples were prepared as a powder. Extraction was performed two times using increasing polarity solvents: n-Hexane, dichloromethane, acetone and methanol for 48 Hours in each extraction, with magnetic agitation at room temperature. After filtration, the filtrate was evaporated under pressure in a rotary evaporator at 40 °C to give the crude extract. Obtained extracts were stored at 4 °C until analysis.

Calculating percent yield

The yield of the raw extract is defined as the ratio between the mass of the dry extract obtained, and the mass of the treated plant material. It is calculated according to the following equation:

$$Y\ (\%) = Me/Mv \times 100$$

where : Y (%): Yield in %; Me: Mass of extract after solvent evaporation; Mv: Mass of vegetal material used for extraction [25].

Determination of total bioactive compounds

Total phenolic contents (TPC)

The total phenolic content of the extracts of *M. vulgare* was determined by multimode plate reader (EnSpire) following the Folin-Ciocalteu method [44] and the results were expressed as micrograms of gallic acid equivalents per milligrams of extract (µg GAE/mg).

Total flavonoid contents (TFC)

The total flavonoid content of the extracts of *M. vulgare* was determined by a multimode plate reader (EnSpire) method described by Topçu *et al.* [47] and the results were expressed as micrograms quercetin equivalents per milligram of extract (µg QE/mg).

Condensed tannin contents (CTC)

The condensed tannin content of the extracts of *M. vulgare* was determined by the Folin-Ciocalteu reagent method [28]. Tannin content was expressed as micrograms tannic acid equivalent per milligram of extract (µg TAE/mg).

Antioxidant activities

DPPH scavenging assay. The DPPH scavenging activity was determined by multimode plate reader (EnSpire) by the method described by Blois [10]. BHA, BHT and α-Tocopherol were used as antioxidant standards for comparison of the activity. The results were given as 50% inhibition concentration (IC_{50}).

ABTS cation radical assay. The ABTS scavenging activity was determined according to the method of Re *et al.* [37], BHA and BHT were used as antioxidant standards for comparison of the activity. The results were given as 50% inhibition concentration (IC_{50}).

Reducing power assay. The reducing power of the extracts of *M. vulgare* was determined according to the method of Oyaizu [33] with slight modification, adapted at microplate-reader. The results were given as absorbance and compared with α-Tocopherol and Ascorbic acid used as antioxidant standards, the results were given as $A_{0.50}$, which corresponds to the concentration producing 0.500 absorbance.

β-carotene/linoleic acid bleaching assay. The antioxidant activity was evaluated by using β-carotene-linoleic acid test according to the method of Marco [29]. BHA and BHT were used as antioxidant standards for comparison of the activity. The results were given as 50% inhibition concentration (IC_{50}).

Cupric reducing antioxidant capacity (CUPRAC). The cupric reducing antioxidant capacity was determined according to the method of Apak [2]. BHA and BHT were used as antioxidant standards for comparison of the activity. The results were given as $A_{0.50}$, which corresponds to the concentration producing 0.500 absorbances.

Ferrous iron chelating (FIC) assay. The metal-chelating activity of the extract via ferrous iron was estimated by the method of Dinis *et al.* [13]. The results were given as 50% inhibition concentration (IC_{50}) and expressed as µg/mL. Ethylenediamine tetra acetic acid (EDTA) was used as a standard.

Phenanthroline assay. The Phenanthroline Assay was determined according to the method of Szydlowska-Czerniaka *et al.* [42]. BHT was used as a

standard antioxidant for comparison of the activity. The results were given as $A_{0.50}$, which corresponds to the concentration producing 0.500 absorbance.

Galvinoxyl radical scavenging assay (GOR). The antioxidant activity was evaluated by the method of Shi *et al*. [43]. The results were given as 50% inhibition concentration (IC_{50}).

Antifungal activity assay

Two phytopathogenic fungi namely: *Fusarium oxysporum* f. sp. *lycopersici* (FOL) f. sp. strain 4287, *Botrytis cinerea*, will be tested for fungi toxicity by evaluating mycelial growth inhibition of phytopathogenic agents:

The inhibitory activity of the various compounds, on the mycelium growth of the two phytopathogenic agents, is determined by measuring the radial growth of the fungus on PDA medium (Potato Dextrose Agar), containing the complex to be tested. Thus, a volume of 1 mL of DMSO solution containing 5 mg of the freeze-dried product was added to 100 mL of PDA medium at 60 °C previously sterilized and then distributed in 4 Petri dishes. Similarly, 1 mL of DMSO was added to 100 mL of PDA medium, and was considered as a negative control. [45].

Experimentally, a disk of 5 mm in diameter is taken from a young fungal culture and is deposited aseptically in the center of the petri dish containing the PDA medium and the complex to be tested. The experiment is replicated 4 times for each treatment. After 6 days of incubation at 25 °C, the mycelial growth of the phytopathogenic agent is measured at millimetric scale. Results were expressed as the percentage of growth inhibition of each fungus by each complex with respect to the mean colony diameters of each fungus grown in control medium. Thus, the inhibition activity was expressed as a percentage and was calculated according to the formula: $I = ((C-T)/C) \times 100$ [14]. Where I = inhibition rate in %; C = radial growth of phytopathogenic agent in mm on PDA medium with DMSO (control); T = the radial growth in mm of the phytopathogenic agent on PDA medium containing the complex to be tested.

Statistical analysis

All data and results were performed in triplicate excepting antifungal activity in quadruplicate, the results were expressed as mean standard deviation (S.D.) using the GraphPad prism 8 program. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined using Tukey's test; $p < 0.05$ were regarded as significant.

RESULTS

Yields of different extracts

The extraction series, we carried out allowed us to obtain four crude extracts (Table1).

The extraction carried out by increasing polarity solvents showed that the methanol extract has the highest yield, followed successively by the dichloromethane extract and then the hexanic extract. However, the weakest yield is obtained with the acetone solvent.

Total bioactive contents

The results of total phenolic and flavonoid contents of *M. vulgare* extracts indicated differences in all components (Table 2). The highest phenolic and flavonoid contents were found in the acetone extract 171.45±3.38 µg GAE/mg, 78.33±0.29 µg QE/mg of plant extract respectively (Table 1), while methanolic extract contains 94.29±2.35 µg GAE/mg, 40.62±0.14 µg QE/mg compared with dichloromethane extract (68.61±0.00 µg QE/mg, 16.15±0.16 µg GAE/mg,). The hexane extract exhibited the lowest contents respectively (22.77±0.14 µg QE/mg, 0.27±0.16 µg GAE/mg of plant extract).

Regarding the tannins content of *M. vulgare* extracts, the highest level was measured in the hexane extract (90±0.01 µg EC/mg) followed by the methanolic extract (60±0.01 µg TAE/mg), while the lower level was obtained with the acetone and dichloromethane extracts (50±0.00 µg TAE/mg).

Antioxidant properties

The antioxidant and free radical scavenging potential of the extracts are given in Table 3, and expressed in terms of IC_{50} and $A_{0.5}$.

Table 1. Different characteristics of the extracts obtained

Extracts	Mass (g)	Yield (%)	Aspect	Color
Hexane	0.09	2.57	Viscous	Yellowish
Dichloromethane	0.11	3.14	Solid	Greenish
Acetone	0.05	1.42	Gel	Greenish
Methanol	0.18	5.14	Oily	Greenish

Table 2. Total phenolics, flavonoids and tannins content of *M. vulgare* extracts

Extracts	TPC (µg GAE mg^{-1} E)	TFC (µg QE mg^{-1} E)	CTC (µg TAE mg^{-1} E)
Hexane	22.77±0.14	0.27±0,16	90±0.01
Dichloromethane	68.61±0.00	16.15±0,16	50±0.00
Acetone	171.45±3.38	78.33±0.29	50±0.00
Methanol	94.29±2.35	40.62±0.14	60±0.01

TPC: Total phenolics content; TFC: Total flavonoids content; CTC: Condensed tannins content.
Results are expressed as means±SD (n=3). The values of TPC, TFC, CTC are no significantly different ($p > 0.05$).

Table 3. Antioxidant activity of various extracts of *M. vulgare*

Extracts	DPPH· Assay IC_{50} µg/mL	$ABTS^+$ assay IC_{50} µg/mL	Reducing power assay $A_{0.50}$ µg/mL	β-Carotene linoleic acid assay IC_{50} µg/mL	CUPRAC assay $A_{0.50}$ µg/mL	Ferrous ions chelating assay IC_{50} µg/mL	Phenanthro line assay $A_{0.50}$ µg/mL	GOR assay IC_{50} µg/mL
Hexane	>800	16.13±0.45	>200	>800	0.17±0.03	Na	0.23±0.02	>800
Dichloromethane	5.25±0.36	20.13±0.38	>200	27.02±0.87	0.16±0.06	Na	0.24±0.02	6.71±1.07
Acetone	33.03±0.14	37.91±0.4	>200	33.7±1.21	0.23±0.01	Na	0.23±0.01	36.82±0.21
Methanol	29.02±0.35	40.10±0.35	>200	38.33±1.49	0.1±0.04	>800	0.26±0.01	32.03±0.25
BHA[b]	6.14±0.41	1.81±0.1	-	1.05±0.03	5.35±0.71	-	Nt	Nt
BHT[b]	12.99±0.41	1.29±0.3	-	0.91±0.01	8.97±3.94	-	Nt	Nt
α-Tocopherol[b]	13.02±5.17	-	34.93±2.38	-	-	-	-	-
Ascorbic acid[b]	-	-	6.77±1.15	-	-	-	-	-
EDTA[b]	-	-		-	-	8.80±0.47	-	-

IC_{50} and $A_{0.50}$ values are defined as the concentration of 50% inhibition percentages and the concentration at 0.50 absorbance respectively. [a]IC_{50} and $A_{0.50}$ were calculated by linear regression analysis and expressed as mean±SD (n=3). Analysis of variance (ANOVA) revealed a highly significant effect ($p < 0.001$) in all antioxidant activities. However non-significant effect ($p > 0.05$) was observed in Phenanthroline activity.
[b]Reference compound: BHA butylatedhydroxyanisole, BHT butylatedhydroxytoluene, EDTA ethylenediamine tetraacetic acid, NT not tested, NA not absorbance.

The results of CUPRAC assay (Table 3) show that the methanolic extract exhibited the best activity (IC_{50}: 0.1±0.04 µg/mL), the same as the activity of dichloromethane and hexane extracts (IC_{50}: 0.16±0.06 µg/mL, 0.17±0.03 µg/mL, respectively) much better than the BHA, BHT (IC_{50}: 5.35±0.71 and 8.97±3.94 µg/mL, respectively). The antioxidant activity using Phenanthroline assay of the extracts was also investigated as shown in Table 3, the highest activity was obtained from the hexane and acetonic extracts (IC_{50}: 0.23±0.02, 0.23±0.01 µg/mL, respectively), the same as the activity of dichloromethane and methanolic extracts (IC_{50}: 0.24±0.02, 0.26±0.01 µg/mL).

The analysis data of the ABTS assay showed that the hexane extract gives the best activity (IC_{50}: 16.13±0.45 µg/mL), but lower than BHA and BHT (IC_{50}: 1.81±0.1, 1.29±0.3 µg/mL, respectively) followed by dichloromethane extract (IC_{50}: 20.13±0.38 µg/mL) furthermore the acetonic and methanol extracts exhibited the low activity (IC_{50}: 37.91±0.40 µg/mL, 40.1±0.35 µg/mL, respectively).

For the DPPH scavenging the dichloromethane extract exhibited the highest antioxidant activity (IC_{50}: 5.25±0.36 µg/mL) compared to BHA, BHT and α-tocopherol (IC_{50}: 6.14±0.41, 12.99±0.41, 13.02±5.17 µg/mL, respectively) and followed by methanolic and acetonic extracts (IC_{50}: 29.02±0.35 µg/mL, 33.03±0.14 µg/mL, respectively) but the hexane extract exhibited week activity at 800 µg/mL.

In addition the results of Galvinoxyl radical (GOR) scavenging assay showed that the dichloromethane extract has the best activity (IC_{50}: 6.71±1.07 µg/mL) followed by methanolic and acetone extracts (IC_{50}: 32.03±0.25, 36.82±0.21 µg/mL, respectively) however the hexane extract exhibited the low activity at 800 µg/mL.

The compared results in Table 3 for the β-carotene assay showed the highest activity of dichloromethane extract (IC_{50}: 27.02±0.87 µg/mL) and followed by acetonic and methanol extracts (IC_{50}: 33.7±1.21, 38.33±1.49 µg/mL, respectively) very lower to BHA, BHT (IC_{50}: 1.05±0.03, and 0.91±0.01 µg/mL, respectively) however the hexane extract exhibited the low activity at 800 µg/mL.

For the Ferrous ions chelating assay only the methanolic extract exhibited a weak activity (IC_{50}: >800 µg/mL) compared with EDTA (IC_{50}: 8.80±0.47 µg/mL) furthermore the other extracts showed being no actives. The result of reducing power assay showed that all extracts exhibited a weak activity at 200 µg/mL, compared with standards: ascorbic acid and α-Tocopherol (IC_{50}: 6.77±1.15 and 34.93±2.38 µg/mL, respectively).

Antifungal activity

The antifungal activity of extracts measured by mycelial growth inhibition against selected phytopathogenic fungus was representing in (Table 4; Figure 2-4).

Table 4. Effect of different extracts of *M. vulgare* on the growth of the fungal strains tested (Inhibition %)

Microorganisms tested	Extracts Hex	Dich	Acé	Mét
Fusarium oxysporum f. sp. *Lycopersici*	17.24	60.34	43.10	24.13
Botrytis cinerea	45.58	60.29	63.23	52.94

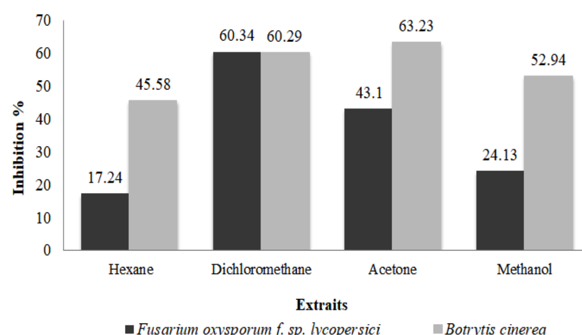

Figure 2. Effect of different extracts of *M. vulgare* on the growth of the fungal strains tested (Inhibition %). Values are mean of four replications and the results indicate statistically no significant differences at $P > 0.05$.

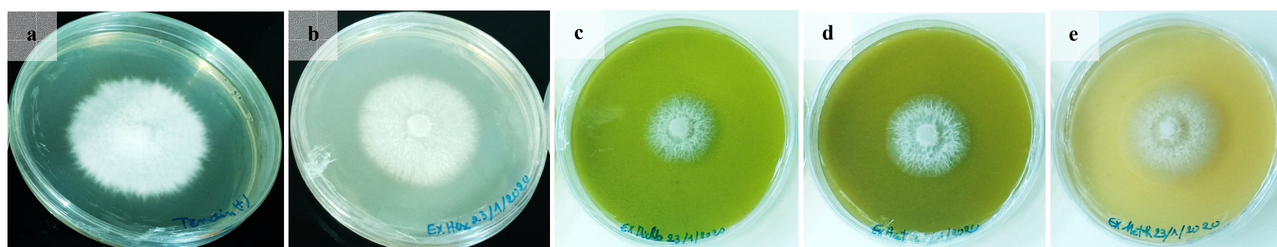

Figure 3. Inhibition rate of *M. vulgare* extracts vis-a-vis *Fusarium oxysporum* f. sp. *lycopersici*, after 5 days of incubation, where: (a): Control, (b): Ex Hexane, (c): Ex Dichloromethane, (d): Ex Acetone, (e): Ex methanol

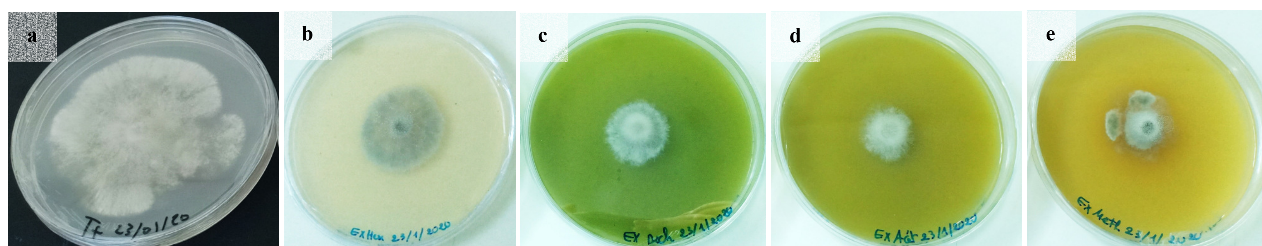

Figure 4. Inhibition rate of *M. vulgare* extracts vis-a-vis *Botrytis cinerea*, after 5 days of incubation, where: (a): Control, (b): Ex Hexane, (c): Ex Dichloromethane, (d): Ex Acetone, (e): Ex methanol

The inhibitory activity of the different extracts of *M. vulgare* against two phytopathogenic fungi *Fusarium oxysporum* f. sp. *lycopersici* (FOL) strain 4287 and *Botrytis cinerea* are presented in (Figure 3 and 4). The results indicate that the extracts studied have antimicrobial activity on the two fungi tested. This sensitivity is different according to the strains and to the extract, with different degrees.

Overall, the dichloromethane extract from the leaves of the plant species *M. vulgare* appears to be more effective than the other extracts against the fungal strains tested with a percent inhibition of growth 60.34% on *Fusarium oxysporum* f. sp. *lycopersici* and 60.29% on *Botrytis cinerea*. While, the acetone and methanolic extract show a fairly high inhibitory effect only on the *Botrytis cinerea* strain with a percent inhibition 63.23% and 52.94%, respectively.

However, the percent inhibition obtained with the acetone extract on *Fusarium oxysporum* f. sp. *lycopersici* was 43.10%. Whereas the hexane extract showed about 45.58% on *Botritus cinerea*. The hexane and methanolic extract showed low activity on the fungal species *Fusarium oxysporum* f. sp. *lycopersici* with a percent inhibition 17.24% and 24.13%, respectively.

DISCUSSION

As a matter of a fact, the study carried out in the province of Souk Ahras (East region of Algeria) on the bioactive compounds and the biological activities of different extracts of *M. vulgare* plant of the region was very interesting. This is the first research performed in this region.

Phenolic compounds are the main class of secondary metabolites in plants, they are ubiquitous showing the extensive diversity of structure [19].

In general, dry extract levels vary not only from one plant to another in the same family, but also depending on the parameters of solid-liquid extraction of polyphenols, extraction solvent, particle size and solvent scattering coefficient. In addition to these quantitative aspects, regardless of the extraction method applied [13].

A yield of 24.34% of the crude metanolic extract was obtained from the aerial part of the same species in a study undertaken by Djahra-Ali [13]. This is higher percentage than that obtained in our case (5.14%). This could be due to the same technique that was used by the author (cold extraction by simple maceration). However Kanyonga *et al*. [22] found a value of 39.2%. This is as much higher as the percentage obtained in our case, by extraction with the Soxhlet and under a temperature of 70 °C, which is not the case in our study.

Regarding the importance of total contents as bioactive products having a wide spectrum of biological activities, the quantification of phenolic, flavonoid and tannin contents were assessed. The results of the total phenolic, flavonoid and tannin contents indicate that the proportions of polyphenols and flavonoids are higher than taninns.

The high content of total phenols of the extracts might explain the strong antioxidant properties of this plant. In terms of the polyphenol content of *M. vulgare*, different results were obtained from the vegetative organs of the same species of *M. vulgare*. A rate of 18.21 mg EAG/mL of extract was advanced by Boudjelal [6]. An extremely high content of total phenolic and flavonoid compounds of *M. vulgare* was noted in a study conducted by Chouaieb *et al*. [9].

Previous studies carried out on various extracts of the species *M. vulgare* including Boudjelal [9] and Ghedadba *et al*. [17] displayed lower levels of polyphenols, flavonoids and a higher levels of tannins [1] compared to our study. The variation of results in total phenolic, flavonoid and tanin contents between species of the same genus is related to several factors

such as genotypic, environmental and climate conditions (abiotic and biotic factors) [30].

Our results revealed that the methanolic extract exhibited remarkable activity towards the DPPH trapping with an IC_{50} of 29.02±0.35 µg/mL. This result was lower than those of Lodhi *et al*. [24] and Boudjelal [9] obtained from the methanolic extract of the aerial parts of *Marrubium vulgare* L. which showed high antioxidant activity with an IC_{50} value of 0.035 µg/mL, 0.49 µg/mL.

In another study undertaken by Yumrutas and Saygideger [50] on the scavenging of DPPH. It was indicated that hexanic and methanolic extract of aerial parts of the species *Marrubium parviflorum* gives a very weak antioxidant activity compared to that found in the methanolic extract of *M. vulgare* with higher IC_{50} values.

In contrast, the work carried out by Orhan *et al*. [34] affirm that the methanolic extract is less active than the acetone extract of the same species. Also, a powerful effect of the methanolic extract of *M. vulgare* (IC_{50} of 0.177 µg/mL) was found from the DPPH trapping during the work carried out by Fathiazad *et al*. [16] and Amessis-Ouchemoukh *et al.* [1].

For the ABTS assay, our results are in agreement with those of Lodhi *et al*. [24]. The methanolic extract of *M. vulgare* leaves exhibited highly potent antioxidant activity of the ABTS assay (IC_{50} = 25 µg/mL). Also Edziri *et al*. [15] recorded a better efficiency of the methanolic extract by an IC_{50} of the order of 0.35±1.2 µg/mL.

Numerous studies have evaluated the Reducing power assay from various plant extracts. The study carried out by Jeong *et al*. [22] shows that the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity. The result of Reducing power showed distinctly a lower activity than the standards (ascorbic acid and α-Tocopherol), our results are in agreement with the study previously reported by Lodhi *et al*. [24].

All extracts have antioxidant activity by the Reducing power assay significantly lower than the standards (ascorbic acid and tocopherol), our results are in agreement with a study previously reported by Lodhi *et al*. [24]. The study reported by Fathiazad *et al*. [16] showed that *M. vulgare* extract has a weak reducing power activity. However, for the ferrous ions chelating assay, all extracts present no activity exceptionally the methanol extract.

Overall, in the present work, the results obtained in β-carotene assay reveal that the dichloromethane, acetone and methanolic extracts are more active than the hexane extract. Our results indicate that the dichloromethane extract has a high activity with IC_{50} of 27.02 ± 0.87 µg/mL. These results are in agreement with those of Pukalskas *et al*. [35] who indicated that the methanol extract of horehound provides remarkable antioxidant activity in the β-carotene assay. Contrary, works carried out by Ghedadba *et al*. [17] affirm that the methanolic extract is more active than the dichloromethane extract of the same species.

Finally, for the CUPRAC, Gor and Phenantroline assays all extracts present very higher activity especially the methanolic extract which exhibited the best activity (IC_{50}: 0.10±0.04 µg/mL) very better than BHA and BHT (IC_{50}: 5.35±0.71, 8.97±3.94 µg/mL). From literature, our results are in accordance with those of previous works, indicating the highest values of CUPRAC with Olah *et al*. [32].

The antifungal activity of the extracts was tested by measuring the radial growth of the fungus. The extracts of *M. vulgare* have shown a strong inhibitory effect against the two fungi tested and the results are in agreement with previous studies on essential oil of *M. vulgare* grown in Tunisia [51, 39].

Several studies attributed the inhibitory effect of plant extracts against pathogenic fungi to their phenolic composition [11, 38]. These results suggest that the antifungal capacity has a good efficiency with crude extracts of *M. vulgare* Purified components may be used as natural antifungals in agrifood systems, as well as to prevent the growth of plant born fungi.

In conclusion and based on the results, it can be concluded that *M. vulgare* plant may be an effective source of phenolic, flavonoid and tannin compounds with high antioxidant activity. Therefore, *M. vulgare* could be useful in the pharmaceutical industry in order to obtain a biofungicides allowing the fight against phytopathogenic fungi, and for the development of additives food in the prevention and treatment of various human diseases.

Abreviations

GAE - gallic acid equivalent
QE - quercetin equivalent
TAE - tannic acid equivalent
DPPH - 2,2-diphenyl-1-picrylhydrazyl.
ABTS - acide 2,2'-azino-bis (3-éthylbenzothiazoline-6-sulphonique)
CUPRAC - cupric reducing antioxidant capacity
GOR - galvinoxyl radical
BHA - butylated hydroxyanisole
BHT - butylhydroxytoluène
IC_{50} - inhibitory concentration 50
$A_{0.50}$ - concentration at 0.5 absorbance
EDTA - ethylenediaminetetraacetic acid
SD - standard deviation
DMSO - dimethylsulfoxide

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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