Study of methods for extracting essential oils from Tunisian *Origanum* spice and their anticandidal activities

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Two different extraction methods were used for a comparative study of the essential oils of the Tunisian majoram leaf: Clavenger and infusion. Clevenger-type hydrodistillation extraction produced high quality essential oils. Comparison of the total essential oil yield quantified by Clevenger-type hydrodistillation of the majoram (0.75%) with the essential oil yields of infusion extracts (varying between 0.021% and 0.036%) revealed that only 20% of the initial oil could be extracted by infusion. With regard to the essential oil composition, we noted significant differences when focusing on the relative proportions of the chemical classes of the constituents determined by comparing both extracts. MIC values of O. majorana essential oil ranged from 0.781mg/mL to 12.5 mg/mL. The most important activities were observed against *C. albicans* and *C. Para psilosis*. EO obtained with the Clevenger method was characterized by the lowest MIC values, due to its richness in bioactive compounds acting in synergy.

Keywords: *Origanum majorana*, Essential oil, Hydrodistillation Clevenger, Infusion, Candida

1. INTRODUCTION

In recent years, interest has been increasing in the biological properties of medicinal plants in order to identify and evaluate their therapeutic potential. Many plant families with potentially beneficial effects attributed to their volatile constituents are used in herbal teas such as the Lamiaceae family. Lamiaceae, also known as the mint family, are flowering plants. Traditionally, they were considered to be closely related to Verbenaceae. The wider Lamiaceae family contains about 236 genera and 6,900 to 7,200 species. The plants are frequently aromatic in all their parts and include many widely used culinary herbs, such as basil, mint, rosemary, sage, savor, marjoram, oregano, thyme, lavender and perilla. *Origanum majorana* L. (marjoram) is a member of the Lamiaceae family and it is one of the western world's most important culinary herbs.
The highly aromatic fresh or dried leaves and flowering tops of marjoram (*Origanum majorana* L.) are widely used to flavour many foods. Its essential oils are used in pharmaceuticals, perfumes and cosmetics [1]. The essential oil obtained by steam distillation contains mainly terpinen-4-ol (>20%) which, with (+)-Cis -sabinene hydrate (3–18%), is responsible for the characteristic flavour and fragrance of marjoram oil. In addition to these compounds, α -terpinene and γ-terpinolene are the other major components [2, 3]. The volatile oil possesses antimicrobial properties effective against food-borne bacteria and mycotoxins.
This aromatic plant is commonly served for consumption in Lebanese, Italian and Mexican cuisines. It is a perennial herb, distributed among temperate, tropical and subtropical regions. Genus *Origanum* is important medicinally since

it has antimicrobial, antifungal, antioxidant, antibacterial, antithrombin, antimutagenic, angiogenic, antiparasetic and antihyperglycaemic properties [4]. Phytochemical investigations of the species of this genus have resulted in the extraction of a number of important bioactive compounds that have many medicinal uses. In fact, marjoram tea (extracts from the leaves and flowers) has been prescribed in folk medicine to relieve the symptoms of hay fever, sinus congestion, indigestion, asthma, stomach pain, headache, dizziness, colds, coughs, and nervous disorders. The plant extract contains mainly terpinenes, aroma-active compounds, carvacrol and thymol, alkaloids, flavonoids, and essential oils [5]. The essential oil of this plant exerts anti-inflammatory and inhibitory activity against bacteria [6], in addition to its widespread use as food additive. In fact, food industries have explored the essential oil of this plant due to its confirmed antibacterial and antifungal properties [7].

In a previous study, it was observed that *O. majorana* Brazilian cultivars have terpinen-4-ol, γ-terpinene, p-cimene and α-terpinene as the main essential oil components, which in turn displayed interesting antibacterial activity against *Escherichia coli,* moderate activity against *Salmonella enteric* and *Enterobacter sakazakii*, but was inactive against *Listeria monocytogenes* [8].

Most natural products have displayed activity predominantly against Gram-positive bacteria. Gram-negative bacteria are intrinsically more resistant to antibacterial agents than Gram-positives due to an additional outer membrane that acts as an effective barrier for amphipathic agents [9] and the overexpression of efflux pumps responsible for innate antimicrobial resistance, such as the AcrAB-TolC efflux system [10]. Therefore, the identification of new antimicrobial agents active against Gram-negative bacteria and/or with the ability to modulate the antibiotic susceptibility of Gram-negative bacteria is of considerable importance to therapeutics. The infusion of *O. majorana* leaves is widely used in folk medicine as well as in food consumption; in fact, infusion extracts are used for the isolation and characterization of therapeutically-active chemical constituents in modern medicines.

Extraction is a crucial step for plant characterization and enhancement. It is very important to select the most suitable extraction methods to achieve the optimal results. Extraction is the separation of medicinally-active portions of plant tissues using selective solvents through standard procedures [11]. The industrial enhancement and application of medicinal and aromatic plants (MAPs) requires this step. Extraction techniques include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction and phytonic extraction.

Extraction is used to recover components from a solid mixture or solution [12]. It is an indispensable step in order to characterize plants by qualitative analysis of their bioactive compounds and to conduct experiments [13]. Plant biomolecules can be initially preserved during a pre-extraction step. Samples can be used either fresh or dried [14]. Extraction involves separating bioactive plant fractions with a selective solvents system and through adequate extraction procedures. In fact, plant extracts are complex mixtures composed of various metabolites. They can take different forms: liquid, semisolid, or dry powder. Solvents are able to spread through solid plant matter and solubilize compounds of similar polarity during the extraction process. The targeted bioactive compounds determine the choice of one solvent rather than another [15].

Hydrodistillation is a very ancient extraction method mainly used to extract volatile essential oils. The sample is placed in a still compartment and covered with a sufficient volume of water, then boiled. Another option is to inject steam directly into the matrix [16]. The steam and oil mixture is condensed by cooling water and oil, thereby separating volatile compounds from the water [17]. Azmir et al. (2013) [18] reported three types of hydrodistillation: direct steam distillation, water and steam distillation, and water distillation (Table I). The major constraint of this technique is that it may cause the loss of some volatile components due to the high extraction temperature. This might limit its use in the case of thermolabile compounds.

As well as maceration, percolation and decoction, infusion is another conventional method of extraction. In infusion and decoction submitted to the maceration principle, samples are plunged into cold or boiled water until the soluble material is dissolved [19]. Infusion requires a longer maceration time than decoction [12]. Fresh samples are likely to deteriorate more rapidly than dried samples [20] (Table I).

The objectives of the research were therefore to extract and compare the two methods for the extraction of *O. majorana* essential oil, to analyze and identify its active compounds and to identify marjoram's antifungal properties.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL

Samples of the dried leaves of marjoram were obtained in May 2014 from the Jendouba region (North West of Tunisia). The herbarium specimen of *O. majorana* was deposited in the National Agronomic Institute of Tunis under the number ≠ 1065. Leaves were dried at room temperature and stored until extraction.

2.2. ESSENTIAL OIL ISOLATION BY THE CLEVENGER METHOD

One hundred grams of dried marjoram leaves were hydro-distilled in a Clevenger type apparatus for 3h. The essential oils were dried over anhydrous sodium sulphate before calculating the amount of essential oil obtained from the plant material. The essential oil was stored in dark-coloured glass bottles and kept at 4°C until analysis.

2.3. ESSENTIAL OIL ISOLATION BY THE INFUSION METHOD

Essential oil was extracted by the infusion method over 10 minutes, using 6, 8 and 10 g of dried aerial parts of *O. majorana*.

We selected three masses (6, 8 and 10 g) to optimize our infusion protocol, which was tested for the first time on *Origanum majorana*. There is a paucity of data on the infusion of medicinal and aromatic plants. Infusion is used to extract the active ingredients of plants without heating to high temperatures, which helps preserve certain heat-sensitive compounds. Extracting essential oils by infusion is a different process to the most common method: hydrodistillation. Infusion consists of macerating plants in a hot liquid (often water or oil) to extract the active ingredients, including essential oils.

However, it is important to note that infusion alone does not allow EOs to be extracted in the same concentrations as hydrodistillation. It results in oil or solvent, which preserves the aromatic and medicinal properties of the plant but the concentration of EOs is much lower. Infusion produces milder extracts and is often used to produce oily macerates or for low-concentration cosmetic and medicinal applications. Despite these limits, infusion has a role to play in some industrial sectors, especially in the pharmaceutical (therapeutic applications) and food industries. Its function and application will depend on the type of product sought and plant-specific properties.

In the pharmaceutical industry, extraction by infusion is mainly used to create “oil macerates” or plant extracts that can be used in the formulation of cosmetic products, skin care, therapeutic oils, and even in some mild medicinal treatments. Infusion produces milder extracts than distilled essential oils, while retaining a wide range of bioactive compounds, albeit in lower concentrations. Examples include St. John’s Wort Oil, used as a massage oil for its anti-inflammatory and calming properties. It is often obtained by infusing St. John’s Wort flowers in a vegetable oil (such as olive oil). Another example is Calendula Oil, reputed to possess soothing properties on irritated and inflamed skin. It is obtained by macerating calendula flowers in a vegetable oil, a method similar to infusion.

In the food industry, infusion is mainly used to extract “natural aromas” or “flavours” from plants, as well as “natural extracts” used as additives or flavours in food and beverage products. Several applications can be reported: mint or camomile infusion can be used to flavour drinks, confectionary, teas, syrups or food preparations due to the unique sweetness. Moreover, lavender extract is incorporated in certain foods, such as confectionary or jams. The infusion also allows the extraction of certain active ingredients with antioxidant or antimicrobial properties. These compounds can improve the shelf life of foodstuffs and provide health benefits.

Infusion extraction has some advantages. Unlike distillation, it does not require intense heat, thereby preserving certain active ingredients that are sensitive to heat. It is a very simple and cheap technique.

Infusion extraction leads to products with lower concentrations of essential oils and bioactive compounds than steam distillation. However, it takes longer and provides lower yields in terms of active extract concentration.

Although infusion extraction is not the most commonly-used methods for obtaining EOs or aromatic

Table I - Comparative table for the two extraction methods: hydrodistillation type Clevenger and infusion

Aspect	**Hydrodistillation (Clevenger)** [18, 20]	**Infusion** [17, 19]
Method Description	Volatile mixtures are extracted by using steam or boiling water and a Clevenger apparatus.	Plant material is macerated into hot water
Equipment	Clevenger apparatus, boiling flask, condenser.	Containers such as beakers or teapots
Extraction Time	Depends on plant part and on plant richness in essential oil	Lasts generally from 5 to 30 minutes, according to plant material and purpose of use.
Efficiency	Very interesting yield of extraction	More adequate to non-volatile compounds than volatile ones
Yield of Extract	Important yield of volatile mixtures	Easier extraction for polyphenols
Temperature Control	High temperature may be a threatening factor for molecules stability, mainly thermolabile ones.	Since water temperature is moderate, the technique is suitable for thermolabile molecules
Applications	The main purpose is flavor compounds, bioactive volatiles and essential oil extraction.	Aims to prepare infusions, teas, and medicinal extracts, mainly those rich in water-soluble compounds.

extracts, it is particularly useful when subtle, gentle extracts are required, suitable for specific cosmetic or food products.
The resulting distillate was extracted with diethyl ether (v/v) and dried anhydrous sodium sulfate. The organic phase was then concentrated at 35°C using a Vigreux column and the resulting EO was subsequently underwent GC-MS analysis. All experiments were done in triplicate.

2.4. GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

The GC-MS analyses were performed on a gas chromatograph HP 6890 (II) interfaced with a HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA) with electron impact ionization (70 eV). A HP-5MS capillary column (60 m x 0.25 mm, 0.25 µm film thickness) was used. The column temperature was programmed to rise from 40°C to 280°C at a rate of 5°C-min-1. The carrier gas was helium, with a flow rate of 1.2 mL. min-1. The scan time and mass range were 1 s and m/z 50-550, respectively. The injected volume was 1 µL, and the total run time was approximately 63 min.

2.5. DETERMINATION OF THE MINIMAL INHIBITORY CONCENTRATIONS

Four yeast strains (*Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida parapsilesis* ATCC 22019 and *Candida krusei* ATCC 6258) were used to study the antifungal activities of *O. majorana* essential oil. The Minimal Inhibitory Concentrations (MICs) were determined using the two-fold serial broth microdilution assay (NCCLS 1990) (National Committee for Clinical Laboratory Standards). Increasing concentrations of the tested EOs were prepared in the sterile Sabouraud Chloramphenicol medium as a 10% solution in dimethyl sulfoxide (DMSO). At this concentration, DMSO did not affect the growth of the studied microorganisms [21]. Briefly, logarithmic phase strain cultures were diluted in Sabouraud Chloramphenicol medium to an absorbance A630 = 0.01 (106 cfu mL-1). The yeasts were grown in 96-well microtitration plates in the presence of 2-fold serial dilutions of the essential oils (0.1 to 50 mg ml-1). Yeast suspensions in the exponential growth phase (50 µL) were mixed with 50 µl of each essential oil concentration in the wells of a microtitration plate. The fungal growth was monitored after overnight incubation at 37°C by measuring the absorbance A630 value using a microplate spectrophotometer (BioTek®, ELx 808). MIC was expressed as the lowest concentration of essential oils that inhibited fungal growth completely and as the average value from three independent experiments, with positive (0.7% formaldehyde) and negative (without extract) inhibition control, and sterility control (H2O).

2.6. STATISTICAL ANALYSIS

The data was presented as average means ± standard deviation (SD). Statistical assessments were conducted using one-way ANOVA, followed by Tukey's post hoc examination for multiple comparisons. Significance among mean values was established at p-values of $p<0.05$. The correlation coefficient (R2) was calculated to determine the relationship between all the parameters and chemical activity was calculated using MS Excel software (CORREL statistical function).

3. RESULTS AND DISCUSSION

3.1. ESSENTIAL OIL YIELD

Marjoram EO extraction was performed in triplicate using the Clevenger method and the average yield for dry plant mass was 0.75 ± 0.11%. EO yield generally ranges from 0.6 to 1.9%. Several previous works have examined *O. majorana* EO. Badee et al. [22] reported a yield of 1.9% for this species. Similarly, Busatta et al., [23] and Romeilah [24] reported, respectively, yields of 1.2% and 1.7%, of dry weight.
Comparing our Clevenger hydrodistillation (0.7% v/w) with that of the different infusions (0.021%; 0.03% and 0.036%), it emerged that only 20% of the initial EO could be extracted. In fact, Green (2000) [25] recommended that herbs containing volatile oils should be added once the vessel (kept covered) has been removed from the heat source to avoid volatile compounds loss. The short preparation time of infusion makes it possible to isolate a small quantity of EOs in addition to the explosion of the secretary glands. Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of crude drugs. It has been reported that in the case of infusion and, depending on the purpose of extract use, the ratio of plant sample to solvent is generally 1:4 or 1:16 [26]. Moreover, the infusion temperature and duration should be carefully controlled to maximize oil yield while preserving the quality and composition of the extracted oil. Time, temperature, and the plant material being infused must be balanced to ensure that bioactive molecules with interesting properties are successfully extracted without any degradation or alteration.
Our results show that the yield varies between 0.021% and 0.036%. The lowest yield was obtained with a mass of 6 g. This increase of EO yield was proportional to the increase of mass which allows the condensation of more volatile substances (R2= 0.986).

3.2. CHEMICAL CONSTITUENTS OF MARJORAM ESSENTIAL OIL

Volatile oils were extracted using the hydrodistillation method. Clevenger EO were fractionated and

identified by using GC/MS (Figure 1). The identified components and their relative proportions are listed in Table II. The major components were terpinen-4-ol (27.67%), γ-terpinene (16.41%), cis sabinene hydrate (13.89%), α-terpinene (11.36%), followed by sabinene (6.32%). They constituted 75.65% of the whole EO, which belongs to terpinen-4-ol/sabinene hydrate chemotype. EOs of *O. majorana* that were reported in the literature as containing high amounts of *majorana* were also rich in terpinen-4-ol [27].

In the literature, two chemotypes characterize *O. majorana* EOs: terpinen-4-ol/sabinene hydrate chemotype [28], and thymol (or carvacrol) chemotype [29]. In medicinal plants, as well as in the other secondary metabolites, EOs biosynthesis and accumulation are significantly impacted by environmental factors [30-32]. Moroccan *O. majorana* from Morocco EOs was reported to belong to the terpinen-4-ol chemotype [33] or the 4-terpinene chemotype [34]. In these oils, linalool (32.68%), sabinene hydrate (14.08%) and *trans*-sabinene hydrate (16.0%) were abundant [35, 36]. According to Komaitis et al. (1992) [37], Greek *Origanum majorana* EO is a terpinen-4-ol chemotype. Furthermore, Daferera et al. (2000) [38] also described a thymol chemotype (14.0%) while Giatropoulos et al. (2018) [39] identified a carvacrol chemotype for Greek *Origanum majorana* EO.

Table II reveals the presence of 24 components, classified as follows: (1) Monoterpene-hydrocarbons

Table II - Essential oil composition (GC/MS) of *Origanum majorana* leaves obtained by Clevenger hydrodistillation

Volatiles compounds	%
α-thujene	1.64 ±0.02
α-pinene	0.8±0.02
Camphene	0.05±0.01
Sabinene	6.32±0.6
β-pinene	0.45±0.2
β-myrcene	1.16±0.4
α-phellandrene	0.66±0.2
α-terpinene	11.36±0.3
p-cymene	2.66±0.2
γ-terpinene	16.41±0.9
trans-sabinène hydrate	1.69±0.2
α-terpinolene	3.47±0.02
cis sabinene hydrate	13.89±0.8
α-terpineol	4.95±0.6
4 terpineol	27.67±0.9
linalyl acetate	1.36±0.01
cis piperitol	0.84±0.03
trans piperitol	0.78±0.02
β- caryophyllene	1.34±0.03
Ahumulene	0.08±0.01
Bicyclogermacrene	0.7±0.02
Spathulenol	0.38±0.03
caryophyllene oxide	0.19±0.02

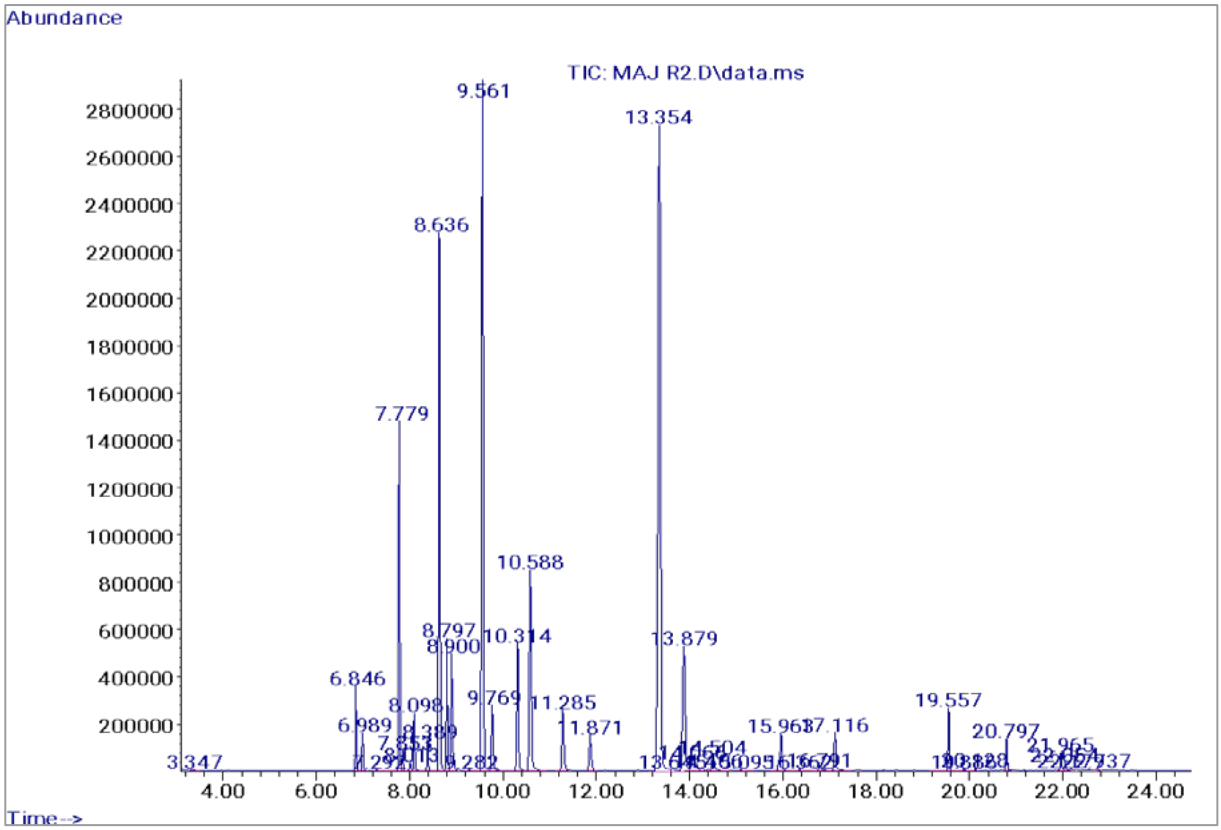

Figure 1 - Chromatographic profile of the essential oil of the marjoram leaf extracted by Clevenger type hydrodistillation.

(54.42%); (2) Oxygenated--monoterpenes (34.24%); (3) Sesquiterpene-hydrocarbons (1.32%); (4) hydrogenated sesquiterpene (2.69%). Similar classification was reported by other authors [40, 41].

In the current study, we selected infusion as the second method for EO extraction by fixing extraction duration to 10min and trying three masses for plant material (6g, 8g and 10 g). Table III shows the chemical compositions of the different infusions.

On considering the chromatograms of the extracts obtained by the infusion and Clevenger methods, we noted significant differences in the relative proportions of the chemical classes.

The significant loss of monoterpene hydrocarbons present in the *O. majorana* infusion may be explained by their greater volatility and lower water solubility (boiling points of about 160°C). Rudulescu et al., (2004) [42] demonstrated that many of the monoterpene hydrocarbons such as tricyclene, myrcene, α-phellandrene, α-terpinene, *p*-cymene, ocimene, terpinolene and the sesquiterpene hydrocarbons *trans*-caryophyllene and α-humulene that occur in rosemary were not detected in the infusion. This loss can be explained by the high temperature of the boiling water. A similar effect was noted for *Salvia officinalis* infusion.

The extract of tea 6 g / 10 min was characterized by low levels of volatile compounds such as 4-terpineol and sabinene hydrate with 2.79% and 3.54%, respectively. The other compounds were detected at trace levels.

The extract of tea 8 g / 10 min had almost the same composition but with larger percentages, especially for major compounds like 4 terpineol (7.1%), cis sabinene hydrate (7.69%).

The extract of tea 10 g / 10 min marjoram leaves was characterized by significant amounts of 4 terpineol, γ-terpinene, cis sabinene hydrate and α-terpinene, respectively (11.61%) (9.48%) (9.08%) and (6.80%) (Table III). This significant accumulation of major compounds could be explained by the increase in plant mass and hydro solubility of these compounds. This chemical composition confirms the effective use of *origanum* infusion as a stimulant, sudorific, emmenagogue and galactagogue and it is also useful in asthma, hysteria and paralysis [6]. Many studies have shown that the infused oil possesses anti-inflammatory activity and accelerates the healing of wounds through the stimulation of epithelial tissue production when applied *ex vivo*, and exhibits gastro-protective effects when taken orally [43, 44].

Table III - *Origanum majorana i*nfusions volatile composition (GC/MS)

Volatiles compounds (%)	6g	8g	10g
α-thujene	0.61±0.02	1.1±0.02	1.02
α-pinene	0.64±0.03	0.9±0.03	1.63±0.4
camphene	-	-	-
Sabinene	1.96±0.04	2.1±0.01	2.61±0.2
β-pinene	0.13±0.03	-	
β-myrcene	0.75±0.03	0.8±0.03	0.82±0.02
α-phellandrene	0.19±0.02	1.6±0.02	2.83±0.6
α-terpinene	0.21±0.01	0.38±0.01	6.80±0.5
p-cymene	0.95±0.03	0.91±0.02	1.69±0.04
γ-terpinene	0.18±0.02	1.38±0.3	9.48±0.9
trans-sabinène hydrate	0.8±0.02	1.39±0.2	1.72±0.6
α-terpinolene	-	0.8±0.02	1.03±0.5
cis sabinene hydrate	3.54±0.3	7.69±0.3	9.08±0.8
α-terpineol	-	1.2±0.4	1.46±0.4
4 terpineol	2.79±0.1	7.1±0.9	11.61±0.9
linalyl acetate	0.11±0.01	-	1.14±0.6
cis piperitol	0.4±0.04	-	0.96±0.23
trans piperitol	0.3±0.02	-	1.56±0.4
β- caryophyllene	1.01±0.2	7.1±0.9	1.3±0.3
α humulene	0.06±0.01	-	-
bicyclogermacrene	-	-	-
spathulenol	-	-	-
caryophyllene oxide	-	-	-

3.3. ANTICANDIDAL ACTIVITY

Candidiasis is a mycosis caused by different *Candida* species, which can promote superficial and systemic opportunist diseases around the world. Candidiasis causes infections that range from the superficial, such as oral thrush [45] and vaginitis, to systemic and potentially life-threatening diseases. The increase of *Candida* infections has developed in parallel with medical advances, such as invasive procedures, immunosuppressive treatments for organ transplants and the widespread use of broad-spectrum antibiotics [46]. In addition, most antifungals currently available for the treatment of different candidiasis clinical forms have limitations that hinder their use. It is therefore essential to conduct research into safe, efficient antimycotic products or molecules. Results of the MIC values of the Tunisian *O. majorana* essential oil are shown in Table IV. The findings demonstrate that the tested EOs provide inhibition against all four *Candida* strains tested. *O.majorana* essential oil has interesting MIC values, ranging from 0.781mg/mL to 12.5 mg/mL. The most important activities were observed against *C. albicans* and *C. para psilosis.* Clevenger EO displays the lowest MIC values, due to its richness in bioactive compounds, compared with EO.

The results confirmed that the highest anticandidal activity of *O. majorana* EO could be correlated to the presence of high levels of Monoterpene-hydrocarbons (54.42%); (2) Oxygenated--monoterpenes (34.24%) constituted by terpinen-4-ol (27.67%), γ-terpinene (16.41%), cis sabinene hydrate (13.89%), α-terpinene (11.36%) and sabinene (6.32%) as its major components.

The antimicrobial action of monoterpenes suggests that they penetrate and damage cell membrane structures [47]. It is known that the antimicrobial action of such molecules depends on their presence in gaseous form, which facilitates their solubilization in cell membranes. Our results indicate that essential oils from *Origanum majorana* could have a practical and promising use in the inhibition of fungal growth.

Furthermore, it has been reported that there is a correlation between the mechanism of action of monoterpenes and the induction of microorganism membrane disruption. These volatile compounds induce fungal cell membrane destabilization. This is facilitated by non-polar nature of the membrane, thereby disrupting fungal lipid structure. Additionally, alcohol moieties, hydroxyl groups, oxygen functions, and delocalized electrons of monoterpenes play a key role in their antimicrobial activity [48-50]. According to Lira et al. (2020) and Kaypetch et al. (2022) [50-51], monoterpenes such as geraniol are able to damage ATPase activity in the plasma membrane, causing mitochondrial dysfunction, and reducing hyphal formation. Clevenger-extracted oil was characterized by greater antifungal properties than the infusion. This may be explained by its richness in monoterpenes acting in synergy to exert a significant antifungal activity.

Future research on Tunisian *Origanum* EO should focus on testing its efficacy in real-world food preservation, where its antimicrobial properties could be harnessed to boost the shelf-life of various foodstuffs. While preliminary studies have demonstrated the potential of these oils in inhibiting fungal growth, it remains crucial to evaluate their practical application in different food matrices under varying storage conditions. Additionally, future investigations could expand the scope of research by exploring the effects of Tunisian *Origanum* EOs against a broader range of fungal pathogens, particularly those responsible for spoilage in perishable food items such as fruits, vegetables, and baked goods. Moreover, the safety and regulatory aspects of using these EOs as natural preservatives require thorough examination, particularly in terms of their sensory impact on food and safety and their long-term effects on human health.

Microbial growth is the main cause of food quality deterioration due to oxidation, and peroxidation. *Origanum majorana* EOs can be used as an alternative to synthetic and chemical additives. They can be used for the preservation of meat and raw mince since they can enhance shelf life and protect the organoleptic qualities.

Exploring novel extraction techniques, such as supercritical CO2 extraction, could also be a highly promising avenue in attempts to enhance the EO yield and the quality of bioactive compounds, further boosting its potential for commercial use and application in the food industry.

Table IV - Antimicrobial Activities (MIC, Milligrams per Milliliter) of the *Origanum majorana,* Essential Oils extracted by Clevenger and infusion methods.

Essential oil	**Minimal inhibitory concentration MIC (mg/mL)**			
	C.albicans	*C.glabrata*	*C.parapsilosis*	*C.krusei*
Clevenger	0.781	3.125	0.781	3.125
Infusion (6 g)	1.562	6.25	3.125	3.125
Infusion (8 g)	1.562	6.25	6.25	6.25
Infusion (10 g)	3.125	12.5	12.5	6.25

4. CONCLUSION

Clavenger and infusion were the two extraction methods used in this comparative study of
essential oils extracted from majoram leaves from Tunisia. The Clevenger hydrodistillation method produces high quality essential oil extraction with essential oil yields of around 0.75%. According to our results, significant differences in the composition of essential oils were revealed based on the relative proportions of the chemical classes of the constituents determined by comparing the two extracts. Due to its richness in bioactive molecules, the Clevenger method displayed the lowest MIC values comparing to the essential oil from the infusion method. This characteristic should contribute to the understanding of the pharmacological activities of the herb. Furthermore, it must be taken into account when the plant is used as an aroma source and in many industrial sectors in relation to the type of volatiles accumulated.

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Conflicts of Interest
The authors state they have no conflicts of interest.

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