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Research article

Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of Northeast of Morocco

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ABSTRACT

Chemical compositions, biological and antioxidant activities of plants are widely affected by several parameters and conditions, such as geographical and climatic conditions, type of extract (aqueous or organic), as well as the polarity of the extracting solvent. Therefore the present study was the first one designed to study the phytochemical composition, the content of polyphenols, tannins and flavonoids, the antioxidant activities and the chemical composition analysis by FTIR spectroscopic of organic (ethanol, methanol, ethyl Acetate, petroleum ether) and aqueous extracts of *Marrubium vulgare* L. leaves, collected from two different sampling localities in the North-East of Morocco: Oulad Daoud Zkhanine and the Cape Three Forks. A phytochemical screening was carried out by specific coloring and precipitation reactions. The colorimetric method Folin- Ciocalteu was used for the quantification of total phenolic content. The method of aluminum chloride was employed for the quantification of total flavonoid content and the method of vanillin for the determination of tannins. The antioxidant power was evaluated by the DPPH and ABTS methods. The chemical composition of the organic extracts was analyzed by the FTIR spectroscopy method. Depending on the sampling location of M.vulgare L., the type of extract (aqueous or organic), the polarity of the extracting solvent, and the phytochemical screening revealed the presence of the following secondary metabolites: catechic tannins, terpenoids, polyphenols and flavonoids. The total concentrations of total polyphenols, flavonoids and tannins varied respectively between 0.27 ± 0.1 and 86.91 ± 1.22 μg gallic acid equivalents/mg,6.08 ± 0.17 and 33.82 ± 0.90 μg quercetin equivalents/mg and 2.73 ± 1.15 and 252.68 ± 4.50 μg catechin equivalents/mg. The antioxidant activity that was evaluated by DPPH and ABTS method showed that ethanol extract, methanol and ethyl acetate extract had the highest percentages of inhibition, unlike petroleum ether extract. The inhibitory concentrations (IC_{50}) ranged from 324.55 ± 0.66 to 980 ± 0.62 μg/ml for DPPH and from 107.85 ± 0.19 to 890.74 ± 0.17 μg/ml for ABTS. FTIR spectroscopic analysis has revealed different characteristic peak values with various functional groups in the extracts such as amide, alcohol, phenol compounds. In general, the organic and aqueous extracts of M.vulgare L. that were harvested from Oulad Daoud Zkhanin were richer in secondary metabolites, and showed higher concentrations of polyphenol, flavonoids and tannins. In addition, they revealed a higher antioxidant capacity than the extracts of M. vulgare L.from the Cape Three Forks.

Overall this study highlighted the potential benefits and richness of M.vulgare L. harvested from the two study areas and suggested it as a potential source of natural antioxidants that could be used in the food and pharmaceutical fields.

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1. Introduction

Marrubium vulgare L. is a perennial herbaceous plant that belongs to the *Lamiaceae* family (Mahmoud et al., 2018), and found natively in Europe, North Africa, Southwest Africa and Central Asia. It is widespread in North-eastern Morocco and is known in the two localities of our study: Oulad Daoud Zkhanine and the Cape Three Forks under the name "Thamariouth". M. vulgare L. is used in traditional medicine for the treatment of various ailments: respiratory, urinary, ear infections, ophthalmia. It is also used against diarrhea, diabetes, rheumatism (Bouterfas et al., 2016) migraine and typhoid fever (Salhi., 2010). In addition, it is traditionally used for its vaso-relaxing, anti-hypertensive, analgesic, anti-inflammatory, anti-hepatotoxic and anti-edematous activities (Boudjelal., 2012; El Bardai et al., 2003; Stulzer.,2006; Ahmed., 2010) emmenagogue, antiseptic, diuretic, anti-typhoid (Benkhnigue et al., 2010),antibacterial (Masoodi et al., 2008), antioxidant (Kadri et al., 2011; Weel.,1999). The activities of marrubium extracts have been pharmacologically demonstrated in several modern studies (Elberry et al., 2015). The aerial part of the M. vulgare L. contains several secondary metabolites such as di-terpenes, including marrubiin that is responsible for most of the biological properties of M. vulgare (Çitoğlu and Aksit, 2002), apigenin, luteolin and their 7-glucosides together with quercetin and its 3-glucoside and 3-rhamnoglucoside (Nawwar., 1989), as well as several phenyl-propanoid esters such as verbascosides (Boudjelal et al., 2012) and tannins (Bouterfas et al., 2016). The composition of the bioactive products of medicinal plants and their biological properties can be affected by several factors namely climatic and geographical conditions (Bouterfas et al., 2016). Previously, the objective of this paper is to study the changes induced the geographical (or geomorphological, altitude, latitude, type of relief) and climatic conditions (temperature, rainfall, humidity) of the locality where the leaves are collected, the type of extract (organic or aqueous), the polarity of the solvents on the phytochemical, antioxidant properties and chemical composition of extracts of M. vulgare L. leaves harvested in two different localities in North-eastern Morocco.

2. Materials and methods

2.1. Biological materials

The leaves of M. vulgare L. identified by Dr. Saadia Belmalha, were collected in June 2019 from two different localities belonging North-eastern Morocco: Oulad Daoud zkhanine and the Cape Three Forks (Table 1). A herbarium has been kept at the Laboratory OLMAN-BGPE.The sampled leaves were washed with distilled water, dried separately in the shade in a dry and ventilated place at room temperature for 15 days until their weight stabilized. A part of these leaves was ground using an electric mill and then sieved in order to obtain a fine vegetable powder, which will be used later for the preparation of aqueous (decocted, macerated and infused) and organic (ethanol,methanol, ethyl acetate, petroleum ether) extracts.

2.2. Preparation of extracts

The infusion was prepared according to the protocol of (SQALLI, 2007) with some modifications, by dissolving 2 g of M. vulgare L. powder in 100 ml boiling distilled water; then, leave to infuse for 6 h; then filtered on a cotton and then on whatman N°1 filter paper. The organic extracts were prepared by maceration of the powder of M. vulgare L. in different organic solvents (methanol; ethyl acetate, petroleum ether, ethanol) with a ratio 10/100 (mass/v), for 24 h at room temperature using a magnetic stirrer. The extracts were filtered and then dried under reduced pressure in a rotary evaporator (Bushi) at a temperature of 60 °C and finally, kept at a temperature of 4 °C in dark bottles.

2.3. Phytochemical screening

The phytochemical screening of the different M.vulgare L. obtained extracts was carried out to ensure the presence of certain chemical families; it was determined by solubility tests, color reactions by characteristic reagents and precipitation. They are carried out on the 2% aqueous and organic extracts. The alkaloids were highlighted by the reagents of Mayer, Dragendorff (Bammou et al., 2015) and by the Reagent of Wagner (Ali et al., 2012), the catechic and gallic tannins by ferric chloride (Karumi et al., 2004), terpenes and sterols by the reaction of Liebermann (Bekro et al., 2008), saponins were determined based on their foam-forming abilities (Mojab et al., 2003),mucilage by the addition of absolute ethanol (Karumi et al., 2004), coumarins by the addition of a few ml of NaOH (Bekro et al., 2008), polyphenolic substances by $FeCl_3$ (Yeo et al., 2011),and the revelation of flavonoids by the reaction with cyanidine (Koffi N'GUESSAN et al., 2009).

2.4. Determination of total polyphenols

The determination of total polyphenols of M. vulgare L. leaves extracts was performed with the Folin-Ciocalteu (FC) reagent according to the method of (Dif et al., 2015) and (Kon, 2009) with some modifications. From a gallic acid stock solution (0.5 g/l), a standard range of methanolic solutions has been prepared (0–200 µg/ml).200 µl plant extract is mixed with 1 ml of the FC reagent (10%), after 20 min incubation in the dark,800 µl Na2CO3 (7.5% (w/v)) is added. The mixture is stirred and incubated in the dark room at temperature for 3 h and the absorbance is measured at 765 nm by a UV spectrophotometer (PerkinElmer). The results are expressed in µg gallic acid equivalent/mg dry plant matter by reference to the calibration curve of gallic acid.

2.5. Determination of total flavonoids

The determination of total flavonoids of M.vulgare L. leaves extracts was determined according to the method described by (MAHMOUDI Souhilaa et al., 2013) with some modifications.

500 µl of each leaves extract is added to 1500 µl of methanol (95 %), 100 µl AlCl3 (10 % (m/v)), 100 µl sodium acetate (1 M) and 2.8 ml distilled water. The mixture is stirred and incubated in the dark room at temperature for 1 h. The absorbance is measured at 415 nm using a UV spectrophotometer (PerkinElmer). The results are expressed in µg quercetin equivalent/mg dry plant matter with reference to the quercetin calibration curve.

2.6. Determination of total catechins tannin

Condensed tannins of *Marrubium vulgare* L. leaves extracts were determined using the vanillin assay described by (Khlifi et al., 2011) and (Belyagoubi-benhammou et al., 2014) with some modifications. To 50 µL

Table 1. Geographical location of sampling sites.

Sites	Location (GPS)	Altitude (mm)	Bioclimat	Rainfall (mm/year)	T° (°C)	Humidity (%)
Oulad Daoud Zkhanine	34°57′51.7″N 2°30′13.7″W	434	Mediterranean	300–400	26.5	57
Cape Three Forks	35°26′18.0″N 2°58′28.0″W	390	semi-arid	250	18	73

of such extract, 1500 µL of vanillin/methanol (4%) solution was added and mixed. Then, 750 µL of concentrated HCl was added and allowed to react at room temperature for 1 h. The absorbance at 550 nm was measured against a blank. The total concentration of condensed tannins was expressed in micrograms of catechin equivalents per milligram dry matter with reference to the catechin calibration curve.

2.7. Antioxidant activity

2.7.1. DPPH radical scavenging activity

Antioxidant activity was assessed by measuring the scavenging power of the DPPH radical. The DPPH test is carried out according to the method described by (Bouterfas et al., 2016) and (Laib, 2012) with certain modifications. 2 ml of the methanolic solutions of leaves extracts of M.vulgare L. prepared from a stock solution (10 mg/ml) at different concentrations (200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml and 1000 µg/ml); are mixed with 2 ml of a methanolic solution of DPPH (0.006%). After 30 min, the absorbance was read at 517 nm against a blank using UV spectrophotometer. The percentage of inhibition of DPPH was calculated as Eq. (1):

$$\% \text{ Inhibition} = [(Abs_{control}\text{-}Abs_{test})/Abs_{test}]*100 \quad (1)$$

The results were expressed as IC_{50}. The lower IC_{50} value is an indication of a more potent antioxidant activity.

2.7.2. ABTS radical scavenging activity

The ABTS test was evaluated according to (Heba Abdel-Hady1 et al., 2018) with some modifications. We prepare two solutions: the ABTS solution (7mM) and the potassium persulfate ($K_2S_2O_8$) solution (2.45mM), the $ABTS^{+}$ cation radical solution was generated by mixing the two solutions at a ratio of (1:0.1).The stock solution was kept in the dark room for 12–16 h, and then diluted to an absorbance of 0.70 ± 0.02 using a spectrophotometer at 734 nm. Then, 200 µl of M.vulgare L. extract at different concentrations (200–1000 µg/ml) was added to 1ml of the prepared solution, and their absorbances were measured after 3 min at 734 nm against a blank. The ABTS scavinging percentage was calculated as Eq. (2):

$$\text{Scanning activity } \% = [(Abs_{Control} - Abs_{sample}) / Abs_{Control}] \times 100 \quad (2)$$

The results were expressed as IC_{50}. The lower IC_{50} value is an indication of a more potent antioxidant activity.

2.8. Analysis by FTIR

To study the chemical composition of leaves extracts of *Marrubium vulgare* L harvested from two different geographical areas, the different extracts were scanned in the wavelength range of 8,300 - 350 cm^{-1} with a resolution of 0.5 cm^{-1} using Spectrum Two FTIR spectrometers PerkinElmer and characteristic peaks and their functional groups were detected. FTIR peak values were recorded. Each analysis was repeated three times for spectrum confirmation.

3. Results

3.1. Phytochemical screening

The phytochemical screening shows that the organic extracts of *Marrubium vulgare* L. of Oulad Daoud Zkhanine have a very high content of catechic tannins, polyphenols and flavonoids, a moderate presence of mucilage and resin and a total absence of alkaloids, gallic tannins, coumarin and saponins. On the other hand, the extracts of M.vulgare L. of the Cap Three Forks reveal a very high content of terpenoids, and an average content of catechic tannins, polyphenols, resin and flavonoids, the other metabolites are absent (Table 2). In general, in the two sampling localities, the organic extracts represent a very high contents comparing to the aqueous extract.

3.2. Total polyphenol (TPC), flavonoid (TFC) and tannin (CTC)

Based on the absorbance values of the various extract solutions, and compared to the equivalent standard solution as described above, the results of the determination of total polyphenols, total flavonoids and total Catechic tannins are summarized in (Table 3). From these results, we can conclude that the polyphenol, flavonoid and tannin content varies according to the, geographical (or geomorphological, altitude, latitude, type of relief) and climatic conditions (temperature, rainfall, humidity) of the locality where the leaves are collected, and the type of extract (organic or aqueous) and the polarity of the solvents.

In fact, M.vulgare L. collected from Oulad Daoud Zkhanine had higher polyphenols, flavonoids and tannins contents than the one collected from the Cape Three Forks. For the both locations, the polyphenol content obtained by the organic extracts was higher than that obtained by the aqueous extract, whose content does not exceed 5.45 ± 0.08 µg EAG/mg. The methanol extract showed the highest polyphenol content with a concentration of 86.91 ± 1.22 µg EAG/mg followed by ethanol, ethyl acetate and petroleum ether extracts (Table 3).Methanol extract was rich in flavonoids with a content of 33.82 ± 0.90 µg EQ/mg followed by ethanol extracts and ethyl acetate extracts, while petroleum ether extracts had lower levels. The flavonoid levels observed in the aqueous extracts were lower compared to that obtained for the organic extracts with a maximum content of 11.74 ± 0.47µg EQ/mg (Table 3).

Table 2. Phytochemical screening results of M.vulgare L. extracts.

Sites	Extract	Chemical compound									
		Alc	TG	TC	Tp	Mc	Coum	Pol	Rs	Sp	Flv
C.T.F	**EAq**	-	-	+	+	-	-	+	+	-	++
	EEp	-	-	+	++	-	-	++	+	-	+
	EEth	-	-	++	++	-	-	++	+	-	++
	EMet	-	-	++	++	-	-	+++	+	-	+++
	EAce	-	-	+++	++	-	-	++	-	-	++
O.D.Z	**EAq**	-	-	+	-	+	-	+	+	-	+
	EEp	-	-	++	-	+	-	++	+	-	++
	EEth	-	-	+++	-	++	-	+++	+	-	+++
	EMet	-	-	+++		++	-	+++	+	-	+++
	EAce	-	-	+++		++	-	+++	+	-	+++

ODZ: Oulad Daoud Zkhanine, **CTF**: Cape Three Forks, **EAq**: aqueous extract, **EEp**: petroleum ether extract, **EEth**: Ethanol extract, **EMet**: Methanol Extract, **EAce**: Ethyl acetate extract,**Alc**: Alkaloids,**TG**: gallic tannins, **TC**: catechic tannins, **Tp**: Terpenoids,**Mc**: Mucilage, **Coum**: Coumarine, **Pol**: Polyphenols, **Rs**: Resin, **Sp**: Saponins, **Flv**: Flavonoides, (-): absence of compound; (+):low compound content (++): medium compound content; (+++): High compound content.

Table 3. Total Polyphenols, Flavonoids and Catechin Tannins content of the Aqueous and organic extracts of M.vulgare L.

Sites	Extract	Flavonoid (µg QE/mg E)	Polyphenol (µg GAE/mg)	Tannin (µg CE/mg E)
ODZ	Petroleum ether	17,06 ± 0,23^{f}	20,6 ± 0,08^{e}	128,24 ± 4,06^{e}
	Ethyl acetate	19 ± 0,23^{g}	23,99 ± 0,83^{f}	252,68 ± 4,50^{h}
	Methanol	33,82 ± 0,90^{i}	86,91 ± 1,22^{h}	108,95 ± 2,29^{c}
	Ethanol	24,59 ± 0,23^{h}	35,41 ± 0,99^{g}	147,46 ± 1,49^{f}
	Aqueous	11,74 ± 0,47^{e}	5,45 ± 0,08^{b}	6,94 ± 0,12^{a}
CTF	Petroleum ether	6,79 ± 0,40^{b}	13,75 ± 0,56^{c}	115,17 ± 2,95^{d}
	Ethyl acetate	7,52 ± 0,23^{c}	19,01 ± 0,46^{d}	230,93 ± 5,66^{g}
	Methanol	10,59 ± 0,11^{d}	24,77 ± 1,36^{f}	125,45 ± 2,29^{e}
	Ethanol	9,95 ± 0,17^{d}	17,6 ± 1,46^{d}	28,98 ± 1,15^{b}
	Aqueous	6,08 ± 0,17^{a}	0,27 ± 0.1^{a}	2,73 ± 1,15^{a}

The values represent the means of three measurements ±standard deviation. Values in the same column with the same letters are not significantly different at $P < 0,05$.

Aqueous extracts present the lowest tannin levels with a concentration of 6.94 ± 0.12 µg EC/mg. For the organic extracts, the ethyl acetate extract had the highest tannin content with a concentration of 252.68 ± 4.50 µg EC/mg, followed by ethanol and then the petroleum ether and methanol extracts (Table 3).

3.3. Antioxidant activity

According to the presented results in the table below (Table 4), the organic and aqueous extracts of *Marrubium vulgare* L. of Oulad Daoud Zkhanin recorded the highest values of antioxidant activity compared to those of Cape Three Forks. For the both localities and among the organic and aqueous extracts tested, ethanol was the most active with a maximum IC_{50} of 324.55 ± 0.66 µg mL-1, for DPPH and of the order of 107.85 ± 0.19 µg mL^{-1} for ABTS followed by methanol with a maximum IC_{50} of 333.58 ± 0.57 µg mL^{-1} for DPPH and 213.8 ± 0.17 µg mL^{-1} for ABTS and ethyl Acetate with a maximum value of 449.21 ± 0.48 µg mL^{-1} for DPPH and 342.35 ± 0.17 µg mL^{-1} for ABTS, followed by the aqueous extract with a maximum of 752.43 ± 0.45 µg mL^{-1} for the DPPH and 658.56 ± 0.19 µg mL^{-1} for the ABTS. The extract prepared with petroleum ether, had fairly low activity with a very high IC_{50}.

3.4. Phytochemical analysis by the FTIR technique

The FTIR spectrum was used to identify the functional group of active components based on the peak value in the infrared radiation region. The results of the most dominant FTIR peak values and functional groups were represented in (Table 5) based on the work of (Movasaghi et al., 2016; Chandra et al., 2017; Kumar and Prasad, 2011). The profile of the FTIR spectra of the different organic and aqueous extracts of *Marrubium vulgare* L. collected from Oulad Daoud zkhanine and the Cap of Three Forks was illustrated in (Figure 1 and Figure 2).

a. Hydrolat

The hydrolat had a characteristic band at 3589 cm^{-1},3246 cm^{-1} characterizing the stretching of (N–H),a band at 1637.8 cm^{-1} for (C=C uracil, C=O) and at 1630.3 cm^{-1} – 700 cm^{-1} for the amide I region, the bands from 707.1 cm^{-1} to 600.1 cm^{-1} characteristic out-of-plane bending CH vibrations, and bands from 591 cm^{-1} to 506.6 cm^{-1} characteristic ring stretching vibrations strongly mixed with CH in the bending plane.

b. Methanol

The methanol extract of M.vulgare L. showed characteristic absorption bands at 3324.8 cm^{-1} and 3309.2 cm^{-1} for N–H amide A,stretching vibrations bands between 2943.7 cm^{-1} and 2832.7 cm^{-1} were assigned for C–H stretching vibrations, characteristic bands of amide I between 1659.2 cm^{-1} and 1610. 8 cm^{-1}, a band at 1562.8 cm^{-1} which characterizes the region of amide II,bands between 1449.2 cm^{-1} and 1413.3 cm^{-1} have been assigned for asymmetric CH3 bending of the methyl groups of the proteins,as well as bands between 1248 cm^{-1} and 1242 cm^{-1} which are characteristic of amide III.

c. Ethyl acetate

Ethyl acetate extract showed characteristic absorption bands at 2984.6 cm^{-1} and 2943.1 cm^{-1} for O–H stretching, at 1737 cm^{-1} for C=O stretching, and absorption bands between 1479.1 cm^{-1} and 607.7 cm^{-1} are characteristic of the amide II region and bands from 536.9 cm^{-1} to 503 cm^{-1} representing CH vibration.

a. Hydrolat

The hydrolat of M.vulgare L. harvested from Cap des Three Forks showed characteristic absorption bands at 3339 cm^{-1} and 3245.9 cm^{-1}

Table 4. Inhibitory concentration 50 (IC_{50}) values for DPPH and ABTS scavenging activities.

Site	Extract	IC50 DPPH (µg/ml)	IC 50 ABTS (µg/ml)
ODZ	Petroleum ether	787.52 ± 0.91	646.53 ± 0.18
	Ethyl Acetate	449.21 ± 0.48	342.35 ± 0.17
	Methanol	333.58 ± 0.57	213.8 ± 0.17
	Ethanol	324.55 ± 0.66	107.85 ± 0.19
	Aqueous	623.15 ± 0.32	489.23 ± 0.12
CTF	Petroleum ether	980 ± 0.62	890.74 ± 0.17
	Ethyl Acetate	507.40 ± 0.65	474.19 ± 0.12
	Methanol	493.75 ± 0.47	325.35 ± 0.16
	Ethanol	431.81 ± 0.51	227.55 ± 0.18
	Aqueous	752.43 ± 0.45	658.56 ± 0.19

Table 5. General band assignments of the FTIR spectra of the different organic and aqueous extracts of M.vulgare L.

Peak	Assignment
472/5 cm-1	Cα = Cα′ torsion and C–OH3 torsion of methoxy group
521 cm-1	Cα = Cα′ torsion and ring torsion of phenyl
600–900 cm–1	CH out-of-plane bending vibrations
700–1000 cm-1	Out-of-plane bending vibrations
900–1300 cm-1	Phosphodiester region
1000–140 cm-1	Protein amide I absorption
1000–200 cm-1	C–OH bonds in oligosaccharides such as mannose & galactose
1000–350 cm-1	Region of the phosphate vibration carbohydrate residues attached to collagen and amide III vibration (in collagen)
1020–50 cm-1	Glycogen
1030 cm-1	Collagen
1105 cm-1	Carbohydrates
1145 cm-1	Phosphate & oligosaccharides
1180–300 cm-1	Amide III band region
1206 cm-1	Amide III Collagen
1244/5 cm-1	PO–2 asymmetric (phosphate I)
1250-400 cm-1	CH2 wagging vibration of the acyl chains (phospholipids)
1255 cm–1	Amide III
1312–1317 cm–1	Amide III band components of proteins collagen
1400-500 cm-1	Ring stretching vibrations mixed strongly with CH in-plane bending
1456 cm–1	CH3 bending vibration (lipids and proteins)
1480–600 cm-1	The region of the amide II band in tissue proteins. Amide II mainly stems from the C–N stretching and C–N–H bending vibrations weakly coupled to the C=O stretching mode
1482 cm–1	Benzene
1504 cm–1	In-plane CH bending vibration from the phenyl rings
1540–650 cm-1	Amide II
1630–700 cm-1	Amide I region
2800–3000 cm–1	C–H Lipid region
3000 cm-1	CH stretching vibrations (remain unaltered by the methoxy and hydroxyl substitution)
3000–600 cm-1	N–H stretching
3000–700 cm-1	O–H stretching (water)
3500–600 cm–1	OH bonds
3506 cm-1	OH stretching (free)
3524/28/42 cm-1	Stretching O–H
3570/77/78/82/90/9 cm-1	Stretching O–H
3611 cm-1	O–H & N–H stretching vibrations

for asymmetrical N–H stretching, a band at 1637.6 cm^{-1} for O–H deformation, the band at 1630.8 cm^{-1} for Amide I: +-sheet,bands from 707.1 cm^{-1} to 600.1 cm^{-1} characteristic out-of-plane bending CH vibrations, and bands from 591 cm^{-1} to 506.6 cm^{-1} characteristic ring stretching vibrations strongly mixed with CH in the bending plane.

b. Methanol

The methanol extract of M.vulgare L. showed characteristic absorption bands at 3325 cm^{-1} for the amide A, bands come from the N–H stretching modes in proteins and nucleic acids, bands at 2918 cm^{-1}, 2850.2 cm^{-1} and 2834.2 cm^{-1} have been assigned for C–H stretching vibrations, the band at 1449.8 cm^{-1} has been assigned to the asymmetric CH3 bending of the methyl groups of the proteins, the band at 1420.1 cm^{-1} characterizes amide II,the band at 1377.2 cm^{-1} was attributed to C–H deformation, the bands from 1377.2 cm^{-1} to 1022.7 cm^{-1} were attributed to asymmetrical and symmetrical stretching vibrations of PO^{-2} and phospholipids, the bands from 630.8 cm^{-1} to 605.8 cm^{-1} were attributed to out-of-plane bending of CH vibrations. The ones from 596.2 cm^{-1} to 504.4 cm^{-1} were attributed to ring stretching vibrations strongly mixed with in-plane bending of CH vibrations.

c. Ethyl acetate

The ethyl acetate extract of M.vulgare L. showed a characteristic absorption band at 2984.1 cm^{-1} that is due to N–H stretching. The bands at about 1737.1 cm^{-1} was attributed to C=O stretching vibration, at 1373.1 cm^{-1} attributed to C–N stretching, the bands from 1300.7 cm^{-1} to 1097. 7 cm^{-1} have been attributed to the asymmetric and symmetric stretching vibrations of PO^{-2} and phospholipids, the band at 1043.7 cm^{-1} represents the mode of stretching of C–O–C of nucleic acids and phospholipids and the bands between 938.3 cm^{-1} and 462.3 cm^{-1} have been attributed to the absorption of the Amide III region.

4. Discussion

The phytochemical composition of *Marrubium vulgare* L. depends both on the geographical (geomorphologic, altitude, latitude, type of relief), climatic conditions (temperature, rainfall, humidity), the type of soil of the locality where the leaves are collected:the soils of Ouled Daoud Zghanin come largely from the limestone and marl geological source rocks; whereas the soils of Cap Three Forks come essentially from a geological substratum of volcano-sedimentary type; according to the

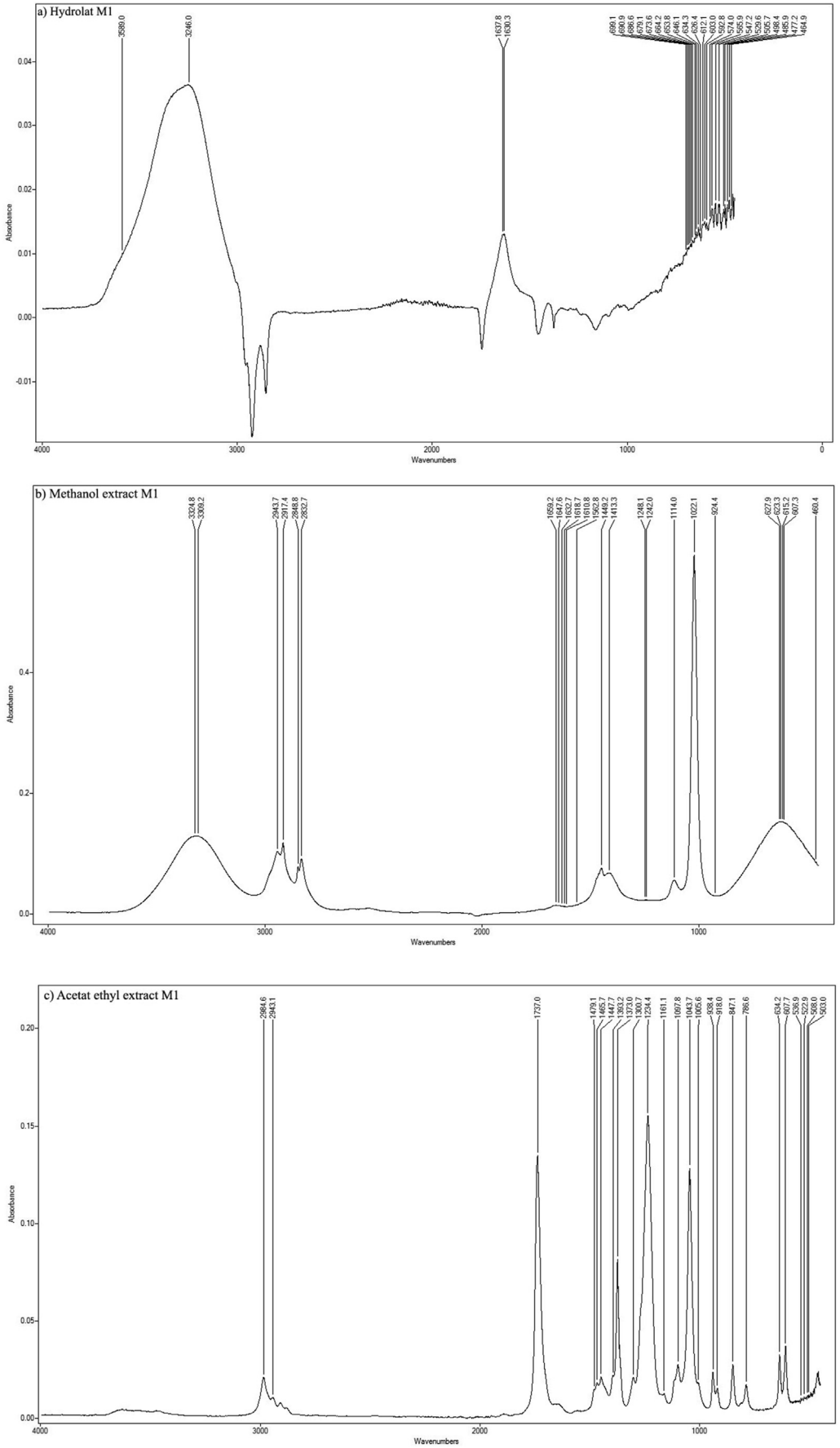

Figure 1. FTIR spectrum of the different extracts of M.vulgare L growing in Oulad Daoud Zkhanine, (a): Hydrolat, (b): methanol extract, (c): acetate ethyl extract.

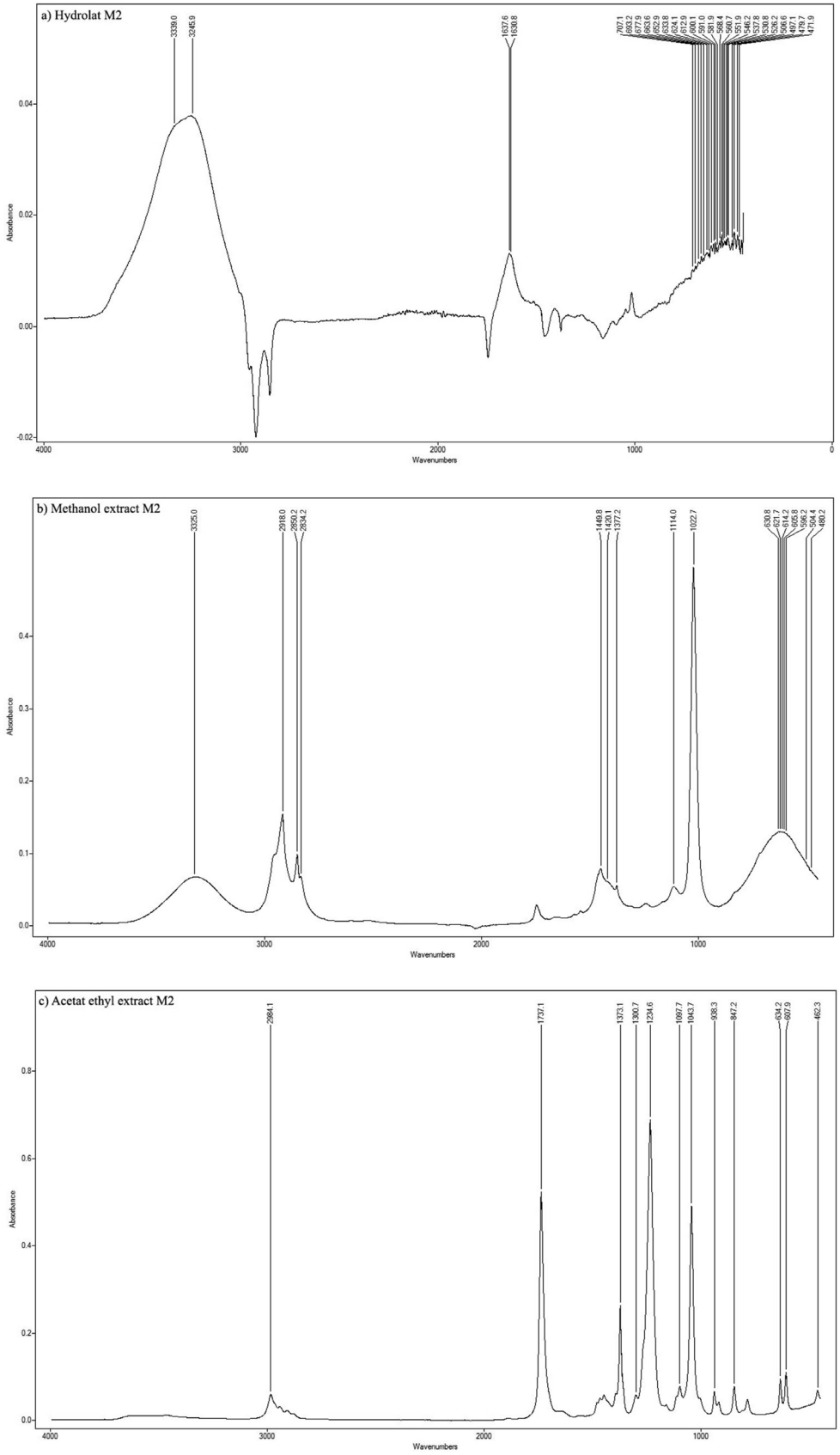

Figure 2. FTIR spectrum of the different extracts of *Marrubium vulgare* L growing in Cap Three Forks (CTF), (a): Hydrolat, (b): methanol extract, (c): acetate ethyl extract.

results obtained, the clay-silt soil of oulad daoud zkhanin is the best soil for the occurrence and growth of M.vulagre L., this is due to the fact that this type of soil is rich and fertile, permeable to water and air, with a high water reserve due to its high silt content and good distribution between microporosity and macroporosity, this water is largely available to the plants, absorbed into the soil by the roots and conducted to all parts of the plant, until it reaches the leaves, where the greatest biochemical reactions and the production of metabolites are achieved; and depends of the extract type (organic or aqueous) and the polarity of the solvents. Indeed, the phytochemical screening shows that the extracts of M. vulgare L. of Oulad Daoud Zkhanine have a very high content of important secondary metabolites such as tannins, flavonoids and polyphenols compared to the extracts of Cap Three Forks. These changes are explained by the climatic and edaphic differences that characterize each geographical region (Connan et al., 2007).Regarding the method of extract preparation, organic extracts maceration always represents the highest content of chemical metabolites compared to infusion. Those results are due of the fact that maceration can accelerates the extraction process and minimizes the contact time of the solvent with the extract while preserving the bioactivity of its constituents. In the same way, this extraction process at room temperature, as well as the exhaustion of the solvent at reduced pressure, allows to obtain the maximum number of compounds and to prevent their denaturation or probable modifications due to the used high temperatures in other extraction methods (Bouterfas et al., 2014).

The variation in the determination of polyphenols, flavonoids and tannins can be explained by the fact that the content of phenolic compounds is influenced by different parameters such as geographical, and climatic conditions of the locality where the leaves are collected, extraction method, solubility and type of solvent used (Naczk and Shahidi, 2004). Indeed, the best extraction rates are obtained from the leaves collected from Oulad Daoud Zkhanine compared to those collected from the Cap Three Forks.

Regarding the nature of the extracting solvent, for the determination of polyphenols and flavonoids of M.vulgare L. of the two sampling localities, methanol always contains the highest yield rates, followed respectively by ethanol and ethyl acetate, while the lowest extraction yields are recorded in petroleum ether. For the dosage of tannins, ethyl acetate shows the highest concentrations followed by ethanol, petroleum ether while methanol gives the lowest concentrations. Those differences can be explained by the polarities of the different compounds present in the leaves of M.vulgare L., such differences have been reported in the literature by (Jayaprakasha et al., 2001), and can be explained by the nature of methanol, which is more polar than the other used solvents and characterized by good solubility for the compounds, thus making it possible to extract many active principles belonging to several chemical classes such as alkaloids, tannins, amino acids (L. R. Snyder et al., 2009), in addition to heteroside flavonoids.

The antioxidant activity of the extracts was proportional to the polarity of the extracting used solvents and was in accordance with the obtained results for the dosage of polyphenol and flavonoids. The prepared extracts by the polar solvent such as ethanol and methanol present the most important results according to the ABTS and DPPH test, followed by those prepared extracts by the moderately polar solvents like ethyl acetate and the petroleum ether lowly polar solvent present the lowest antioxidant activity.

According to (Zohra, 2013) and (Mandadi et al., 2007), those results can be explained by the fact that the antioxidant capacities are directly related to the secondary metabolites present in each extract and fraction depend on the antioxidant substances, their nature, quantity, structure and any molecular interactions that may act synergistically to enhance this activity.

Other studies done by (Gheldof and Engeseth, 2002; Holasova et al., 2002; Kumaran et al., 2007) shows that there is a linear correlation between total polyphenols and antioxidant activity and that most of this activity is due mainly to the presence of polyphenols because they are one of the most effective antioxidant constituents of the plant and effective donors of hydrogen to the DPPH radical, because of their ideal structural chemistry (Turkmen et al., 2007), plus of the polyphenols the other minor phenolic compounds should not be neglected, because the synergy between the different chemicals should be taken into account in the biological activity (Bourgou et al., 2008). Indeed, not only phenolic compounds which are antioxidant substances par excellence but other non-phenolic substances which can be more effective and powerful antioxidants (Zohra, 2013).

Fourier Transform Infrared Spectroscopy (FTIR) is a non-destructive characterization technique that uses infrared radiation to irradiate the sample, and depending on the chemical composition of the sample, the absorbed radiation gives a specific spectrum. The FTIR spectrum results obtained in this study demonstrated absorption signals for multiples wavenumber ranges, which were identified in the Hydrolat, methanol, acetate ethyl extract functional group composition of alcohol and phenols (O–H), carboxylic acids (C–O stretching), methyl and aldehyde group (stretching of C–H bonds), C=O stretching (aldehyde group), alkenes (C=C stretching), amines and amides (N–H bending), nitro (N=O), and aromatics (C–C stretching). The obtained spectrum constitutes the fingerprint of a product, highlighting in the form of characteristic peaks (or bands) of the various chemical bonds and organic groups present in the studied extracts (Nadjib BOUKHATEM et al., 2010; Naumann et al., 2010; Thummajitsakul et al., 2020; Boughendjioua et al., 2017); Mehenni et al. (2016).

5. Conclusion

The study that we conducted on the leaves of *Marrubium vulgare* L. highlighted the impact of geographical and climatic conditions as well as the type of extracting solvent on the nature and content of the chemical components contained in those leaves.

In qualitative terms, several chemical groups were identified: catechic tannins, terpenoids, polyphenols, flavonoids, phenolic acids, alcohols and amides.

The quantitative study of these identified components reveals more or less variable proportions from one sampling site to the other, with high levels of total polyphenols, total flavonoids and tannins contained in M. vulgare L. sampled from both localities.

Evaluation of the antioxidant activity of the various plant extracts of the two localities shows a high scavenging potential of M. vulgare L. in relation to the chemical composition and contents of total polyphenols and minor phenolic compounds.

In fact, the different extracts of M. vulgare harvested from the two study areas (Oulad Daoud Zkhanine and Cap Three Forks) could be considered as a potential source of natural antioxidants that could be used in the food and pharmaceutical fields.

Declarations

Author contribution statement

JAADAN Hayat: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

AKODAD Mustapha, BELMALHA Saadia: Conceived and designed the experiments.

BAGHOUR Mourad, SKALLI Ali, MOUMEN Abdelmajid: Contributed reagents, materials, analysis tools or data.

EZRARI Said: Analyzed and interpreted the data.

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Data availability statement

Data included in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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