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Evaluation of the Antifungal Effects of Phenolic Compounds and Volatile Oils Extracted from *Petroselinum sativum L.* on *Microsporum canis* and *Trichophyton mentagrophytes*

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Abstract

A current study on the volatile oil and phenolic extract of *Petroselinum sativum* L. was conducted to evaluate the antifungal activity of the plant against *Trichophyton mentagrophytes* and *Microsporum canis*, the phytochemical constituents were first examined by preparing an ether-alcoholic extract of the leaves. The antifungal activity of the phenolic extract and volatile oil was evaluated against fungi at different concentrations. At the lowest tested concentration 0.5 mg/mL, the volatile oil produced inhibition zones of 2.23 mm against *M. canis* and 1.12 mm against *T. mentagrophytes* . The phenolic extract showed stronger inhibitory effects. At 1 mg/mL, inhibition were 3.78 mm for *M. canis* and 1.99 mm for *T. mentagrophytes*. At 3 mg/mL resulted in inhibition were of 4.78 mm for *M. canis* and 6.56 mm for *T. mentagrophytes*, while at 8 mg/mL with the same values, suggesting a possible plateau effect at this level. The inhibition area of phenol was 2.12 mm against *Trichophyton mentagrophytes*, 3.78 mm against *Microsporum canis* at 3 mg/mL .At 5 mg/mL, inhibition zones reached 4.98 mm for *M. canis* and 2.98 mm for *T. mentagrophytes*. The highest activities were observed at 10 and 12 mg/mL. At 10 mg/mL, inhibition zones measured 6.12 mm for M. canis and 5.34 mm for *T. mentagrophytes*, while at 12 mg/mL the inhibition zones increased further to 7.34 mm and 5.99 mm, respectively.

Keywords: *Petroselinum sativum*, volatile oil, antifungal, *Trichophyton mentagrophytes*, *Microsporum canis.*

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Introduction

One of the most frequent causes of superficial fungal infections is the keratinophillic known as dermatophytes fungus group [1,2]. Antifungal resistance has been a growing concern in recent decades [3]. Antifungal medicines are losing effectiveness against pathogenic fungi, which cause invasive and superficial infections [4]. Complementary medicine, particularly the use of medicinal plants, has gained increasing prominence in the fields of pharmacy and medicine due to their potential therapeutic properties and growing acceptance in modern healthcare [5]. Volatile oils, secondary metabolites derived from plants' essential oils, have emerged as promising candidates in the development of alternative antimicrobial agents [6]. The results revealed a synergistic impact of essential oils and Cecropin A on both bacteria, indicating the necessity for more study into the efficacy of natural extracts, these chemicals have the potential for use in a variety of sectors, including medicine, food, and animal feed [7]. Parsley (*Petroselinum crispum*) is a biennial herb belonging to the Apiaceae family, which also includes carrots. It has been cultivated worldwide for thousands of years and widely utilized as a culinary flavoring agent, a source of essential oils, and a component of traditional remedies. The major bioactive constituents of parsley include apiole, myristicin, α-pinene, β-pinene, and elemicin, which contribute to its characteristic aroma and potential therapeutic properties, the species or cultivars, as well as the cultivation conditions, including soil type, weather, irrigation, pruning, and other horticultural activities, all affect the amount of each of these chemical elements [8]. In addition to being used as food, *P. sativum L.* has essential medicinal uses as a hypoglycemic, diuretic, antispasmodic, carminative, laxative, antibacterial, uterine tonic, and hypotensive, the percentage of volatile oils (5%) [9, 10, 11]. The use of these novel drugs, which are not derived from synthetic agents, has been proposed as a promising strategy to overcome antibiotic resistance [12]. The aim of this study was to identify the secondary metabolites of parsley through preliminary screening, isolate the phenolic compounds from its extract, and evaluate the antifungal activity of both the volatile oil and the phenolic extract against *Trichophyton mentagrophytes* and *Microsporum canis.*

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Plant's collection: Fresh parsley (*Petroselinum sativum L.*) plants were collected from local fields in Karbala, Iraq, at the peak of the flowering stage on August 18, 2022. The taxonomic identification of the plant was confirmed according to standard botanical references. Fresh leaves were carefully separated, washed with cold distilled water to eliminate dust and surface contaminants, and then air-dried under shade at room temperature (25 ± 2 °C) for 24 hours to prevent degradation of bioactive compounds. The dried plant material was subsequently ground into a fine powder using a mechanical grinder. Coarse residues were removed by sieving through sterile gauze to obtain a uniform particle size suitable for extraction procedures [13,14].

Volatile oil Extraction: A total of 200 g of dried parsley leaves was placed in a small Soxhlet extractor. The plant material was extracted for 24 hours using 250 mL of 70% hexane in a 1000 mL round-bottom flask. After extraction, the obtained solvent was removed under reduced pressure at 45°C using a rotary evaporator to yield the volatile oil [15].

Alcoholic extracts: The alcoholic extract was prepared using a rotary evaporator to dissolve 100 g of the powdered material in 100 mL of 70% ethanol solvent in a 500 mL flask using a Soxhelet apparatus for one full day [15].

Separation: The ethanolic extract of parsley was acidified using 2 M HCl to a pH below 3. The acidified extract was transferred to a separator funnel and washed with chloroform ($CHCl_3$). The mixture was thoroughly shaken, and after phase separation, the lower (chloroform) layer was collected. This procedure was repeated two to three times to ensure complete extraction of the phenolic compounds. The collected phenolic fraction was then dried in an oven at 20–30 °C and stored at 4 °C until further use. This method was performed as previously described [16].

Chemical analysis of plant extracts' active components

1. Phenols

a. Ferric Chloride reagents: Three to four drops of a ferric chloride solution were added to the extracts Phenols are present when a bluish black color forms.

b. Lead acetate: Using distilled water to the extract (50 mg) was dissolved in 5 mL. 10% of lead acetate was then added to it. The presence of phenol compounds was indicated by a large white precipitate.

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2. Glycoside: Five mL of Benedict reagent were combined with 1 mL of each part's extract. The presence of lowering sugar is indicated by the presence of crimson sediment.

3. Flavonoids: Five mL of each extract was treated with 1 mL of potassium hydroxide alcohol, the development of yellow sediment is an indication of the presence Flavonoids.

4. Sapiens : The appear once of foam for a long time as a result of stirring the aqueous solution of plant in test tube indicated saponins existence.

5. Tannins: After being heated at 80–100 °C for 10 minutes in a water bath, the mixture was filtered, and then 5–6 drops of 1% ferric chloride were added. The dark green hue of the mixture indicates the presence tannins [17, 18].

Antifungal activity volatile oils and phenol extract of leaf

Two dermatophyte isolates, *Microsporum canis* and *Trichophyton mentagrophytes*, were obtained from the Microbiology Laboratory at the University of Karbala. The antifungal activity of both the volatile oil and the phenolic extract from parsley leaves was evaluated against these isolates using standard in vitro assays following the protocols described in previous studies [19]. The medium was Prepared by dissolving 65 g SDA, 1 liter of distilled water autoclaving it for 15 minutes at 121℃. The effect of using antifungal activity was studied by mixing the medium at a temperature of 45 to 55 degrees Celsius in clean Petri dishes. After that, the fungi were taken and cultured on the required medium after hardening and then incubated at a temperature of 28 degrees Celsius for 7 to 10 days, after that It was compared with controls (grown only on SDA). Using the equation 1, the zone of inhibition (mm) of the fungus was estimated [16, 20].

$$\textbf{\textit{Rate inhibit}}\ (\%) = \frac{\textbf{\textit{Diameter of fungi control}} - \textbf{\textit{diiameter of fungi treated}}}{\textbf{\textit{Diameter of fungi conterol}}} \times \mathbf{100} \qquad \ldots (1)$$

Statistical

The tests were carried out as factorial experiments with three replications using a completely randomized design (CRD), and the Least Significant difference was used to determine whether two or three factors were significant (L.S.D. at 1% likelihood (P 0.01).

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Results and Discussion

Chemical identification of plant extracts' active compounds:

The results of the study showed the presence of active chemicals in parsley extract, as shown in Table (1). Through screen analysis, phenols, glycosides, saponins, and flavonoids showed positive results, while the tannins showed negative results.

Table 1. phytochemical screen of *N. sativa* extract.

Active Compounds	Extract
Glycoside	+
Phenol	+
Tannins	-
Flavonoid	+
Saponins	+

The results also showed that the concentrations of different volatile oil components (0.5, 1,3, and 8 mg/mL) enhance the inhibitory zone against fungi. As shown in Table (2).

Table 2. The various concentrations volatile oil and their effects on fungi development.

Concentrations (mg/mL)	*Microsporum canis* inhibition zone mm	*Trichophyton mentagrophytes* inhibition zone mm	Control
0.5	**2.23**	**1.12**	**0**
1	**3.78**	**1.99**	**0**
3	**4.78**	**2.67**	**0**
8	**6.56**	**4.78**	**0**
LSD =	**2.77**	**1.98**	

The antifungal activity of parsley volatile oil and phenolic extract was evaluated against *Trichophyton mentagrophytes* and *Microsporum canis* using the disc diffusion method. At a concentration of 0.5 mg/mL, the volatile oil produced inhibition zones of 1.12 mm against *T. mentagrophytes* and 2.23 mm against *M. canis*. Increasing the concentration to 1 mg/mL resulted in inhibition zones of 1.99 mm for *T. mentagrophytes* and 3.78 mm for *M. canis.* At 3 mg/mL,

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the inhibition zones measured 2.67 mm and 4.78 mm for *T. mentagrophytes* and *M. canis*, respectively, while at 8 mg/mL, the zones were 4.78 mm for *T. mentagrophytes* and 6.56 mm for *M. canis*. *Parsley*, a member of the Umbelliferae family, contains a wide range of volatile oil components, including apiole, myristicin, and furanocoumarins, which are believed to contribute to its antifungal properties. The disc diffusion results demonstrate that both the volatile oil and the phenolic extract exhibit inhibitory effects against these dermatophytes, with varying efficacy depending on the concentration. These findings align with the established applications of parsley, which are largely attributed to its essential oil content [21]. It has been discovered that *P. crispum* contains a wide range of bioactive chemicals, including phenolic compounds, particularly flavonoids (such as apigenin, apiin, and 6"-Acetylapiin) essential oil constituents (mainly myristicin and apiol), coumarins, and furocoumarins [22]. These results suggested that phenolic extract exhibited stronger antibacterial than antifungal properties, which could be explained by its capacity to enters cell walls [23]. Polyphenols are classified as bioactive chemicals. The "hydroxyl groups" and phenolic rings in the molecules have the ability to link with proteins and bacterial membranes to form complexes, which is related to their biological processes[24].

Table 3. Effect the different concentrations of phenol on fungi development.

Concentrations mg/mL	*Microsporum canis*	*Trichophyton mentagrophytes*	Control
3	**3.78**	**2.12**	**0**
5	**4.98**	**2.98**	**0**
10	**6.12**	**5.34**	**0**
12	**7.34**	**5.99**	**0**
LSD =	**1.70**	**1.81**	**-**

The results were seen in table (3) that showing the effect of various concentrations (3, 5.10 and 12 mg/mL) of phenol compounds which increased the inhibition area against fungi. The inhibition area of phenol was 2.12 mm against *Trichophyton, mentagrophytes*, 3.78 mm against *Microsporum canis* in concentration 3 mg/mL, while concentration 5 milligrams at of milliliter in *Trichophyton mentagrophytes* 2.98 mm, *Microsporum canis* 4.98mm, while concentration 10 mg/mL in *Trichophyton, mentagrophytes* 5.34mm, *Microsporum canis* 6.12 mm, concentration

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12 mg/mL in *Trichophyton mentagrophytes* 5.99, *Microsporum canis* 7.34 mm. Simplest bioactive phytochemicals include single-substituted phenolic rings that are highly oxidized. The common herbs have phenols, which are good at fighting bacteria [25].Common herbs contain natural antibacterial compounds, primarily phenols, while their volatile oils also exhibit antimicrobial activity by interfering with the synthesis of genetic material (DNA and RNA) in bacterial cells [26]. Additionally, enzymes may be inhibited through oxidation or by interactions with sulfhydryl groups, and more generally, phenolic compounds can react with proteins, leading to toxicity against microorganisms [27]. Carboxylic acids are found in many plants metabolite molecular structures they have previously been found to be a potent antibacterial agent and have been connected to a number of antimicrobial as well as antifungal actions [28- 30].

Conclusions

The present this study the volatile oil and phenol extract from parsley has antifungal, The volatile oil compounds extract from leaf parsley were tested to check if it could inhibit the growth of the fungi *M.m canis* and *T. mentagrophytes* by the disc diffusion inhibition test.

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