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Investigation on chemical composition, antioxidant and antimicrobial potential of *Pulicaria mauritanica* essential oil applied by direct addition or vapor contact

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Abstract
Analysis of essential oil (EO) obtained from *Pulicaria mauritanica* in southeast of Morocco allowed the identification of 23 components, which accounted for 91.6% of the total oil in which carvotanacetone was the predominant constituent (55.1%), followed by (*E*)-Phytol (6.8%), β-caryophyllene (6.6%) and Thujopsene (4.9%). Antimicrobial potential of *P. mauritanica* oil in liquid and vapor phase against different fungal strains (*Penicillium expansum*, *Alternaria* sp., *Rhizopus stolonifer*, *Fusarium oxysporum* f. sp. *albedinis* and *Aspergillus brasiliensis* ATCC 16404) and bacterial strains (*Staphylococcus* aureus ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Salmonella abony* NCTC 6017) was determined by agar dilution method and disc volatilization method. *S. abony* was the most sensitive bacteria, providing the lowest growth with an MIC equal to 0.039 mg mL^{-1}. *Alternaria* sp., *R. stolonifer* and *A. brasiliensis* were found to be fungal susceptible to the EO of *P. mauritanica* and were completely inhibited in the presence of the liquid oil at 0.25 µL mL^{-1}. However, the mycelium growth was totally inhibited in the presence of the vapor generated by 0.125 µL mL^{-1} air for *Alternaria sp.*, *F. oxysporum albedinis*, *A. brasiliensis* and *R. stolonifer*. Furthermore, the *P. mauritanica* EO showed an important antioxidant property determined by 1, 1-diphenyl-1-picrylhydrazyl (DPPH) assay and by β-carotene bleaching test.

Keywords: *Pulicaria mauritanica*, Essential oil, antibacterial, antifungal, antioxidant, chemical composition

1. Introduction
The *Pulicaria* genus, which consists of 100 species, is well distributed from Europe to North Africa, particularly around the Mediterranean basin, as well as in Asia [1].
The extracted oils are a mixture of different compounds, which play a key role in the protection of the plants as antifungals, antibacterials, antivirals, insecticides and anti-herbivores which reduce their appetite for such plants [2].
Pulicaria mauritanica, which belongs to the important Asteraceae family, is an aromatic and endemic plant growing wild in southeast of Morocco. It is an herb endowed with a very strong odor; the flowers are yellow where it blooms in March-May.
P. mauritanica is locally known in Morocco by the Berber vernacular names of "Ifanzi n' Oudaden". In the Moroccan traditional medicine, the plant are used to treat diseases such as digestive and circulatory [3]. In Algerian traditional medicine, the local herbalists traditionally use this wild plant to treat inflammation related diseases as well as for treatment of intestinal disorders and headache [4].
This study deals with the valorization of medicinal and aromatic plants of Moroccan oasis flora, in order to find new bioactive natural products. The aims of this work were to examine the chemical composition of the essential oil obtained from aerial part of *P. mauritanica* originated from Southern Moroccan Sahara and investigate its antioxidant activities and antimicrobial properties by poison food (PF) technique and the volatile activity assay (VA) against fives agricultural pathogenic fungi and four pathogenic bacteria.

2. Materials and Methods
2.1 Plant material
The aerial part of *P. mauritanica* was collected in Errachidia (Morocco), during the flowering

period. The voucher specimen has been deposited under the number A 12/2015 at the Biochemistry of Natural Products Laboratory, Department of Biology, Faculty of Sciences & Techniques, Errachidia, Morocco.
The dried plant material was stored in the laboratory at room temperature (25°C) and in the shade before the extraction.

2.2 Hydrodistillation apparatus and procedure
The extraction of essential oil (EO) of the aerial part of *P. mauritanica* was conducted using distillation with water vapor drag in Clevenger type apparatus [5]. The obtained EO was dried using anhydrous sodium sulfate and stored in an amber vial at −20 °C until test and analysis.

2.3 Chromatographic analysis
The EO was analyzed using gas chromatography coupled to the mass spectrometry (GC / MS Trace-Polaris Q, Thermo Finnigan), fitted with a TRACE TR-5 capillary column (30 m × 0.25 mm ID × 0.25 µm, Thermo). The carrier gas was helium; the program was 2 min isothermal at 40 °C, then the temperature increased by 5 °C/min to 280 °C. The injection port and transfer lines temperature were 220 and 280 °C, respectively.
The individual compounds were identified by comparing their retention indices relative to C8-C26 *n*-alkanes (Kovat's indices, KI) and by comparing their mass spectra and retention times with data already available in the NIST (National Institute of Standardization and Technology) library and literature [6].

2.4 Antioxidant activity
2.4.1 DPPH radical scavenging activity: Radical scavenging activity of EO against stable 1,1-diphenyl-1-picrylhydrazyl (DPPH) was evaluated [7] with slight modifications. The reaction mixture contained 50 µL of diluted essential oil at different concentrations (2.5, 5, 10, 20, 40 µg mL^{-1}) and 2 mL of methanolic DPPH (60 µM). The result mixtures were left at room temperature for 30 min and the absorbance was measured at 517 nm. The IC_{50} (concentration providing 50% inhibition) values were calculated from the plotted graph of scavenging activity against the concentrations of essential oil. BHT (butylatedhydroxytoluene) a stable antioxidant was used as a synthetic reference.

$$\%\ inhibition = \frac{(\text{Abs (control)} - \text{Abs (sample)})}{\text{Abs (control)}} X100$$

Abs control is the absorbance without extract; Abs sample is the absorbance of the essential oil or standard.

2.4.2 β-Carotene bleaching assay: The β-carotene bleaching inhibition method was carried out [8]. Briefly, β-carotene (2 mg) were dissolved in chloroform (10 mL) of which 4 mL of solution was pipetted into a round-bottom flask, which contains linoleic acid (40 mg) and of Tween 40(500 mg). The chloroform was then, evaporated under vacuum at 40°C and 100 mL of oxygenated water was added and vigorously shaken to yield an emulsion. 1 mL of the emulsion was transferred into test tubes containing 100 µL of diluted EO and incubated in a water bath at 50°C then the absorbance was measured at 470 nm immediately (t = 0 min) and after 120 min of incubation against a blank which contains the emulsion without β-carotene. The antioxidant activity of plants was compared to the positive control, which was BHT in this assay. The β-carotene bleaching inhibition (%) of the analyzed solution was calculated via the following formula:

$$\beta\text{ carotene bleaching inhibition }(\%) = \frac{\beta\text{ carotene content after 2 h assay}}{\text{Initial }\beta\text{ carotene content}} \text{x } 100$$

2.5 Antibacterial activity
2.5.1 Bacterial strains: The antibacterial activity was evaluated against four selected Gram-positive and Gram-negative species: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Salmonella abony* NCTC 6017 and *Escherichia coli* ATCC 8739. Microorganisms were obtained from the culture collection of the National Institute of Hygiene (Rabat).

2.5.2 Solid diffusion tests: The qualitative antimicrobial essay of the EO was carried out using the disc diffusion method [9]. It was performed using culture growth at 37°C for 18h and adjusted to approximately 10^8 CFU mL^{-1} of the micro-organism under study. The culture medium used for the bacteria was Mueller Hinton Agar (MHA). 100 µL of the inocula were spread over plates containing MHA and a Whatman paper disc (6 mm) impregnated with 5, 10 and 15 µL of the EO was placed on the surface of the media. The plates were left 30 min at room temperature to allow the diffusion of the EO. They were incubated 24 h at 37 °C. After incubation period, the inhibition zone obtained around the disc was measured. Two controls were also included in the test, the first was involving the presence of microorganisms without test material and the second was standard antibiotic. The developing inhibition zones were compared with those of reference discs.

2.5.3 Vapor diffusion tests: Vapor diffusion assay was conducted [10]. Briefly solidified medium was inoculated with 100 µL of bacterial suspension containing 10^8 CFU mL^{-1} of the microorganism under study. A sterile filter discs (diameter 6 mm) was laid in the centre of the inside surface of the upper lid. Then, 5, 10 and 15 µL of EO were added to each disc. The plate inoculated with microorganisms were immediately inverted on top of the lid and sealed with parafilm to prevent leakage of EO vapor. Plates were incubated at 37°C for 24 h and the diameter of the resulting inhibition zone in the bacterial lawn was measured.

2.5.4 Dilution method: The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of tested volatile fractions were determined using the Mueller Hinton broth (MHB) dilution method [11] with slight modification.
Bacterial strains were cultured overnight in MHB supplemented with agar (0.2%) at 37 °C. Tubes of MHB containing various concentrations of volatile fractions were inoculated with 10 µL of 10^8 CFU mL^{-1} of standardized microorganism suspensions. Control tubes without tested samples were essayed simultaneously. The MIC was defined as the lowest concentration preventing visible growth [12]. The MBC is the lowest concentration without colony growth on the agar plates. All experiments were performed in triplicate.
The MBC/MIC ratio is a parameter that reflects the bactericidal capacity of the analyzed compound. When this ratio is less than or equal to 4, the EO is qualified to be bactericidal and when it is greater than 4, the EO is called bacteriostatic [13].
The tolerance level of bacterial strains toward the EO was calculated from the respective MIC and MBC values.

2.6. Antifungal activity

2.6.1 Fungal strains: Five agricultural pathogenic fungi were selected for their implication in the contamination and the deterioration of vegetables and fruits. The fungal species used in the experiments were *Alternaria* sp., *Pencillium expansum*, *Rhizopus stolonifer*, *Fusarium oxysporum* f. sp. *albedinis* and *Aspergillus brasiliensis* ATCC 16404. These fungi were obtained from the culture collection at Faculty of Sciences & Technology, Errachidia.

2.6.2 Poison food (PF) technique: The antifungal activity of EO against mycelial growth of fungi was evaluated by poisoned food technique (PF) with minor modifications [14]. Concisely, the EO was dispersed as an emulsion in sterile agar suspension (0.2%) and added to PDA immediately before it was emptied into the glass Petri dishes (90 × 20 mm) at 45 °C to achieve final concentrations of 0.062 to 0.5 µL mL^{-1}. The controls received the same quantity of sterile agar suspension (0.2%) mixed with PDA. The tested fungi were inoculated with 6 mm mycelial plugs from 7-days-old cultures cut with a sterile cork borer and incubated for 3 days for *Rhizopus stolonifer* and 6 days for *Alternaria* sp., *P. expansum*, *F. oxysporum* f. sp. *albedinis* and *A. brasiliensis* at 25±2 °C.

2.6.3 Volatile activity assay: The antifungal activity of EO against mycelial growth was determined by following the volatile activity assay (VA) [15]. The Petri dishes were filled with 20 mL of potato dextrose agar (PDA) medium and then seeded with a mycelial disc (6 mm diameter), cut from the periphery of 7-days-old mycelium culture of the tested fungi. The Petri dishes (90 × 20 mm, which offer 80 mL air spaces after addition of 20 mL PDA), were inverted and sterile filter paper discs (9 mm in diameter) impregnated with different concentrations of EO (i.e., 0.062-0.5 and 1µL mL^{-1} air) were deposited on the inverted lid and incubated for 3 days for *Rhizopus stolonifer* and 6 days for *Alternaria* sp., *P. expansum*, *F. oxysporum* f. sp. *albedinis* and *A. brasiliensis* at 25±2°C.

In both types of experiments, triplicate plates were inoculated for each treatment and the radial growth was recorded for each plate by calculating the average of two perpendicular diameters. Fungitoxicity of essential oil was expressed in terms of percentage of mycelial growth inhibition (IP %) and calculated following the formula [16].

Percentage of mycelial growth inhibition (IP)

$$= \left(1 - \left(\frac{Dc}{Dt}\right)\right) \times 100$$

Where

D_c: Average diameter (in mm) of mycelial in control and D_t: Average diameter (in mm) of mycelial in treatment.

The fungistatic–fungicidal nature of the EO was tested by observing revival of growth of the inhibited mycelial disc following its transfer to non-treated PDA. A fungicidal effect was where there was no growth, whereas a fungistatic effect was where temporary inhibition of microbial growth occurred.

2.7 Statistical analysis

Statistical analysis was performed using StatView 5.0 software. The results are reported as mean ± SD (standard deviation) (n=3).

3. Results & Discussion

3.1 Chemical Composition of the Essential Oil

The chemical composition of the essential oil extracted from *P. mauritanica* was analyzed by GC-MS. The essential oil components their percentage as well as their retention times (RT) are summarized in Table 1 and Figure 1. Hydrodistillation of plant material yielded approx 0.55 % of yellowish oil. Twenty-two compounds were identified in this sample representing 91.56% of the total oil. The most abundant components were carvotanacetone (55.12%), (*E*)-phytol (6.75 %), β-caryophyllene (6.59 %) and thujopsene (4.94 %).

Table 1: Percentage composition of the essential oils of *P. mauritanica*

N°	Retention Time (min)	Chemical constituents	KI	%Area
1	12.73	α-Pinene	933	0.37
2	15.46	Carene	1002	0.39
3	15.79	*o*-Cymene	1042	1.31
4	16.85	γ-Terpinene	1061	0.61
5	20.85	*cis*-2-Menthenol	1121	0.44
6	21.44	Terpinen-4-ol	1162	1.4
7	21.61	Borneol	1167	0.9
8	23.48	Carvotanacetone	1226	55.12
9	24.62	Carvenone	1233	1.33
10	26.74	Thymol	1272	0.51
11	27.01	Elixene	1354	0.36
12	27.58	Thujopsene	1424	4.94
13	29.12	β-Caryophyllene	1438	6.59
14	30.15	Germacrene D	1486	1.68
15	31.62	δ-Cadinene	1513	0.64
16	31.89	Alloaromadendrene	1517	2.67
17	33.12	Caryophyllene oxide	1570	2.85
18	34.30	τ-Cadinol	1624	0.5
19	36.76	α-Cadinol	1634	0.4
20	37.08	β-Eudesmol	1637	0.43
21	42.45	Anthracene	1795	1.37
22	48.77	(*E*)-Phytol	1949	6.75
	Total			91.56

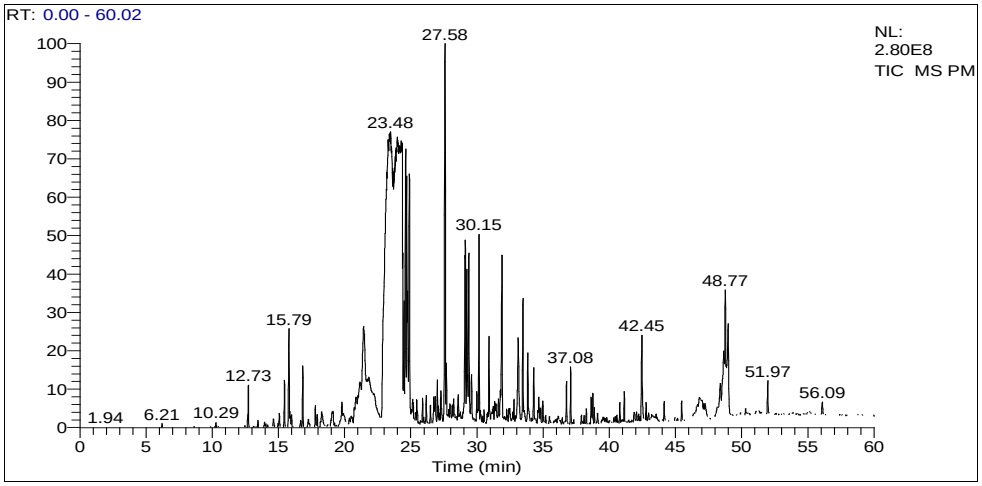

Fig 1: Chromatogram of essential oil of aerial parts of *P. mauritanica* from Morocco

These results are similar with those previously reported by Cristofari *et al.* [17] and have identified twenty-five compounds strongly dominated by carvotanacetone (87.3%) in oil of *P. mauritanica* grown in Morocco. In addition, Gherib *et al.* [18] have identified 22 compounds with carvotanacetone constituting 89.2% of the oil. The differences recorded in EO chemical composition might be due to several factors in particular soil composition, plant tissue, vegetative cycle phase, climate, genetics, Physiological age of the plant in addition to the method of oil isolation [19].

3.2 Antioxidant activities

In this study, the antioxidant activity of EO was investigated by two complementary tests: scavenging of DPPH• free radicals and the β-carotene bleaching test. The results are shown in Table 2. The DPPH antioxidant activity of EO is presented by its IC_{50} value.

Comparison of the DPPH scavenging activity of the EO (232.43 ± 0.62 µg ml^{-1}) and those expressed by the synthetic antioxidant BHT (47.82 ± 0.13µg ml^{-1}) showed that the EO exhibited weaker antioxidant effects than BHT; the antioxidant effect of the oil was about 5 times lower than that of the BHT.

Table 2: Antioxidant capacity of *P. mauritanica* essential oil.

	DPPH IC_{50} (µg mL^{-1})	β-carotene/linoleic acid inhibition (%)
P. mauritanica EO	232.43 ± 0.62	69.84 ± 0.38
BHT	47.82 ± 0.13	79.52 ± 0.26

The results are expressed in mean ± standard error (n = 3)

As can be seen from Table 2, the potential of EO to inhibit lipid peroxidation was evaluated using the β-carotene/linoleic acid bleaching test, with a value of 69.84 ± 0.38% and 79.52 ± 0.26%, obtained for the oil and the positive control BHT, respectively at the same concentration of 100 µg mL^{-1}. This activity was moderately lower than that of BHT.

The antioxidant activity of this EO can be attributed to the presence of appreciable amount of antioxidant compounds such as of β-caryophyllene [20], α-pinene and thymol [21].

3.3 Antibacterial activity

The results of susceptibility of strains tested are indicated in Table 3. Standard antibiotics presented an important activity against Gram+ bacteria compared to Gram- bacteria, except penicillin which is inactive against *E. coli*. The *in vitro* antibacterial activities of the EO against the four bacteria species tested were assessed in liquid as well as in vapor phase by the presence or absence of inhibition zone diameters, and MIC values.

Table 3: Susceptibility of the bacterial strains tested against *P. mauritanica* essential oil

	Antibiotics					
Microorganisms	**P_{10}**	**C_{30}**	**CN_{10}**	**S_{10}**	**AMC_{30}**	**CIP_5**
E. coli ATCC 8739	NA	26.33±0.57	21.67±0.57	12.00±0.00	17.00±1.00	12.33±0.57
S. aureus ATCC 6538	34.33±1.15	29.00±1.00	24.66±0.57	20.00±0.00	33.66±0.57	15.33±0.57
S. abony NCTC 6017	15.33±0.57	26.33±0.57	21.00±1.00	16.66±0.57	23.33±0.57	14.67±0.43
B. subtilis ATCC 6633	34.66±0.57	35.33±1.15	30.66±0.57	21.33±0.57	29.66±1.15	21.67±0.57

P_{10}: Penicillin (10µg/disc); C_{30}: Chloramphenicol (30µg/disc); CN_{10}: Gentamicin (10µg/disc); S_{10}: Streptomycin (10µg/disc); AMC_{30}: Amoxycillin/Calvulanic Acid (30µg/disc); CIP_5: Ciprofloxacine (5µg/disc).

The EO showed higher activity in the liquid phase (Table 4). Despite the fact that vapor has the advantage of being able to treat large areas and do not require direct contact with liquid oils, which can make them more suitable for use as disinfectants [22]. *B. subtilis* was the only microorganism that was better inhibited when in vapor contact. At low concentration (5 µL/disc), the EO in the liquid phase, showed inhibition zones against all bacteria. A strong antibacterial activity was observed against *S. abony* (18.00±0.00 mm), the size of the inhibition zone generally increased in the following order: *B. subtilis* (14.33±1.15 mm) followed by *E. coli* (12.33±0.58 mm), and *S. aureus* (10.33±0.58 mm), the latter was the most resistant bacteria.

Table 4: Antibacterial activity of *P. mauritanica* essential oil against select bacterial strains

	Liquid contact EO (µl/disc)			Vapor contact EO (µl/disc)		
	Inhibition zone diameter [a]					
Microorganisms	5	10	15	5	10	15
Gram-						
E. coli ATCC 8739	12.33±0.58	16.67±0.58	23.00±1.00	NA	NA	NA
S. abony NCTC 6017	18.00±1.00	37.00±1.00	47.67±1.15	NA	14.33±0.58	26.00±1.00
Gram-						
S. aureus ATCC 6538	10.33±0.58	13.67±0.58	18.00±0.00	NA	NA	NA
B. subtilis ATCC 6633	14.33±1.15	23.33±0.58	36.33±1.53	18.75±0.50	22.67±0.58	33.00±1.00

[a] Diameter of the zone of inhibition (mm) including disk diameter of 6 mm; NA: Not active

However, no difference was observed in antibacterial activity against Gram-positive or Gram-negative bacteria and the activity of the EO was ascertained. The essential oil of *P. odora* L. was more effective against *Streptococcus C, Bacillus cereus, Enterococcus faecalis* and *Proteu svulgari* [23].Whereas, oils from *Thymus satureioides* L. were reported more effective than those from *Mentha pulegium* L. on human mycosis causing agents [24]. *Artemisia herba alba* inhibit some microorganism growth like *Streptococcus agalactiae, Salmonella enteritidis.* However, it had no effect on *Pseudomonas aeruginosa* [25]. It was also reported that *Origanum vulgare* and *Thymus zygis* essential oils were more effective on *E. coli* compared to those from *Rosmarinus officinalis, Lavandula* sp. and *Thymus vulgaris,* whereas, *Origanum vulgare* was the only one effective on *Staphylococcus aureus* [26].

The inhibition activity depends on plant taxonomy as well as on oil concentration and types of chemical radicals of the molecules in the oils [24].

The noteworthy antibacterial activity of the EO was supported by the values of the MIC (0.039 - 0.312 mg mL^{-1}) and MBC (0.078 - 1.248 mg mL^{-1}) against all tested bacterial strains (Table 5). Regarding the MIC values, *S. abony* are the most sensitive microorganisms, providing the lowest growth of the microorganism with an MIC equal to 0.039 mg mL^{-1}, followed by *B. subtilis* (0.078 mg mL^{-1}), *E. coli* (0.156 mg mL^{-1}) and finally *S. aureus* (0.312 mg mL^{-1}).

The MBC/MIC ratio is used to determine the bactericidal capacity of the analyzed compound against the tested organism. In all strains, the tolerance level was <4 when charged with EO. Bactericidal agents kill bacteria, whereas bacteriostatic agents simply inhibit the bacterial growth or reproduction. When MBC/MIC ratio is >4 for bacterial strains, the agent is considered bacteriostatic and when this ratio is ≤4, the agent is considered bactericidal [13].

Table 5: The MIC and MBC values of *P. mauritanica* essential oil against select microorganisms

Microorganisms	MIC (mg mL^{-1})	MBC (mg mL^{-1})	MBC/MIC	Effect
Gram-negative bacteria				
Escherichia coli ATCC 8739	0.156	0.624	4	bactericidal
Salmonella abony NCTC 6017	0.039	0.078	2	bactericidal
Gram+ positive bacteria				
Staphylococcus aureus ATCC 6538	0.312	1.248	4	bactericidal
Bacillus subtilis ATCC 6633	0.078	0.312	4	bactericidal

The significant antibacterial activity of EO against these strains is probably due to the major compound carvotanacetone (55%), an oxygenated monoterpene. Generally, bioactivity of monoterpenes such as ketones, alcohols and aldehydes have been demonstrated against pathogens [27].

3.4 Antifungal activity

The antifungal activity of the EO was determined by *in-vitro* testing against four microorganisms, as seen in Figures 2, 3 and Table 6. The vapor exposure or direct addition of EO, the percent inhibition increased with EO concentration. Using the PF assay, the results showed that *Alternaria* sp., *R. stolonifer* and *A. brasiliensis* were found to be susceptible to the EO and were completely inhibited in the presence of the EO at 0.25 µL mL^{-1} (Figure 2). At same concentration, *F. oxysporum albedinis* and *P. expansum* with the IP were 87.04±0.64 % and 81.85±1.7 %, respectively. Furthermore, the successful effects correlate with EO concentration [24] as well as with the type of fungal species. For example, with *M. pulegium*, 10 µL was necessary to inhibit growth of *Penicillium expansum* and *Alternaria alternata* [28] whereas 20 µL was needed for *Penicilium. sp* and 2 µg mL^{-1} for mycosis agents [24].

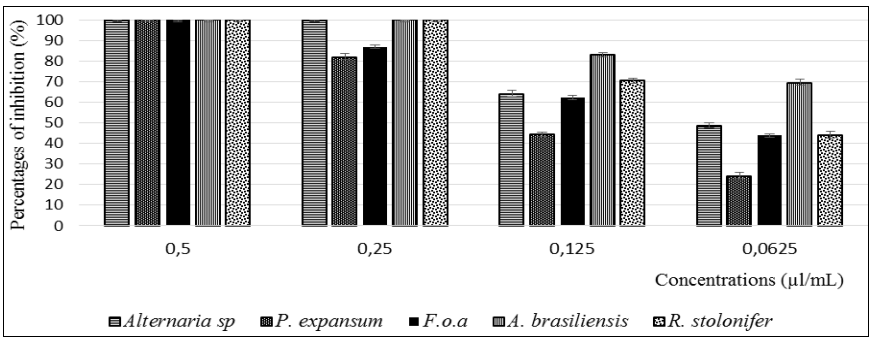

Fig 2: Antifungal potential of *P. mauritanica* essential oil liquid as percent inhibition.

Using VA assay, the results (Figure 3) showed that the activity of EO vapor was more pronounced for all strains tested. The mycelium growth was totally inhibited (100±0.0 %) in the presence of the vapor generated by 0.125 µl mL^{-1} air for *Alternaria* sp., *F. oxysporum albedinis*, *A. brasiliensis* and *R. stolonifer*. Moreover, the mycelium growth of *P. expansum* was only partially inhibited (79.6±0.7 %) at same concentration.

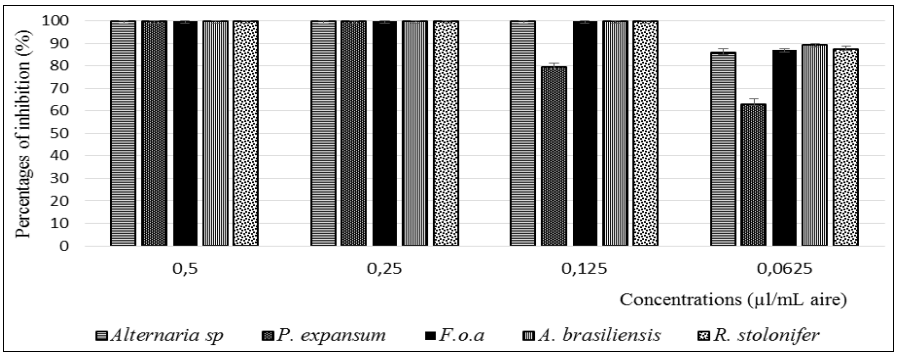

Fig 3: Antifungal potential of *P. mauritanica* essential oil vapor as percent inhibition.

The fungitoxic nature of this EO vapor against all fungal strains tested was required for suitable applications. To confirm this, the mycelial discs where growth inhibition was complete by EO vapor into the PDA medium not containing this oil was transferred. The EO was shown to have antifungal activity at a minimum inhibitory dose of 0.125 µL mL^{-1} air (Table 6). All fungal species failed to restore growth even after incubation for six days without this EO, indicating a fungicidal activity of the EO at 0.25 µL mL^{-1} air, except of *P. expansum* where the MFC was 0.5 µL mL^{-1} air.

Table 6: Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) values of essential oil in µL mL^{-1} air

	Direct method µl/mL		**Vapor method µl/mL air**	
Strains	MIC	MFC	MIC	MFC
Alternaria sp.	0.25	0.5	0.125	0.25
P. expansum	0.5	0.5	0.25	0.5
F. oxysporum albedinis	0.5	1	0.125	0.25
A. brasiliensis (ATCC 16404)	0.25	0.5	0.125	0.25
R. stolonifer	0.25	0.5	0.125	0.25

The antifungal activity of EO probably related to the high concentration of carvotanacetone and cadinol that were reported as effective antifungal agents [29]. Bouchra *et al*. [30] have reported that pulegone and 1, 8-cineole require higher concentration to inhibit mycelia growth than when used as total essential oil. Thus, the activity of the EO probably results from the combination of all major compounds as well as from a synergic effect of the less dominant ones [30].

According to the results of this study, EO vapor has better antifungal activity against tested fungal strains compared to that observed using the PF technique. The efficacy of essential oils in vapor state was probably attributable to the direct deposition of essential oils on lipophilic fungal mycelia together with an indirect effect via adsorption through the culture medium [31]. Recently, there have been several studies confirming that vapor phases of EOs are more effective antifungal than their liquid phases including *Rosmarinus officinalis* [32], *Citrus sinensis* [33] and a range of others including thyme and fennel [34].

4. Conclusions

Essential oils (EO) were successfully distilled from *P. mauritanica* and were assessed for their *in-vitro* antimicrobial/antifungal and antioxidant properties. The EO was rich in carvotanacetone (55%) and possesses good antifungal activity against *Alternaria* sp., *Pencillium expansum*, *Rhizopus stolonifer*, *Fusarium oxysporum* f. sp., a *lbedinis* and *Aspergillus brasiliensis*. In addition, the EO is highly effective in the liquid phase and could potentially be used to combat bacterial pathogens (*Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella abony* and *Escherichia coli*). The EO vapor may be considered as a potential agent for preventing phyto-pathogenic fungi. Further studies are warranted to confirm the antifungal activity of EO for food preservation and/or extended shelf life of raw and processed food and in animal models.

References

1. Williams CA, Harborne JB, Greenham JR, Grayer RJ, Kite GC, Eagles J. Variations in lipophilic and vacuolar flavonoids among European Pulicaria species. Phytochemistry. 2003; 64(1):275-283.
2. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils–a review. Food and chemical toxicology. 2008; 46(2):446-475.
3. Ouhaddou H, Boubaker H, Msanda F, El Mousadik A. An ethnobotanical study of medicinal plants of the Agadir Ida Ou Tanane province (Southwest Morocco). Journal of Applied Biosciences. 2015; 84(1):7707-7722.
4. Xu T, Gherib M, Bekhechi C, Atik-Bekkara F, Casabianca H, Tomi F *et al*. Thymyl esters derivatives and a new natural product modhephanone from Pulicaria mauritanica Coss. (Asteraceae) root oil. Flavour and Fragrance Journal. 2015; 30(1):83-90.
5. Clevenger J. Apparatus for the determination of volatile oil. Journal of the American Pharmaceutical Association. 1928; 17(4):345-349.
6. Adams RP, Sparkman OD. Review of Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. J Am Soc Mass Spectrom. 2007; 18:803-806.
7. Andrade MA, das Graças Cardoso M, de Andrade J, Silva

LF, Teixeira ML, Valério Resende JM *et al*. Chemical composition and antioxidant activity of essential oils from Cinnamodendron dinisii Schwacke and Siparuna guianensis Aublet. Antioxidants. 2013; 2(4):384-397.
8. Bouhlali EDT, Sellam K, Bammou M, Alem C, FIlali-Zehzouti Y. *In vitro* Antioxidant and anti-inflammatory properties of selected Moroccan medicinal plants. Journal of Applied Pharmaceutical Science. 2016; 6(5):156-162.
9. Bammou M, Bouhlali EDT, Sellam K, Ibijbijen J, El Rhaffari l, Nassiri L. Liquid and vapour-phase bioactivity of *Hertia maroccana* (Batt.) Maire essential oil: an endemic Asteraceae from Morocco. Journal of Applied Pharmaceutical Science. 2016; 6(3):131-136.
10. Lopez P, Sanchez C, Batlle R, Nerin C. Solid-and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. Journal of agricultural and food chemistry. 2005; 53(17):6939-6946.
11. Amezouar F, Badri W, Hsaine M, Bourhim N, Fougrach H. Chemical Composition, Antioxidant and Antibacterial Activities of Leaves Essential Oil and Ethanolic Extract of Moroccan *Warionia saharae* Benth. & Coss. Journal of Applied Pharmaceutical Science. 2012; 2(05):212-217.
12. Kuppusamy P, Yusoff MM, Parine NR, Govindan N. Evaluation of *in-vitro* antioxidant and antibacterial properties of *Commelina nudiflora* L. extracts prepared by different polar solvents. Saudi journal of biological sciences. 2015; 22(3):293-301.
13. Joubert T, Chambon P, Gattefosse M. Détermination du pouvoir bactériostatique et bactéricide des essences pures et mélanges. Bull. Tech. Gatte. 1958; 56:7-16.
14. Perrucci S, Mancianti F, Cioni P, Flamini G. *In vitro* antifungal activity of essential oils against some isolates of Microsporum canis and Microsporum gypseum. Planta medica. 1994; 60(2):184-186.
15. Soylu EM, Kurt Ş, Soylu S. *In vitro* and *in vivo* antifungal activities of the essential oils of various plants against tomato grey mould disease agent Botrytis cinerea. International Journal of Food Microbiology. 2010; 143(3):183-189.
16. Pandey D, Tripathi N, Tripathi R, Dixit S. Fungitoxic and phytotoxic properties of the essential oil of *Hyptis suaveolens*. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz. 1982; 89:344-349.
17. Cristofari G, Znini M, Majidi L, Bouyanzer A, Al-Deyab S, Paolini J *et al*. Chemical composition and anti-corrosive activity of Pulicaria mauritanica essential oil against the corrosion of mild steel in 0.5 MH_2SO_4. Int. J Electrochem. Sci. 2011; 6:6699-6717.
18. Gherib M, Bekhechi C, Bekkara FA, Bighelli A, Casanova J, Tomi F. Chemical Composition and Antimicrobial Activity of the Essential Oil from Aerial Parts of Algerian Pulicaria mauritanica. Natural product communications. 2016; 11(1):109-112.
19. Teixeira B, Marques A, Ramos C, Neng NR, Nogueira JM, Saraiva JA *et al*. Chemical composition and antibacterial and antioxidant properties of commercial essential oils. Industrial Crops and Products. 2013; 43:587-595.
20. Calleja MA, Vieites JM, Montero-Meterdez T, Torres MI, Faus MJ, Gil A *et al*. The antioxidant effect of β-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. British journal of nutrition. 2013; 109(3):394-401.
21. Ebrahimzadeh M, Nabavi S, Nabavi S. Antioxidant activities of methanol extract of *Sambucus ebulus* L. flower. Pakistan Journal of Biological Sciences. 2009; 12(5):447.
22. Křůmal K, Kubátková N, Večeřa Z, Mikuška P. Antimicrobial properties and chemical composition of liquid and gaseous phases of essential oils. Chemical Papers. 2015; 69(8):1084-1092.
23. Hanbali FE, Akssira M, Ezoubeiri A, Mellouki F, Benherraf A, Blazquez AM *et al*. Chemical composition and antibacterial activity of essential oil of *Pulicaria odora* L. Journal of Ethnopharmacology. 2005; 99(3):399-401.
24. Ouraïni D, Agoumi A, Ismaïli-Alaoui M, Alaoui PK, Cherrah Y, Amrani M *et al*. Étude de l'activité des huiles essentielles de plantes aromatiques à propriétés antifongiques sur les différentes étapes du développement des dermatophytes. Phytothérapie. 2005; 3(4):147-157.
25. Yashphe J, Feuerstein I, Barel S, Segal R. The antibacterial and antispasmodic activity of Artemisia herba alba Asso. II. Examination of essential oils from various chemotypes. International journal of crude drug research. 1987; 25(2):89-96.
26. Kaloustian J, Chevalier J, Mikail C, Martino M, Abou L, Vergnes MF. Étude de six huiles essentielles: composition chimique et activité antibactérienne. Phytothérapie. 2008; 6(3):160-164.
27. Pauli A. Antimicrobial properties of essential oil constituents. International Journal of Aromatherapy. 2001; 11(3):126-133.
28. Satrani B, Ghanmi M, Hmiri S, Rahouti M, Habib Z, El Ajjouri M. Évaluation du potentiel antifongique des huiles essentielles de Mentha pulegium et d'Eucalyptus Camaldulensis dans la lutte biologique contre les champignons responsables de la détérioration des pommes en conservation. Bulletin de la Société Royale des Sciences de Liège. 2011; 80:824-836.
29. Ho CL, Liao PC, Wang EI, Su YC. Composition and antifungal activities of the leaf essential oil of *Neolitsea parvigemma* from Taiwan. Natural product communications. 2011; 6(9):1357-1360.
30. Bouchra C, Achouri M, Hassani LI, Hmamouchi M. Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against Botrytis cinerea Pers: Fr. Journal of Ethnopharmacology. 2003; 89(1):165-169.
31. Edris AE, Farrag ES. Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase. Molecular Nutrition & Food Research. 2003; 47(2):117-121.
32. Tyagi AK, Malik A. Antimicrobial potential and chemical composition of *Eucalyptus globulus* oil in liquid and vapour phase against food spoilage microorganisms. Food Chemistry. 2011; 126(1):228-235.
33. Survilienė E, Valiuškaitė A, Snieškienė V, Stankevičienė A. Effect of essential oils on fungi isolated from apples and vegetables. Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian, University Of Agriculture, Sodininkyst Ir Darininkyst. 2009; 28:227-234.
34. Tullio V, Nostro A, Mandras N, Dugo P, Banche G, Cannatelli M *et al*. Antifungal activity of essential oils against filamentous fungi determined by broth microdilution and vapour contact methods. Journal of applied microbiology. 2007; 102(6):1544-1550.