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Effects of *Centaurea cineraria* extract on the growth of some dermatophytes

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ABSTRACT

This research was carried out in the Imam Al-Hussein Center, Karbala Governorate, Iraq for Researcher Care and Manuscript Restoration to assess the effects of active ingredient extracted from the *Centaurea cineraria* methanolic on inhibiting the growth of the cutaneous fungus *Trichophyton rubrum* and *Microsporum canis* and determining its appropriate concentration. The plant was chemically tested in the experiment to diagnose active chemicals utilizing qualitative analysis of compounds in the plant sample using the GC-MS technique. Different treatments (concentrations) of 10, 20, 30, 40, 50 and 60% of the methanolic extract were prepared, and the results were obtained as a function of colony diameter on SDA medium along with the colony weight on SD broth medium after 14 days of incubation, and also observing the shape of fungal colonies on the culture medium. The concentrations of the methanolic extract affected the studied fungi, where $T_{60\%}$ on *Trichophyton* exhibited to be the most effective, with a diameter of 9.67 mm, while $T_{10\%}$ on *Microsporum* displayed the lowest effect with a diameter of 77.67 mm. As for the dry weight of the fungal colony, $T_{60\%}$ was more effective on *Trichophyton*, where the average dry weight was 0.106 g, while the $T_{10\%}$ on *Microsporum* exhibited the lowest effect with a dry weight rate of 0.346 g.

Keywords: *Centaurea cineraria*, *Trichophyton*, *Microsporum*, Methanolic extract, Dermatophyte.
Article type: Research Article.

INTRODUCTION

Centaurea is the fourth biggest genus in the Asteraceae family, with over 600 species found throughout the world, primarily in Western Asia and the Mediterranean (Heywood *et al.* 1978; Garcia Jacas *et al.* 2000). Plant extracts have been utilized for thousands of years for a number of purposes (Jones 1996; Porusia & Septiyana 2021; Naser AL-Isawi 2022; Salih *et al.* 2022; Al-Shurait & Al-Ali 2022). The Mediterranean coastal areas, the Red Sea, and Egypt's Nile regions are home to about 17 *Centaurea* species. Its species are used as an antipyretic, anti-diarrheal febrifuge, and for the treatment of liver problems in folk medicine (Reyhan *et al.* 2004). Antimicrobial and anti-diabetic compounds are found in flowering branch extracts (Soumyanath 2005). Among the fungi that cause communicable diseases are dermatophytes. They are a collection of related keratinophilic fungi that infect human and animal surface keratinized tissues (Shehata *et al.* 2008). They cause the majority of external fungal infections, especially in the skin, hair, and nails, and the lifetime risk of contracting a dermatophyte infection is estimated to be between 10% and 20% (Aly 1994; Drake *et al.* 1996). Dermatophytes prefer moist places of the skin with skin wrinkles, according to common observation. *Microsporum*, *Trichophyton* and *Epidermophyton* are some of the most commonly associated genera (Magill *et al.* 2007). Even with commercially available pharmacological medications, treating dermatophytic infections is tough due to the requirement for long-term treatment and frequent failures (Nowrozi *et al.* 2008). Plant-derived drugs could be effective in treating infections and combating side effects. Herbal medicine has grown in popularity as a type of treatment. It should, however, be tested for efficacy using traditional trial methods. Several herbal extracts have been shown to be effective in treating various ailments (Firenzouli & Gori 2007). *Centaurea* chemical composition varies widely depending on the species and

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field. Sesquiterpene lactones (Krasnov *et al.* 2012; Grienke *et al.* 2018), flavonoids, lignans, alkaloids (Lockowandt *et al.* 2019), phenolic compounds (Albayrak *et al.* 2017; Özcan *et al.* 2019), steroids, terpenes, etc. are the most physiologically-active components (Ayad & Akkal 2019); Guvensen *et al.* 2019; Naeim *et al.* 2020). *Centaurea* L. species obtained in Turkey showed a wide spectrum of antibacterial activity to varied degrees. Ethyl acetate extracts of *C. odyssei* and *C. kurdica*, in particular, showed substantial antibacterial and anticandidal activity and could be employed as antimicrobial agents in new therapeutic medicines (Güven *et al.* 2005). As a result, the study's goal was to see if methanol extraction of *Centaurea cineraria* might stop the fungi, *Trichophyton rubrum* and *Microsporum canis* from growing.

MATERIALS AND METHODS

The fungi used in the study

The two fungal isolates used in this study, *Trichophyton rubrum* isolate IQT-No.1 and *Microsporum canis* isolate IQM-No.3 were obtained from the Graduate Studies Laboratory, College of Education for Pure Sciences, University of Karbala, Iraq, then were identified and registered in GenBank as MK167434. 1 and MK167439.1 respectively. The two isolates were activated and grown on SDA medium (Sandven & Lassen 1999), and their phenotypic and microscopic characteristics were examined before and after the treatments.

Plant sample collection

The plant (*Centaurea cineraria)* during the flowering season in July, was harvested from one of the Karbala's local nurseries, where the used plant parts (leaves) for the aim of obtaining vegetable powder, were washed with ordinary water, then distilled water, air dried, and finally processed with an electric grinder.

Preparation of methanolic extracts

Fifty g of the dry plant powder was weighed and mixed with 500-mL 70% methyl alcohol in a 1000 mL glass beaker sealed with cotton and aluminum paper and left at room temperature for 24 hours. Then the mixture was filtered using several layers of medical gauze for disposal. The plankton was centrifuged at 3000 rpm for 10 minutes, and the extract was filtered using 0.1 µm Whatman filter papers to make a transparent solution for GC-MS (Gas Chromatography-Mass Spectrometry) (Hernández Pérez *et al.* 1994).

GC-MS technology for qualitative and quantitative study of chemical components in plant samples

Gas chromatography-mass spectrometry was used to determine the active component content of leaves.

Active compound diagnostics

By comparing the obtained spectrum of the unknown component with known stored components in the National Institute of Standards and Technology (NIST) library, components were discovered using the NIST database. This part of study was carried out at Mass Spectrometry Gas Chromatography Laboratory, Environment and Water Department, the Ministry of Science and Technology, Iraq.

Culture media

Sabouraud dextrose agar (SDA)

SDA was prepared by dissolving 65 g of the prepared medium powder in an amount of distilled water, then completing the volume to 1000 mL according to the instructions of the manufacturer (HIMEDIA), followed by pouring into the petri dishes according to the purpose of the experiment.

Sabouraud Dextrose broth (SD broth)

SD broth was prepared by dissolving 30 g of the ready-made powder in a quantity of distilled water, then completing the volume to 1000 mL, according to the instructions of the manufacturer (OXOID), followed by pouring into the tubes according to the purpose of the experiment.

Sterilization of culture media

The antibiotic chloramphenicol was added at a rate of 250 mg L^{-1} to all culture media, then sterilized with osmosis at a temperature of 121 °C and a pressure of 15 atmospheres for 20 minutes and then left to cool.

The following characteristics were examined

1-Average colony diameter of fungi (mm) on SDA

SDA medium: The method of (Kady & El Maraghy 1993) was followed, where the methanolic extract was mixed with the SDA culture medium before hardening, with six concentrations (treatments) of 10%, 20%, 30%, 40%, 50% and 60% ($T_{10\%}$, $T_{20\%}$, $T_{30\%}$, $T_{40\%}$, $T_{50\%}$ and $T_{60\%}$,). A hole was drilled in the center of each dish with a cork borer with a diameter of 7 mm, at a rate of three duplicates for each concentration, in addition to the control treatment (cultivar medium solely without any addition) and after medium hardening. After two weeks, the dishes were inoculated with the investigated fungi cultured on SDA medium by planting a disc with a diameter of 7 mm in the hole formed in the middle of the dish for each of them. The diameter of the developing colony was measured by two perpendicular diameters and the findings were recorded after two weeks of incubation at 25 °C (Khanzada *et al.* 2006).

2- Average dry weight (g) of growth on liquid SD medium

The methanolic extract was used to examine the effect of the extracts on the dry weight of the fungi. It was mixed with the sterile SD broth after it was taken out from the autoclave at a temperature of 50 °C with six concentrations (treatments) of 10%, 20%, 30%, 40%, 50% and 60% ($T_{10\%}$, $T_{20\%}$, $T_{30\%}$, $T_{40\%}$, $T_{50\%}$ and $T_{60\%}$,). For each concentration, three replicates were performed. Aside from the control therapy, (cultivar medium only without any addition) 70 ml tubes were used, each with 20 ml of culture medium. The fungus vaccine was introduced into the tubes by producing a disc with a diameter of 7 mm.

The tubes were incubated for two weeks at a temperature of 25 °C. Then the dry weight was calculated after filtering the liquid cultures through a filter paper with a known weight. Afterward, the filter papers were dried with the fungi in an oven at a temperature of 40 °C until the weight got stable. The dry weight of fungus was calculated using a sensitive electric balance with four decimal places using the following equation (Arey 2010).

Weight of mycelium = (weight of filter paper + weight of mycelium) – (weight of filter paper)

Statistical analysis

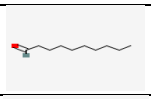
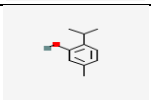
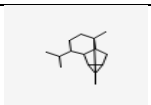
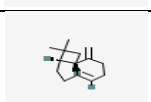
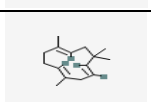
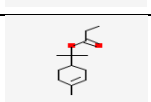
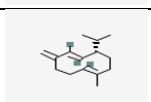
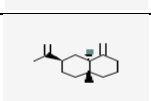
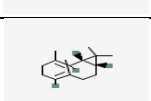
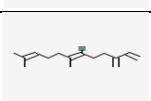
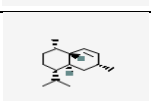
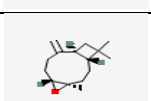
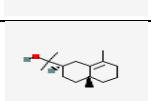
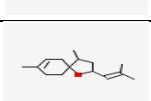
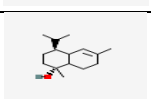
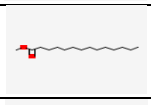
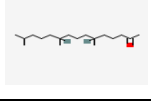
The researchers used a Complete Randomized Design (CRD). The least significant difference (LSD) was used to compare the averages of the coefficients at a probability level of 0.05 (d Steel *et al.* 1996) and the statistical analysis was performed using the Genstat program.

RESULTS AND DISCUSSION

Table 1 show the GC-MS analysis of methanolic extraction of *Centaurea cineraria* the main compound in the methanolic extraction was Germacrenes D (22%). It is a class of volatile organic hydrocarbons, specifically, sesquiterpenes. Germacrenes are typically produced in a number of plant species for their antimicrobial activities, containing palmitic acid (20.8%), β-Caryophyllene (8.6%), phenolic compound in the 5-methyl-2-propan-2-ylphenol (0.9%), Guaiacol (1.6%), 4-Vinylguaiacol (2.7%), Eugenol 2.1% in addition to a number of organic compounds and volatile oils. Table 2 depicts that *Trichophyton* was most affected by different methanolic extract of *Centaurea* than *Microsporum*, with significant differences, where the average diameters were 44.86 and 57.48 mm, respectively. As for the effect of the methanolic extract concentration, $T_{60\%}$ was more inhibiting with a diameter of 16.83 mm compared to the control treatment, while $T_{10\%}$ was less effective compared to the control with a diameter of 77.00 mm.

As for the bilateral interaction between the concentration and the type of fungus, $T_{60\%}$ was more effective on inhibiting the growth of *Trichophyton*, so that, the average diameter reached 9.67 mm, while $T_{60\%}$ exhibited lowest effect on *Microsporum*, with an average diameter of 77.67 mm. Table 3 illustrates that *Trichophyton* was most affected by different methanolic extract concentrations of *Centaurea* than *Microsporum* in the dry weight of the fungal colonies and with significant differences, where it recorded an average dry weight of 0.267 and 0.299 g, respectively.

Table 1. Components of methanolic extract of *Centaurea cineraria* using GC-MS.

Area %	Compound name	Molecular Formula	Structure Formula
1.3	Decanal aldehyde	$C_{10}H_{20}O$	
0.9	5-methyl-2-propan-2-ylphenol	$C_{10}H_{14}O$	
1.5	Cyclosativene	$C_{15}H_{24}$	
8.6	β-Caryophyllene	$C_{15}H_{24}$	
1.7	α-Humulene	$C_{15}H_{24}$	
3.4	α-Terpinyl propionate	$C_{13}H_{22}O_2$	
22.o	Germacrene D	$C_{15}H_{24}$	
1.4	α-Selinene	$C_{15}H_{24}$	
1.7	Bicyclogemacrene	$C_{15}H_{24}$	
1.3	Farnesene	$C_{15}H_{24}$	
1.1	δ-Cadinene	$C_{15}H_{26}$	
3.2	Caryophyllene oxide	$C_{15}H_{24}O$	
1.2	β-Eudesmol	$C_{15}H_{26}O$	
1.4	α-Bisabolene oxide	$C_{15}H_{24}O$	
0.8	α-Cadinol	$C_{15}H_{26}O$	
1.1	Methyl myristate	$C_{15}H_{30}O_2$	
1.2	6,10,14-Trimethyl-2-pentadecanone	$C_{18}H_{36}O$	

Countiue Table 1. Components of methanolic extract…

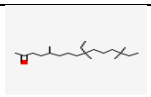
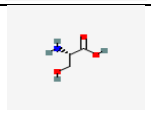
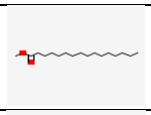
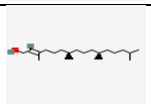
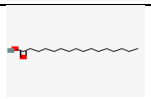
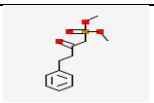
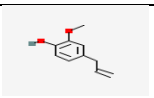
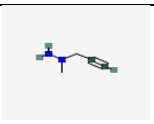
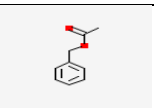
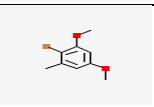
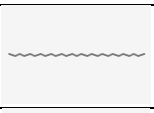
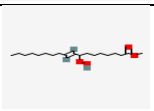
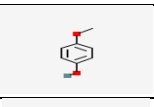
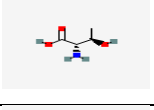
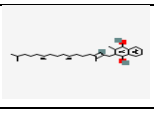
Area %	Compound name	Molecular Formula	Structure Formula
1.9	(E,E)-Farnesyl acetone	$C_{21}H_{42}O$	
1.7	(2S)-2-amino-3-hydroxypropanoic acid	$C_3H_7NO_3$	
1.2	Methyl palmitate	$C_{17}H_{34}O_2$	
1.3	Phytol	$C_{20}H_{40}O$	
20.8	Palmitic acid	$C_{16}H_{32}O_2$	
0.29	1-dimethoxyphosphoryl-4-phenylbutan-2-one	$C_{12}H_{17}O_4P$	
2.1	Eugenol	$C_{10}H_{12}O_2$	
3.4	1-methyl-1-prop-2-ynylhydrazine	$C_4H_8N_2$	
2.2	Vinyl guaiacol	$C_9H_{10}O_2$	
2.17	2-bromo-1,5-dimethoxy-3-methylbenzene	$C_9H_{11}BrO_2$	
3.5	Heptacosane	$C_{27}H_{56}$	
2.4	9-octadecenoic acid, methyl ester (E)	$C_{19}H_{36}O_4$	
1.6	Guaiacol	$C_7H_8O_2$	
0.44	(2S,3R)-2-amino-3-hydroxybutanoic acid	$C_4H_9NO_3$	
1.2	Phytonadiol	$C_{31}H_{48}O_2$	

Table 2. Effect of different concentrations of methanolic extract of *Centaurea* on average diameter (mm) *Trichophyton* and *Microsporum.*

Concentration Average	*Microsporum*	*Trichophyton*	fungus concentration
78.83	78.00	79.67	%0 Cont.
77.00	77.67	67.33	%10
62.83	70.33	55.33	%20
50.33	59.67	41.00	%30
28.00	38.33	17.67	%40
44.33	54.33	34.33	%50
16.83	24.00	9.67	%60
	57.48	44.86	Average fungus
4.137 interaction	1.564 fungus	2.925 concentration	L.S.D 0.05

Table 3. Effect of different concentrations of methanolic extract of *Centaurea* on average dry weight (g) of *Trichophyton* and *Microsporum.*

Concentration Average	*Microsporum*	*Trichophyton*	Fungus concentration
0.496	0.520	0.473	0% Cont.
0.338	0.346	0.330	10%
0.306	0.310	0.303	20%
0.275	0.280	0.270	30%
0.233	0.250	0.216	40%
0.190	0.210	0.170	50%
0.141	0.176	0.106	60%
	0.299	0.267	Fungus average
0.0204 interaction	0.0077 fungus	0.0144 concentration	L.S.D 0.05

In the case of the methanolic extract concentrations, $T_{60\%}$ was more effective in inhibiting with a dry weight of 0.141 g compared to the control group, while $T_{10\%}$ was less effective compared to the control with a dry weight of 0.338 g. In the case of the bilateral interaction between the concentration and the type of fungus, $T_{60\%}$ was more effective on inhibiting the *Trichophyton* growth, as the average dry weight was 0.106 g, while $T_{60\%}$ exhibited $_{\text{lowest}}$ effect on *Microsporum* with an average dry weight of 0.346 g. Many phenolic compounds and active compounds containing hydroxyl groups, carbonyl groups, and double bonds were found in GC-MS analysis, which are known to have various biological activities such as antioxidants and inhibiting the microorganism growth in addition to their chemical protective importance of the active compounds (Senatore *et al.* 2003). The biological activities of these compounds are an indication of the medicinal potential of these plants that have been studied (Reed 1995). Some researchers believe that phenolic compounds' antifungal activity is a result of the synergy of different types of phenolic compounds, rather than being attributed to a single component (Arnous *et al.* 2002; Lee *et al.* 2003); fatty acids such as palmitic acid can act as anionic surfactants and have antifungal properties (Pohl *et al.* 2011; Bruno *et al.* 2018; Hayes& Berkovitz 1979). The inhibition mechanism of plant extracts may occur because they contain biologically active substances that help and induced by the interaction of the active chemical compounds in the extract with the lipid layer in the membranes, resulting in damage or leakage of intracellular substances

(Webster *et al.* 2008). Cells of fungi (the outer and inner membrane) (Chen *et al.* 2003) or perhaps the extract are stimulated by the osmotic pressure of water, which causes the cell to swell more and leads to death, or perhaps the extracts inhibit the synthesis of the protein of the fungal cell DNA (Abd Elaah &Ahmed 2005). The fungi varied in their resistance to the effect of the alcoholic extract, where *M. canis* was more resistant than the fungus *T. rubrum.*

This may be due to the genetic difference between the two fungi and the difference in the thickness of the cell wall as well as the composition of the plasma membrane, and also difference in the degree of hydrophobicity of its cell walls, where the more the cell hydrophobicity, the more easily the adherence to the surfaces of the host cells (hence being hydrophobic), and therefore the greater the resistance (Sentandreu *et al.* 2004). This result is consistent with (Yassin & Mohammed 2021) who found that dermatophytes varied in their resistance to the antifungal due to the difference in their genetic makeup.

CONCLUSION

The results of the current study showed that the methanolic extract of *Centaurea* contains a number of compounds such as (Caryophyllene oxide،Palmitic acid، Germacrene D، β-Caryophyllene ،α-Terpinyl propionate) that have activity against dermatophytes, and that the active compounds in the extract affected differently in the studied fungi, where *Microsporum canis* was more resistant than *Trichophyton rubrum.*

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