

**CHEMICAL COMPOSITION, ANTIBACTERIAL AND ANTIOXIDANT
ACTIVITIES OF ESSENTIAL OIL EXTRACTED FROM SCHINUS MOLLE
OF ALGERIA**

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ABSTRACT

The work in hand was aimed mainly to highlight the antioxidant and anti-inflammatory efficacy of the essential oils and the hydrolate of the aromatic plant *Schinus molle*. The findings confirmed that both extracts of the studied species involve important biological actions on the tested pathogenic germs.

The essential oil resulted from hydro-distillation has undergone GC/MS technique analysis. 28 components were detected representing 99.98% of the total oil. The foremost components are: 1, 4, 6, 7, 8, 9-hexahydro-2-methoxy-3-methyl-6-6-diispropylnaphthalene 16.03%, β -phelandrene 13.17%, α -phelandrene 12.83%, δ -cadiene 6.48%, camphene 6.04%, β -myrcene 5.20%, α -pinene 5.10%, Elemol 4.74%, α -cadinol 4.09% presenting 73.68% of the total oil.

The good miscibility of the essential oil shows the opportunity to use it in cosmetics and pharmaceutical preparations.

Key words: *Schinus molle*, hydrodistillation, essential oil, GC/MS, anti-inflammatory, Activity, MIC.

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1. INTRODUCTION

Mankind has used plants since antiquity to relieve and alleviate ailments [1]. The use of plants in folk medication is very old and is currently experiencing an improved attention worldwide. According to the World Health Organization (WHO) approximately 65-80% of the world's population relies on traditional medicine to meet primary therapeutic needs, due to scarcity and deficiency to access to modern medicine basic medication needs [2-3]. In terms of economic contribution, global sales of herbal remedy amounted to about US \$ 60 billion in 2000 [4].

Algeria has one of the largest diverse and original flora in the Mediterranean. This flora involves around 3.139 species (653 endemic) belonging to 150 families, with an endemism rate of about 12.6%. Considering the phytogeographic sector of Oran, it preserves about 1780 plant species representing 57% of the total Algerian flora, and almost 95% of the Maghreb Mediterranean flora (this later accounts 1 865 species) [5].. About 14% (250 species) of this floristic patrimony were listed in the flora as strictly subservient to the cultivated plots [6].

The species *Schinus molle* L. (Anacardiaceae), is a small, graceful tree with a dense and drooping silhouette, indigenous to Central and South America, introduced in most tropical and subtropical regions [7]. It is very widespread in Ecuador and Peru. It adapts to all climates, but it is generally well bespoke to the Mediterranean littoral [8].

In North Africa, *Schinus molle* and *Schinus terebinthifolius* were introduced as ornamental species in the late 1900s by the colonists. Their successful introduction into a non-native area is accredited to their high drought and heat tolerance [9]. In their native region (South America), these highly aromatic species have been the subject of numerous investigations to appraise their antibacterial, antifungal, anti-inflammatory, insecticidal and allelopathic activities [10]. Pharmacological studies performed with the essential oil and hydrolate of *Schinus molle* have exposed that this plant has several biological effects, such as hypotensive [11], analgesic [12], antispasmodic [13], antitumoral [14] and antifungal [15,16]. The aim of this work is to study the antibacterial, anti-inflammatory, and antioxidant activity of essential oil and hydrolate of the leaves and fruits of the aromatic *Schinus molle* cultivated in the city of Ouargla.

2. MATERIALS AND METHODS

2.1. Vegetal material: The Leaves of *Schinus molle* were collected in June, 2014 in the surroundings of Ennacer city Ouargla (Algeria), during a hot period (40°C at the collection

time). The plant was identified by Pr Abdelmadjid Chahma, a botanist in Biology Department, Ouargla University, Algeria. A specimen was deposited at the herbarium of the University under the number GO2014-1.

2.2. Hydrodistillation: The extraction of essential oil was done by hydrodistillation; in a Clevenger apparatus by immersing 100g of dry leaves in 600 ml distilled water, and then distilled for 3h (fig .1). The resulting essential oil was dried with Calcium chlorate (CaCl_2) and stored in the dark at $+4^\circ\text{C}$.



Fig.1. Hydrodistillation of *Schinus molle*. Installation of hydrodistillation (Clevenger apparatus) [17]

The distillation was carried out with a recycling cohobage commonly known as described in the (Ph.Eur) [17,18].

The essential oil yield from fresh plant material are defined as follows [19]:

$$\text{RHEa} = \frac{\text{HE mass}}{\text{Mass dry plant material}}$$

2.3. Gas chromatography-mass spectrometry

The essential oil was analyzed in CRAPC (scientific and technical research center in physico-chemical analysis, Algiers), by using an HP Agilent 2890 Gas chromatography coupled to an Agilent 5975B mass selective detector with electron impact ionization (70 eV) and an Agilent Chemstation software (Agilent Technologies, Palo Alto, USA). Separation of oil components was performed on HP-5MS; 5% Phenyl-95%-dimethyl-poly-siloxane capillary column (30m x 0.25mm, film thickness 0.25 μm) in the split mode (1:80) at 250°C . The oven temperature was fixed at 60°C for 8 min, then raised to 250°C at 2°C min^{-1} and finally kept at this temperature for 10 min. Helium was employed as transporter gas at a flow of 0.5 ml min^{-1} . Linear Retention Indices (RI) for all compounds was performed by matching their mass

spectral fragmentation patterns with corresponding data (NIST 2014 and Wiley 2014 libraries) and by the laboratory database.

2.4. Antibacterial activity

2.4.1. Bacterial strains: Microbiological bits and pieces consist of three pathogenic strains in charge for some infectious diseases. They have been provided by Microbiology Laboratory Mohamed Boudiaf Hospital and the Microbiology Laboratory of University Kasdi Merbah, Ouargla. These bacteria are: *Escherichia Coli*, *Staphylococcus* and *Streptococcus*.

2.4.2. Disc diffusion method: The appraisal of the antibacterial action was done using the disc diffusion system with respect to the NCCLS reference [20].

The disc system is a procedure of distributing samples to test from a paper disc that can qualitatively measure the sensitivity of antimicrobial effects [21]. This method has been selected for its reliability and simplicity. It provides preliminary results on the sensibility of the strains vis-à-vis the product through the diameters of inhibition appearing around the discs.

The bacterial strains were spread on the Mueller Hinton Agar (MHA) Disc (6mm; Whatman N°3) impregnated in the essential oil were placed on the surface of such media and hatched at 37°C for 24 h. All assays were performed three times.

2.4.3. Determination of the Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentration (MIC) of oil was determined by disk diffusion in Mueller-Hinton according to Benabderrahmane [22] with some modification. Because of the unmixability of essential oils in water, it was diluted in DMSO (dimethyl sulfoxide) giving rise to a concentration interval of 1-0.01 mg mL⁻¹ and then integrated into discs of 6.0 mm diameter by 0.01 mL. The equal amount of DMSO was used as control.

The microbial suspensions were calibrated with respect to the standards (0.5 Mc Farland equivalent that are (10⁸CFU mL⁻¹) [23], 0.1 mL of inoculum was immunized into the agar instantly.

The discs holding diverse concentrations of oil were placed straight on the surface of the agar. Durations and incubation temperatures were 24 h and 37°C. The MIC was the lowly concentration of essential oil requisite to fully restrain the development of the tested microorganisms around the discs.

2.5. Antioxidant activity

2.5.1. Free radical scavenging effect:

Scavenging activity was estimated by measuring the antiradical effect on the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical, by means of the method illustrated in the bibliography [24-25], with a minor modification. Various dilutions of essential oil and DPPH radical solution were set in analytical ethanol. One milliliter of every sample concentration was assorted with a similar volume of 0.1 mM DPPH. The reaction was evidenced at 517 nm. Combination of 1 mL of DPPH solution and 1 mL of ethanol was taken as a blank. Ascorbic acid was taken as a positive control. Inhibition of DPPH free radical in percent (I%) was estimated as follows :

$$I(\%) = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

Where, A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance of the tested essential oil or ascorbic acid. Extract concentration showing 50% inhibition (IC_{50}) was deduced from the graph scheming inhibition proportion in opposition to extract concentration. Tests were accomplished in triplicate.

2.6. Anti-inflammatory activity

The anti-inflammatory potential of the diverse extracts of the plant was evaluated by measuring the inhibition of 5-lipoxygenase [26]. 0.5 ml of extract was dissolved in a mixture of dimethylsulfoxide (DMSO) and Tween 20 (V / V = 1), then 1.8 ml of potassium phosphate buffer (0.1 M, pH 6.3) and 100 UM of linoleic acid were added. Reaction is started by adding 1 ml of the diluted 5-lipoxygenase in a phosphate buffer and stored at + 4 ° C. after 10 minutes the emergence of the reaction product by UV spectrometry at 234 nm. Then, we compare the initial rate of the enzymatic reaction without addition and with the addition of increasing amounts of extract to be evaluated. The inhibition result in a decrease in the reaction rate and the concentration (in mg.mL⁻¹) corresponds to a 50% inhibition of the initial rate (IC_{50}). NDGA (Nordihydroguaiaretic acid) is used as a reference product.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of *Schinus molle* essential oil and extract

3.1.1. Hydrodistillation

This is the first report on *Schinus molle* volatile oil cultivated in Ouargla, Algeria. The plant gave yield of 2.0% of extracted essential oil, with clear yellow color and very strong and unrelenting odor of pepper.

3.1.2. GC/MS analysis

The chemical composition including the retention index and the relative content of each constituent, are presented in Table1.

Table 1. Chemical composition of essential oil of *Schinus molle* from Ouargla (Algeria)

Compounds	RI	Area (%)
tricyclene	8536	0.75
α -pinene	9201	5.10
camphene	10040	6.04
β -pinene	11684	2.11
β -myrcene	12753	5.20
α -phelandrene	13606	12.83
P-cymene	14990	3.90
β -phelandrene	15284	13.17
β -elemene	40220	1.04
α -gurjunene	41164	1.25
β -caryophyllene	41815	1.20
α -humulene	43974	0.99
Germacrene D	45767	3.70
α -muurolene	46967	0.64
Y-cardiene	47810	1.09
δ -cadiene	48442	6.48
Elemol	50220	4.74
Germacrene D-4-ol	51574	0.41
1, 4, 6, 7, 8, 9-hexahydro-2-methoxy-3-methyl-6-6-diiispropylnaphtalene	53357	16.03
2, 4, 5, 6, 7, 7 α -hexahydro -3-(-1-methylethyl-7 α -methyl-1H-2-indenone	54586	0.69
Y-eudesmol	54789	1.21
T-muurolol	55454	2.17
β -eudesmol	55883	0.95
α -eudesmol	56042	0.95
α -cadinol	56230	4.09
Hexenylcyclopentanone	58076	1.51
Iso calamendiol	61025	1.02
Trans-z-alpha bisaboleneepoxide	64433	0.72
	Total	99.98

RI : Retention indices

A total of Twenty-two compounds were identified, representing approximately 99.98 % of the total composition. The major compounds are:

1,4,6,7,8,9-hexahydro-2-methoxy-3-methyl-6,6-diispropylnaphtalene 16.03 % , β -phellandrene 13.17%, α -phellandrene 12.83%, δ -cadiene 6.48%, camphene 6.04%, β -myrcène 5.20%, α -pinene 5.10%, Elemol 4.74%, α -cadinol 4.09 % P-cymene ,3.90% , Germacrene D 3.70 % . According to preceding information as regards this species, this grouping of most important compounds were not reported in other regions from the world.

The major components recognized in the essential oil of *S. molle* of Resistencia city (Argentina) were α -pinene 11.51%, β -pinene 14.71%, limonene 9.18%, α -ocimene 3.17%, germacrene D3.57%, γ -cadinene 6.87%, δ -cadinene 4.90 % and epi-bicyclo-sesquiphellandrene 18.60 % . However, the compositions of these oils differ in their major constituents matched up to to data collected from extra bases such as Liguria (Italy), whose main components are α -phellandrene 30.24% and elemol 13.25% [27]. Uruguay with 29.20% of Bicyclgermacreno [28], state of Rio Grandedo Sul in southern Brazil with 41.87% of limonene [29] and Santa Fe (Argentina) whose major component is limonene (3.80%) [30].

It is reported that the disparity in chemical composition of essential oil could be attributed to the environmental origin of the plant, the extraction technique, harvest time and climatic factors in one part [31] and to genetic factors in other part [32], that should not be considered in explaining the chemo-variation of essential oils.

3.2. Antimicrobial activity

3.2.1. The Antimicrobial activity of the essential oil and the hydrolate of the plant *Schinus molle* of Ouargla region was evaluated against the following bacterial strains: *Escherichia Coli*, *Staphylococcus*, and *Streptococcus*, showed an inhibition zone from 12.25 mm to 13.5 mm for the essential oil and from 11.75 mm to 13 mm for the hydrolate. These values have a pronounced activity between 8-14 mm [33] and minimal inhibitory concentration (1.0, 0.5 and 0.125) $\mu\text{g}/\text{mL}^{-1}$, respectively.

The hydrolate has an effect almost equal to that given by the essential oil, which is a true scientific revolution, because for a yield of 100% in hydrolate compared to the dry mass of the plant, while the quantities extracted from hydrolate exceed 100 ml for 1 ml of oil.

Indeed, the essential oil and the hydrolate of *Schinus molle* showed an important inhibitory effect against the microorganisms studied.

The microorganisms most sensitive to this essential oil and hydrolate were *Escherichia Coli* with a diameter of 13.5 mm and 13 mm respectively. The results are displayed in figure 2.

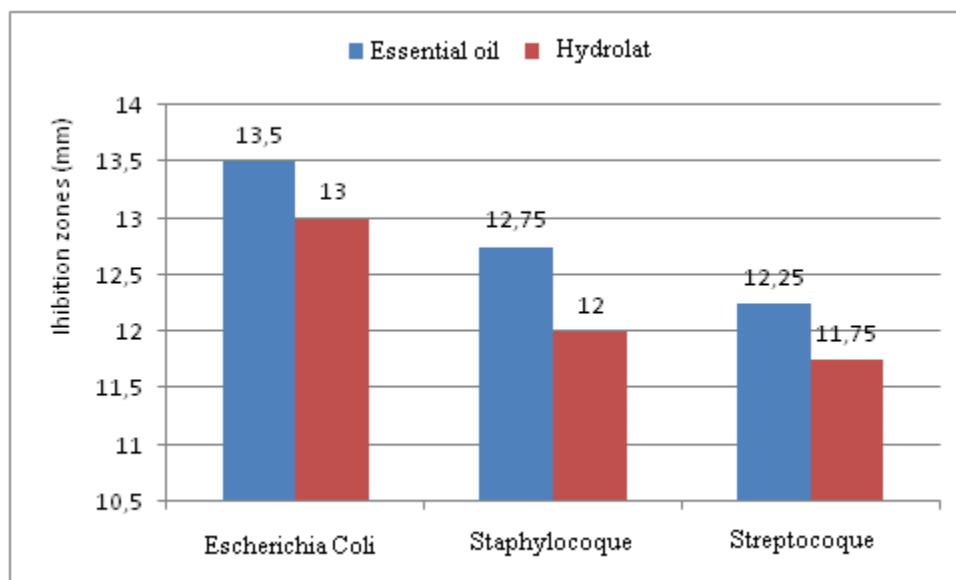


Fig.2. Microbial activity of essential oil and hydrolat of the plant *Schinu molle*

The total reducing force of *Schinus molle* extracts is 1.27 mg / ml for Essential Oil and 0.02 mg / ml for hydrolate compared to ascorbic acid. This high reducing control observed in *Schinus molle* is likely due to the high number of hydroxyl groups that the aromatic constituents contain [34].

The *Schinus molle* soft extracts show a considerable anti-inflammatory activity with an IC_{50} anti-inflammatory potential of 15.31 mg .mL and IC_{50} of 61.04 mg.mL⁻¹ for the essential oil and their hydrolate respectively.

4. CONCLUSION

The findings demonstrate that the plant *Schinus molle* presents important contents of water varying between 43 to 63%, which confirms their water richness. Extraction of the essential oil by the hydro-distillation gave an oil yield of 1.9996 % and a quantity of 100 ml of hydrolate for each ml of essential oil.

The essential oil of *Schinus molle* is composed mainly of 1, 4, 6, 7, 8, 9-hexahydro-2-methoxy-3-methyl-6-6-diispropylnaphthalene 16.03%, β -phelandrene 13.17%, α -phelandrene 12.83%. The qualitative estimation of the antibacterial results of the extracts show a bacterial sensitivity towards the strains tested. It should be noted that the antibacterial power of HE was bigger than that of hydrolate. The essential oil of *Schinus molle* has a very important antioxidant and anti-inflammatory power.

The hydrolate has a bacterial effect almost equal to that given by the oil, which is a scientific revolution, because it was obtained a yield of 100% in hydrolate relative to the mass of the dry plant.

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