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ANTIBACTERIAL, ANTIOXIDANT, AND REPELLENCY POTENTIAL OF THE ESSENTIAL OIL FROM SPARTIUM JUNCEUM L. GROWN IN LEBANON

Hawraa Zahrddin
Department of Biology, Faculty of Science, Beirut Arab University, hawraa.zahreddine@liu.edu.lb

Mahmoud Khalil
Department of Biology, Faculty of Science, Beirut Arab University, m.khalil@bau.edu.lb

Akram Hijazi
Department of Biology, Faculty of Science, Lebanese University, akram.hijazi@ul.edu.lb

1. INTRODUCTION

Spartium junceum L. belongs to the Fabaceae family. This species is native to the Mediterranean in southern Europe, southwest Asia and northwest Africa. It possesses various applications in many domains (Bischof and Kovacevic, 2013; Piorkowski et al., 2014; Ghasemi et al., 2015). In a study done by Cerchiara et al. (2013) the antimicrobial, antioxidant and cytotoxic activities of the aromatic water of this plant were investigated. It was found that no antibacterial activity against tested microorganisms has been detected. However, a good antioxidant and antitumor activity was demonstrated. Also, flowers of *S. junceum* have been reported to possess mild sedative and diuretic activities (Baytop, 1984), antiulcer (Yesilada et al., 2000), anti-inflammatory, analgesic (Menghini et al., 2006) and antitumor (Cerchiara et al., 2012).

Essential oils are defined as aromatic compounds obtained from different plant parts using various methods like hydrodistillation, steam distillation, expression, and so on. In addition, the amount of these oils differs according to the plant used and also to the plant parts.

Due to the high demand on natural products, essential oils have been used more widely as additives (Nerio et al., 2010). They demonstrated antioxidant and antimicrobial activities, and thereby serving as natural additives in foods and food products (Nerio et al., 2010). They are commonly used as flavouring agents in food products, drinks, perfumeries, pharmaceuticals and cosmetics (Bakkali et al., 2008).

By definition, repellent is a substance that acts locally or at a distance, avoiding an insect from flying to, landing on or biting human or animal skin (Blackwell et al., 2003; Choochote et al., 2007). In general, it works by producing a vapor barrier in order to obviate insects from approaching the surface (Brown and Hebert, 1997).

For the first time, in our study, the repellent capacity of the essential oil obtained from the *Spartium junceum* grown in Lebanon was evaluated in addition to its antioxidant and antibacterial effects.

2. MATERIEL AND METHODS

2.1 Essential Oil Extraction

The flowers of the used plant were collected very early in the morning from south Lebanon (700 m of altitude) during the spring of 2022, in order to preserve the integrity of their volatile molecules. Essential oil was obtained by hydrodistillation using a Clevenger type apparatus. Briefly, flowers (100 g) were completely immersed in water, followed by boiling for 3 hours. After that, the steam and the essential oil vapor were condensed to an aqueous fraction and the essential oil was collected and stored into dark glass bottles in the fridge, until usage.

2.2 Yield of Essential Oils

The yield of essential oil was calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{Amount of extracted oil (g)}}{\text{Amount of dry vegetal matter mass (g)}} \times 100$$

2.3 Organoleptic characterization

Naturally, the most crucial sense for physically testing an essential oil is our sense of smell. However, the color, texture, thickness, and taste can also reveal important information about its quality. In our study we focused on three elements: the appearance, color and smell.

2.4. DPPH Free Radical Scavenging Assay

The method of Rammal et al. (2012) has been used in the determination of the antioxidant capacity of the obtained essential oil. Briefly, 1 mL of different dilutions (2%, 1.6%, 0.8%, 0.4%, 0.2%, 0.1% and 0.05%) of the essential oil was added to 1 mL of DPPH

(0.15 mM in ethanol) and at the same time, a control consisting of 1 mL DPPH with 1 mL ethanol was prepared. The tubes were mixed by hand and then allowed to stand in dark at room temperature for 30 minutes. After this period, the absorbance was measured at 517 nm.

The gallic acid was used as a positive control and the ethanol was used as blank. The scavenging ability was calculated using the following equation:

$$\%\ Scavenging\ \text{activity} = [(A_{control} - A_{sample})]/ (A_{control})] \times 100$$

2.5. Determination of the Repulsive/Insecticidal Effect of S. junceum Essential Oil

A volume of 5 mL of different dilutions of the EO was placed in glass jars containing fruit flies (drosophila). Also a negative control consisting of 5 mL of pure ethanol was prepared.

The time required for total drosophila mortality was observed and recorded. If a mortality was observed after a very short term (< 5 minutes), an increase in the dilution of the essential oil was performed.

2.6. Evaluation of the Antibacterial Activity

Bacterial strains: Gram positive bacteria (*Staphylococcus aureus* ATCC 25923) and Gram-negative strains (*Escherichia coli* ATCC 35218) were used in this study.

MIC and MBC assays: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the essential oil of *S. junceum* were determined by broth microdilution assay, as recommended by the Clinical Laboratory and Standard Institute (CLSI) (Wikler et al., 2006). In a 96-well plate (200 µl/well) (Greiner Bio-One, Essen, Germany), a serial of two-fold dilutions in MHB of the different dilutions was done. The wells were inoculated with 5×10^5 bacteria/mL. After incubating the plates at 37°C for 24 hours, the MIC was determined. Moreover, wells with no visible growth were plated on BHA in order to determine the MBC. The Petri plates were incubated overnight at 37°C, and the MBC was determined.

3. Results and Discussion

3.1 Yield of Essential Oil

After three hours, the obtained essential oil was collected, and its yield was estimated at 1.33 %.

3.2 Organoleptic Characterization

The characterization of the essential oil was focused on three factors (Table 1). The organoleptic characteristics of our obtained essential oil are comparable to those of MPIANA KIBWELA (2020). In general, the climate, altitude, growing conditions, expertise of the distiller, age of the oil and numerous other factors can impact the essential oil quality and the aroma of an essential oil.

Also, inspecting the color and clarity can be helpful. Some oils become darker as they age. Aging of an essential oil may also appear cloudy and may become more vicious (thick).

Table 1: Organoleptic feature of *Spartium junceum* essential oil

	Aspect	Color	Odor
Essential Oil	Liquide	Pale Yellow	Strong and Fresh

3.3 Antiradical DPPH Assay

The results indicated an evolution in the antioxidant activity for the obtained essential oil in a concentration dependent manner to reach a value of 82 % with an IC_{50} of 0.95 %. These results are in accordance with those obtained by Habibatni et al. (2016) who demonstrated that *S. junceum* extracts display a good antioxidant efficacy using the DPPH test.

3.4 Repellent /Insecticidal Activity

In our study, the essential oil from *S. junceum* was used to evaluate its repellent capacity. The obtained results indicated that after the addition of this essential oil into the jar, the insects have moved away quickly, dispersed and spread to avoid coming close to it. They died few minutes later depending on the dilution used as presented in Table 2.

Table 1 : Necessary time to kill drosophila according to essential oil percentage

Percentage %	2	1.6	0.8	0.4	0.2	0.1	0.05
Time in Min	1.5	3	8	10	15.5	24.5	38

The results presented in the Table above showed that the time required to kill drosophila has increased with the increase in dilutions of essential oil.

3.5 Antibacterial Activity

The obtained results showed that the essential oil of *S. junceum* has an antibacterial effect against both *E. coli* and *S. aureus* (Table 3).

Table 2: MIC & MBC of essential oil of *Spartium junceum*

	MIC		MBC	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
Essential Oil	0.5%	1.5%	0.5%	1%

These results are in contrast with those obtained by Cerchiara et al. (2013) who found that the *Spartium junceum* aromatic water did not possess an antimicrobial activity against fungal organisms (Aspergillus brasiliensis; Candida albicans), Gram-positive (Enterococcus hirae; Staphylococcus aureus) and Gram-negative (Escherichia coli; Pseudomonas aeruginosa) bacteria.

On the other hand, our results are in accordance with those obtained by Habibatni et al. (2016) who demonstrated that S. junceum extracts display antimicrobial efficacy against Gram-positive bacteria, whereas they were not active against the Gram-negative.

4. CONCLUSION

The objective of our work was to evaluate, for the first time, the possible repellent capacity, in addition to the antibacterial and antioxidant efficacy of essential oil of *Spartium junceum* grown in Lebanon. The results show that this oil has important antioxidant and antibacterial activities allowing it to be used for therapeutic applications, knowing that antioxidants contribute very effectively in the prevention of some diseases related to oxidative stress. In addition, the essential oils demonstrated an insecticidal effect and an antibacterial effect.

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