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Evaluation of Biological Activity of Essential Oil from *Teucrium polium* L.

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ABSTRACT

Background: *Teucrium polium* L. has an essential place in traditional medicine throughout the world. Their use, based on empirical knowledge passed down from generation to generation, has helped to treat a multitude of illnesses and relieve a variety of symptoms. These traditional practices have laid the foundations for modern research, which seeks to understand the significant antibacterial properties. The bioactive compounds present in the plant, such as essential oils and polyphenols, have demonstrated antimicrobial activity against a wide range of pathogenic bacteria. This includes bacteria responsible for gastrointestinal, skin and respiratory infections.

Methods: The focus of this study was the extraction of essential oils from the aerial portions of *Teucrium polium L. via* the hydrodistillation method, their anti-oxidant effect using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay method and iron reduction and antimicrobial activity of these essential oils was evaluated against seven ATCC bacterial strains: *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC10428, *Pseudomonas aeruginosa* ATCC27853, *Enterococcus faecalis* ATCC29212, *Klebsiella pneumoniae* ATCC700603 and *Bacillus subtilis* ATCC7033. In addition, the minimum inhibitory concentrations (MICs) were determined by incorporation method. These tests were carried out at the Laboratoires de the analytical chemistry laboratory at the Department of Pharmacy, Badji Mokhtar University, Annaba, Algeria.

Result: The hydro distillation method yielded an essential oil of 0.45%. Antimicrobial activity was tested by the sensitivity test, which revealed inhibition zone diameters ranging from 11.5 mm to ≤20 mm. In addition, minimum inhibitory concentrations (MICs) were determined, showing variations between bacterial strains, suggesting potential therapeutic importance for some strains. Additionally, the antioxidant activity of the essential oils was assessed using the iron-reducing capacity assay and the DPPH free radical scavenging assay. Although the results indicated notable antioxidant potential, the IC50 values obtained were relatively high when compared to ascorbic acid, registering 107.38 mg/ml for iron reduction and 91.62 mg/ml for DPPH reduction. These findings contribute to the exploration of the therapeutic potential of *Teucrium polium* L. essential oils for their antimicrobial and antioxidant properties.

Key words: Bacteria, Biology activity, Essential oil, *Teucrium polium* L.

INTRODUCTION

Algeria boasts a rich diversity of medicinal plants thriving in the fertile Sahel regions as well as the arid Highlands and desert zones. The focus of this study is the tomentose germander (*Teucrium polium* L.), a species belonging to the Lamiaceae family, collected from the Oued Melegue Daïra Ouenza region of Tebessa, in eastern Algeria.

The Teucrium genus encompasses over 300 generally aromatic species distributed across various parts of the globe. It is widely represented in the Mediterranean basin, with 21 species in Algeria alone (Andary, 1988). *Teucrium polium* L. is a flowering plant abundant in the Mediterranean region, spanning Europe, northern Africa and northwest Asia. This species typically inhabits arid and semi-arid bioclimates, preferring well-drained soils and sun exposure, often found on hillsides and sandy areas (Esmaeil, 2010).

Chemical studies on the Teucrium genus have revealed these plants to be rich sources of active principles, particularly essential oils (Rahmouni, 2021). Backed by scientific research, *Teucrium polium* L. has been utilized in traditional medicine for over 2,000 years (Mahmoudi, 2013) with experimental evidence supporting its efficacy. Infusions prepared from the aerial parts of *Teucrium polium* L. are renowned for their anti-inflammatory (Muhammed *et al.*, 2011), anti-jaundice, anti-anorexic, anti-spasmodic (Shahraki *et al.,* 2007) and anti-gastric colic properties, as well as their anti-diabetic, antioxidant, antispasmodic, anti-inflammatory (Parisa *et al.,* 2007) and antibacterial activities.

This study had three main objectives: firstly, to extract essential oils from the plant material through the hydrolysis

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method; secondly, to assess the antibacterial activity of the obtained oil samples against seven strains of gram-positive and gram-negative bacteria; and thirdly, to examine the antioxidant capacity of the oils by employing two protocols, namely the DPPH and FRAP assays.

MATERIALS AND METHODS

Extraction of essential oils (E.O)

Hydro distillation is a method that facilitates the extraction of essential oils (EO) from fresh or dried plant material. The process involves placing 100 g of the dried plant mass into a large glass flask and adding an adequate amount of distilled water, leaving sufficient headspace to prevent overflow during boiling. The flask is then heated using a heating mantle, bringing the mixture to a boil.

As essential oils have a lower density compared to water, they float on the surface of the aqueous mixture. The essential oil layer is carefully collected and stored in well-sealed, opaque bottles at low temperature (4-5°C) to ensure preservation.

Microbial activity

Determination of antibacterial activity by the disc method

The sensitivity of bacterial strains to the essential oil (EO) was evaluated using the disk diffusion method. Bacterial suspensions were prepared in physiological saline from young colonies (18-24 hours old) by adjusting the turbidity to 0.5 McFarland standard. These suspended bacteria were then swabbed onto the dry surface of Mueller-Hinton agar plates using sterile swabs. Sterile Whatman No. 3 paper discs (6mm in diameter) were placed on the inoculated agar and impregnated with 10 µL of the essential oil (Esmaeil, 2010; Darshana, 2015).

The plates were incubated at 37°C for 24 hours. The bacterial strain was considered non-sensitive to the natural substance if the inhibition zone diameter was less than 8 mm, moderately sensitive between 8 and 14 mm, sensitive between 14 and 20 mm and highly sensitive if the diameter exceeded 20 mm (Ameur *et al*., 2009; Dalila, 2019).

MICs determination of essential oils

A dilution series ranging from 2.5 to 20µL/mL was prepared by dissolving the essential oil in dimethyl sulfoxide (DMSO). For each dilution, 2 mL was mixed with 18 mL of Mueller-Hinton agar cooled to 45°C. The mixtures were then poured into 90mm diameter Petri dishes and allowed to solidify at room temperature for 24 hours (Kempf, 2011).

Bacterial suspensions were prepared in physiological saline from young 18-24-hour colonies, with the turbidity adjusted to 0.5 McFarland standard. A 2-µL aliquot of each suspension was spot-inoculated onto the surface of the essential oil-incorporated agar plates and allowed to dry. The inoculated plates were subsequently incubated at 37°C for 24 hours (Benslimani, 2011). The minimum concentration preventing visible bacterial growth was recorded as the MIC.

In vitro anti oxydant activity

Capacity of trapping of the free radical "2,2-diphenyl-1-picrylhydrazyl" (DPPH)

The free radical scavenging capacity of the essential oil was evaluated against 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method described by (Boulila, 2015).1 mL of the essential oil at various concentrations was mixed with 2 mL of a methanolic DPPH solution (0.04 g/L). After incubating in the dark for 60 minutes, the absorbance was measured at 517 nm using a JENWAY 6300 spectrophotometer, with methanol used as the blank.

The percentages of inhibition of the DPPH were calculated according to the formula:

$$\%\text{ of inhibition DPPH} = \frac{A_0 - Aeq}{A_0} \times 100$$

Aeq represents the absorbance of the methanolic DPPH solution after the addition of the antioxidant sample, once equilibrium is reached. A_0 denotes the absorbance of the DPPH solution with only methanol added in the same proportions. A curve plotting the percentage of DPPH inhibition against the antioxidant concentration in micrograms per milliliter (µg/mL) enabled the determination of the median inhibitory concentration (IC50), defined as the antioxidant concentration required to reduce the initial DPPH concentration by 50%. Ascorbic acid was used as a positive control for comparison (Dridi, 2016).

Reduction of ferric ion

The reducing power was evaluated following the method described by (Oyaizu, 1986). 1 mL of essential oil concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL in methanol) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution. The mixtures were incubated at 50°C for 30 minutes. Subsequently, 2.5 mL of 10% trichloroacetic acid was added to the solutions, which were then centrifuged at 3000 rpm for 10 minutes.

After centrifugation, 2.5 mL of the supernatant from each concentration was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution. The absorbance was measured at 700 nm using a JENWAY 6300 spectrophotometer. Ascorbic acid was used as a positive control, following the same experimental conditions.

The result is expressed as the effective concentration EC50, defined as the concentration of essential oil in micrograms per milliliter of the mixture (EC50 in µg/mL) required to achieve a given reducing capacity.

RESULTS AND DISCUSSION

Extraction and activity of E.O

Extraction of E.O

The essential oil extracted from *T. polium* exhibited a light-yellow color and a strong, pleasant aroma. It was observed to be less dense than water. The average yield obtained

Table 1: The anti-bacterial activity.

Strain	Inhibition diameter of E.H. of *Teucrium polium* L. (mm)	Minimum inhibitory concentration ul/ml
Bacillus subtilis ATCC7033	12	10
Eschirichilia coli ATCC25922	10.5	15
Enterococcus feacalis ATCC 29219	<20	1
Klebsiella pneumoniae ATCC10428	15	1
Pseudomonas aeroginosa ATCC27853	12	15
Salmonella typhymurium ATCC10428	11	5
Staphylococus aureus ATCC25923	22	2

from the plant's dry matter was 0.45 ml of essential oil per 100 grams.

Microbial activity

Antibacterial activity

The inhibition zone diameters for the *T. polium* essential oil ranged from 10.5 mm to 20 mm, indicating that most of the tested bacterial strains were sensitive or highly sensitive to the oil's antimicrobial effects.

Comparing with (Belmekki, 2013) on *T. polium* essential oil tested against two of the present strains, a larger inhibition zone (15 mm) was observed for *E. faecalis* in their study, whereas the current work found a larger diameter for P. *aeruginosa* (15 mm). Hammoudi (2013) reported inhibition zones of 12 mm for *S. aureus*, 10 mm for *E. coli* and 9 mm for *P. aeruginosa*.

The essential oil's minimum inhibitory concentrations (MICs) varied across the different bacterial strains. For *E. faecalis* and *S. aureus*, (Belmekki, 2013) obtained MICs of 5 µg/mL and 3 µg/mL, respectively, while the present study showed MICs of 1 µg/mL, 15 µg/mL and 2 µg/mL for these strains. Belmekki (2013) found a lower MIC for E. coli compared to the current results (Hammoudi, 2013). The study reported MICs of 4.9 mg/mL for S. aureus, 12.25 mg/mL for *E. coli* and 12.25 mg/mL for *P. aeruginosa*.

The broad-spectrum antimicrobial effects observed can be attributed to the rich biochemical composition of essential oils, containing numerous active compounds that synergistically limit the risk of bacterial resistance development, as previously reported by Elaissi (2009).

Antioxidant activity

The data presented in Table 2 reveals that the antioxidant activity of the essential oil (E.O.) derived from *Teucrium polium* L. is considered less potent when compared to ascorbic acid, a highly effective antioxidant.

Other studies, such as Hammoudi, (2013), reported an EC50 value of 9200 µg/mL for this essential oil, while (Chabane, 2020) found an EC50 of 14.6 ± 0.71 µg/mL.

According to both the theoretical and practical results obtained in our study, the antioxidant substance demonstrated a variable antiradical activity, but it was comparatively weaker than ascorbic acid.

Table 2: Antioxidant activity expressed in IC 50 (ug/ml) of essential oil of *Teucrium polium* L.

	Capacity of trapping of the free radical DPPH ug/ml	Reduction of Ferric ion ug/ml
E.O	91.62	107.38
Ascorbic acid	0.37	1.961

To assess the reducing potential of the Teucrium essential oil, the FRAP method was employed. This method involves the reduction of the ferric-tripyridyltriazine complex to its colored ferrous form in the presence of antioxidants (Hammoudi, 2013). Our findings from this test indicated an average antioxidant (reducing) potential compared to ascorbic acid.

CONCLUSION

Medicinal plants remain an enthralling field that continually captures the curiosity of researchers. In the pursuit of developing natural substances, this study endeavored to investigate the species *Teucrium polium* L., found in the Meguessemia region of Guelma, renowned for its numerous medicinal virtues.

Through the hydrodistillation extraction technique, the essential oil obtained from this plant exhibited significant antibacterial effects against various gram-positive and gram-negative bacterial strains. This potent antibacterial activity can be attributed to the chemical composition of the essential oil, comprising natural compounds with inherent antibacterial properties. The findings of this study contribute to the ongoing exploration and validation of traditional medicinal plants, paving the way for their potential integration into modern therapeutic applications.

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Informed consent

All participants provided written informed consent after being fully informed about the study.

Conflict of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper's content.

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