Egypt. J. Chem. Vol. 67, SI: M. R. Mahran, pp. **1661 - 1664** (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Antioxidant and Antibacterial Properties of Essential Oil of *Schinus Molle L.* From Southwest of Algeria

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Abstract

This study aimed to evaluate the antioxidant and antibacterial potential of *Schinus molle* essential oil in southwest Algeria.The antioxidant activity of the essential oil was assessed using the DPPH free radical scavenging method, while the antibacterial activity was determined using the disc diffusion method against *Staphylococcus aureus* and *Escherichia coli* strains, with chloramphenicol as the positive control.The results showed that the Schinus molle oil exhibited a high antioxidant potential, with 50% free radical inhibitory concentrations (IC_{50}) of 0.41 mg/ml. Additionally, the oil demonstrated potent antibacterial properties against Gram-positive *Staphylococcus aureus* and weaker activity against Gram-negative *Escherichia coli*. These findings suggest that the essential oil of *Schinus molle* possesses promising biological potential, warranting further research into compounds derived from this species for the development of new antimicrobial drugs

Keywords: Schinus molle; Natural Product; Antioxydants Effects; Biological Activities.

1. Introduction

Essential oils and aromatic extracts are commonly used in pharmaceutical products and dietary supplements [1]. These natural substances have demonstrated antioxidant properties that help combat the formation of free radicals and other oxidizing compounds [2]. Moreover, essential oils have shown significant antimicrobial effects in vitro against pathogenic agents and foodborne bacteria responsible for diseases [3]. The *Schinus molle* L., a tree belonging to the Anacardiaceae family, is indigenous to the subtropical regions of South America [4]. In traditional medicine, this plant is valued for its antibacterial, antiviral, topical antiseptic properties, as well as for its antifungal, antioxidant, anti-inflammatory, anticancer, antispasmodic, analgesic, stimulant and antidepressant virtues [4,5]. It is also used to treat dental pain, rheumatic disorders, menstrual problems, and respiratory and urinary infections [6].The present study aims to extract the essential oils from *Schinus molle* steam, evaluate their antioxidant properties through DPPH free radical scavenging activity, and examine their antimicrobial activities against two pathogenic bacteria.

2. Experimental

2.1. Plant Material

The stem cuttings of the *Schinus molle* plant were harvested in March 2019 from the university campus in Bechar, a town situated in southwestern Algeria. The botanical authentication of this species was conducted by the local National Agency for Nature Protection (ANN).

2.2. Essential Oil Extraction

With the aim of extracting the aromatic oil, a quantity of one kilogram of dehydrated *Schinus molle* steams underwent a hydrodistillation process fractionated into three 5-hour cycles. For this, a Clevenger-type apparatus was utilized, following the detailed methodology outlined in the European Pharmacopoeia guide [7]. After distillation, the oil phase was carefully separated from the remaining aqueous phase, then dehydrated using anhydrous sodium sulfate. The purified oil samples were subsequently transferred into airtight vials and stored at low temperature (4°C) pending their further analysis.

2.3. Assessment of Antioxidant Capacity by DPPH Method

The evaluation of the antioxidant potential of the essential oil was conducted by measuring its scavenging ability against the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). Briefly, 100 µL of different concentrations of each extract in methanol were added to 1.9 mL of a 0.004% methanolic DPPH solution. The mixture was vigorously shaken and then allowed to stand for 30 minutes at room temperature. A solution of 100 µL methanol and 1.9 mL DPPH served as the control. The DPPH radical scavenging activity was expressed as a percentage inhibition according to the following equation [8]:

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Receive Date: 06 May 2024, Revise Date: 01 July 2024, Accept Date: 12 July 2024

DOI: 10.21608/ejchem.2024.287294.9680

$$\%\ \boldsymbol{Inhibition} = [\frac{(\boldsymbol{AB} - \boldsymbol{AS})}{\boldsymbol{AB}}] \times \mathbf{100}$$

Where AB represents the absorbance of the control reaction (containing all reagents except the tested compound) and AS is the absorbance of the tested compound. Ascorbic acid, a reference antioxidant, was used as a positive control.

2.4. Assessment of Antibacterial Activity

The disc diffusion method was employed to investigate the antibacterial potential of the essential oil against four bacterial strains: Gram-positive *Staphylococcus aureus* (ATCC 29213) and Gram-negative *Escherichia coli* (ATCC 25922). Chloramphenicol was used as a positive control. The maintenance of bacterial cultures on nutrient agar slants was ensured through monthly subculturing with incubation at 37°C for 18 to 24 hours.

3. Results and Discussion

3.1. Extraction Yield and Antioxidant Activity

The essential oil extracted from the stems of Schinus molle L. was obtained with a yield of 2.41%. It presented as a viscous liquid, pale yellow in color, emitting an aromatic peppery odor with spicy notes.

The evaluation of the antioxidant potential was carried out using the DPPH assay, with the

results illustrated in Figures 1 and 2, showing the variation of antioxidant power as a function of the concentration of each extract.

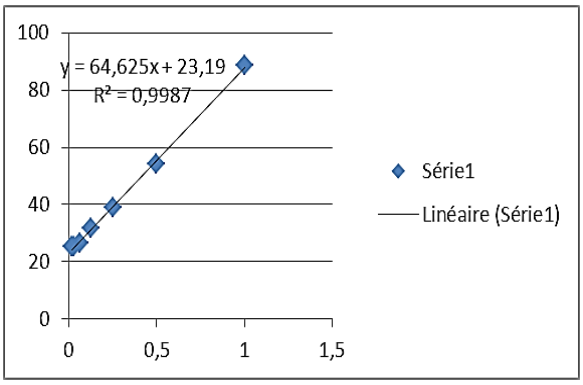

Figure 1: Antioxidant activity of the essential oil from stems

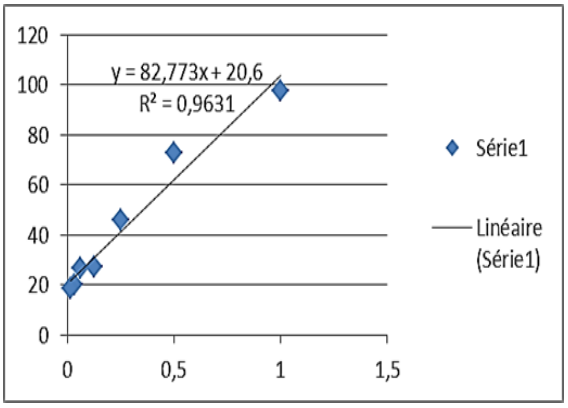

Figure 2: Antioxidant power of ascorbic acid

The antioxidant activity of this essential oil is expressed by the IC_{50} factor, defined as the inhibitory concentration causing a 50% loss of DPPH activity. The lower the IC_{50} value, the higher the antioxidant activity of the compound. According to the DPPH test, the IC_{50} for the different samples was 0.414 mg/mL (Table 1).

Table 1. IC_{50} values for EOs and ascorbic acid

Samples	IC_{50} (mg/ml)
Steams	0.41
Ascorbic acid	0.32

Eos: Essential oil

While the synthetic antioxidant, ascorbic acid, exhibited the strongest activity with an IC_{50} of 0.32 mg/mL, the antioxidant capacity of the tested oils remained relatively lower. However, it is noteworthy that the essential oils derived from the aerial parts of *Schinus molle* L. displayed significant anti-radical activity, despite their lower potency compared to ascorbic acid. These findings highlight the promising free radical scavenging properties of these aromatic essences, as confirmed by the study of their DPPH reducing power [9].

The comparison of our study with that of Díaz et *al*, (2008) [10], using the same test (DPPH) with essential oil extracted from the leaves of the same Costa Rican plant, showed that the IC_{50} value is equal to 36.3µg/ml, which means that the antioxidant power of our oil is 110 times lower than the power of the oil tested in that study.

On the other hand, our results are 9 times higher than the results obtained by Kasmi et *al*, (2016) [11], in a study carried out in Tunis on leaf (IC_{50}= 3.586mg/ml), stem (IC_{50}= 3.559mg/ml) and fruit (IC_{50}= 3.586mg/ml) essential oils. This difference in antioxidant power is probably due to the chemical composition of the essential oils. The presence of antioxidant activity in the essential oil can be attributed to its chemical composition, more specifically to oxygenated compounds that possess a hydroxyl group (OH) capable of yielding its proton to be captured by the DPPH free radical, such as Linalool, Eudesmol, α-Terpineol, Geraniol and α-Cadinol. It is not only the majority of compounds in essential oils that are responsible for this antioxidant activity, but there may also be other minority compounds that can interact in a synergistic or antagonistic way to create an effective system against free radicals [12]. Amarti et *al*. (2011) found that the antioxidant power of an essential oil is much higher when its different parts work together [09].

3.2. Antibacterial Properties of the Essential Oil

The antibacterial activity tests of the essential oil from *Schinus molle* stems, performed using the disc diffusion method, revealed inhibition zones ranging from 16.3 to 20 mm in diameter depending on the

bacterial strain tested. The studied microorganisms did not exhibit the same sensitivity towards this oil (Table 2).

According to Table 1, the essential oil showed strong activity against *Staphylococcus aureus* (20 mm) and *Escherichia coli* (16.3 mm), as illustrated in Figure 3.

Table 2. Antibacterial activity of 10 µl essential oil from *Schinus molle* Steams

Organisms	Inhibition diameters (mm)
Escherichia coli	16.3
Staphylococcus aureus	20

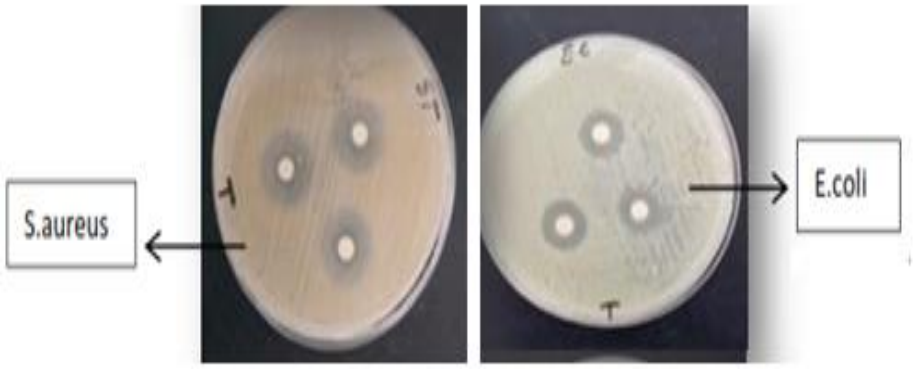

Figure 3: Antibacterial activity of *Schinus molle* essential oil.

The most sensitive strain was *Staphylococcus aureus* (Gram-positive), followed by *Escherichia coli* (Gram-negative), which exhibited moderate sensitivity. Our results align with the literature indicating that Gram-positive bacteria are generally more susceptible to essential oils [13]. These findings surpass those of Tamer et *al*. (2017), who reported inhibition zones of 12 mm against *Staphylococcus aureus* and 13 mm against *Escherichia coli* for an oil extracted from seeds of the same plant in Turkey [14]. However, our data coincide with those of Rouibi et *al*. (2010), who studied the antimicrobial power of essential oils from *Schinus molle* leaves harvested in the Blida region of Algeria, revealing strong activity against *Staphylococcus aureus* (22 mm) and *Escherichia coli* (14 mm) [15].

This bioactivity of the studied essential oil is likely related to its chemical composition or one of its constituents. Indeed, Oussalah et *al*. (2006) reported that the antibacterial effect of essential oils is attributed to monoterpenes, particularly phenols [16].

4. Conclusion

The essential oils derived from stems of *Schinus molle* exhibited promising antioxidant and antimicrobial properties against Gram-positive and Gram-negative bacteria. This suggests potential applications in the fields of biotechnology, food, and/or pharmaceuticals. Moreover, these essential oils will undergo further studies on different human tumor cell lines to assess their effect on cell proliferation, viability, and apoptosis, and explore their potential as therapeutic agents.

These works deserve to be further investigated along several lines, such as:

- An in-depth spatio-temporal study to analyze the influence of ecological factors on the content and chemical composition of *Schinus molle* essential oils.
- A deeper investigation into the constituents responsible for the antimicrobial activity, with the aim of characterizing the involved molecule(s) and elucidating their molecular mode of action.
- Evaluation of potential synergistic or inhibitory activities of the active compounds, either among themselves or with other antimicrobial agents; broadening the range of tested microorganisms (more pathogenic bacterial species).

5. Conflict of Interest

The authors declare no conflict of interest.

6. Ethics Committee Approval

The authors declare that the ethics committee approval is not required for this study.

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