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Chemical composition, anti-fungal and cytotoxic effects of *Ferula macrecolea* essential oil against *Candida albicans* resistant and sensitive strains

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ABSTRACT

Introduction: Candidiasis therapy is a complicated concern because of the occurrence of resistance to antifungal agents. We studied the anti-fungal effects of *Ferula macrecolea* essential oil (FME) against *Candida albicans* resistant and sensitive strains, as well as its cytotoxic effects against normal and cancer cell lines.

Methods: The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *F. macrecolea* essential oil against *C. albicans* ATCC 5027 and *C. albicans* ATCC 76616 were studied by broth-microdilution approach. The cytotoxicities of FME on HGF1-PI (normal gingival cell line) and HepG2 (liver cancer cell line) cells were also studied.

Results: The main components of essential oil were terpinolene (71.25%), n-nonanal (6.32%), and linalool (3.95%), respectively. The MIC and MFC of FME on *C. albicans* sensitive to nystatin were 1.6 and 2.0 µg/mL, respectively. The MIC and MFC of FME on nystatin-resistant strains were 3.3 and 4 µg/mL, respectively. The MIC and MFC of terpinolene on *C. albicans* sensitive to nystatin were 0.8 and 1.0 µg/mL, respectively. The MIC and MFC of terpinolene on nystatin-resistant strains were 2 and 2.4 µg/mL, respectively. The essential oil and terpinolene had no significant cytotoxic effects against normal cells.

Conclusion: We revealed the promising antifungal effect of *F. macrecolea* essential oil and its main component, terpinolene, against *C. albicans* sensitive and resistant to nystatin with no significant toxicity on normal cells.

Implication for health policy/practice/research/medical education:
Our findings revealed the promising antifungal effects of *F. macrecolea* essential oil and terpinolene (main composition) against *C. albicans* sensitive and resistant to nystatin with no significant cytotoxic effect on normal cell lines. Hence, they might be used against candidiasis.

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Introduction

Candida albicans exists in different forms, such as yeast, pseudohyphae, and true hyphae, being able to reproduce in the pH range between 2 and 8, as well as in anaerobic and even aerobic conditions (1). Candidiasis therapy is a complicated concern because of the adverse effects and the occurrence of resistance to antifungal agents (2-4). Therefore, the necessity to find the novel antifungal drugs with potent efficacy against *Candida* and the strains that are resistant to available antifungals has increased (4).

Polyenes (nystatin) and azoles (miconazole) used topically are the most common synthetic drugs for superficial candidiasis (5). In addition, systemic azole antifungal drugs can also be used against superficial

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candidiasis and chronic forms of infection (5). Antifungals are also frequently used in the treatment of oral candidiasis in immunosuppressed people such as AIDS and leukemia patients (6). The study of natural products against candidiasis has recently increased considerably with research on herbs, where the natural products derived from plants may be potentially lead to the emergence of new compounds that can act on these fungi (7,8).

Ferula macrecolea is one of the genera of *Ferula* plants, which has more than 150 plant species widely used to treat various diseases (9). Previous surveys have reported that *Ferula* spp. has numerous therapeutic benefits, such as anti-inflammatory, antioxidant, anticancer, and antimicrobial ones (10,11). So, we intended to study the anti-fungal effects of essential oil of *F. macrecolea* against *C. albicans* resistant and sensitive strains, as well as its cytotoxic effects against normal and cancer cell lines.

Materials and Methods

Plant collection

In this experimental-laboratory study, the aerial parts were obtained from Kermanshah province (West of Iran) in June 2022. Then, the herbarium specimen was confirmed by the botanist and was kept at the herbarium of the Lorestan University of Medical Sciences, Khorramabad, Iran (No. 1400.2276).

Extraction of *Ferula macrecolea* essential oil

Essential oil was obtained through the water distillation method according to standard protocols (12). Materials were chopped and 600 mL of water was added to it, the resulting suspension was placed in a Clevenger device for 4 hours. Finally, it was dehydrated by sodium sulfate and kept at 4°C until testing.

Fungi

Candida isolates, including *C. albicans* ATCC 5027 (sensitive to nystatin) and *C. albicans* ATCC 76616 (resistant to nystatin) were used.

Gas chromatography-mass spectrometry (GC/MS) of Ferula macrecolea essential oil

Compounds were detected by a gas chromatography device connected to mass spectrometry (Hewlett-Packard 6890) with an HP-5MS column (30 m × 0.25 mm; film thickness, 0.25 mm), which was applied to perform the phytochemical analysis (13).

Antifungal effects of essential oil

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *F. macrecolea* essential oil against Candida isolates were determined by the broth-microdilution method based on the Clinical and Laboratory Standards Institute (CLSI) instructions (14, 15). In this test, using Sabouraud Dextrose Broth (SDB) (Merck, Germany) and fungal suspension with 0.5 McFarland turbidity, different concentrations of essential oils were evaluated in 96-well plates. The lowest concentration of the extract that had no fungal growth and turbidity was considered as MIC. Fungal suspension in the SDB culture medium without the presence of essential oil was used as a positive control and *F. macrecolea* essential oil and SDB culture medium without the presence of fungi were used as a negative control. To determine MFC, 20 µL of pre-MIC wells were subsequently cultured in Sabouraud Dextrose Agar (Merck, Germany) and kept at 35°C for 24 hours.

Cytotoxicity effects of *Ferula macrecolea* essential oil on normal and cancerous oral cells

In order to investigate the effect of the essential oil of *F. macrecolea* on the growth and proliferation of normal gingival cell line (HGF1-PI cells) and liver cancer cells (HepG2), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid) colorimetric was used (16,17). This technique is based on breaking down the tetrazolium salt by the mitochondrial enzyme succinate dehydrogenase. In this method, after 72 hours of incubation of culture medium containing 10^5 cells and different concentrations of essential oil in 96-well microtiter plates and adding 5 mg/mL MTT solution, diluted DMSO solution was added to each well. Finally, the absorbance of each well was read at 490 nm by an ELISA plate reader.

Statistical analysis

Statistical analysis of the data was done using SPSS 2019 software and ANOVA and *t* test methods. A significance level was considered at $P < 0.05$.

Results

GC/MS analysis of the essential oil of *Ferula macrecolea*

In GC/MS analysis (Table 1), 18 compounds were compromised 97.85% of essential oil. The main compounds were terpinolene (71.25%), n-nonanal (6.32%), and linalool (3.95%), respectively.

Antifungal effects of *Ferula macrecolea* essential oil

MIC and MFC, after 3 repetitions, for the essential oil of *F. macrecolea*, the main component of its essential oil (terpinolene), and nystatin as a control are shown in Table 2. *F. macrecolea* essential oil had an acceptable anti-candida effect on *C. albicans* sensitive to nystatin with MIC and MFC of 1.6 and 2.0 µg/mL. However, this difference was not significant compared to nystatin. On the other hand, in nystatin-resistant strains, *F. macrecolea* essential oil showed a much better anti-candida effect than the control, so the MIC and MFC of this essential oil against nystatin-resistant strains were calculated as 3.3 and 4 µg/mL, respectively. Compared to nystatin, this difference

Table 1. Chemical compounds of the essential oil of *Ferula macrocolea* analyzed by GC/MS

Chemical compounds	Retention index (RI)	Percentage
Allo-ocimene	1198	0.50
Benzeneacetaldehyde	1032	1.15
Camphor	1148	0.21
Di-sec-butyl disulfide	1212	1.16
Geijerene	1098	0.55
Limonene	1036	1.25
Linalool	1139	3.95
Methyl carvacrol	1076	1.90
Myrtenal	1196	1.25
n-Nonanal	1102	6.32
p–Cymene	1010	0.23
Piperiton	1252	1.1
Terpinolene	1094	71.25
Thuj-3-en-lo-al	1186	0.30
α–Campholenal	1125	0.32
α–Pinene	936	1.11
α–Thujene	1035	3.92
β–Phellandrene	1028	1.30
Total		97.85

was significant ($P<0.001$). In addition, terpinolene as the main compound in the essential oil of the *F. macrecolea* on *C. albicans* sensitive to nystatin had a much better anti-candida effect compared to the essential oil and nystatin on this strain with MIC and MFC of 0.8 and 1.0 µg/mL. Also, in nystatin-resistant strains, terpinolene showed a stronger anti-candida effect than the control and essential oil, so the MIC and MFC of this compound on nystatin-resistant strains were calculated as 2 and 2.4 µg/mL, respectively ($P<0.001$).

Cytotoxicity effects of *Ferula macrecolea* and terpinolene

The MTT results showed that the 50% cytotoxic concentrations (CC_{50}) of the essential oil against HGF1-PI1 and HepG2 cells were 349.6 and 168.4 µg/mL, respectively. The toxicity activity of terpinolene as the main compound of this essential oil was studied on HGF1-PI normal and cancerous HepG2 cells after 72 hours of incubation. MTT results showed that the CC_{50s} of terpinolene against HGF1-PI1 and HepG2 cells were 279.6 and 108.4 µg/mL, respectively.

Discussion

Candidiasis therapy is a complicated concern due to the emergence of *Candida* strains that are resistant to commonly used antifungal agents (1). Here, we investigated the anti-fungal and cytotoxicity effects of *F. macrecolea* essential oil against *C. albicans* resistant and sensitive strains.

We observed that the main constituents of *F. macrecolea* essential oil were monoterpenes such ass terpinolene (71.25%), n-nonanal (6.32%), and linalool (3.95%), respectively. It is also known that monoterpenes are the main component in many plant essential oils (18). Rustaiyan et al (19) analyzed the compounds of *F. macrecolea* and identified 55 main compounds such as β-pinene (15.9%), α-pinene (10.4%), and β-caryophyllene (8.6%). Akhgar et al reported the main compounds of *F. macrecolea* oil as α-pinene (19.2%), nonan (13.2%), and β-pinene (13.0%) (20), which are consistent with the results of our study.

Ferula macrecolea essential oil had an acceptable anti-*Candida* effect on *C. albicans* sensitive to nystatin with MIC and MFC of 1.6 and 2.0 µg/mL; however, this difference was not significant compared to nystatin. On the other hand, in nystatin-resistant strains, *F. macrecolea* essential oil showed a much better anti-*Candida* effect than the control; however, compared to nystatin, this difference was significant ($P<0.001$). In addition, terpinolene as the main compound in the essential oil of the *F. macrecolea* on *C. albicans* sensitive to nystatin had a much better anti-candida effect compared to the essential oil and nystatin on this strain. Also, in nystatin-resistant strains, terpinolene showed a stronger anti-*Candida* effect than the control and essential oil.

Ferula macrecolea essential oil at 150 and 300 µg/mL showed good antiparasitic effects against *Echinococcus granulosus* protoscoleces (21). In another study, the effects of this essential oil and terpinolene displayed a strong antiparasitic effect on *Leishmania tropica* (22). The results of this study are consistent with the results of our study. Since the antifungal effects of *F. macrecolea* essential oil have not been recorded so far, this study can be an idea

Table 2. The effect of essential oil on *Candida albicans* sensitive and resistant to nystatin by minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC)

	MIC (µg/mL)		MFC (µg/mL)	
	***C. albicans* ATCC 5027**	***C. albicans* ATCC 76616**	***C. albicans* ATCC 5027**	***C. albicans* ATCC 76616**
Essential oil of *F. macrecolea*	1.6±0.57	3.3±1.54*	2.0±0.0	4.0±0.0*
Terpinolene	0.8±0.34	2.0±0.69*	1.0±0.34	2.4±0.0
Nystatin	1.33±0.57	>32	1.66±0.57	>32

*$P<0.001$ compared with the control group. *C. albicans* ATCC 5027 (Sensitive to nystatin); *C. albicans* ATCC 76616 (Resistance to nystatin).

for continuing research in the field of preparation of antifungal agents.

Nowadays, due to the spread of drug resistance, extensive research is being done in the field of using plant extracts and essential oils to control candidiasis. In the study of Sharifzadeh et al, the MIC value of ginger essential oil for *C. albicans* strains, resistant and sensitive to fluconazole, was reported to be 2500 µg/mL (23). In Amiri Karladani et al study, the MIC of *Rosa damascena* essential oil was 8-62 µg/mL for *Candia spp.* (24). In addition, Ghasemi et al, in Iran reported the antifungal activity of *F. gummosa* against *C. albicans* and *C. kefyr* (25).

Effective drugs for use in chemotherapy are compounds with cytotoxic properties (26). For this purpose, cytotoxicity was investigated in the present study by MTT method. The MTT results showed that the CC_{50s} of the essential oil against HGF1-PI1 and HepG2 cells were 349.6 and 168.4 µg/mL, respectively. The CC_{50} values of terpinolene against HGF1-PI1 and HepG2 cells were 279.6 and 108.4 µg/mL, respectively, indicating the essential oil and terpinolene were safe for normal cells and toxic for HepG2 cancer cells. Similarly, Mahmoudvand et al have reported the CC_{50} of *F. macrecolea* essential oil and terpinolene on J774-A1 macrophage cells as 471.3 and 207.3 µg/mL, respectively (22).

Conclusion

The results of investigating the cytotoxicity on KB cells and antifungal effect of *F. macrecolea* essential oil and terpinolene (the main compound) against *C. albicans* sensitive and resistant to nystatin showed that both compounds, especially terpinolene, had a significant effect in inhibiting *C. albicans*. Examining the effect of cytotoxicity also indicated that terpinolene had a greater effect than the *F. macrecolea* essential oil. Therefore, with a general look at the findings of the present study, considering the increasing prevalence of fungal diseases caused by *C. albicans*, it can be hoped that in the future, with clinical evaluation, *F. macrecolea* essential oil and terpinolene might be used in the preparation of new compounds to remove *C. albicans* infection and improve oral cancer cells.

Authors' contribution

NS and HS designed the experiments. AA, and AS performed experiments and collected data. SG and DNM discussed the results and strategy. SG supervised, directed, and managed the study. All authors approved the final version to be published.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

This study was approved by the ethics committee of Lorestan University of Medical Sciences, Khorramabad, Iran, with the ethics number of IR.LUMS.REC.1401.034.

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