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Research Article
Chemical Composition and Antifungal Activity of the Essential Oils of Algerian *Vitex agnus-castus* and *Artemisia Herba-alba*

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Abstract

Background and Objective: The essential oils of *Artemisia herba-alba* and *Vitex agnus-castus* have been the subject of several studies in different countries including Algeria but never been studied together. The available data showed a great variability in the chemical composition. The present work deals with the composition of the essential oils of both plants and their antifungal activity against two pathogenic fungi (*Aspergillus niger* and *Scedosporium apiospermum*). The comparison of the two oils aims at determining the substances responsible for the inhibitory activity. **Materials and Methods:** Essential oils were isolated from aerial parts by steam distillation and their chemical composition was evaluated by GC-MS. The *in vitro* antifungal activity was evaluated by measuring the mycelial radial growth diameters on PDA medium and by calculating fungal inhibition rate compared to the control. **Results:** The main components of essential oils were 1,8-Cineole (17.54%), γ-Elemene (10.47%) and α-Pinene (9.03%) in *V. agnus-castus* and α-Thujone (20.36%), Verbenone (9.40%) and β-Thujone (7.60%) in *A. herba-alba*. The effect on the tow mold species is manifested by a delay in growth. Both oils showed high inhibition rates on *S. apiospermum* especially that of Artemisia. **Conclusion:** The study suggested that the significant activity of *Artemisia* essential oil against *S. apiospermum* can be assigned to 1,8-Cineole and Thujone that are highly similar in structure.

Key words: Essential oil, *Artemisia*, *Vitex*, antifungal, cineole, thujone

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Algerian flora is very rich in aromatic species. They are widespread in diversified climatic conditions (humid, sub-humid, semi-arid, arid and desert). Presently they are used only traditionally, therefore they have certainly a higher economic interest. The white wormwood *Artemisia herba-alba* Asso (Asteraceae family), synonyme *Artemisia inculata* Del.; *Artemisia sieberii* Besser; *Seriphidium herba-album* (Asso) Sojak *fide* GHA and RIZ. Common names: Apsinthos in Greek, Armoise blanche in French, Shih in Arabic (Algeria); Izri in Berber (Algeria)[1], is a small perennial wooly bush growing extensively in North African and Middle Eastern countries[2]. *A. herba-alba* is widely used in Algerian folk medicine for treatment of gastric disorders such as diarrhea, abdominal cramps and for healing external wounds, it is recommended for neurological disorders[3]. The decoction of *A. herba-alba* was effective in reducing blood glucose[4]. The flavonoids detected in *A. herba-alba* also show considerable structural diversity ranging from common flavon and flavonol glycosides to more unusual highly methylated flavonoids[5,6]. The Chaste Berry (*Vitex agnus-castus* L.) (Lamiaceae family formerly Verbenaceae)[7] synonyme *Vitex arbora* Desf., *Vitex bicolor* Willd, *Vitex chinensis* Mill.[8], is a shrub producing violet-colored flowers and very dark red berries. Once found only around the Mediterranean, it is now cultivated in various subtropical areas of the world[9]. Common names: Vitex, Monk's pepper in English[10], le gattilier or poivre sauvage in French[11], Kef Meriam in Arabic[12], in Biskra (Algeria) it's called Khzama. This plant is widely used in Europe for premenstrual syndrome[13], helpful with insulin resistance[14]. Vitex is known to contain essential and fixed oils that have antimicrobial activity[15], diterpenoids, iridoid glycosides (aucubin and agnuside), which have been found to have anti-inflammatory activity[16].

Aspergillus niger (Trichocomaceae family), the most important member of the genus *Aspergillus*[17], is a worldwide saprophyte in soil which occurs on decaying organic matter and causes diseases in plants and animals (contamination from mouldy hay, straw, grain and other foods)[18], it is the causal agent of black molds[19]. This pathogen is dark brown to black in culture, produces spherical black spiny dry conidia (2-5 μM in diameter) readily dispersed in the air[20]. Inhalation of *Aspergillus* spores is the usual mode of infection in humans. *Scedosporium apiospermum* (Microascaceae family) is a saprophytic mould found in soil, polluted water, sewage, decaying vegetation and manure[21], it is found also in biological air purification systems[22]. *Scedosporium* species cause a broad spectrum of human diseases, ranged from transient colonization of the lungs to localized subcutaneous or deep-tissue infection and widespread dissemination infection[20]. *Scedosporium* infection is an opportunistic mycosis, this mould is one of the causal agents of cutaneous and subcutaneous mycoses in human[23].

To find new bioactive natural products, this work focuses on the valorization of Algerian aromatic plants[24]. This study aims to compare the chemical composition of essential oils of these two plants harvested in the northern Algerian Sahara and determinate the components possessing antifungal properties.

MATERIALS AND METHODS

This research was performed during 14 months from June, 2012 to August, 2013. The GC-MS analysis was performed in the Laboratory of Physical Measurements (LMP), Institute of Biomolecules Max Mousseron (IBMM), University of Montpellier, France. The antifungal activity essays were performed in the laboratory of the Department of Nature and Life Sciences, University of Biskra, Algeria.

Plant materials: Aerial parts of the two plants were collected at the flowering stage, *V. agnus-castus* (cultivated shrub) in June, 2012 at Biskra city and *A. herba-alba* (steppic spontaneous wooly bush) in September, 2012 from the steps of Djammoura (Department of Bikra). Voucher specimens are deposited in the herbarium of the Department of Nature and Life Sciences, University of Biskra, Algeria, under the codes LAM-020-6-2012 and AST-003-9-2012, respectively. After drying at room temperature, only leaves and flowers/inflorescences were used.

Essential oils extraction: The essential oils were extracted by steam distillation method (for 5 h) using a Clevenger apparatus, a flask (2000 mL of distilled water) communicating with a funnel containing the plant material (1000 g), the steam then passes through the Clevenger apparatus. Oils were recovered directly without adding any solvent and stored at 4°C.

Fungi species: *Aspergillus niger* was brought from the collection of plant pathogenic fungi of the Department of Botany ENSA, El-Harrach, Algiers. *Scedosporium apiospermum* was obtained from the collection of fungal isolates of the Laboratory of Microbiology, Department of Nature and Life Sciences, University of Biskra, Algeria.

Gas chromatography/ mass spectrometry (GC/MS) analysis: The composition of the oils was analyzed by GC/MS using a Focus GC (Thermo) gas chromatograph, equipped with BR5-MS column (5% phenyl methyl siloxane) 30 m×0.25 mm internal diameter, film thickness 0.25 μM (Bruker). This GC is coupled to a DSQII (Thermo) mass spectrometer worked in EI mode at 70 eV. The mass spectrum is recorded between 40 and 500 Da (m/z equivalent unit). Injections are given in split mode n1/100. Carrier gas: Helium at a linear velocity 1.2 mL min^{-1}. Injection volume: 1 μL. Column temperature was initially 70°C and then gradually increased to 300°C at 10°C min^{-1}. The samples were diluted in chloroforme $CHCl_3$. Identification of compounds was achieved by comparison of their recorded mass spectra with those of a computer library (NIST 2008 v2.0/Xcalibur data system) provided by the instrument software. Quantification is based on relative area percentages.

Antifungal assay: Young mycelia fungi (6 days) from a pure culture were tested. Fungal strains were cultured on Potato Dextrose Agar (PDA, DIFCO, Le Pont de Claix, France) at 28°C. The essential oils are not miscible in PDA, addition of extracts to the growth media was carried out according to the method of Hassane *et al.*[25]. To obtain a homogeneous distribution of essential oil in the medium and for maximum contact between fungi and substances, the dilutions were prepared in test tubs by adding volumes of essential oil to a PDA solution (vol/vol) to obtain 2 mL (0.05/1.95, 0.1/1.90, 0.2/1.80 and 0.5/1.50 mL mL^{-1}), the dilutions were shaken strongly to obtain an emulsion. About 0.1 mL of each solution were added aseptically to 19.9 mL of sterile PDA still liquid (+50°C) in test tubes. The final concentrations (vol/vol) obtained were 1/4000, 1/2000, 1/1000 and 1/400 (vol/vol). The mixture (still liquid) in each tub (20 mL) is stirred vigorously to ensure homogeneous distribution of essential oil before being poured into Petri dishes (9 cm of diameter). After solidification, the agar dishes were inoculated with a mycelial disc (5 mm) taken from the center of the young pre-cultivated mycelium and placed aseptically in the center of the agar surface. The cultures were incubated for 7 days at 28°C, this period allows the fungi to colonize all the agar surface[26]. The diameters of the mycelial radial growth area were measured using a digital caliper. The effect on fungi was assessed by the inhibition rate calculated according to the following equation[27]:

$$T_i = \frac{N_0 - N_c}{N_0} \times 100$$

Where:

T_i : Inhibition rate of mycelial growth (%)
N_0: Diameter (mm) of mycelial radial growth area (mm) in the control
N_c: Diameter of mycelial radial growth area (mm) in the presence of the extract

Statistical analysis: For each fungus, factorial arrangement of treatments is adopted with 3 replications in a completely randomized design. The data obtained on the fungal growth diameters were subject to two-way ANOVA and the averages are compared using the Fischer LSD test to determining significant differences at $p = 0.05$ level.

RESULTS

Chemical composition of the essential oils: The oil yield obtained from *V. agnus-castus* calculated from the dry weight is low (0.68%) compared to that of *A. herba-alba* (1.93%). All the identified compounds of *V. agnus-castus* and *A. herba-alba* essential oils are listed, respectively in Table 1 and 2 in order of their elution from the BR5-MS capillary column, along with their relative percentages.

The main similar components in the two essential oils were 1,8-Cineole, α-Pinene, Verbenone, Camphene and α-Terpinen. For the other non-common compounds, *V. agnus-castus* oil is rich on γ-Elemene (10.47%), (E)-β-Farnesene (6.42%), α-Terpinyl acetate (5.14%), β-Caryophyllene (4.54%) and Spathulenol (4.46%) while the essential oil of *A. herba-alba* is characterized by high percentages of α-Thujone (20.36%), Verbenone (9.40%), β-Thujone (7.60%) and Myrtenyl acetate (3.90%).

Antifungal activity: The antifungal properties of the characterized essential oils were evaluated by measuring the mycelial radial growth diameters on PDA and by calculating fungal inhibition rate compared to the control. As shown in Table 3, statistically, the radial growth diameter of both fungi decreases significantly by increasing concentration of oils in the medium. The results showed differences in the sensitivity of the tested mold species. The less diluted oils have a high inhibitory effect compared to the high dilution.

Both oils showed high inhibition levels on *Scedosporium apiospermum* compared to *Aspergillus niger* (Fig. 1), in particular that of *A. herba-alba* which gave a nearly total inhibition (90.86%) at 1/400 dilution, whereas it is less than 18% on *A. niger*. For the Vitex oil, at the same dilution, the inhibition rate reaches 59.45% for *S. apiospermum* whereas it does not exceed 15% for *A. niger*.

Table 1: Chemical composition of the essential oil of *Vitex agnus-castus* cultivated in Biskra (Algeria)

RT	Area (%)	Constituents	Previous works on *Vitex agnus-castus* essential oil composition
3.81	0.17	α-Thujene	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Hamid *et al.*[30], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34] and Kustrak *et al.*[35]
3.92	9.03	α-Pinene	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Hamid *et al.*[30], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Kustrak *et al.*[35], Taziki *et al.*[36], Sarikurkcu *et al.*[37] and Lucks *et al.*[38]
4.12	0.46	Camphene	Galletti *et al.*[32], Senatore *et al.*[33] and Ghannadi *et al.*[39]
4.58	1.01	Myrcene	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Hamid *et al.*[30], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Kustrak *et al.*[35], Taziki *et al.*[36], Sarikurkcu *et al.*[37] and Borges *et al.*[40]
4.41	3.64	β-Phellandrene	Hamid *et al.*[30], Novak *et al.*[31], Senatore *et al.*[33] and Taziki *et al.*[36]
4.48	0.47	α-Terpinen	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Hamid *et al.*[30], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Sarikurkcu *et al.*[37] and Borges *et al.*[40]
4.91	0.32	δ-3-Carene	Kuruuzum-Uz *et al.*[16] and Senatore *et al.*[33]
5.09	1.40	p-Cymene	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Kustrak *et al.*[35], Taziki *et al.*[36], Lucks *et al.*[38] and Abbas[41]
5.21	17.54	1,8-Cineole	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Hamid *et al.*[30], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Kustrak *et al.*[35], Sarikurkcu *et al.*[37], Lucks *et al.*[38], Borges *et al.*[40] and Abbas[41]
6.09	0.41	Linalool	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Hamid *et al.*[30], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Taziki *et al.*[36] and Sarikurkcu *et al.*[37]
5.68	0.47	cis-p-Menth-2-en-1-ol	Kuruuzum-Uz *et al.*[16], Stojkovic *et al.*[29], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33] and Galletti *et al.*[34]
6.13	0.45	trans-p-Menth-2-en-1-ol	Kuruuzum-Uz *et al.*[16], Stojkovic *et al.*[29], Galletti *et al.*[32], Senatore *et al.*[33] and Galletti *et al.*[34]
6.85	0.92	Camphor	Kustrak *et al.*[35] and Ghannadi *et al.*[39]
7.29	0.96	Terpinene-4-ol	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Hamid *et al.*[30], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Kustrak *et al.*[35], Taziki *et al.*[36], Sarikurkcu *et al.*[37] , Lucks *et al.*[38] and Ghannadi *et al.*[39]
7.46	3.43	α-Terpineol	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Hamid *et al.*[30], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Kustrak *et al.*[35], Taziki *et al.*[36], Sarikurkcu *et al.*[37], Lucks *et al.*[38], Ghannadi *et al.*[39], Borges *et al.*[40] and Abbas[41]
6.53	1.08	Verbenone	Stojkovic *et al.*[29]
7.90	0.46	β-Citronellol	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Hamid *et al.*[30], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34] and Abbas[41]
8.21	0.21	Carvone	Stojkovic *et al.*[29]
8.80	0.22	Bornylacetate	Stojkovic *et al.*[29], Hamid *et al.*[30], Senatore *et al.*[33], Taziki *et al.*[36] and Sarikurkcu *et al.*[37]
9.54	0.22	exo-2-Hydroxycineole acetate	Stojkovic *et al.*[29]
9.64	5.14	α-Terpinylacetate	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Kustrak *et al.*[35], Sarikurkcu *et al.*[37], Lucks *et al.*[38] and Ghannadi *et al.*[39]
10.54	1.14	α-Gurjunene	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34] and Kustrak *et al.*[35]
10.68	4.54	β-Caryophyllene	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Hamid *et al.*[30], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Kustrak *et al.*[35], Taziki *et al.*[36], Sarikurkcu *et al.*[37], Lucks *et al.*[38], Ghannadi *et al.*[39] and Abbas[41]
10.79	0.24	α-trans-Bergamotene	Kuruuzum-Uz *et al.*[16], Stojkovic *et al.*[29], Novak *et al.*[31], Senatore *et al.*[33], Taziki *et al.*[36] and Ghannadi *et al.*[39]
11.65	10.47	γ-Elemene	Galletti *et al.*[32]
10.88	0.46	(Z)-β-Farnesene	Kuruuzum-Uz *et al.*[16], Stojkovic *et al.*[29], Novak *et al.*[31], Senatore *et al.*[33], Taziki *et al.*[36] and Sarikurkcu *et al.*[37]
10.99	6.42	(E)-β-Farnesene	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Hamid *et al.*[30], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Kustrak *et al.*[35], Taziki *et al.*[36], Sarikurkcu *et al.*[37], Lucks *et al.*[38], Ghannadi *et al.*[39] and Borges *et al.*[40]
16.85	0.60	β-Bisabolene	Kuruuzum-Uz *et al.*[16], Stojkovic *et al.*[29], Hamid *et al.*[30] and Taziki *et al.*[36]
11.84	0.28	γ-Cadinene	Stojkovic *et al.*[29], Galletti *et al.*[32], Galletti *et al.*[34], Taziki *et al.*[36] and Ghannadi *et al.*[39]
12.27	0.17	Cis-Sesquisabinene hydrate	Stojkovic *et al.*[29]
12.31	0.16	E-Nerolidol	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Senatore *et al.*[33] and Abbas[41]
12.54	0.60	Palustrol	Duymus *et al.*[28], Stojkovic *et al.*[29] and Taziki *et al.*[36]
12.64	4.46	Spathulenol	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Kustrak *et al.*[35], Taziki *et al.*[36] and Ghannadi *et al.*[39]
12.72	1.85	Caryophyllene oxide	Kuruuzum-Uz *et al.*[16], Duymus *et al.*[28], Stojkovic *et al.*[29], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Kustrak *et al.*[35], Taziki *et al.*[36], Sarikurkcu *et al.*[37] and Ghannadi *et al.*[39]
12.96	1.66	Viridiflorol	Kuruuzum-Uz *et al.*[16], Stojkovic *et al.*[29], Hamid *et al.*[30], Senatore *et al.*[33] and Taziki *et al.*[36]
13.33	3.87	τ-Cadinol	Kuruuzum-Uz *et al.*[16], Stojkovic *et al.*[29], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34], Kustrak *et al.*[35] and Abbas[41]
16.68	0.90	α-Bisabolol	Duymus *et al.*[28], Stojkovic *et al.*[29], Senatore *et al.*[33] and Taziki *et al.*[36]
13.37	0.43	T-Muurolol	Galletti *et al.*[32] and Galletti *et al.*[34]
15.97	0.75	Manool	Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34] and Taziki *et al.*[36]
16.63	0.38	Manoyloxide	Duymus *et al.*[28], Novak *et al.*[31], Galletti *et al.*[32], Senatore *et al.*[33], Galletti *et al.*[34]

RT: Retention time

DISCUSSION

The essential oils analysis performed in this study identified some major components such as 1,8-Cineole, γ-Elemene and α-Thujone. In addition, many similar components are present in the two plants, some with a high percentage in Vitex and others in Artemisia. The observation of the present analysis results confirmed the

Table 2: Chemical composition of the essential oil of *Artemisia herba-alba* grown in the steps of Djammoura, Algeria

RT	Area (%)	Constituents	Previous works on *Artemisia herba-alba* essential oil composition
3.79	0.27	Tricyclene	Neffati *et al.*[47], Mighri *et al.*[48], Paolini *et al.*[49], Salido *et al.*[50] and Mohsen and Ali[51]
3.92	2.57	α-Pinene	Neffati *et al.*[47], Mighri *et al.*[48], Paolini *et al.*[49], Salido *et al.*[50], Kadri *et al.*[52], Mighri *et al.*[53], Dob and Benabdelkader[54], Belhattab *et al.*[55] and Feuerstein *et al.*[56]
4.13	3.52	Camphene	Neffati *et al.*[47], Mighri *et al.*[48], Paolini *et al.*[49], Salido *et al.*[50], Kadri *et al.*[52], Mighri *et al.*[53], Dob and Benabdelkader[54], Belhattab *et al.*[55] and Feuerstein *et al.*[56]
4.41	1.42	α-Terpinen	Neffati *et al.*[47], Mighri *et al.*[48], Paolini *et al.*[49], Salido *et al.*[50], Mohsen and Ali[51], Mighri *et al.*[53], Dob and Benabdelkader[54], Belhattab *et al.*[55], Feuerstein *et al.*[56] and Bezza *et al.*[57]
4.48	0.57	β-Pinene	Mighri *et al.*[48] and Belhattab *et al.*[55]
4.58	0.64	Myrcene	Neffati *et al.*[47], Mighri *et al.*[48], Salido *et al.*[50] and Belhattab *et al.*[55]
4.82	0.11	α-Fellandrene	Neffati *et al.*[47], Salido *et al.*[50], Mohsen and Ali[51], Dob and Benabdelkader[54], Belhattab *et al.*[55]
5.09	1.44	p-Cymene	Neffati *et al.*[47], Mighri *et al.*[48], Paolini *et al.*[49], Salido *et al.*[50], Mohsen and Ali[51], Mighri *et al.*[53], Dob and Benabdelkader[54], Belhattab *et al.*[55], Feuerstein *et al.*[56] and Bezza *et al.*[57]
5.21	7.13	1,8-Cineole	Neffati *et al.*[47], Mighri *et al.*[48], Paolini *et al.*[49], Salido *et al.*[50], Mohsen and Ali[51], Kadri *et al.*[52], Mighri *et al.*[53], Dob and Benabdelkader[54], Belhattab *et al.*[55], Feuerstein *et al.*[56], Bezza *et al.*[57] and Tilaoui *et al.*[58]
5.69	0.34	trans-p-Ment-2-en-1-ol	Paolini *et al.*[49], Salido *et al.*[50], Mohsen and Ali[51], Dob and Benabdelkader[54], Belhattab *et al.*[55] and Bezza *et al.*[57]
5.99	0.19	Terpinolene	Mighri *et al.*[48], Salido *et al.*[50], Mohsen and Ali[51], Dob and Benabdelkader[54], Belhattab *et al.*[55] and Feuerstein *et al.*[56] and Bezza *et al.*[57]
6.31	20.36	α-Thujone	Mighri *et al.*[48], Paolini *et al.*[49], Mohsen and Ali[51], Kadri *et al.*[52], Mighri *et al.*[53], Dob and Benabdelkader[54], Belhattab *et al.*[55], Bezza *et al.*[57], Tilaoui *et al.*[58] and Vernin *et al.*[59]
6.44	7.60	β-Thujone	Mighri *et al.*[48], Paolini *et al.*[49], Mohsen and Ali[51], Kadri *et al.*[52], Mighri *et al.*[53], Dob and Benabdelkader[54], Belhattab *et al.*[55], Bezza *et al.*[57], Tilaoui *et al.*[58] and Vernin *et al.*[59]
6.57	9.40	Verbenone	Paolini *et al.*[49], Dob and Benabdelkader[54] and Bezza *et al.*[57]
6.80	1.50	cis-Sabinol	Mohsen and Ali[51] and Tilaoui *et al.*[58]
7.06	1.58	(S)-cis-Verbenol	Paolini *et al.*[49], Mohsen and Ali[51], Belhattab *et al.*[55] and Tilaoui *et al.*[58]
7.11	1.08	Pinocarvone	Neffati *et al.*[47], Mighri *et al.*[48], Paolini *et al.*[49], Salido *et al.*[50], Mohsen and Ali[51], Mighri *et al.*[53], Dob and Benabdelkader[54], Belhattab *et al.*[55] and Bezza *et al.*[57]
7.16	1.43	Borneo Camphor	Mighri *et al.*[48], Paolini *et al.*[49], Salido *et al.*[50], Mohsen and Ali[51], Kadri *et al.*[52], Mighri *et al.*[53], Dob and Benabdelkader[54], Belhattab *et al.*[55], Feuerstein *et al.*[56] and Bezza *et al.*[57]
7.30	0.43	Terpinen-4-ol	Mighri *et al.*[48], Paolini *et al.*[49], Salido *et al.*[50], Mohsen and Ali[51], Dob and Benabdelkader[54], Belhattab *et al.*[55], Feuerstein *et al.*[56], Bezza *et al.*[57] and Tilaoui *et al.*[58]
7.37	0.14	Thymol	Salido *et al.*[50], Mohsen and Ali[51] and Dob and Benabdelkader[54]
7.47	0.20	L-α-Terpineol	Paolini *et al.*[49], Salido *et al.*[50], Mohsen and Ali[51] and Bezza *et al.*[57]
7.57	0.58	Myrtenal	Mighri *et al.*[48], Paolini *et al.*[49], Mohsen and Ali[51], Benabdelkader[54] and Belhattab *et al.*[55]
7.70	0.18	trans-Piperitol	Paolini *et al.*[49], Salido *et al.*[50], Mohsen and Ali[51] and Dob and Benabdelkader[54]
8.22	0.15	Carvone	Neffati *et al.*[47], Paolini *et al.*[49], Mohsen and Ali[51], Dob and Benabdelkader[54], Belhattab *et al.*[55] and Bezza *et al.*[57]
8.37	0.30	Piperitone	Mohsen and Ali[51], Dob and Benabdelkader[54], Belhattab *et al.*[55] and Tilaoui *et al.*[58]
8.61	0.09	Isopiperitenone	Paolini *et al.*[49], Mohsen and Ali[51] and Bezza *et al.*[57]
8.85	3.90	Myrtenylacetate	Bezza *et al.*[57]
10.30	0.72	cis-Jasmone	Mohsen and Ali[51] and Bezza *et al.*[57]
10.68	0.08	Caryophyllene	Neffati *et al.*[47], Mighri *et al.*[48], Paolini *et al.*[49], Salido *et al.*[50], Kadri *et al.*[52], Dob and Benabdelkader[54], Feuerstein *et al.*[56] and Bezza *et al.*[57]
11.45	0.61	D-Germacrene	Neffati *et al.*[47], Paolini *et al.*[49], Mohsen and Ali[51], Kadri *et al.*[52], Mighri *et al.*[53], Dob and Benabdelkader[54] and Bezza *et al.*[57]
11.92	0.17	δ-Cadinene	Neffati *et al.*[47], Mohsen and Ali[51], Kadri *et al.*[52], Belhattab *et al.*[55] and Bezza *et al.*[57]
12.63	0.43	Espatulenol	Neffati *et al.*[47], Mighri *et al.*[48], Paolini *et al.*[49], Salido *et al.*[50], Mohsen and Ali[51], Dob and Benabdelkader[54], Belhattab *et al.*[55] and Bezza *et al.*[57]

RT: Retention time

Table 3: Mycelial radial growth diameters (cm) of *Aspergillus niger* and *Scedosporium apiospermum* treated by different dilutions of *Vitex agnus-castus* and *Artemisia herba-alba* essential oils

		Essential oil concentrations (vol/vol)							
		Vitex agnus-castus				*Artemisia herba-alba*			
Fungal species	Control	1/4000 §	1/2000	1/1000	1/400	1/4000	1/2000	1/1000	1/400
A. niger	9.00±0.00 a*	8.65±0.05 b	8.3±0.09 cd	8.07±0.02 de	7.68±0.01 fg	8.15±0.00 cd	8.39±0.25 bc	7.79±0.11 ef	7.40±0.05 g
S. apiospermum	7.43±0.15 a	4.79±0.03 b	4.68±0.09 b	4.21±0.18 bc	3.01±0.16 d	5.65±0.07 e	4.66±0.15 b	3.53±0.03 df	0.68±0.06 g

Data expressed as Mean±Standard error (n = 3). § Final concentrations in 20 mL of PDA agar media. *Values with same letters in the same line are not significantly different (Fisher LSD, p = 0.05)

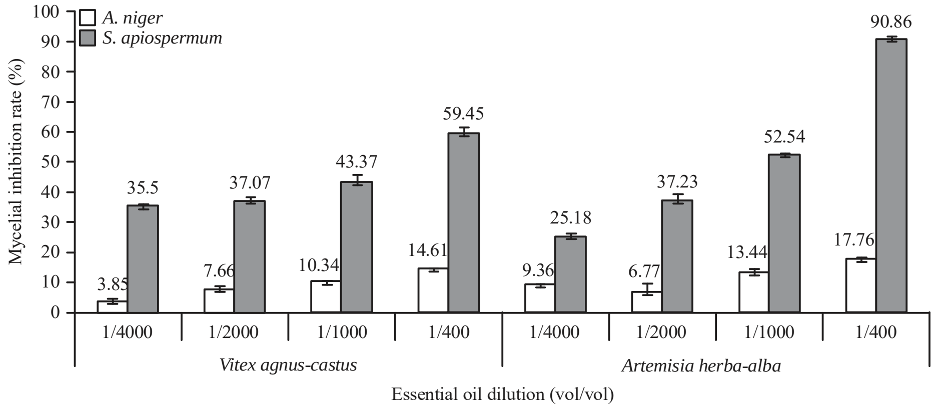

Fig. 1: Fungal inhibition rate of *Vitex agnus-castus* and *Artemisia herba-alba* essential oils
Error bars represent the standard error of the means (n = 3)

obvious differences between the literature data[28-58]. This heterogeneity can be explained by the fact that the previously analyzed samples are either different parts (leaves, flowers or fruits) or geographically distant or still harvested at different seasons.

In most researches on *V. agnus-castus* essential oil, 1,8-Cineole, a natural monoterpene also known as Eucalyptol, was found as the major compound, usually followed by α-Pinene and β-Caryophyllene[29,40,41]. Recent clinical essays based on antioxidant and anti-inflammatory properties have shown early evidence of the use of 1,8-Cineole as a long-term therapy in the prevention of exacerbations of chronic obstructive pulmonary disease and improve asthma control[42]. The γ-Elemene is of great importance because of its anti-proliferation effects against certain types of cancer cells[43,44]. α-Pinene is a therapeutic agent of interesting pharmacological properties, such as anti-inflammatory, bronchodilator, hypoglycemic, antioxidant and antiulcerogenic activities[44-46]. Various secondary metabolites have been isolated from *A. herba-alba*, the most important are the sesquiterpene lactones[59]. α-Thujone is the active ingredient of wormwood oil and some other herbal medicines, it is also the toxic agent in absinthe and is reported to have antinociceptive, insecticidal and anthelmintic activity[60].

The effect of essential oils on the mold species was manifested by a delay in growth, this is certainly due to one or more inhibitory substances. The significant activity of Artemisia essential oil against *S. apiospermum* compared to Vitex can be assigned to 1,8-Cineole and Thujone (α and β) that are major compounds. The two compounds can target the same site and have the same mode of action[61] given their similarity in structure and physical properties[62].

In eukaryotic cells (fungi), volatile substances can most often target intracellular membranes, especially the inner mitochondrial membrane, where key steps in the respiratory chain occurs[63]. Mechanisms of the antifungal activity of some volatile compounds occurs by a breakdown of Ca^{2+} and H^{+} and blocks the regulation of gene transcription associated with this process. Other compounds cause the stress of nutrient deprivation, disrupt the integrity of the membrane and alter the biosynthesis of some vital substances for the fungus[64]. Whatever the mechanism of inhibitory action of essential oils, it always depends on their chemical compositions and the dilution used, it also depends on microorganisms[65]. The resistance of *Aspergillus niger* can be due to their metabolic properties; a number of volatile compounds can be metabolized and bio-transformed by various species of fungi as a result of enzymatic activity. Some fungi such as *Aspergillus niger* and *Aspergillus cellulosae* produce metabolites that are totally different from those of the substrate[66].

CONCLUSION

In order to discover new natural molecules of therapeutic uses, it is of great scientific importance to study simultaneously the chemical composition and the bioactivity of various essential oils. This study provided interesting data on the chemical composition and antifungal activity of *Vitex agnus-castus* and *Artemisia herba-alba*. The essential oil of *A. herba-alba* showed a significant activity against *Scedosporium apiospermum* that causes severe human diseases. This inhibitory action can be assigned to the major compounds 1,8-Cineole and Thujone that are highly similar in structure. However, further studies should be conducted on the mode of action, structural similarity and synergic effects of the compounds present in the volatile mixture. In addition, the sensitivity of other pathogenic micro-organisms to this oil, including bacteria, fungi and protozoan must also be verified.

SIGNIFICANCE STATEMENT

This study discovered the sensibility of the harmful fungus (*Scedosporium apiospermum*) to the essential oils of *Vitex agnus-castus* and *Artemisia herba-alba*. With the phytochemical provided data, the results may represent an important tool for more accurate treatments.

This study will help the researchers to uncover the chemical similarity in the composition of the essential oils of these plants and of other plants. A new theory on biological activity, structural similarity and synergetic effect of some active constituents may be arrived at a good agreement between the volatile found components, especially those in higher amounts and those usually reported to possess *in vitro* antifungal properties.

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