Research Article

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Physicochemical characterization, antioxidant and antifungal activities of essential oils of *Urginea maritima* and *Allium sativum*

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Abstract: This study aims to contribute to the valorizing of aromatic and medicinal plants of Morocco by studying the antioxidant and antifungal properties of the essential oils (EOs) of *Allium sativum* (AS) (garlic) and *Urginea maritima* (Syn. *Drimia maritima*) tested *in vitro*. After the hydrodistillation of EOs, we determined their physicochemical indices according to an international standard (Moroccan ISO standard), and the chemical composition of the oils was studied by gas chromatography coupled with mass spectrometry (GC-MS). Then, the antioxidant properties were determined using the free radical scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl and the ferric-reducing antioxidant power of these oils. The evaluation of the antifungal activity was carried out by the direct contact method against two fungal strains, *Fusarium oxysporum* and *Fusarium equiseti*. GC-MS analysis revealed a significant percentage of terpenes in *U. maritima* EO, with terpinolene (21.82%) and estragole (6.24%) as the main compounds. Biological tests have demonstrated the remarkable antioxidant activities of this EO compared with AS EO, with an IC_{50} of 1.03 mg/mL and an $RP_{0.5}$ of 1.60 mg/mL. On the other hand, while *A. sativum* (garlic) EO showed exceptional antifungal activities, with 100% inhibition and MICs of 0.5 µL/mL for *F. equiseti* and 2.5 µL/mL for *F. oxysporum*. Also, regardless of its antioxidant properties being moderate, this EO stands out for its efficacy against fungal infections of the *Fusarium* genus. In summary, the results suggest that *U. maritima* EO could be used as a natural source of antioxidants, while garlic EO could be considered a potentially effective treatment against fungal infections caused by *Fusarium* fungi.

Keywords: *Allium sativum*, *Urginea maritima*, essential oil, physicochemical index, antioxidant, *Fusarium*

1 Introduction

Since ancient times, aromatic and medicinal herbs have been utilized to treat a variety of illnesses and infections. Medicinal plants were utilized by numerous civilizations, such as the Egyptian, Greek, Roman, and Chinese, to alleviate illnesses and advance well-being [1]. Over time, knowledge has been transmitted from generation to generation and has been enriched with new plants and treatment methods. These plants have been the origin of all drug discoveries in the world. As secondary metabolites, they possess a significant biological activity required for health [2,3], leading to traditional knowledge about the use of plants that are becoming a source of inspiration for scientists seeking to develop new remedies.

Generally, aromatic plants constitute a natural wealth of bioactive compounds whose valorization requires a perfect knowledge of the properties to be developed. Morocco is endowed with exceptional ecological and floristic diversity, thanks to its geographical position, which makes it a real phytogenetic reserve [3–5]. Among these species, we found *Allium sativum* (AS) and *Urginea maritima* (UM). The first is a perennial plant belonging to the *Allium* genus and

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the second is a wild plant belonging to the *Urginea* genus. Both plants are members of the Liliaceae family and are frequently used in traditional medicine to treat a variety of illnesses [6–8]. The use of plants has historically involved the application of extracts or essential oils (EOs), the latter being the volatile fractions responsible for their characteristic aromas. The specific use of these compounds varies according to factors such as the source of the plants, the quality of the materials, and the extraction methods employed [9]. Generally, EOs are known for their remarkable biological effects, including their antimicrobial capacity [4,10], their antioxidant effect [1], and their effectiveness against fungi [5]. Owing to these effects, EOs are more appreciated in various industrial sectors such as the pharmaceutical industry, food industry, cosmetics, and agronomy.

Lipid peroxidation is a major problem in the food sector, as it is the primary cause of unpleasant flavors and odors that render food unfit for consumption. It also brings about other alterations that may have an impact on the nutritional value of food due to the breakdown of essential fatty acids and liposoluble vitamins, as well as the food's safety and integrity. Similarly, peroxidation of unsaturated lipids in biological systems (cell membranes) can damage the membrane, disrupt metabolite exchange mechanisms, and lead to cell death. This peroxidation phenomenon may be responsible for premature aging, cardiovascular diseases, immune system decline, and brain dysfunction [11–13].

Furthermore, cryptogamic diseases like Fusarium, which seriously harm several plant species globally and subsequently lower agricultural output yields, have an impact on food quality [1,5]. As a result, the use of antioxidants for lipid peroxidation and fungicides for cryptogamic diseases is necessary. According to the literature, synthetic antioxidants and chemical fungicides have many disadvantages, including being toxic [14,15], harmful to the soil, leading to deviation from the normal system of flora and fauna, pathogen resistance, and environmental pollution [16–18]. Due to these problems, new safe and efficient treatments for oxidative stress and fungal illnesses must be developed immediately [16,19].

Numerous research studies have documented the utilization of EOs as safer alternatives to toxic compounds in biological pesticide control [20,21], microscopic fungi [22], and oxidative stress [23,24] as an alternative to harmful products. It is in this context that we sought to evaluate the antioxidant and antifungal potency of UM and AS EOs, which have the advantage of being environmentally friendly products. The main objective of our work is to valorize two plants from the Moroccan flora by studying their chemical compositions and their antioxidant and antifungal properties.

2 Materials and methods

2.1 Plant material

AS bulbs were collected in the region of Agourai, Meknes, while UM L. bulbs were collected in September 2021 in the region of Sidi Taibi, located in the province of Kenitra, Morocco. Professor Lahcen ZIDANE of the Laboratory of Plant, Animal Productions and Agro-industry at Ibn, Tofail University, confirmed that the specimens were deposited in the herbarium of the Laboratory of Biotechnology, Environment, Agroalimentary, and Health at the Faculty of Sciences, University Sidi Mohamed Ben Abdellah in Fez, Morocco, under the reference numbers BPRN/TG/F/17 for UM (L.) and BPRN52 for AS L. The bulbs were identified and then kept at room temperature in the laboratory until they were needed.

2.2 Extraction of EOs

The EOs from UM and AS were extracted according to the protocol described in previous studies [25,26]. First, 600 g of fresh, cleaned, and peeled bulb was mixed with distilled water using a domestic blender. The obtained mixture was transferred to a 2 L flask, which was heated under gentle boiling. After 3 h of heating, the EO was collected in amber glass bottles and stored in the refrigerator until analysis.

2.3 Chemicals and reagents

The reagents and chemicals used in this study were purchased from the following suppliers: Lobachemie (Mumbai, India), Oxfordlab (Maharashtra, India), Sigma-Aldrich (Darmstadt, Germany), and ScienceMed (Bucharest, Romania). These chemical compounds included ethanol, potassium hydroxide, $FeCl_3$, potassium iodide, ascorbic acid, gallic acid, sodium thiosulfate, acetic acid, hydrochloric acid, chloroform, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, and phenolphthalein.

2.4 Physicochemical analysis

In order to evaluate the quality and purity of the extracted EO, physicochemical analysis, including ethanol miscibility, density, refractive index, acid index (AI), peroxide index (PI), and ester index (EI), was performed.

2.4.1 Determination of physical indices

2.4.1.1 Relative density at 20°C

The relative density at 20°C of the EO was determined according to ISO 279: 2008. Using a 5 mL pycnometer, equal volumes of oil and water were weighed at a temperature of 20°C. The mass ratio of EO to water gives the density of our oil sample. This physical quantity is given by the following formula:

$$d_{\frac{20}{20}} = \frac{m_2 - m_0}{m_1 - m_0}, \quad (1)$$

where m_0, m_1, and m_2 represent the mass of the empty pycnometer, water, and EO studied, respectively.

2.4.1.2 Refraction index

The ratio of the sinusoidal angle of incidence to the sinusoidal angle of refraction of a ray of light of a specific wavelength entering the EO from air kept at a constant temperature is known as the refractive index. The refractive index was read directly using a Comecta-Ivymen refractometer in accordance with NM ISO 280 (2008).

2.4.2 Determination of chemical indices

2.4.2.1 Miscibility to ethanol

According to ISO 875:2008, ethanol miscibility was ascertained at a temperature of 20°C.

2.4.2.2 AI

The AI is the amount of potassium hydroxide (in mg) required to neutralize the free acids in 1 g of EO. In accordance with ISO 1242: 2008, the assay was carried out by adding 2 g of EO to a 100 mL flask, followed by 5 mL of 95% ethanol and five drops of a 2 g/L phenolphthalein solution. The mixture was continuously stirred while being titrated against KOH (0.1 M) until a persistent pink tint was achieved. The acid value was then determined as

$$\text{AI} = V \times C \times \frac{56.11}{m}, \quad (2)$$

where m, V, and C represent the mass of the test sample in g, the volume of KOH solution added per mL, and the KOH concentration, respectively.

2.4.2.3 PI

The amount of oxygen milliequivalents per kilogram of fat and oxidizing potassium iodide is known as the PI. The methodology involves soaking a test sample in a potassium iodide solution along with acetic acid and chloroform, and then titrating the iodine with a standard sodium thiosulfate solution. The procedure described by Boukeria et al. [27] was used to determine this parameter. Thus, the following formula is used to calculate the PI:

$$\text{PI} = \frac{(S - B) \times N \times 1{,}000}{W}, \quad (3)$$

where PI is the Meq peroxide per 100 g sample, S is the volume of the titrant (cm^3) for the sample, B is the titrant volume (cm^3) for the blank, N is the molarity of $Na_2S_2O_3$ solution (mEq/cm^3), 1,000 is the unit conversion (g/kg), and W is the weight of the oil sample.

2.4.2.4 EI

The quantity of acids generated during the hydrolysis of esters in an EO is measured with the EI. We decided to use the method outlined by NM ISO (Moroccan Standard ISO) to ascertain the EI of our EOs: We placed 2 g of EO in a flask and then filled it with 25 mL of potassium hydroxide solution. In addition, fragments of porcelain or pumice were added, and the flask was heated for an hour using a reflux heater. We filled the flask with 20 mL of water and five drops of phenolphthalein solution were added when it had cooled. A solution containing 0.5 M hydrochloric acid was used to titrate the excess potassium hydroxide. We utilized the following formula to obtain the EI:

$$\text{EI} = \frac{N \times (V_0 - V_1) \times 56.1}{M}, \quad (4)$$

where V_0 is the volume in mL of the HCl solution used for the blank, and V_1 is the volume in mL of the HCl solution used to determine the EI of the EO, N is the normality of the hydrochloric acid solution, M is the mass of EO used, and 56.1 is the molecular weight of potassium hydroxide.

2.5 GC-MS analysis

Chromatographic analysis of the EO of UM was performed using a 456-GC gas chromatograph coupled to an EVOQ TQ mass spectrometer at the Centre of Analysis, Expertise, Technology Transfer and Incubator CUA2TI of the IBN TOFAIL University of Kenitra. With an electrical shock applied at 70 eV, fragmentation was accomplished. The Rxi-5Sil MS capillary column (30 m × 0.25 mm ID × 0.25 µm df) was used. The column's temperature was initially set for 8 min at 40°C, increasing by 2°C every minute, until it reached 200°C, where it remained for 20 min. The carrier

gas, helium, was employed at a constant flow rate of 1.50 mL/min. One microliter of injection was used, and the injection temperature was kept at 250°C. The device, which was operated by Ms Data Review software and linked to a computer system that maintained a library of mass spectra from NIST MS Search2.0, allowed users to track the development of the chromatographic analyses. By comparing the retention times of components with those of reference compounds in the computerized database, components were identified (NIST 2014).

2.6 DPPH test

The DPPH test is one of the most used colorimetric techniques for assessing a sample's capacity to scavenge free radicals. We employed the protocol as reported by Olugbami et al. [28] and modified by Haida et al. [29], wherein 0.1 mL of a sample generated at various concentrations (0–2 mg/mL) was mixed with 2 mL of a 76 µM DPPH solution (0.03 mg/mL). After stirring the mixture quickly, it was allowed to rest at room temperature in the dark. The absorbances of the solutions were measured at 517 nm after 30 min of incubation using a spectrophotometer (UV-2005, Selecta).

Ascorbic acid was prepared as a standard under the same conditions, replacing the EO with ascorbic acid solutions at different concentrations. The experiments were repeated three times. The results are expressed as the percentage of $DPPH^{\cdot}$ inhibition, calculated as

$$\text{Inhibition percentage}(\%) = \frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{test}}}{\text{Abs}_{\text{blank}}} \times 100. \quad (5)$$

Using the equation of the lines for each sample, the IC_{50} value – which represents the concentration of the sample needed to neutralize 50% of the DPPH free radical – was calculated.

2.7 Ferric reducing antioxidant power (FRAP) test

The colorimetric test known as FRAP, or FLAP, measures a chemical species' antioxidant capacity to transfer electrons from a yellow iron(III) complex containing Fe^{3+} to a blue iron(II) complex having Fe^{2+} [30]. The assay was carried out in accordance with the methodology of Haida et al. [29]. Here, 2.5 mL of potassium ferricyanide ($K_3Fe(CN)_6$) solution and 2.5 mL of phosphate buffer solution (0.2 M at pH 6.6) were mixed with 1 mL of the samples (EO or reference) at various concentrations (0–1 mg/mL). For 20 min, the mixture was incubated at 50°C. To stop the reaction, 2.5 mL of a 10% trichloroacetic acid solution was added. After centrifuging the tubes for 10 min at 3,000 rpm, 2.5 mL of the supernatant was mixed with 0.5 mL of $FeCl_3$ (0.1%) and 2.5 mL of distilled water. A spectrophotometer (UV-2005, Selecta) was used to measure the absorbance of the solutions at 700 nm in comparison to a blank sample that was generated in the same way.

2.8 Evaluation of antifungal activity

2.8.1 Fungal material

The antifungal activity of EOs was also evaluated against the following fungal strains: *Fusarium oxysporum* and *Fusarium equiseti*. These were collected in the Mycotheca of the Laboratory of Plant, Animal, and Agroindustrial Production of the Faculty of Science of Kenitra, where they are regularly grown on a culture medium PDA (agar potato dextrose). The identification of the strains was done by observing their morphological characteristics.

2.8.2 Antifungal activities of EOs

The direct contact approach [1] was utilized to assess the antifungal activities of EOs. This involved adding a volume V of EOs to 20 mL of PDA medium in test tubes. After shaking, the medium was transferred to glass Petri dishes measuring 9 cm in diameter. In the center of each dish, a mycelial disk from a culture was grown for 3–7 days, with a diameter of roughly 0.5 cm. For every concentration, three duplicates were carried out, with a Petri dish containing 20 mL of PDA free of inhibitor serving as the control. Following a 7-day incubation period at 25 ± 4°C and accounting for the growth of the control mycelium, we calculated the antifungal index using the following formula:

$$\text{Antifungal index}(\%) = \frac{D_{\text{b}} - D_{\text{a}}}{D_{\text{b}}} \times 100, \quad (6)$$

where D_{a} is the diameter of the growth zone in the presence of the studied product and D_{b} is the diameter of the growth zone of the control.

2.8.3 Sporulation study

The effect of EO on sporulation was also studied. The obtained spores were diluted in distilled water and their

number was counted with a Malassez cell. The results were expressed as spore concentration/mL. The number of spores was determined according to the following formula:

$$\text{Spore concentration/mL} = \text{The average spore concentration} \times 10^5 \times \text{Dilution factor.} \quad (7)$$

2.9 Statistical analysis

The statistical analysis was performed by ANOVA with the SPSS 26 software and using Tukey's *post hoc* test at $p < 0.05$. In addition, the graphPad Prism 8.4.3 was used to perform the one-way ANOVA analysis.

3 Results and discussion

3.1 Yield study and organoleptic characteristics

The evaluation of the organoleptic characteristics (appearance, odor, color) of the two EOs obtained, after 3 h of extraction by hydrodistillation indicates that both EOs have an oily aspect of yellow for AS and pale yellow to colorless for UM; moreover, both oils have a pungent odor, undesirable for the case of AS. Also, the EOs obtained gave average yields of 0.43% ± 0.01% and 0.20% ± 0.01% for AS and UM, respectively. These data show that AS bulbs have high EO content compared to UM. Comparing these results with those of the literature, we note that the yield obtained for UM EO is double that reported by Tahri et al. [25], which may be because grinding in distilled water before hydrodistillation leads to increased contact area. It is important to note that the latter is the only study carried out on the extraction of EO from the UM bulb. The yield of AS EO obtained in our case is higher than that obtained by other studies. We note that the yield (0.4%) obtained by the study of Lawrence et al. [31,32] is the closest to our case. This variation in the EO content of the same species is mainly due to factors such as the nature of the soil, climate, year, and harvest period [33].

3.2 Physicochemical parameters

The physicochemical characteristics of AS and UM EOs were evaluated and summarized in Table 1. For AS, we compared our results with those of two other EOs of the same species grown in Morocco [27] and Algeria [34]. For UM, we compared the physicochemical properties of its oil with those of AS because these two plants belong to the same order *Asparagales* according to the APGIII classification. It should be noted that these parameters were determined for the first time for the EO of UM.

The results of the physicochemical tests of EOs are more or less close to those found in the literature [27,34].

The relative density of the two EOs was measured. A higher density than water was observed for AS, allowing them to be underwater during extraction. As for the value obtained for UM, it was lower, but close to that of water, allowing them to be above with a more or less miscible fraction. It was also found that the EO of AS was less miscible than that of UM when their miscibility was tested with ethanol.

It should be noted that the refractive index is influenced by the chemical composition of the sample and varies mainly according to the content of oxygenates and monoterpenes, a high content of the latter leading to a high index. The refractive indices obtained for the two EOs are approximately 1.47 and 1.42, respectively. These values are similar to those reported by Boukeria et al. [27] and Othman et al. [34].

Table 1: Physicochemical parameters recorded for AS and UM EOs

	Physical index			Chemical index		
	Relative density	**Refractive index**	**Miscibility to ethanol**	**Indices of acids**	**PI**	**EI**
AS						
This study	1.02 ± 0.00	1.47 ± 0.00	1V/5V	0.56 ± 0.01	4.012 ± 0.01	168.3 ± 0.22
Boukeria et al.	1.03	1.47	2.75	6.63	7.97	86.24
Othman et al.	ND	1.45	ND	ND	ND	ND
UM						
This study	0.93 ± 0.00	1.42 ± 0.00	1V/1V	1.12 ± 0.01	2.75 ± 0.00	188.63 ± 0.15

AI is an indicator of the free acid content in EOs. According to Juru De Cliff and Harerimana [35], an AI < 2 indicates a low amount of free acids and therefore a good preservation of the oils. The results of our study show that the AI values for AS and UM are 0.56 and 1.12, respectively, confirming the good quality and shelf life of our EOs.

The peroxide value and the ester value are two indicators used to evaluate the quality of EOs. A low peroxide value is a sign of the freshness of the oil, while a high ester concentration indicates the high quality of the sample. According to a study conducted by Rached et al. [1], the EOs of AS and UM had low peroxide values and high ester content compared to those obtained by Boukeria et al. [27]. These results indicate that the tested samples are fresh and of better quality.

3.3 GC-MS analysis of the EO of UM

In order to identify the chemical composition of the EO of the UM bulb, a GC-MS analysis was performed. This analysis identified the presence of 52 different compounds in the EO, representing 76.55% of its total composition. Figure 1 and Table 2 show the chemical composition of the EO.

GC-MS analysis indicates that the composition of this EO is mainly composed of monoterpenes (36.45%), such as compound 23, which is the major compound, Terpinen-4-ol (29), Terpineol (31), Linalol (35) as well as compound 42, sesquiterpenes (3.33%), such as α-bisabolol (50), *trans*-sesquisabinene hydrate (48), and cedrelanol (49), and other aromatic compounds such as compound anisaldehyde (37). The two main compounds are terpinolene (21.82%) and estragole (6.24%).

It is important to highlight that not much research has been done on the chemical composition of the EO found in UM flowers and bulbs [25,36]. Regarding the EO extracted from bulbs, we identified only one study conducted by Tahri et al. [25], which revealed the presence of 16 compounds, representing 80.08% of detected molecules, of which the major compounds were carvacrol and eugenol. Comparing the results of our study with those of Tahri et al., it is important to note that the majority composition differs considerably, with a low content of carvacrol detected in our EO. It is also interesting to note that some compounds, such as eucalyptol (17), terpinen-4-ol (29), and geraniol (36), which were identified in the EO extracted from the bulbs studied by Tahri et al., were detected. However, similarities were noted between the composition of the EO of UM flowers and our case, including the presence of 4-vinyl-guaiacol (40) and terpineol (31). Nevertheless, the percentage of these components in our oil is lower than that detected in the flowers [25]. These variations can be attributed to different factors such as geographical origin, harvesting period, extracted plant part, etc.

Concerning the EO of AS evaluated in our study, it was extracted and analyzed its chemical composition was determined and published in our previous paper [26]. The main compounds present in the oil are given in Table 3.

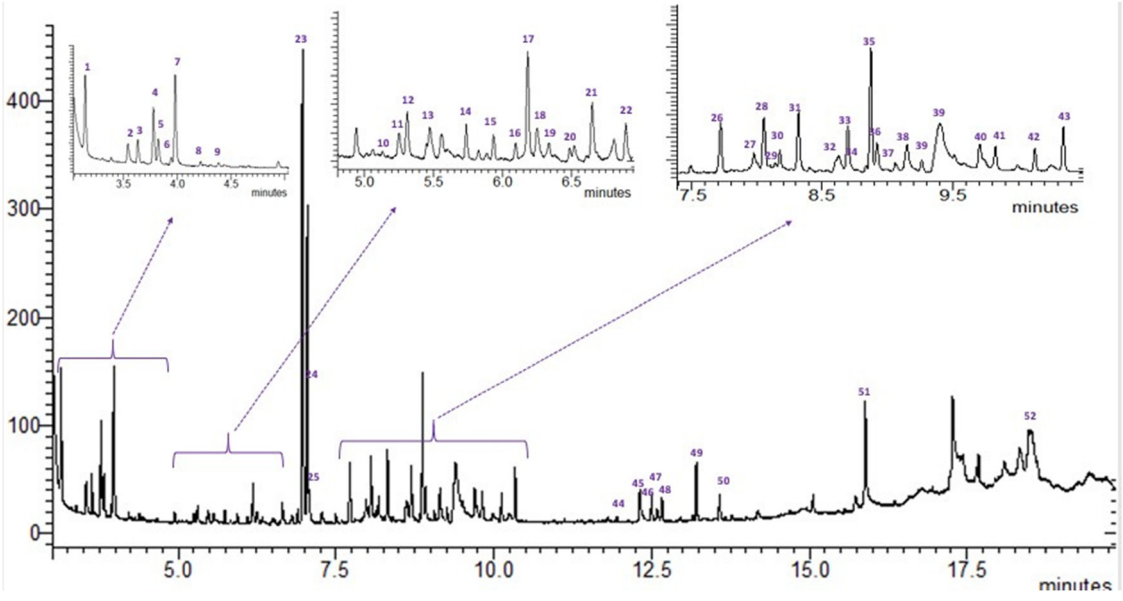


Figure 1: Chromatographic profile of EO from UM.

Table 2: Chemical compounds identified in the EO of UM

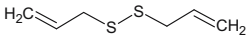
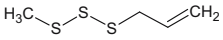
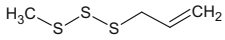
Num	Retention time (min)	Compound	*m/z* (%)	Chemical class	Percentage
1	3.145	Hexanal	44 (100), 41 (90), 56 (70), 43 (62), 57 (51)	Aliphatic compound	2.96
2	3.542	Furfural	32 (100), 39 (75), 95 (80), 96 (78), 39 (76),	Aromatic aldehyde	0.92
3	3.633	Tyranton	43 (100), 59 (50), 32 (42), 101 (15), 83 (14)	Aliphatic compound	0.96
4	3.778	(*E*)-2-Hexenal	41 (100), 55 (80), 39 (75), 69 (60), 83 (58)	Aliphatic compound	2.42
5	3.823	3-Hexen-1-ol, (*E*)-	41 (100), 67 (89), 32 (85), 55 (43), 39 (38)	Aliphatic compound	1.21
6	3.944	2-Hexen-1-ol, (*E*)-	32 (100), 57 (54), 41 (33), 83 (27), 85 (20)	Aliphatic compound	0.28
7	3.980	1-Hexanol	56 (100), 43 (60), 41 (59), 55 (59), 69 (35)	Aliphatic compound	4.02
8	4.221	2-Heptanone	32 (100), 43 (62), 58 (32), 83 (81), 85 (22)	Aliphatic compound	0.15
9	4.384	Heptanal	32 (100), 83 (35), 85 (22), 43 (19), 41 (18)	Aliphatic compound	0.16
10	5.251	Benzaldehyde	32 (100), 77 (43), 106 (32), 105 (32), 83 (31)	Aromatic aldehyde	0.27
11	5.313	1-Heptanol	32 (100), 41 (56), 70 (52), 55 (49), 56 (46)	Aliphatic compound	0.49
12	5.473	2,3-Octanedione	43 (100), 32 (80), 83 (24), 41 (22), 71 (20)	Aliphatic compound	0.54
13	5.557	2-Octanone	32 (100), 43 (52), 83 (31), 58 (29), 85 (22)	Aliphatic compound	0.25
14	5.738	Octanal	32 (100), 41 (42), 43 (41), 83 (32), 57 (30)	Aliphatic compound	0.35
15	5.935	Di-*t*-butylacetylene	32 (100), 81 (37), 83 (28), 123 (24), 41 (19)	Aliphatic compound	0.22
16	6.096	Ethylhexanol	32 (100), 57 (43), 83 (37), 41 (25), 43 (23)	Aliphatic compound	0.14
17	6.183	Eucalyptol	43 (100), 32 (66), 81 (54), 71 (40), 83 (36)	Oxygenated monoterpene	1.02
18	6.253	Benzylalcohol	32 (100), 79 (66), 77 (43), 108 (38), 83 (30)	Aromatic alcohol	0.40
19	6.488	(*E*)-Oct-2-enal	32 (100), 83 (37), 41 (18), 85 (17), 55 (15)	Aliphatic compound	0.12
20	6.519	2,3 Octanedione	32 (100), 43 (55), 83 (28), 99 (20), 41 (21)	Aliphatic compound	0.18
21	6.652	1-Octanol	32 (100), 41 (73), 56 (68), 55 (63), 43 (52)	Aliphatic compound	0.59
22	6.894	2-Dodecanone	32 (100), 41 (50), 39 (29), 83 (26), 43 (24)	Aliphatic compound	0.39
23	6.972	Terpinolene	55 (100), 43 (66), 83 (53), 41 (37), 45 (21)	Monoterpene	21.83
24	7.078	Nonanal	43 (100), 32 (88), 41 (82), 55 (68), 57 (60)	Aliphatic compound	0.70
25	7.281	Benzyl carbinol	32 (100), 91 (79), 92 (48), 83 (30), 65 (20)	Aromatic alcohol	0.38
26	7.723	Norcamphor	95 (100), 41 (76), 81 (76), 55 (52), 32 (48)	Monoterpene	1.71
27	7.979	*n*-Caprylic acid	32 (100), 60 (76), 73 (58), 42 (57), 43 (42)	Fatty acid	0.59
28	8.057	Endo-Borneol	95 (100), 41 (24), 32 (20), 43 (18), 67 (17),	Monoterpene	1.92
29	8.143	Terpinen-4-ol	32 (100), 71 (45), 43 (44), 41 (36), 83 (26)	Monoterpene	0.07
30	8.177	2-Dodecanone	43 (100), 58 (91), 32 (50), 41 (38), 71 (29)	Aliphatic compound	0.64
31	8.319	Terpineol	59 (100), 93 (54), 43 (48), 81 (38), 121 (31)	Monoterpene	1.96
32	8.400	*o*-Cumenol	32 (100), 68 (23), 41 (21), 83 (21), 55 (16)	Aromatic compound	0.14
33	8.626	*Cis*-Geraniol	32 (100), 41 (88), 69 (70), 83 (32), 68 (29)	Monoterpene	1.15
34	8.834	Pulegone	32 (100), 81 (34), 67 (29), 41 (28), 83 (21)	Monoterpene	0.17
35	8.872	Linalol	93 (100), 43 (90), 41 (55), 80 (47), 69 (34)	Monoterpene	3.66
36	8.918	Geraniol	69 (100), 41 (90), 32 (42), 68 (24), 39 (23)	Monoterpene	1.07
37	9.058	Anisaldehyde	32 (100), 41 (30), 135 (29), 83 (24), 55 (22)	Aromatic aldehyde	0.33
38	9.148	Nonanoic acid	60 (100), 32 (80), 73 (80), 41 (71), 57 (58)	Fatty acid	1.45
39	9.396	Estragole	148 (100), 77 (66), 147 (60), 32 (50), 105 (45)	Phénylpropanoides	6.24
40	9.701	4-Vinylguaiacol	77 (100), 135 (82), 32 (72), 150 (72), 107 (61)	Phenylpropanoides	0.97

(Continued)

Table 2: *Continued*

Num	Retention time (min)	Compound	*m/z* (%)	Chemical class	Percentage
41	9.821	Carvacrol	107 (100), 32 (72), 133 (58), 77 (36), 105 (28)	Monoterpene phenol	0.81
42	10.123	Nerol	69 (100), 32 (77), 41 (76), 43 (61), 68 (47)	Monoterpene	0.71
43	10.339	Neryl acetate	69 (100), 41 (74), 43 (65), 68 (44), 32 (32)	Monoterpene ester	1.39
44	11.804	2,4-Di-*tert*-butylphenol	32 (100), 191 (21), 57 (20), 83 (18), 41 (14)	Alkylated phenols	0.13
45	11.957	α-Acorenol	32 (100), 83 (17), 43 (13), 41 (13), 91 (11),	Sesquiterpene	0.12
46	12.317	Oleic acid	32 (100), 41 (84), 73 (74), 43 (70), 60 (65)	Fatty acids	0.92
47	12.668	Caryophylene oxide	32 (100), 41 (55), 43 (50), 79 (50), 91 (31)	Sesquiterpene	0.72
48	13.156	*trans*-Sesquisabinene hydrate	32 (100), 41 (19), 83 (16), 43 (14), 91 (12)	Sesquiterpene	0.17
49	13.211	Cedrelanol	43 (100), 161 (90), 81 (66), 32 (61), 105 (62)	Sesquiterpene	1.61
50	13.578	α-Bisabolol	32 (100), 43 (72), 69 (55), 41 (52), 109 (32)	Sesquiterpene	0.71
51	15.890	*n*-Hexadecanoic acid	43 (100), 41 (92), 73 (90), 60 (85), 55 (80)	Fatty acid	3.66
52	18.517	Abietinol	55 (100), 43 (60), 41 (58), 115 (55), 32 (57)	Diterpene	3.02
Chemical class				Monoterpenes	36.45
				Oxygenated monoterpenes	1.02
				Diterpenes	3.02
				Sesquiterpenes	3.33
				Other aroma compounds	9.78
				Aliphatic compounds	16.73
				Fatty acids	6.62
Total yield					

Table 3: The key compounds found in AS EO

Compound	Diallyl disulfide	Trisulfide, methyl-2-propenyl	Trisulfide, di-2-propenyl
Structure			
Percentage	26.62	16.46	34.10

3.4 Antioxidant activities of EOs

The DPPH test and the FRAP test, which evaluate a compound's capacity to release either an electron or a proton, are the two techniques used to determine the antioxidant activities of the EOs of the two plants.

3.4.1 DPPH assay

The IC_{50} value, or the concentration needed to lower 50% of the DPPH radical, is used to assess the antioxidant properties of EOs. The stronger the antioxidant potency, the lower the IC_{50} value [37]. Figure 2 represents the percentages of DPPH radical inhibition according to the concentrations of the EOs studied.

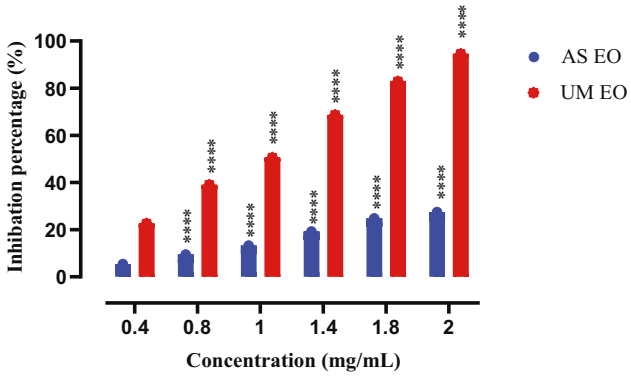


Figure 2: Evolution of antioxidant potency as a function of the concentration of EOs. ****$p < 0.0001$.

Figure 2 shows that the antioxidant activities of the studied samples are concentration-dependent, with an increase in DPPH radical inhibition when the amount of EO is increased in the medium. The EOs of UM and AS showed inhibition percentages of 94.48 and 27.53%, respectively. The IC_{50} index was used to compare the antioxidant properties of the samples against a reference. The trend line equations, R regression coefficients, and IC_{50} values are presented in Table 4.

Table 4 shows the IC_{50} values for the different samples. Ascorbic acid showed a very strong antiradical potency with an IC_{50} of 0.09 mg/mL, while the EO of UM showed better inhibitory properties than that of AS EO, with IC_{50} values of about 1.03 and 3.63 mg/mL, respectively.

The comparison of these values with those reported in the literature shows a variability in the values obtained. In the case of AS EO, Lawrence and Lawrence [31] reported that the EO of garlic grown in northern India showed an IC_{50} value of 0.5 mg/mL, similar to the results reported by Ncir et al. [3], who deduced the same value. This may be due to the presence of eugenol with 15% in their EO, which is known for its strong antioxidant properties. However, Ndoye et al. and Herrera-Calderon et al. [39,40] showed that AS EO shows excellent inhibitory properties with IC_{50} of 0.19 and 124.60 µg/mL, respectively. Herrera-Calderon et al. [40] attributed the strong antioxidant properties to the high diallyl trisulfide content in their EOs. Other works reported lower antioxidant potencies than previously, with IC_{50} of 7.67 and 63.58 mg/mL reported by Mnayer et al. [23] and Süfer and Bozok [41], respectively. The results determined by Mnayer et al. [23] Ndoye Foe et al. [31] and Süfer and Bozok [41] were also analyzed, and it was noticed that the antioxidant properties of the

Table 4: Free radical scavenging properties of AS and UM EOs, represented by IC_{50} values

Products	Equation	Coefficient of regression R	IC_{50} (mg/mL)
Ascorbic acid	$y = 521.29x + 4.3143$	0.9931	0.09 ± 0.0002^{a}
EO AS	$y = 13.907x - 0.444$	0.9969	3.63 ± 0.0006^{c}
EO UM	$y = 46.1x + 2.4292$	0.9966	1.03 ± 0.0003^{b}

Tukey's multiple range test indicates that values in the same column separated by the same letter do not significantly differ ($p < 0.05$).

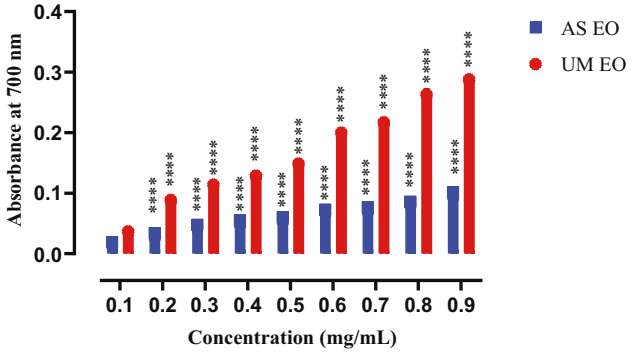


Figure 3: Evolution of absorbance as a function of the concentration of EOs. ****$p < 0.0001$.

EOs are related to the content of diallyl trisulfide, which confirms the data reported by Herrera-Calderon et al. [40].

In addition, Tahiri et al. [25] reported that the EO of UM exhibits strong antioxidant properties with an IC_{50} of 40.04 and 50.41 µg/mL for bulbs and flowers, respectively. In contrast, in our case, the EO shows an IC_{50} of 1.03 mg/mL. This difference is due to the variation in chemical composition between the EO analyzed by Tahri et al. and our case.

3.4.2 FRAP assay

The results of the FRAP test show an increase in absorption with increasing concentration of EO, as shown in Figure 3. According to Kallel et al. and Narendhirakannan and Rajeswari [42,43], this increase in absorbance indicates an increase in the reducing power of the test material. The results indicate that the EO of UM has a stronger reducing antioxidant power than the EO of AS. In order to make a meaningful comparison, the reducing capacity of the EOs was expressed in milligrams of ascorbic acid equivalent per gram (mg EAA/g) and $RP_{0.5AU}$, which is defined as the effective concentration at which the absorbance is 0.5 [42]. The results obtained are shown in Table 5.

Table 5: Reducing power of AS and UM EOs

	FRAP (mgAAE/g)	$RP_{0.5AU}$ (mg/mL)
Ascorbic acid	—	0.09 ± 0.0004^a
EO of UM	51.19 ± 0.004^b	1.60 ± 0.0009^b
EO of AS	11.36 ± 0.002^a	4.84 ± 0.0001^c

Tukey's multiple range test indicates that values in the same column separated by the same letter do not differ significantly ($p < 0.05$).

From Table 5, it is confirmed that the EO of UM shows a higher reducing power than AS with values of $RP_{0.5AU}$ = 1.60 mg/mL; 51.19 mgAAE/g and $RP_{0.5AU}$ = 4.84 mg/mL; 11.36 mgAAE/g, respectively. The results obtained for the case of AS are superior to those found by Barbara and Florentine [31,44]; the latter indicates that the reducing antioxidant power of AS EO is estimated to be 5.33 µg AAE/mg. As for the EO of UM, we had not identified any literature study that evaluated their reducing effect.

In general, FRAP and DPPH tests performed on the EOs showed that UM EO has a higher antiradical and reducing power than AS EO. This difference in performance could be explained by the chemical composition of the two EOs, which reveals that the EO of UM is richer in monoterpenes and sesquiterpenes, known for their antioxidant effects, than the EO of AS.

3.5 Antifungal activities

3.5.1 Effect on mycelial growth

Table 6 shows the results of the individual *in vitro* tests conducted on the chosen fungal strains (*F. oxysporum* and *F. equiseti*) to determine the antifungal activities of the EOs of AS and UM. The latter groups the percentages of antifungal inhibition noted for each concentration of EO tested on the seventh day of incubation.

Table 6: Antifungal index of EOs of AS and UM

	I% (AS EO)		*I*% (UM EO)	
Concentration (µL/mL)	*F. oxysporum*	*F. equiseti*	*F. oxysporum*	*F. equiseti*
0.08	10.94	12.78	24.06	37.84
0.25	24.06	33.42	24.38	39.31
0.50	56.56	100.00	27.81	39.56
2.50	100.00	100.00	28.75	40.05
5.00	100.00	100.00	31.25	40.54
12.50	100.00	100.00	31.25	45.21
CMI	0.50	0.25	—	—

Table 7: *In vitro* evaluation of the EOs studied against sporulation of *F. oxysporum* and *F. equiseti*

	AS EO				UM EO			
	F. oxysporum		*F. equiseti*		*F. oxysporum*		*F. equiseti*	
Concentration (µL/mL)	Mean sporulation (spores/mL)	Rate of sporulation reduction (%)	Mean sporulation (spores/mL)	Rate of sporulation reduction (%)	Mean sporulation (spores/mL)	Rate of sporulation reduction (%)	Mean sporulation (spores/mL)	Rate of sporulation reduction (%)
0.08	00.0×10^5	100	$5.8 \times 10^5 \pm 0.01$	78.52	$9.3 \times 10^5 \pm 0.01$	75.40	00.0×10^5	100
0.25	00.0×10^5	100	$2.4 \times 10^5 \pm 0.01$	91.11	$8.1 \times 10^5 \pm 0.01$	78.57	00.0×10^5	100
0.50	00.0×10^5	100	00.0×10^5	100	$6.3 \times 10^5 \pm 0.01$	83.33	00.0×10^5	100
2.50	00.0×10^5	100	00.0×10^5	100	$4.2 \times 10^5 \pm 0.004$	88.88	00.0×10^5	100
5.00	00.0×10^5	100	00.0×10^5	100	$1.25 \times 10^5 \pm 0.002$	96.69	00.0×10^5	100
12.50	00.0×10^5	100	00.0×10^5	100	00.0×10^5	100	00.0×10^5	100
Blank	$3.78 \times 10^6 \pm 0.01$	—	$2.7 \times 10^6 \pm 0.004$	—	$3.78 \times 10^6 \pm 0.01$	—	$2.7 \times 10^6 \pm 0.004$	—

The results indicate that at low concentrations (0.08–0.25), the percentage of inhibition for the EO of UM is higher than the percentage obtained for the EO of AS for the two strains studied. In contrast, beyond these concentrations, the percentage of inhibition increases remarkably with regard to AS to reach 100%. According to Hajji et al. [45], the tested product shows a weak antifungal activity if the inhibition percentage (*I*%) is between 30 and 40%, a moderate activity for *I*% varying between 50 and 60%, a good activity for *I*% between 60 and 70%, and an excellent activity when the percentage of inhibition is higher than 70%. From these data, we can infer that the EO of UM has weak antifungal activity toward *F. oxysporum* and moderate activity toward *F. equiseti*. In contrast, the EO of AS shows excellent antifungal activity toward both strains with minimum inhibitory concentrations of 0.50 µL/mL for *F. oxysporum* and 0.25 µL/mL for *F. equiseti*. These results can be explained by the presence of organosulfur compounds such as diallyl trisulfide known for strong antifungal activities [46–48]. It is concluded that the presence of allicin-derived organosulfur compound increases the antifungal potency of AS EO compared to UM EO.

3.5.2 Effect of sporulation

The results of the study on the sporulation of our fungi in the absence and presence of the EOs are summarized in Table 7.

Table 7 shows that both EOs exert an effect on the sporulation of both fungal strains. In addition, a decrease in sporulation of the samples is closely related to the increase of the EO concentration.

However, the analysis of Table 7 shows an overall inhibition of sporulation in the case of AS toward *F. oxysporum* and for the EO of UM toward *F. equiseti*.

The EO of AS showed a higher antifungal activity than that of UM, inhibiting not only the mycelial growth but also the sporulation of the studied fungal strains. The antifungal activity of 12 medicinal plants was reported in a previous study by Singh et al. [49], which also found that AS's EO had outstanding antifungal properties against *F. oxysporum*. These results are consistent with that work.

4 Conclusions

Overall, the results of this study clearly demonstrate that the GC-MS analysis revealed a significant percentage of terpenes in UM EO, with terpinolene (21.82%) and estragole (6.24%) as the main compounds. Moreover, UM EO showed

remarkable antioxidant activities compared to AS EO, with an IC_{50} of 1.03 mg/mL and an $RP_{0.5}$ of 1.60 mg/mL. These properties suggest that it could play a significant role in the pharmaceutical and food industries as a natural antioxidant agent. In contrast, AS EO demonstrated potent antifungal activities, particularly against the *Fusarium* strains studied, with MICs of 0.5 µL/mL for *F. equiseti* and 2.5 µL/mL for *F. oxysporum*. These antifungal properties offer interesting prospects for their potential use in the agricultural field, notably against fungal infections.

Abbreviations

GC-MS	gas chromatography and mass spectrometry
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	ferric reducing antioxidant power
IC_{50}	concentration causing 50% inhibition of the DPPH radical
RP0.5	concentration corresponding to absorbance 0.5
MICs	minimal inhibitory concentration
AI	acid index
PI	peroxide index
EI	ester index
PDA	potato dextrose agar
APG	angiosperm phylogeny group
UM	*Urginea Maritima*
AS	*Allium Sativum*
EO	essential oil

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Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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