



## Phytochemical Investigation of *Thymus zygis* L. and *Salvia officinalis* L. Collected from Fez-Meknes Region, Morocco

Asmae Benabderrahmane<sup>(1, 2)</sup>, Majid Atmani<sup>(1)</sup>, Abdellatif Boutagayout<sup>(2, 3)</sup>,  
Wijdane Rhioui<sup>(1, 2)</sup>, Saadia Belmalha<sup>(2)</sup>

<sup>(1)</sup>Laboratory of Functional Ecology and Environmental Engineering, Faculty of Sciences and Techniques, Sidi Mohamed Ben Abdellah University, Fez, Morocco;

<sup>(2)</sup>Department of Plant Protection and Environment, National School of Agriculture, Meknes, Morocco; <sup>(3)</sup>Environment and Soil Microbiology Unit, Faculty of Sciences-Moulay Ismail University, Meknes, Morocco.



CrossMark

**T**HYMUS and *Salvia* are among the most popular plants both in traditional medicine and in the culinary arts. This study has the aims to detect the chemical composition of the extracts and the powder of *Thymus zygis* L. (T) and *Salvia officinalis* L. (S) collected from the Fez-Meknes region. Two extracts were prepared: aqueous and essential oil. Phytochemical tests were performed to qualitatively evaluate the presence or absence of phytoconstituents using standard methods. The essential oils were analyzed by gas chromatography-mass spectrometry (GC/MS). Two powder analyses were performed: Fourier transform infrared spectroscopy (FTIR) analysis and elemental analysis. The drying of both plants took a similar amount of time with a noticeable loss in weight for *Salvia*. The phytochemical screening revealed the abundant presence of terpenoids, catechic tannins, steroids and sterols in the two plants. GC/MS analysis showed richness in carvacrol for *Thymus zygis* L. and in thujone for *Salvia officinalis* L. The analysis by FTIR showed characteristic peak readings of various functional groups in the powders, citing proteins, aliphatic compounds, carbonyl compounds and aromatic rings. In the elemental analysis, there is a high carbon content for *Thymus* and *Salvia* (T: 66.70%, S: 53.34%), followed by oxygen (T: 36.45%, S: 37.88%) and hydrogen (T: 6.08%, S: 5.61%). Altogether, this study highlights the richness of these two species in chemical compounds that can be used in the pharmaceutical industry.

**Keywords:** Elemental analysis, FTIR, Phytochemical screening, *Salvia officinalis* L., *Thymus zygis* L.

### Introduction

Mankind has used various plants found in the environment to treat and cure many diseases. They represent a huge reservoir of primary and secondary metabolites (amino acids, polyphenols, flavonoids) with a wide range of biological activities with or without a direct function in the growing and development of plants (Crozier et al., 2008). Currently, despite the remarkable development of modern medicine, traditional medicines and pharmacopoeia remain a basic

solution in the relief of many health problems (Abouri et al., 2012).

Morocco is a country well-known for its floral diversity due to its geography and climate, and it is one of the countries whose population is accustomed to the use of herbal remedies (Hammada et al., 2004; Msanda et al., 2005).

The *Lamiaceae* family is one of the most represented plant families in popular medicine

#Corresponding author email: [asmae.benabderrahmane@usmba.ac.ma](mailto:asmae.benabderrahmane@usmba.ac.ma)

Received 18/12/2022; Accepted 01/03/2023

DOI: 10.21608/ejbo.2023.181580.2215

Edited by: Dr. Hamdy Zahran, National Research Centre, 12622 Dokki, Cairo, Egypt

©2023 National Information and Documentation Center (NIDOC)

in Morocco (Abouri et al., 2012; El-Ghazouani et al., 2021). It is full of valuable aromatic plants used in both folk and contemporary medicine, and they represent an important part of the food and pharmaceutical industries. The most popular plants of this family are used for the treatment of gastro-colitis, dermatitis, respiratory diseases, and many other infections and inflammations (Nieto, 2017). Particularly, several species of the genus *Thymus* and *Salvia* have been used in many parts of Morocco to fight against various diseases and to contribute to the culinary arts. Parts of these plants are used to make decoctions, infusions, hydro alcoholic extracts, and essential oils (EOs) for oral administration or external application (Bodalska et al., 2021). Essential oils are liquid, and at the same time volatile, clear, and rarely colored. They are soluble in lipids and organic solvents and generally have a low density compared to water (Bakkali et al., 2008). Antimicrobial, antifungal, and antioxidant properties have been attributed to EOs, but the use of the whole plant, powder, or another type of extract seems to have a beneficial impact as well (Hou et al., 2022).

Among the 1000 species of the genus *Salvia* and 215 species of the genus *Thymus*, we found *Thymus zygis* L. and *Salvia officinalis* L. (Khiya et al., 2019; Fakchich & Elachouri, 2021), which have been considered as medicines since antiquity. *Salvia officinalis* L. is mainly used in the oropharyngeal sphere (laryngitis, pharyngitis, and tonsillitis) and pulmonary areas (bronchitis and bronchiolitis). *Thymus zygis* L. is broadly applied for its antiseptic, antispasmodic, carminative, and antitussive properties (Bodalska et al., 2021).

It is important to highlight the presence of secondary metabolites and to prove the biological effects of *Thymus zygis* and *Salvia officinalis* extracts which are the most traditionally used by Moroccan people. For this purpose, the objectives of this study include a qualitative analysis of the aqueous extracts by phytochemical tests, a quantitative analysis of the essential oils of the two plants by GC/MS, and finally, FTIR and elemental analysis of the powder samples to identify the purity of characteristic groups.

## **Materials and Methods**

### *Biological material*

*Thymus zygis* L. and *Salvia officinalis* L. were first gathered in June 2021 in two distinct

localities in the region of Fez-Meknes: El Menzel (*Thymus zygis* L.) and El Haj Kaddour (*Salvia officinalis* L.), with the geographic and climatic characteristics listed in Table 1. Plants were shade-dried separately in a well ventilated dry area at room temperature until weight settled (Nurhaslina et al., 2022). The aerial parts were crushed using a FRITSCH electric mill type PULVERISETTE 11, then sifted to obtain a finely ground vegetable powder.

### *Extract preparation*

#### *Aqueous extract*

The aqueous extracts were prepared according to the protocol of Jaradat et al. (2015), with some modifications. The infusion was done by dissolving about 2g of powder of each plant in 80mL of boiling distilled water and then leaving it to infuse for 24h. The decoction was prepared by mixing 2g of powder of each plant in 80mL of distilled water, and then the mixture was heated on a hot plate at 30-40°C and continuously stirred for 20min. The resulting mix was filtered through Whatman filter paper No.4, and the filtrate was used for subsequent phytochemical analysis (Jaradat et al., 2015).

#### *Essential oil*

Essential oils were prepared using aerial plant material without grinding and subjected to hydro distillation in a Clevenger-type apparatus connected to an essential oil extractor. *Thymus zygis* L. and *Salvia officinalis* L. (100g) were put in a vial and heated for 3 hours using a flask heater at 1kW h (Liu et al., 2022). Essential oils were stored at 4°C in the dark until analyzed (Karahisar et al., 2022). The average yield was obtained after 3 extractions and was expressed as a percentage according to the following formula:

$$\frac{EOw}{PW} \times 100,$$

where, EOw is the weight of the essential oil, and PW is the initial weight of the plants (Cutillas et al., 2018).

### *Preliminary phytochemical tests*

To evaluate the presence or absence of phytoconstituents in *Thymus zygis* L. and *Salvia officinalis* L., some standard tests were performed as listed in Table 2.

**TABLE 1. Characteristics of the harvest locations**

	Sample location	GPS	Altitude (m)	Climat	T (°C)	Humidity (%)	Precipitations (mm)
<i>Thymus zygis</i> L.	El Menzel	33°50'41.6N-4°35'08.7W	809	Hot Mediterranean	17.1	70	468.2
<i>Salvia officinalis</i> L.	El Hajkaddour	33°840738N-5°477390W	663	Hot Mediterranean	17.1	56	511

**TABLE 2. Qualitative tests for preliminary phytochemical screening**

Compounds	Test or reagents	Positive sign
Terpenoids	Salkowski's test (Adusei et al., 2019)	Reddish-brown precipitate
Alkaloids	Hydrochloric acid+ Dragendroff reagent (Fannang et al., 2021)	Orange or red precipitate
Flavonoids	Alkaline reagent test (Jaradat et al., 2015)	Dark yellow coloration
Glycosides	Keller Kilian test (Jaradat et al., 2015)	Red coloration
Catechic tanins	FeCl <sub>3</sub> (1%) (Benzeggouta, 2017).	Greenish coloration
Gallic tanins	FeCl <sub>3</sub> (1%) (Benzeggouta, 2017)	Blackish blue coloration
Condensed tanins	Hydrochloric acid (Benzeggouta, 2017)	Persistent red color
Coumarins	NaOH (Benzeggouta, 2017)	Yellow fluorescence
Saponins	Foam test (Adusei et al., 2019)	Persistent foam with a height ≥1 cm
Organic acid	Bromothymol Blue (Benzeggouta, 2017)	Canary yellow
Steroids	Liebermann Burchard (Fannang et al., 2021)	Red coloration
Unsaturated sterols	Sulfuric acid (Benzeggouta, 2017)	Red coloration

#### GC/MS analysis

GC/MS was executed by gas chromatography type GC/MS-TQ8040 NX (Shimadzu) equipped with a triple quadrupole detector and an apolar Rxi-5MS capillary column (30m x 0.25mm ID x 0.25µm). The carrier gas of the GC/MS was helium. The analysis was carried out by injection of 1µL of essential oil in splitless mode under a pressure of 37.1KPa. The column temperature was programmed to increase from 50 to 250°C at a rate of 4°C/min. The ionization energy was set to 70eV. The ion source temperature was 200°C and the interface temperature was 200°C. The essential oils were diluted with hexane solvent. The compounds were identified based on a comparison of their mass spectra with the 11<sup>th</sup> edition (2017) of the National Institute of Standards and Technologies, Mass Spectra Libraries (NIST) (Zaim et al., 2015; Lemjallad et al., 2019).

#### Fourier transform infrared spectroscopy (FTIR) analysis

Each powdered sample was analyzed in the 450cm<sup>-1</sup> and 4000cm<sup>-1</sup> infrared region by the "Perkin-Elmer LS 55" spectrophotometer, which

was coupled to "PerkinElmer Spectrum TM 10" software that allows for presenting the results as spectra. These were then read and interpreted with the help of the 2021 "OriginLab" software. Each analysis was repeated thrice to confirm the spectrum pics (Jaadan et al., 2020).

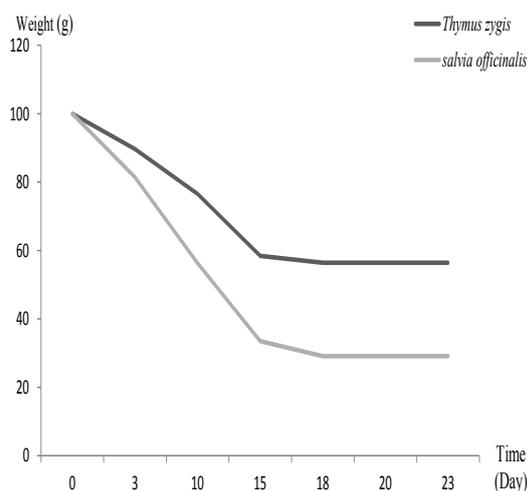
#### Elemental analysis

CHNS/O elemental analysis was used to evaluate the carbon (C), hydrogen (H), nitrogen (N), sulfur (S), and oxygen (O) content of powder samples from two plants (*Thymus zygis* L. and *Salvia officinalis* L.). Organic elemental analysis was performed using a Flash Smart CHNS/O Thermo Fisher Scientific Organic Elemental Analyzer. The CHNS analysis was performed at a furnace temperature of 950°C. The samples were combusted in the presence of 240mL/min oxygen flow, and the gases produced (CO<sub>2</sub>, H<sub>2</sub>O, SO<sub>2</sub> and N<sub>2</sub>) were carried by a constant flow of carrier gas (helium) at 100ml/min and then separated by gas chromatography at an oven temperature of 65°C. Oxygen was determined using a pyrolysis furnace at a temperature of 1050°C with the same helium flow (100mL/min) (Malik et al., 2015).

## Results and Discussion

### Drying process and essential oil yield

The drying of both plants took a similar amount of time: 18 days for *Thymus zygis* L. and 20 days for *Salvia officinalis* L., with more weight loss for *Salvia officinalis* L., (Fig. 1) proving that it has a higher water content.



**Fig. 1. Drying curve of *Salvia officinalis* L. and *Thymus zygis* L.**

The average EO yield of *Thymus zygis* L. and *Salvia officinalis* L. was 1.63 % and 2.2%, respectively. Yield and quality of essential oils are affected by a number of factors including cultivation techniques, plant age, genetic factors, geographical location, soil type, climatic conditions, post-harvest handling practices, and drying methods. Furthermore, the influence of the extraction method and analysis of data compounds can also have an effect (Thamkaew et al., 2020; Tibaldi et al., 2022). Shade drying provides a superior yield of essential oil compared to other high-temperature processes and is considered the best method for drying herbs to preserve the majority of its constituents

(Nurhaslina et al., 2022). The yield of *Thymus zygis* L. obtained remains satisfactory in comparison with the lower yields obtained by Rodrigues et al. (2019) in Portugal (1.2%) and Cutillas et al. (2018) (from 0.4 to 0.8%) and Carrasco et al. (2015) (from 0.2 to 1.5%) in Spain. In Morocco, the study conducted by Bouymajane et al. (2022) in Ifrane gave a lower yield than ours (1.5%). On the other hand, studies of Radi et al. (2021) (yielding 5.25%) in Ifrane and Drioiche et al. (2022) (yielding between 2.68 and 4.12%) in three localities, Tigrigra, Ain Aghbal and Timahdite had notably higher yields.

In this study, *Salvia officinalis* presented a better yield than those obtained in studies carried out in Algeria by Dob et al. (2007) (0.9%) and Adrar et al. (2016) (0.97%) and in Italy by Vergine et al. (2019) (0.7%). However, it remains close to the studies in Tunisia (1.8%), in France (2.05%), in Portugal (2.9%), and in Romania (2.3%) mentioned by Fella et al. (2006). Also, a study by Khiya et al. (2019) showed the highest *Salvia officinalis* EO yield of 4.13%.

### Phytochemical compounds

Phytochemical screening is the conventional method for identifying major families of secondary metabolites. The aqueous extract was used to simulate human consumption in the form of herbal tea to search for beneficial functional groups. The presence of organic acids and starch was detected by decoction, whereas the other chemical groups were identified by infusion. The results shown in Table 3 reveal the abundant presence of terpenoids, catechic tannins, steroids, and sterols in the two plants. Gallic tannins, organic acids, carotenoids, and coumarins were absent in both plants. Condensed tannins were absent in *Thymus zygis* L. and present in moderate traces in *Salvia officinalis* L. Flavonoids were not abundant in the two aqueous extracts.

**TABLE 3. Phytochemical screening of *Thymus zygis* L. and *salvia officinalis* L. aqueous extracts**

	T	Alc	Flv	Glc	Tca	Tg	Tco	Ao	S	Su	Ct	Cm	Sp
<i>Thymus zygis</i> L.	+++	++	+	++	+++	-	-	-	+++	+++	-	-	+
<i>Salvia officinalis</i> L.	+++	+++	+	++	+++	-	++	-	+++	+++	-	-	++

T:terpenoid, Alc: alkaloids, Flv: flavonoids, Glc: glycosides, Tca: catechic tanins, Tco: condensed tanins, Ao: organic acids, S: steroids, Su: unsaturatedsterols, Ct: carotenoids, Cm: coumarins, Sp: saponins. +++: abundant, ++: medium, +: traces, -: absent.

The results of *Salvia officinalis* are consistent with those cited by Khiya et al. (2019) in the Khenifra region, except for gallic tannins (+) and saponins (-). The results of preliminary tests for *Thymus zygis* L. are lacking, but in comparison with other genera, there is variance in the compounds with the presence of some and absence of others (Sayout et al., 2015).

#### GC/MS

GC/MS analysis in Table 4 showed richness in p-cymen-2-ol (carvacrol) for the *Thymus zygis* L. On the other hand, *Salvia officinalis* L. presented richness in thujone. Carvacrol has many bioactive properties including antimicrobial, antitumor, antimutagenic, analgesic, antispasmodic, anti-inflammatory, antiparasitic, antiplatelet, insecticidal and hepatic protection. This monoterpenic phenol isomeric with thymol has shown good results in the treatment of gastrointestinal diseases as well as in the breeding of bees (Husnu Can Baser, 2008; Sharifi-Rad et al., 2018). Thujone is found in many medicinal plants. It is a natural terpenoid with antioxidant, anti-diabetic, and anti-tumorigenic properties. It is also used as a food ingredient and a cosmetic additive (Lee et al., 2020). Several studies have reported neurotoxicity at very high doses (Pelkonen et al., 2013). After carvacrol as a major EO component for *Thymus zygis* L. comes  $\gamma$ -terpinene followed by o-cymen, thymol and linalool. Regarding *Salvia officinalis* L., after thujone there are camphor, eucalyptol, caryophyllene and humulene, respectively.

*Thymus zygis* L. from Portugal presented the same majority of components, but with a dominance of p-cymene. Carvacrol came in third place after thymol (Rodrigues et al., 2019). A review edited by Coimbra et al. (2022a) found that carvacrol was dominant in three samples, followed by thymol. The three other major components of these essential oils are  $\gamma$ -terpinene, p-cymene and camphene (Coimbra et al., 2022a). Another study identified thymol as the main component (43%), with carvacrol and p-cymene as the next most important compounds (Coimbra et al., 2022b). The study on Moroccan *Thymus zygis* L. from the Ifran region edited by Drioiche et al. (2022) also revealed a predominance of carvacrol in the essential oil.

The Algerian *Salvia officinalis* L. has the same compounds as cited but with a prevalence of camphor followed by thujone (Dob et al., 2007). However, Koubaa-Ghorbel et al. (2020) presents eucalyptol as the main component without any mention of thujone. The study done by Tardugno et al. (2018) on *Salvia officinalis* L. essential oil also shows a composition rich in thujone as a major component, followed by camphor and eucalyptol.

This difference in the order of major components can be because of differences in either the natural conditions relative to the plant (soil, climate, etc.) or to the technical conditions before obtaining the essential oil (harvest, drying, etc.) and during its analysis (GC/MS conditions, equipment, etc.).

**TABLE 4. The main chemical components revealed by the GCMS analysis**

	Compounds name	Retention time	Peak area (%)	Molecular formula	Molecular weight
<i>Thymus zygis</i> L.	Carvacrol	19.167	62.81	C <sub>10</sub> H <sub>14</sub> O	150.21
	$\gamma$ -Terpinene	11.591	10.95	C <sub>10</sub> H <sub>16</sub>	136.23
	O-cymene	10.560	11.61	C <sub>10</sub> H <sub>14</sub>	134.21
	Thymol	18.748	5.81	C <sub>10</sub> H <sub>14</sub> O	150.22
	Linalool	12.803	3.67	C <sub>10</sub> H <sub>18</sub> O	154.25
<i>Salvia officinalis</i> L.	Thujone	13.377	30.79	C <sub>10</sub> H <sub>16</sub> O	152.23
	Camphor	14.258	17.48	C <sub>10</sub> H <sub>16</sub> O	152.23
	Eucalyptol	10.805	18.67	C <sub>10</sub> H <sub>18</sub> O	154.24
	Caryophyllene	23.380	7.61	C <sub>15</sub> H <sub>24</sub>	204.35
	Humulene	24.558	4.75	C <sub>15</sub> H <sub>24</sub>	204.35

### Fourier transform infrared spectroscopy (FTIR) analysis

In the single band area (2500-4000 $\text{cm}^{-1}$ ), the broad absorption bands, as shown in Figs. 2 and 3, with peaks between 3300 and 3500 $\text{cm}^{-1}$  indicate a hydrogen bond, which means the presence of a hydrate ( $\text{H}_2\text{O}$ ), a hydroxyl (-OH), an ammonium, or an amino. This wide band is representative of the cellulose components (Sahu et al., 2020). For both plants, two clear peaks reflect the presence of aliphatic compounds in two narrow bands, namely the peaks at 2917 and 2849 $\text{cm}^{-1}$  for thyme, and the peaks at 2919 and 2850 $\text{cm}^{-1}$  for sage. The triple band zone (2000-2500 $\text{cm}^{-1}$ ) contains peaks around 2200  $\text{cm}^{-1}$  (2210.49 and 2190.47 for sage and 2201.29 for thyme) indicating the presence of an absorption band of  $\text{C}\equiv\text{C}$ . In the area of the double band (1500-2000 $\text{cm}^{-1}$ ), peaks such as 1686.54 $\text{cm}^{-1}$  and 1733.83 $\text{cm}^{-1}$  for sage and 1685.38 $\text{cm}^{-1}$  and 1724.34 $\text{cm}^{-1}$  for thyme give information about the presence of carbonyl compounds. In particular, the peaks 1980.2 $\text{cm}^{-1}$  for sage and 1871 $\text{cm}^{-1}$ , 1903 $\text{cm}^{-1}$ , and 1980.7 $\text{cm}^{-1}$

for thyme indicate the presence of active carbonyl groups such as anhydrides, halogenated acids, or halogenated carbonyls or cyclic carbonyls, such as lactones or organic carbonates. Peaks between 1750 and 1700 $\text{cm}^{-1}$  (sage: 1733.83  $\text{cm}^{-1}$  and thyme: 1724.34 $\text{cm}^{-1}$ ) refer to simple carbonyl compounds such as ketones, aldehydes, esters, and carboxyls. All peaks below 1700 $\text{cm}^{-1}$  (sage: 1686.54 $\text{cm}^{-1}$ , 1604.93 $\text{cm}^{-1}$ , and 1516.89 $\text{cm}^{-1}$ ; thyme: 1685.38 $\text{cm}^{-1}$  and 1606.78 $\text{cm}^{-1}$ ) indicate amide or carboxylate functional groups. The high intensities detected at 1606.78 $\text{cm}^{-1}$  (T) and 1604.93 $\text{cm}^{-1}$  (S) indicate the existence of aromatic compounds (aromatic rings), which is corroborated by the presence of numerous low intensities between 1700 and 2000 $\text{cm}^{-1}$  noticed for both plants. The fingerprint area (450-1500 $\text{cm}^{-1}$ ) is a complex area with many bands. It contains a large number of low-intensity peaks, making it difficult to identify individual peaks. Moreover, the peaks detected for both plants have many possible interpretations (Table 5).

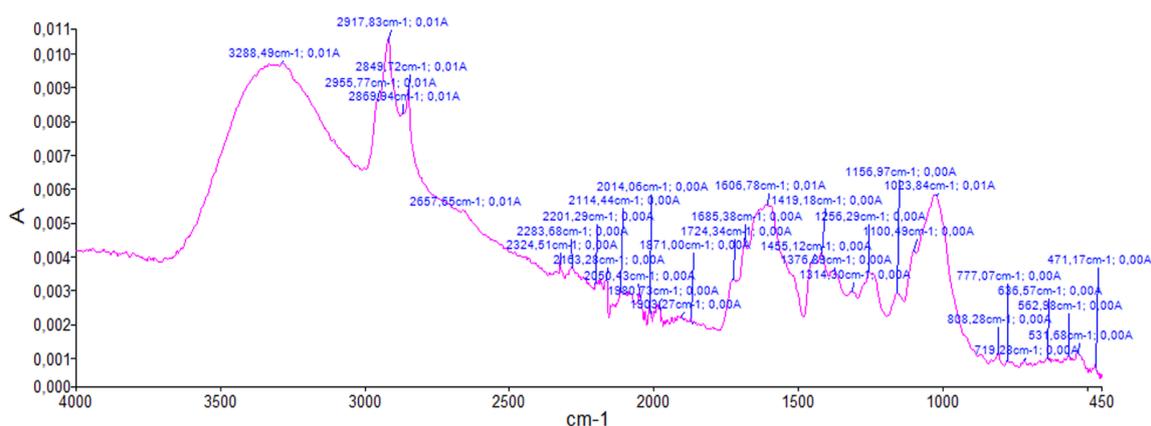


Fig. 2. FTIR spectrum of *Thymus zygis* L.

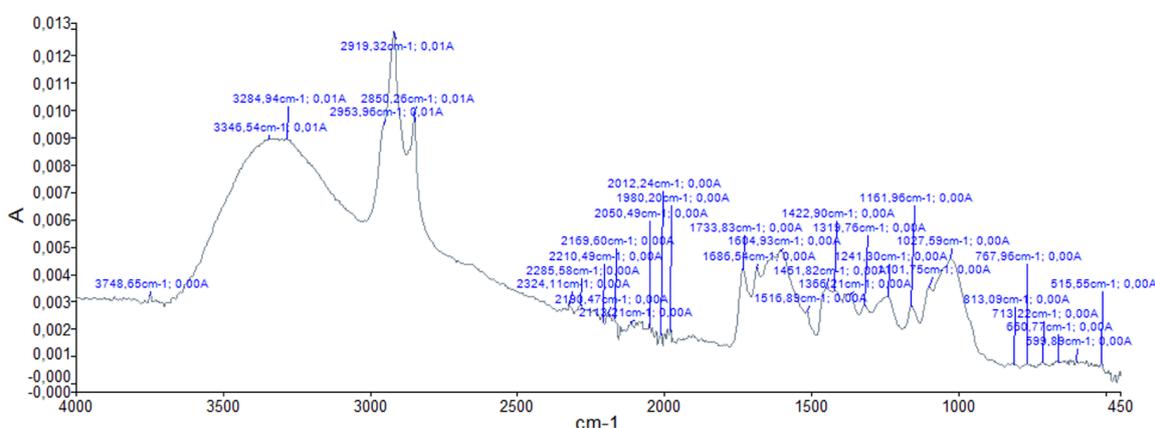


Fig. 3. FTIR spectrum of *Salvia officinalis* L.

**TABLE 5. FTIR peaks, general bands and assignments of the FTIR spectra of *Thymus zygis* and *Salvia officinalis* from references: Coates (2000), Movasaghi et al. (2008), Kumar et al. (2016), and Talari et al. (2016)**

Peaks value (cm <sup>-1</sup> )		Wavenumber range (cm <sup>-1</sup> )	Assignments
<i>Thymus zygis</i> L.	<i>Salvia officinalis</i> L.		
3288.49	3284.94	3300-3200	-N-H str. of proteins (amide A) and O-H stretching (str)
	3346.54	3500-3300	-O-H str. of the hydroxyl group -O-H, N-H, C-H
2955.77	2953.96	2956	-Asymmetric stretching vibration of CH <sub>3</sub> of acyl chains (lipids)
		2960-2955	-Mainly from C-H str. (asym) of CH <sub>3</sub> from lipid acyl chains and also little from proteins
2917.83	2919.32	3000-2890	-C-H str. of methane groups
		2917	-Cholesterol ester
		2917/8/9	-Stretching C-H
2869.94		2875-2868	-C-H str. (sym) of CH <sub>3</sub>
		2870	-CH <sub>3</sub> symmetric stretching: protein side chains, lipids, with some contribution from carbohydrates and nucleic acids
2849.72	2850.26	2855-2845	-C-H str. (sym) of CH <sub>2</sub> from lipid acyl chains
		2850	-C-H stretching bands -vs CH <sub>2</sub> , lipids, fatty acids -CH <sub>2</sub> symmetric
2657.65		2633/678	-Stretching N-H (NH <sub>3</sub> <sup>+</sup> )
2201.29	2210.49	2260-2190	-C≡C Medial alkyne (disubstituted)
	2190.47		
2163.28	2169.6	2175-2140	-Thiocyanate (-SCN)
2114.44	2113.21	2140-2100	-C≡C Terminal alkyne (monosubstituted)
2050.43	2050.49	2150-1990	-Isothiocyanate (-NCS)
2014.06	2012.24	2200-2000	-Cyanide ion, thiocyanate ion, and related ions
1980.73	1980.2	2100-1800	-Transition metalcarbonyls
1903.27			-Active carbonyl groups
		1871	
1724.34	1733.83	1740-1720	-Aldehyde group
		1745-1730	-Ester group (lipid)
		1725- 1745	-C=O stretching band mode of the fatty acid
1685.38	1686.54	1695-1680	-β-turns also β-sheets (anti)
		1685	-Amide I (disordered structure-non-hydrogen bonded) -Amide I β-turns of proteins
1606.78	1604.93	1680-1590	-C = C str
		1605	-vas (COO-) (polysaccharides, pectin)
		1604/1606	-Adenine vibration in DNA

TABLE. 5. Cont.

Peaks value (cm <sup>-1</sup> )		Wavenumber range (cm <sup>-1</sup> )	Assignments
<i>Thymus zygis</i> L.	<i>Salvia officinalis</i> L.		
	1516.89	1519-1514	-CC and C-H vibration from Tyrosine
		1517	-Amide II
1455.12	1451.82	1451	-Asymmetric CH <sub>3</sub> bending modes of the methyl groups of proteins
		1455/6	-δ[(CH <sub>3</sub> )] asymmetric
			-Δ <sub>as</sub> CH and δ <sub>as</sub> CH <sub>2</sub>
		1430-1470	-Methyl C-H asym/sym bend
	1422.9	1470–	-Methyl C-H asym./sym. Bend
		1430/1380–	
1419.18		1370	-vs(COO-) (polysaccharides, pectin
		1419	
		1410-1420	-vinyl C-H in plane bend
1376.89		1370-1420	-Organic sulfate
	1366.21	1340-1365	-Sulfonate
		1365-1370	-Gem-dimethyl or iso- (doublet)
1314.3	1319.76	1314	-Amide III (protein), νP=O (lipid, nucleic acid)
		1300-1335	-Dialkyl/aryl sulfones
		1310-1360	-Aromatic tertiary amine, CN stretch
1256.29	1241.3	1256	-PO <sub>2</sub> <sup>-</sup> asymmetric (Phosphate I)
		1270-1220	-PO <sub>2</sub> <sup>-</sup> antisymmetric str
		1241	-PO <sub>2</sub> <sup>-</sup> asymmetric (Phosphate I)
			-Phosphate band
			(phosphate stretching modes originate from the phosphodiester groups of nucleic acids and suggest an increase in the nucleic acids in the malignant tissues)(Generally, the PO <sub>2</sub> <sup>-</sup> groups of phospholipids do not contribute to these bands)
			-Phosphate stretching bands from phosphodiester groups of cellular nucleic acids
			-vas Phosphate
			-Amide III (protein), νP=O (lipid, nucleic acid)
1156.97	1161.96	1200-900	-C-O, C-C str., C-O-H, C-O-C def (carbohydrate)
		1130-1190	-Secondary amine –CH stretch
		1161/2	-Mainly from the C-O stretching mode of C-OH groups of serine, threonine & tyrosine of proteins)
			-ν(CC), δ(COH), ν(CO) stretching
		1162	-Stretching modes of the C-OH groups of serine, threonine, and tyrosine residues of cellular proteins
			-δ(C-O-C), ring (polysaccharides, cellulose)

TABLE. 5. Cont.

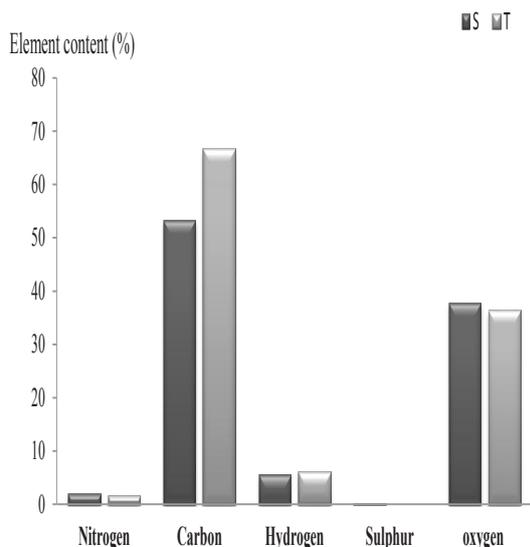
Peaks value (cm <sup>-1</sup> )		Wavenumber range (cm <sup>-1</sup> )	Assignments
<i>Thymus zygis</i> L.	<i>Salvia officinalis</i> L.		
1100.49	1101.75	1200-900	-C-O, C-C str., C-O-H, C-O-C def (carbohydrate)
		1101	-Methyl-carbon stretching (polypeptides)
1023.84	1027.59	1200-900	-C-O, C-C str., C-O-H, C-O-C def (carbohydrate)
		1000-1100	-Phosphate ion
		1028	-Glycogen absorption due to C-O and C-C -stretching and C-O-H deformation motions
808.28	813.09	700-1000	-Out-of-plane bending vibrations
		813	-Ring CH deformation
777.07	767.96	700-1000	-Out-of-plane bending vibrations
719.28	713.22	700-1000	-Out-of-plane bending vibrations
		700-1300	-Skeletal C-C vibrations
		670-900	-Aromatic C-H out of plane bend (several)
		670-715	-Aryl thioether (C-S stretch)
636.57	660.77	600-900	-CH out-of-plane bending vibrations
		630-660	-Thioethers, CH <sub>3</sub> -S- (C-S stretch)
		600-620	-Disulfide (S-S stretch)
		610-680	-Alkyne C-H bend
562.98	599.89	500-600	-Aliphatic iodo compound
531.68		590-720	-Alcohol, OH out of plane bending
471.17	515.55	430-500	-Aryl disulfide (S-S stretch)
		470-500	-Polysulfides (S-S stretch)

#### Elemental analysis

In the current study, the values of carbon, hydrogen, nitrogen, and sulfur detected in the sample of *Thymus zygis* L. were 66.70, 6.08, 1.65, and 0%, respectively. The percentage of oxygen content was 36.45%. *Salvia officinalis* L. presented as 53.34 % carbon, 5.6 % hydrogen, 2.11% nitrogen, 0.23% sulfur and 37.88% oxygen (Fig. 4).

Carbon is the most abundant element, which with high hydrogen content, indicates the presence of hydrocarbons (aliphatic, olefinic, and aromatic), which are the basic elements of many metabolites, as well as secondary metabolites (Kupareva et al., 2013; Mandal et al., 2017). Both plant samples had a lower H/C ratio, indicating high aromaticity (Sahu et al., 2020). The high oxygen and hydrocarbon contents provide several

functional groups (Braun & Pantano, 2014). Another important element, nitrogen, is present in almost all the amino acids and in nucleic acid (DNA). It is also an essential nutrient for plant growth (Anjum et al., 2019). *Thymus zygis* has a higher C/N ratio than *Salvia officinalis*, reflecting a greater resistance of the cell wall (Mandal et al., 2017). The rate of sulfur is very low or even absent; it is an element that also affects the growth of the plant; its presence in the soil is primordial, and it ensures numerous functions in the enzymatic reaction and synthesis of proteins (Mandal et al., 2017). The results obtained by this analysis correlated with those of the FTIR analysis. The latter method is more informative. Close values have been reported for other plants in the study of Anjum et al. (2019), and as far as we know, these plants have never been the subject of an elemental analysis.



**Fig. 4.** CHNS/O elemental analysis of *Thymus zygis* L. (T) and *Salvia officinalis* L. (S)

### Conclusion

The Mediterranean area, especially Morocco, is characterized by the quality of its medicinal plants. This study reveals a multitude of beneficial chemical compounds for numerous uses, whether in aqueous extracts or in essential oils of *Thymus zygis* and *Salvia officinalis* from the region of Fez-Meknes. Furthermore, Fourier-transform infrared spectroscopy and elemental analysis have highlighted the richness of both plants in functional molecules and macroelements. Through this study, a contribution has been made to the phytochemical valorization of these plants.

**Acknowledgements:** The authors would like to thank the central research laboratory of national school of agriculture, Meknes for carrying out FTIR analysis. Thanks are also addressed to Pr. Fathallah Laghrib from The Regional Center of Interface, University of Sidi Mohamed Ben Abdellah, Fez for GCMS and elemental analysis.

**Competing interests:** The authors report no conflicts of interest regarding this work.

**Authors' contributions:** All co-authors contributed to the design and implementation of the research, the analysis of the results and the writing of the manuscript.

**Ethics approval:** Not applicable.

### References

- Abouri, M., El Mousadik, A., Msanda, F., Boubaker, H., Saadi, B., Cherifi, K. (2012) An ethnobotanical survey of medicinal plants used in the Tata Province, Morocco. *International Journal of Medicinal Plants Research*, **1**(7), 99-123.
- Adrar, N., Oukil, N., Bedjou, F. (2016) Antioxidant and Antibacterial Activities of *Thymus numidicus* and *Salvia officinalis* essential oils alone or in combination. *Industrial Crops and Products*, **88**, 112-119.
- Adusei, S., Otchere J.K., Oteng, P., Mensah R.Q., Tei-Mensah, E. (2019) Phytochemical analysis, antioxidant and metal chelating capacity of *Tetrapleura Tetraptera*. *Heliyon*, **5**(11), e02762.
- Anjum, S., Bazai Z.A., Rizwan, S., Benincasa, C., Mehmood, K., Siddique, N., Shaheen, G., Mehmood, Z., Azam, M., Sajjad, A. (2019) Elemental characterization of medicinal plants and soils from Hazarganji Chiltan National Park and nearby unprotected areas of Balochistan, Pakistan. *Journal of Oleo Science*, **68**(5), 443-461.
- Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. (2008) Biological effects of essential oils – A review. *Food and Chemical Toxicology*, **46**(2), 446-475.
- Benzeggouta, N. (2017) Evaluation des effets biologiques des extraits aqueux de plantes médicinales seules et combinées. *Ph.D. Dissertation*, Mentouri-Constantine University, Constantine, Algeria.
- Bodalska, A., Kowalczyk, A., Fecka, I. (2021) Quality of herbal medicinal products based on Sage and Thyme preparations. *Acta Poloniae Pharmaceutica - Drug Research*, **78**(4), 539-561.
- Bouymajane, A., Rhazi Filali, F., Ed-Dra, A., Aazza, M., Nalbone, L., Giarratana, F., Alibrando, F., Miceli, N., Mondello, L., Cacciola, F. (2022) Chemical profile, antibacterial, antioxidant, and anisakicidal activities of *Thymus Zygis* Subsp. *gracilis* essential oil and its effect against *Listeria monocytogenes*. *International Journal of Food Microbiology*, **383**, 109960.
- Braun, E. I., Pantano, P. (2014) The importance of an extensive elemental analysis of single-walled

- carbon nanotube soots. *Carbon*, **77**, 912-919.
- Carrasco, A., Tomas, V., Tudela, J., Miguel M.G. (2015) Comparative study of GC-MS characterization, antioxidant activity and ayaluronidase inhibition of different species of *Lavandula* and *Thymus* essential oils. *Flavour and Fragrance Journal*, **31**(1), 57-69.
- Coates, J. (2000) Interpretation of Infrared Spectra, A practical approach. In: "Encyclopedia of Analytical Chemistry", pp. 10815-10837, R. A. Meyers (Ed.), John Wiley & Sons, Chichester, UK.
- Coimbra, A., Ferreira, S., Duarte, A.P. (2022a) Biological Properties of *Thymus Zygis* essential oil with emphasis on antimicrobial activity and food application. *Food Chemistry*, **393**, 133370.
- Coimbra, A., Miguel, S., Ribeiro, M., Coutinho, P., Silva, L., Duarte, A.P., Ferreira, S. (2022b) *Thymus zygis* essential oil: Phytochemical characterization, bioactivity evaluation and synergistic effect with antibiotics against *Staphylococcus Aureus*. *Antibiotics*, **11**(2), 146.
- Crozier, A., Clifford, M.N., Ashihara, H. [Eds.] (2008) *Plant secondary metabolites: occurrence, structure and role in the human diet*. Blackwell Publishing, John Wiley & Sons, Oxford, UK.
- Cutillas, A.B., Carrasco, A., Martinez-Gutierrez, R., Tomas, V., Tudela, J. (2018) Thyme essential oils from Spain: Aromatic profile ascertained by GC-MS, and their antioxidant, anti-lipoxygenase and antimicrobial activities. *Journal of Food and Drug Analysis*, **26**(2), 529-544.
- Dob, T., Berramdane, T., Dahmane, D., Benabdelkader, T., Chelghoum, C. (2007) Chemical Composition of the essential oil of *Salvia officinalis* from Algeria. *Chemistry of Natural Compounds*, **43**(4), 491-494.
- Drioiche, A., Radi, F.Z., Ailli, A., Bouzoubaa, A., Boutakiout, A., Mekdad, S., AL Kamaly, O., et al. (2022) Correlation between the chemical composition and the antimicrobial properties of seven samples of essential oils of endemic Thymes in Morocco against multi-resistant bacteria and pathogenic fungi. *Saudi Pharmaceutical Journal*, **30**(8), 1200-1214.
- El-Ghazouani, F., El-Ouahmani, N., Teixidor-Toneu I., Yacoubi, B., Zekhnini, A. (2021) A survey of medicinal plants used in traditional medicine by women and herbalists from the city of Agadir, southwest of Morocco. *European Journal of Integrative Medicine*, **42**, 101284.
- Fakchich, J., Elachouri, M. (2021) An overview on ethnobotanico-pharmacological studies carried out in Morocco, from 1991 to 2015: Systematic review (Part 1). *Journal of Ethnopharmacology*, **267**, 113200.
- Fannang, S. V., Tankeu, S.E., Etame Loe G.M.M., Yinyang, J., Ngouondjou Foze, T., Bamal, H.D., Mbida Mvomo, B.D. (2021) Phytochemical screening and study of the acute oral toxicity of the aqueous extract of the leaves of *Diospyros hoyleana* F.white (Ebenaceae). *Saudi Journal of Medical and Pharmaceutical Sciences*, **7**(5), 230-235.
- Fellah, S., Romdhane, M., Abderraba, M. (2006) Extraction et étude des huiles essentielles de la *Salvia officinalis* L. cueillie dans deux régions différentes de la Tunisie. *Journal de la Société Algérienne de Chimie*, **16**(2), 193-202.
- Hammada, S., Dakki, M., Ibn Tattou, M., Ouyahya, A., Fennane, M. (2004) Analyse de la biodiversité floristique des zones humides du Maroc: flore rare, menacée et halophile. *Acta Botanica Malacitana*, **29**, 43-66.
- Hou, T., Sana, S.S., Li, H., Xing, Y., Nanda, A., Netala, V.R., Zhang, Z. (2022) Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: A review. *Food Bioscience*, **47**, 101-716.
- Husnu Can Baser, K. (2008) Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. *Current Pharmaceutical Design*, **14**(29), 3106-3120.
- Jaadan, H., Akodad, M., Moumen, A., Baghour, M., Skalli, A., Ezrari, S., Belmalha, S.. (2020) Phytochemical screening, polyphenols, flavonoids and tannin Content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of northeast of Morocco. *Heliyon*, **6**(11), e05609.
- Jaradat, N., Hussen, F., Al Ali, A. (2015) Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata decne*. *Journal of Materials and Environmental Science*, **6**(6), 1771-

- 1778.
- Karahisar, E., Köse, Y.B., İşcan, G., Kürkçüoğlu, M., Tugay, O. (2022) Chemical composition and anticandidal activity of essential oils obtained from different part of *Prangos heyniae* H. Duman & M. F. Watson. *Records of Natural Products*, **16**(1), 74-83.
- Khiya, Z., Hayani, M., Gamar, A., Kharchouf, S., Amine, S., Berrekhis, F., Bouzoubae, A., Zair, T., El Hilali, F. (2019) Valorization of the *Salvia officinalis* L. of the Morocco bioactive extracts: Phytochemistry, antioxidant activity and corrosion inhibition. *Journal of King Saud University - Science*, **31**(3), 322-335.
- Koubaa-Ghorbel, F., Chaâbane, M., Turki, M., Makni-Ayadi, F., El Feki, A. (2020) The protective effects of *Salvia officinalis* essential oil compared to simvastatin against hyperlipidemia, liver, and kidney injuries in mice submitted to a high-fat Diet. *Journal of Food Biochemistry*, **44**(4), e13160.
- Kumar, S., Lahlali, R., Liu, X., Karunakaran, C. (2016) Infrared spectroscopy combined with imaging: A new Developing analytical tool in health and plant science. *Applied Spectroscopy Reviews*, **51**(6), 466-483.
- Kupareva, A., Mäki-Arvela, P., Grénman, H., Eränen, K., Sjöholm, R., Reunanen, M., Murzin, D.Y. (2013) Chemical characterization of Lube oils. *Energy & Fuels*, **27**(1), 27-34.
- Lee, J. Y., Park, H., Lim, W., Song, G. (2020)  $\alpha$ ,  $\beta$ -Thujone Suppresses Human Placental Choriocarcinoma Cells via Metabolic Disruption. *Reproduction*, **159**(6), 745-756.
- Lemjallad, L., Sayah, M.Y., Yassir, Z., Errachidi, F., El Ghadraoui, L., Chabir, R., Ouzzani Chahdi, F., Kandri Rodi, Y. (2019) Study of biological activities of essential oil and extracts from the hydrodistillation residue of anise (*Pimpinella Anisum*). *International Journal of Pharmacognosy and Phytochemical Research*, **11**(3), 205-213.
- Liu, Z., Li, H., Zhu, Z., Huang, D., Qi, Y., Ma, C., Zou, Z., Ni, H. (2022) Cinnamomum Camphora fruit peel as a source of essential oil extracted using the solvent-free microwave-assisted method compared with conventional hydrodistillation. *Lwt-Food Science and Technology*, **153**, 112549.
- Malik, D.S., Jain, C.K., Yadav, A.K. (2015) Preparation and characterization of plant based low cost adsorbents. *Journal of Global Biosciences*, **4**, 1824-1829.
- Mandal, M., Misra, D., Ghosh, N.N., Mandal, V. (2017) Physicochemical and elemental studies of *Hydrocotyle javanica* Thunb. for standardization as herbal drug. *Asian Pacific Journal of Tropical Biomedicine*, **7**(11), 979-986.
- Movasaghi, Z., Rehman, D., Dr. Rehman, I. (2008) Fourier Transform Infrared (FTIR) Spectroscopy of biological tissues. *Applied Spectroscopy Reviews*, **43**(2), 134-179.
- Msanda, F., El Aboudi, A., Peltier, J.P. (2005) Biodiversité et biogéographie de l'arganeraie marocaine. *Cahiers Agriculture*, **14**(4), 357-364.
- Nieto, G. (2017) Biological activities of three essential oils of the *Lamiaceae* family. *Medicines*, **4**(3), 63.
- Nurhaslina, C.R., Bacho S.A., Mustapa, A.N. (2022) Review on drying methods for herbal plants. *Materials Today: Proceedings*, **63**, S122-S139.
- Pelkonen, O., Abass, K., Wiesner, J. (2013) Thujone and thujone-containing herbal medicinal and botanical products: Toxicological assessment. *Regulatory Toxicology and Pharmacology*, **65**(1), 100-107.
- Radi, F.Z., Bouhrim M., Mechchate, H., Al-zahrani, M., Qurtam, A.A., Aleissa, A.M., Drioiche, A., Handaq, N., Zair, T. (2021) Phytochemical analysis, antimicrobial and antioxidant properties of *Thymus zygis* L. and *Thymus wilddenowii* Boiss. essential oils. *Plants*, **11**(1), 15.
- Rodrigues, V., Cabral, C., Evora, L., Ferreira, I., Cavaleiro, C., Cruz, M.T., Salgueiro, L. (2019) Chemical composition, anti-inflammatory activity and cytotoxicity of *Thymus zygis* L. Subsp. *sylvestris* (Hoffmanns. & Link) Cout. essential oil and its main compounds. *Arabian Journal of Chemistry*, **12**(8), 3236-3243.
- Sahu, A., Sen, S., Mishra, S.C. (2020) Processing and properties of *Calotropis gigantea* bio-char: a wasteland weed. *Materials Today: Proceedings*, **33**, 5334-5340.
- Sayout, A., Bahi, F., Ouknin, M., Arjouni, Y., Majidi, L., Romane, A. (2015) Phytochemical screening

- and antioxidant activity of four Moroccan *Thymus* species: *T. Leptobotrys* Murb. , *T. Pallidus* Batt. , *T. Broussonetti* Boiss. and *T. Maroccanus* Ball. *Arabian Journal of Medicinal & Aromatic Plants*, **1**(2), 117-128.
- Sharifi-Rad, M., Varoni, E.M., Iriti, M., Martorell, M., Setzer, W.N., del Mar Contreras, M., Salehi, B., Soltani-Nejad, A., Rajabi, S., Tajbakhsh, M., Sharifi-Rad, J. (2018) Carvacrol and human health: A comprehensive review. *Phytotherapy Research*, **32**(9), 1675-1687.
- Talari, A.C.S., Garcia Martinez, M.A., Movasaghi, Z., Rehman, S., Rehman, I. (2016) Advances in Fourier Transform Infrared (FTIR) spectroscopy of biological tissues. *Applied Spectroscopy Reviews*, **52**(5), 456-506.
- Tardugno, R., Pellati, F., Iseppi, R., Bondi, M., Bruzzesi, G., Benvenuti, S. (2018) Phytochemical composition and in vitro screening of the antimicrobial activity of essential oils on oral pathogenic bacteria. *Natural Product Research*, **32**(5), 544-551.
- Thamkaew, G., Sjöholm, I., Gómez Galindo, F. (2020) A review of drying methods for improving the quality of dried herbs. *Critical Reviews in Food Science and Nutrition*, **61**(11), 1763-1786.
- Tibaldi, G., Hazrati, S., Hosseini, S.J., Ertani, A., Bulgari, R., Nicola, S. (2022) Cultivation techniques and drying process can affect the inflorescence essential oil composition of three selections of *Salvia officinalis*. *Industrial Crops and Products*, **183**, 114923.
- Vergine, M., Nicoli, F., Negro, C., Luvisi, A., Nutricati, E., Accogli, R., Sabella, E., Miceli, A. (2019) Phytochemical profiles and antioxidant activity of *Salvia* species from southern Italy. *Records of Natural Products*, **13**(3), 205-215
- Zaim, A., Benjelloun, M., El Harchli, E.H., Farah, A., Meni Mahzoum, A., Alaoui Mhamdi, M., El Ghadraoui, L. (2015) Chemical composition and bactericidal properties of the Moroccan *Tanacetum annuum* L. essential oils. *International Journal Of Engineering And Science*, **5**, 13-19.

### استقصاء كيميائي نباتي عن *Thymus zygis* L. و *Salvia officinalis* L. التي تم جمعها من منطقة فاس- مكناس ، المغرب.

أسماء بن عبد الرحمان<sup>(1,2)</sup>، مجيد عثمان<sup>(1)</sup>، عبد اللطيف بوتاكوت<sup>(2,3)</sup>، وجدان الرحيوي<sup>(1,2)</sup>، سعدية بلملحة<sup>(2)</sup>

<sup>(1)</sup>معمل البيئة الوظيفية والهندسة البيئية - كلية العلوم والتقنيات - جامعة سيدي محمد بن عبد الله - فاس - المغرب،  
<sup>(2)</sup> قسم حماية النبات والبيئة - المدرسة الوطنية للزراعة - مكناس - المغرب، <sup>(3)</sup> وحدة ميكروبيولوجيا البيئة والتربة - كلية العلوم - جامعة مولاي إسماعيل - مكناس - المغرب.

يعتبر الزعتر والمريمية من أشهر النباتات في الطب التقليدي وفن الطهي. تهدف هذه الدراسة إلى الكشف عن التركيب الكيميائي لمستخلصات ومسحوق *Thymus zygis* L. (T) و *Salvia officinalis* L. (S) المقطوفة من منطقة فاس- مكناس. تم تحضير مستخلصين: مائي وزيت أساسي. تم إجراء اختبارات كيميائية نباتية لتقييم وجود أو عدم وجود المكونات النباتية نوعياً باستخدام الطرق المتداولة. تم تحليل الزيوت الأساسية بواسطة مقياس الطيف الكتلي اللوني للغاز (GC/MS). تم إجراء تحليلين للمسحوق: تحليل فورييه للتحويل الطيفي بالأشعة تحت الحمراء (FTIR) والتحليل الأولي. استغرق تجفيف كلا النباتين وقتاً مشابهاً تقريباً مع خسارة كبيرة في وزن المريمية. كشف الفحص الكيميائي النباتي عن وجود تربينويدات، ومضادات التانينات، والستيرويدات، والستيرولات بكثرة وبطريقة متساوية تقريباً في النباتين. أظهر تحليل GC/MS ثراء *Thymus zygis* L. في carvacrol و ثراء *Salvia officinalis* L. في thujone. أظهر التحليل بواسطة FTIR قراءات ذروة مختلفة ومميزة لمجموعات وظيفية مختلفة في المساحيق، مستشهدين من بينها: البروتينات والمركبات الأليفاتية ومركبات الكربونيل والحلقات العطرية. في التحليل الأولي، يوجد محتوى عالٍ من الكربون للزعتر والمريمية (S: 53.34%، T: 66.70%)، يليه الأكسجين (S: 37.88%، T: 36.45%)، والهيدروجين (S: 5.61%، T: 6.08%). باختصار، تسلط هذه الدراسة الضوء على ثراء هذين النوعين بالمركبات الكيميائية التي يمكن استخدامها في صناعة الأدوية.