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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Evaluation of antifungal activities of the essential oil and various extracts of *Nigella sativa* and its main component, thymoquinone against pathogenic dermatophyte strains

Évaluation de l'activité antifongique de l'huile essentielle et de divers extraits de Nigella sativa *et de son composant principal, la thymoquinone, contre des dermatophytes pathogènes*

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KEYWORDS
Black cumin;
Microsporum canis;
Microsporum gypseum;
Trichophyton mentagrophytes

Summary
Objective. — Plant extracts and plant-derived compounds are valuable sources as folk medicine for the treatment and prevention of a wide range of diseases including infectious diseases. In the present study, the antifungal activities of the essential oil and various extracts *Nigella sativa* and its active principle, thymoquinone against *Trichophyton mentagrophytes*, *Microsporum canis* and *Microsporum gypseum* as pathogenic dermatophyte strains have been evaluated. In addition, the cytotoxic effects of *N. sativa* against murine macrophage cells were determined.
Materials and methods. — In this study, the antifungal activity was studied by disk diffusion method and assessment of minimum inhibitory concentration (MIC) of extracts using broth

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macrodilution method. In addition, the cytotoxic activity of *N. sativa* was evaluated by colorimetric assay (MTT). The components of the *N. sativa* essential oil were also identified by gas chromatography/mass spectroscopy (GC/MS) analysis.
Results. — The results showed that the essential oil and various extracts of *N. sativa* particularly thymoquinone have potent antifungal effects on *T. mentagrophytes*, *M. canis* and *M. gypseum* as pathogenic dermatophyte strains. In the assessment of the cytotoxicity activity, it could be observed that *N. sativa* had no significant cytotoxicity in the murine macrophages at low concentrations. While, thymoquinone in comparison with essential oil and various extracts of *N. sativa* showed higher cytotoxicity on murine macrophage cells. In the GC/MS analysis, thymoquinone (42.4%), *p*-cymene (14.1%), carvacrol (10.3%) and longifolene (6.1%) were found to be the major components of *N. sativa* essential oil.
Conclusion. — The findings of this study suggest a first step in the search of new antidermatophytic drugs and aid the use of *N. sativa* seeds in the traditional medicine for dermatophytic infections.

MOTS CLÉS
Thymoquinone ;
Nigella sativa ;
Microsporum canis ;
Microsporum gypseum ;
Trichophyton mentagrophytes

Résumé
Objectif. — Des extraits de plantes et des composés d'origine végétale sont des sources précieuses pour la médecine traditionnelle dans le traitement et la prévention d'un large éventail de maladies, notamment des maladies infectieuses. Dans la présente étude, les activités antifongiques de l'huile essentielle et de divers extraits de *Nigella sativa* et de son principe actif, la thymoquinone contre *Trichophyton mentagrophytes*, *Microsporum canis* et *Microsporum gypseum*, souches de dermatophytes pathogènes ont été évaluées. En outre l' activité cytotoxique de *N. sativa* sur les macrophages murins a été determinée.
Matériels et méthodes. — Dans cette étude, l'activité antifongique a été étudiée par la méthode de diffusion de disques et l'évaluation de la concentration minimale inhibitrice (CMI) des extraits par la méthode de macrodilution en milieu liquide. En outre, l'activité cytotoxique de *N. sativa* a été évaluée par analyse colorimétrique (MTT). Les principaux composants de l'huile essentielle de *N. sativa* ont été analysés par chromatographie gazeuse/spectrométrie de masse en phase gazeuse (GC/MS).
Résultats. — Les résultats ont montré que l'huile essentielle et divers extraits de *N. sativa* surtout thymoquinone ont des effets antifongiques puissants sur *T. mentagrophytes*, *M. canis* et *M. gypseum*, souches de dermatophytes pathogènes. Dans l'évaluation de l'activité de cytotoxicité, *N. sativa* n'a pas montré une cytotoxicité significative sur les macrophages murins à de faibles concentrations. Alors que la thymoquinone en comparaison avec l'huile essentielle et divers extraits de *N. sativa* présentait une cytotoxicité élevée sur les macrophages murins. Dans l'analyse GC/MS, la thymoquinone (42,4 %), le *p*-cymène (14,1 %), le carvacrol (10,3 %) et le longifolène (6,1 %) se sont révélés être les principaux composants de l'huile essentielle de *N. sativa*.
Conclusion. — Les résultats de cette étude sont une première étape dans la recherche de nouveaux médicaments antidermatophytiques et confortent l'utilisation de graines de *N. sativa* dans la médecine traditionnelle pour les infections à dermatophytes.

Introduction

One of the most important groups of fungi, which causes worldwide human and animals infections are the dermatophytes. They have the ability to invade keratinized tissues, such as hair, skin and nails to produce an infection, commonly referred to as ringworm. There are different forms of the disease such as tinea corporis, tinea pedis, capitis, barbae, cruris, manum and onychomycosis [31]. At present, there are many antidermatophytic drugs including imidazoles and terbinafine for topical treatment and triazoles, griseofulvin and terbinafine as oral antifungal for systemic treatment of dermatophytosis which exhibited some problems such as prolonged systemic therapy, fungal resistance, high toxicity and high cost [10,30,31]. For these reasons, development of new drugs or combination therapy for treatment of dermatophytosis is urgent. Various studies have shown that plant extracts and plant-derived components have high availability, less side effects and low cost. They are rich and valuable sources that are currently used to treat a wide range of diseases, such as infectious diseases [12,21,25].

Nigella sativa Linn. (family Ranunculaceae), generally known as black seed, cumin noir or black cumin, is commonly grown in the Middle East, Eastern Europe and Western and Middle Asia. It has been traditionally used as a folk remedy to treat a number of diseases and conditions that include asthma, hypertension, diabetes, inflammation, cough, bronchitis,

headache, eczema, fever, dizziness and influenza worldwide [4]. So far, several pharmacological effects such as antioxidant, anti-inflammatory, anticancer and antimicrobial have been related to *N. sativa* or its active principles which include thymoquinone, thymohydroquinone, dithymoquinone, carvacrol, ρ-cymene and thymol [4,24]. At present, in various studies, antibacterial and antiviral effects of this plant have been proven [3,20,26]. Moreover, previous studies revealed that *N. sativa* seeds have potent antifungal and antiparasitic activity against some pathogenic fungal and parasitic strains [5,14,16,23]. The present study was aimed to evaluate in vitro antifungal activities of the essential oil and various extracts of *N. sativa* and its active principle, thymoquinone against *Trichophyton mentagrophytes*, *Microsporum canis* and *Microsporum gypseum* as pathogenic dermatophyte strains.

Materials and methods

Chemicals

Crude powder of fluconazole and ketoconazole as control drugs were obtained from Sigma-Aldrich Chemical Company, GmbH, Riedstr. Penicillin and streptomycin were obtained from Alborz Pharmacy, Karaj, Iran and were stored at room temperature (25 °C) until testing. Thymoquinone, MTT powder [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide], RPMI-1640 medium with L-glutamine and Sabouraud dextrose broth (SDA) were purchased from Sigma-Aldrich, (St. Louis, MO, USA). Also potato dextrose agar (PDA) was prepared from Oxoid, Basingstoke, Hampshire, United Kingdom. All other chemicals and solvents were of analytical grade.

Antifungal agent dilution

Two hundred milligrams of thymoquinone were dissolved in 4 mL of dimethyl sulphoxide (DMSO) and serial dilution was subsequently made to obtain thymoquinone at 0.0625 to 1 mg/mL. For the preparation of dilutions of the fluconazole and ketoconazole, 1 mg of the crude powder was dissolved in 2 mL of distilled water and DMSO, respectively. Then, serial dilutions were subsequently made to obtain drugs at 0.004–0.5 mg/mL.

Collection of fungi

A standard strains *T. mentagrophytes* (PTCC 5054), *M. canis* (PTCC 5069) and *M. gypseum* (PTCC 5070) were obtained from the center for Persian Type Culture Collection (Tehran, Iran) and were incubated in Sabouraud dextrose agar (SDA) at 30 °C for 7–10 days.

Preparation murine macrophage cells

Murine macrophages were collected from male BALB/c mice (Pasteur Institute of Iran, Tehran, Iran) by injecting 3–5 mL of cold RPMI-1640 medium into mouse peritoneal cavity and then aspirated macrophages were washed twice and resuspended in RPMI-1640 medium [17]. The experimental procedures carried out in this survey were in compliance with the guidelines of the Kerman University of Medical Science (Kerman, Iran) for the care and use of laboratory animals.

Collection of plant materials

The seeds of *N. sativa* were collected from rural regions of Bam district (Kerman province, Iran) in September 2012. The plant was identified by a botanist of the Botany Department of Shahid Bahonar University, Kerman, Iran. Voucher specimen (KF575) has been deposited in the Herbarium of Department of Pharmacognosy, School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.

Isolation of the essential oil

Crushed seeds were extracted with light petroleum (petroleum ether, BP 40–60 °C) using a Soxhlet apparatus. The solvent was removed under vacuum and the brownish residue was steam distilled. Extraction of the aqueous distillate with *n*-hexane and removal of the solvent gave the essential oil. The essential oil (EO) was stored in sealed vials at 2–8 °C until testing.

Preparation of various extracts

The dried seeds (100 g) of *N. sativa* were grinded and extracted by percolation method with methanol (80%) and water successively for 72 h at room temperature. The extracts were passed through filter paper (Whatman No. 3, Sigma, Germany) to remove plant debris. The extracts were finally concentrated in vacuum at 50 °C using a rotary evaporator (Heidolph, Germany) and stored at −20 °C, until use.

Gas chromatography/mass spectrometry (GC/MS) analysis of EO

GC analysis

In this study, GC analysis was carried out by a Hewlett-Packard 6890 with a HP-5MS column (30m × 0.25 mm, film thickness 0.25 mm). The column temperature was maintained at 60 °C for 3 min and programmed to 220 °C at a rate of 5 °C per min, and kept constant at 220 °C for 5 min. Injector and interface temperatures were 220 °C and 250 °C, respectively. The flow rate of Helium as carrier gas was (1 mL/min C.F). The percentages were calculated by electronic integration of FID peak areas without the use of response factors correction. Linear retention indices for all components were determined by co-injection of the samples with a solution containing homologous series of C8–C22 *n*-alkanes.

GC/MS analysis

GC–MS analysis was performed using a Thermoquest- Finnigan gas chromatograph equipped with fused silica capillary DB-5 column (30m × 0.25 mm, film thickness 0.25 mm) coupled with a TRACE mass (Manchester, UK). Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 220 °C and 250 °C, respectively. Mass range was from 40 to 400u. Oven temperature program was the same given above for the GC.

Identification of the EO components

The components of the EO were identified by comparison of their relative retention time and mass spectra with those of standards Wiley 2001 library data of the GC–MS system or with those of reported in the literature data [2].

Antidermatophytic activity of the N. sativa

Antidermatophytic activity of the EO and various extracts of *N. sativa* and also thymoquinone were carried out by disk diffusion

method and evaluation of minimum inhibitory concentration (MIC) by broth macrodilution method.

Disk diffusion method

Antidermatophytic activity of *N. sativa* by disk diffusion was evaluated according to the method described elsewhere with some modifications [13,22]. Briefly, fresh culture of *T. mentagrophytes*, *M. canis* and *M. gypseum* after 21 days were spread on Sabouraud dextrose agar (SDA). Then filter paper disks (5 mm) were loaded with 1 mg/disk of various extracts and EO of *N. sativa* and 0.1 mg/disk of thymoquinone. Also, ketoconazole (0.01 mg/disk), dimethyl sulphoxide (DMSO 1%) were used as positive and negative control, respectively. After evaporation of solvents, the disks were placed on the SDA plates. They were incubated at 30 °C for 7—14 days and were measured for the development of inhibition zones diameter around the disks. Finally, the zone diameter of inhibition was measured and the results were recorded. All experiments were repeated in triplicate.

Evaluation of MIC by broth macrodilution method

Evaluation of MIC *N. sativa* was determined by broth macrodilution method, according to the protocol M38-A2 of the Clinical and Laboratory Standards Institute (CLSI) for filamentous fungi with some modifications [7]. Prior to testing, dermatophyte strains were sub-cultured on PDA slants and incubated at 30 °C for 7 to 10 days. Mature colonies were covered with 2 mL of sterile physiological saline (0.85%), suspensions were prepared by gently probing the colony with the tip of a sterile Pasteur pipette and transferred to a sterile conical tube, the final volume being adjusted to 5 mL with saline. The resulting mixture of conidia and hyphae was vortexed, mixed for 15 seconds and the heavy particles allowed to settle for 5—10 minutes. The upper homogeneous suspension was used for further testing. The resulting conidia suspension was counted in a Neubauer chamber and standardized to concentrations of 1×10^5 to 5×10^5/mL. This suspension was further diluted 1:10 with RPMI-1640 medium broth with L-glutamine and without sodium bicarbonate to final concentrations of 1×10^4 to 5×10^4/mL. For the broth macrodilution method, 0.9 mL of the final conidia suspensions were mixed with 0.1 mL of the different concentrations of various extracts and essential oil of *N. sativa* (1—16 mg/mL) and thymoquinone (0.062—0.5 mg/mL) in test tubes and incubated at 30 °C for 7 days. The positive control tube contained 0.9 mL of conidial suspension and 0.1 mL of RPMI-1640, and the negative one contained 1 mL of RPMI-1640 only. The minimum concentrations at which no visible growth was observed were defined as the MIC, which were expressed in mg/mL.

Cytotoxicity effects on murine macrophage cells

Cytotoxicity effects of the EO, extracts of *N. sativa* and thymoquinone were determined by cultivating murine macrophages (5×10^5 cells/mL) with different concentrations of extracts and thymoquinone in 96-well microtiter plates, at 37 °C for 48 h. The viability of the macrophages was checked using the MTT assay. The CC_{50} (cytotoxicity concentration for 50% of cells) values were evaluated by Probit test in SPSS software [18,28].

Statistical analysis

SPSS Software ver. 17 (SPSS Inc., Chicago) was used for data entry and statistical analysis and the differences between groups were determined by using one way analysis of variance (ANOVA) test. Moreover, to compare the CC_{50} values of groups, *t*-test was performed. *P*-value of less than 0.05 was considered to be statistically significant.

Results

GC-MS analysis of *N. sativa* EO

In the present study, EO of *N. sativa*, which was obtained by Soxhlet extraction, was analyzed using GC/MS. Table 1 shows the identified compounds and percentage obtained by GC/MS. The main components were thymoquinone (42.4%), *p*-cymene (14.1%), carvacrol (10.3%), longifolene (6.1%) and 4-terpineol (5.1%).

Antidermatophytic activity

The present study was designated to determine the antidermatophytic activity of EO and various extracts of *N. sativa* and its active principle, thymoquinone against some pathogenic dermatophyte strains including *T. mentagrophytes*, *M. canis* and *M. gypseum* by disk diffusion method and assessment of MIC. The results showed that all the aforementioned extracts and particularly thymoquinone

Table 1 GC-MS analyze of the *N. sativa* essential oil. *Composition de l'huile essentielle de* N. sativa *identifiée par GC-MS.*

No.	Compound	Percentage
1	Camphene	0.06
2	t-Anethole	2.3
3	β-Pinene	0.03
4	α-Pinene	0.04
5	γ-Terpinene	0.4
6	β-Myrcene	0.05
7	α-Terpinene	0.01
8	Limonene	1.7
9	1,8-Cineole	0.16
10	Sabinene	1.3
11	ρ-Cymene	14.1
12	α-Terpinolene	0.01
13	ρ-Cymene-8-ol	0.42
14	Carvacrol	10.3
15	Longipinene	0.4
16	Camphor	1.5
17	Linaloolcis	0.21
18	Sabinenehydrate	0.2
19	Longifolene	6.1
20	Bornylacetate	0.53
21	Thymol	1.2
22	4-Terpineol	5.1
23	Borneol	0.14
24	Carvone	0.08
25	Thymoquinone	42.4
26	2-Tridecanone	0.8
27	Thujone	1.5
28	ρ-Anisaldehyde	0.1
29	2-Undecanone	0.21
30	Unknown peak	3.1
	Total	96.6

Table 2 Antifungal effect of essential oil and various extracts of *N. sativa* and its main component, thymoquinone against *T. mentagrophytes*, *M. canis* and *M. gypseum* by disk diffusion method. Data are expressed as the mean ± SD ($n = 3$).
Effet antifongique des huiles essentielles et extraits divers de N. sativa *et sa principale composante, thymoquinone contre* T. mentagrophytes, M. canis *et* M. gypseum *par la méthode de diffusion en disque. Les données sont exprimées en moyenne ± écart-type* (n = *3).*

Dermatophytes	*Nigella sativa* (mm)					
	Essential oil	Methanolic extract	Aqueous extract	Thymoquinone	Fluconazole	Ketoconazole
T. mentagrophytes	31.6 ± 2.8	24.3 ± 1.52	22 ± 2.8	56.3 ± 2.52[a]	25.3 ± 2.17	28.6 ± 1.17
M. canis	40.3 ± 2.5	31 ± 2.5	28.3 ± 2.52	65.3 ± 2.08[a]	34.6 ± 2.15	37.6 ± 1.52
M. gypseum	33.3 ± 1.5	27.6 ± 1.52	23.3 ± 1.17	58.6 ± 2.15[a]	26.6 ± 1.52	31.6 ± 1.52

[a] The difference in antidermatophytic effects with the standard drugs is statistically significant ($P < 0.05$); *T. mentagrophytes*: *Trichophyton mentagrophytes*; *M. canis*: *Microsporum canis*; *M. gypseum*: *Microsporum gypseum*.

had remarkable antidermatophytic effects against *T. mentagrophytes*, *M. canis* and *M. gypseum*. In disk diffusion method, thymoquinone significantly ($P < 0.05$) was much more effective than various extracts of *N. sativa* and control drugs once it exhibited wider zone diameter of inhibition (> 50 mm) for all the tested dermatophyte strains. In addition, among the tested extracts of *N. sativa*, EO (from 31.6 to 40.3 mm) revealed higher antidermatophytic effects than methanolic (from 24.3 to 31 mm) and aqueous extracts. While the lowest antidermatophytic effect was related to aqueous extract (from 22 to 28.3 mm) of *N. sativa*. However, the difference in antidermatophytic effects between the extracts and the standard drugs was not statistically significant ($P > 0.05$), whereas, this difference was statistically significant ($P < 0.05$) between thymoquinone and the standard drugs. Our findings also showed that *M. canis* and *T. mentagrophytes* were the most sensitive and resistant strains to various extracts of *N. sativa* and thymoquinone, respectively. Negative controls had also not shown any inhibition zone against the tested dermatophyte strains (Table 2).

Table 3 indicates results of MIC values of various extracts of *N. sativa* and thymoquinone by broth macrodilution method. It could be observed that MIC values similar to disk diffusion method confirmed that thymoquinone followed by EO had more antidermatophytic effects than various extracts of *N. sativa* with the lower MIC values for all the tested dermatophyte strains. However, the MIC values of tested extracts were significantly lower compared to the control drugs ($P < 0.05$). These results also exhibited that thymoquinone had significantly better ($P < 0.05$) antidermatophytic activity against tested dermatophytes than fluconazole, but, this activity was significantly lower ($P < 0.05$) than that of ketoconazole.

Cytotoxicity effects

In evaluating cytotoxic effects of various extracts of *N. sativa* and thymoquinone on murine macrophages by MTT assay, it could be observed a dose-dependent response; with increasing concentrations of extracts. In this stage, thymoquinone as the active constituent showed a more cytotoxic effect on murine macrophages in comparsion with various extracts of *N. sativa*. Whereas, the lowest cytotoxicity effect was related to aqueous extract of *N. sativa*. The CC_{50} values of various extracts of *N. sativa*, thymoquinone and control drugs were shown in Table 4.

Discussion

Plants extracts and plant-derived compounds have been used as a valuable source of natural remedy for centuries [21,25]. Emerging of synthetic antimicrobials drugs in the end of the last century caused lack of interest in plants as a rich and valuable resource for antimicrobial agents [8]. But the emergence of some limitations in the use of these synthetic drugs led to change in situation and the field of ethnobotanical

Table 3 Minimum inhibitory concentration (MIC) of essential oil and various extracts *Nigella sativa* and its main component, thymoquinone against *T. mentagrophytes*, *M. canis* and *M. gypseum* by broth macrodillution method.
Concentration minimale inhibitrice (CMI) d'huile essentielle et de divers extraits Nigella sativa *et son principal composant : la thymoquinone contre* T. mentagrophytes, M. canis *et* M. gypseum *par la méthode de macrodillution en milieu liquide*.

Dermatophytes	MIC (mg/mL)					
	Essential oil	Methanolic extract	Aqueous extract	Thymoquinone	Fluconazole	Ketoconazole
T. mentagrophytes	4	8	16	0.125	0.250	0.008
M. canis	4	4	8	0.062	0.125	0.004
M. gypseum	4	8	16	0.125	0.250	0.016

T. mentagrophytes: *Trichophyton mentagrophytes*; *M. canis*: *Microsporum canis*; *M. gypseum*: *Microsporum gypseum*.

Table 4 Comparison of the mean CC_{50} values among with various extracts of *Nigella sativa* seeds and thymoquinone on murine macrophage cells after 48 h incubation. Data are expressed as the mean ± SD (n = 3).
La comparaison des valeurs moyennes parmi CC_{50} avec divers extraits de graines et Nigella sativa *et la thymoquinone sur les cellules macrophages murines après 48 h d'incubation. Les données sont exprimées sous forme de moyenne ± SD (*n = *3).*

Sample	CC_{50} (μg/mL)
Essential oil	641.6 ± 5.1
Methanolic extract	827.6 ± 8.4
Aqueous extract	1218 ± 13.2
Thymoquinone	36.3 ± 2.15
Fluconazole	256.6 ± 5.1
Ketoconazole	173.3 ± 4.01

research has expanded [19]. Up to now, in the various studies, antibacterial, antiviral and antiparasitic effects of *N. sativa* and its derivatives have been demonstrated [4]. Our findings in the present study showed the high potential *N. sativa* and particularly its active principle, thymoquinone as a natural source for the production of new antidermatophytic drugs.

In disk diffusion method, thymoquinone significantly ($P < 0.05$) revealed better antidermatophytic activity than control drugs. While, the difference between antidermatophytic effects of extracts and standard drugs was not statistically significant ($P > 0.05$). In the evaluation of the MIC values, thymoquinone significantly ($P < 0.05$) had higher antidermatophytic activity than fluconazole against tested dermatophytes. However, this activity was significantly lower ($P < 0.05$) than that of ketoconazole. Furthermore, antidermatophytic effects of various extracts of *N. sativa* were significantly ($P < 0.05$) lower than control dugs.

Our results similar to antidermatophytic effects of ketoconazole and fluconazole showed that various extracts of *N. sativa* and thymoquinone were more effective against *M. canis* (MIC values varying from 4 to 8 mg/mL), while, the most resistant one among tested dermatophytes was *T. mentagrophytes* (MIC values varying from 4 to 16 mg/mL). In line with these findings, Aljabre et al. revealed that *T. mentagrophytes* (MIC 40 mg/mL) and *M. canis* (MIC 10 mg/mL) were the most resistant and sensitive dermatophytes to the ether extract of *N. sativa* seeds [5].

In the case of antifungal effects of *N. sativa*, Khan et al. showed that the aqueous extract of *N. sativa* exhibits inhibitory effect against *Candida albicans* in mice with candidiasis [14]. In the other investigation, Aljabre et al. reported that ether extract of *N. sativa* seed and its active principle thymoquinone significantly were able to inhibit the growth of eight species of dermatophytes: four species of *T. rubrum* and one each of *T. interdigitale*, *T. mentagrophytes*, *Epidermophyton floccosum* and *M. canis* [5]. In addition, they demonstrated that thymoquinone as active principle of *N. sativa* extract has a better antidermatophytic effect than ether extract of *N. sativa*. Therefore, all these results are consistent with the results of the present study. In the present study, we found that the main compound of the *N. sativa* essential oil was thymoquinone (42.4%), when being analyzed by GC/MS. Similarly, Ali and Blunden [4] indicated that the most biological activity of the *N. sativa* seeds was due to thymoquinone, the major compound of the EO obtained using soxhlet extraction. Thus, high antidermatophytic activity of *N. sativa* EO could be due to higher content of thymoquinone in comparison with methanolic and aqueous extracts, as previously described elsewhere [5]. However, various study reported potent antifungal effects of other components of EO such as carvacrol and thymol, against *C. albicans* strains [29], *Cladosporium* sp., *Aspergillus* sp. [1] and *T. rubrum* strains [9].

Since the exact mechanisms of the antimicrobial activity of thymoquinone are not clear, further studies are required to investigate these mechanisms. However, it has been proven that *N. sativa* oil can inhibit DNA synthesis by inhibiting histone deacetylase (HDAC) enzyme interacting with the chromosomes [27]. Findings of this investigation demonstrated that the extracts of *N. sativa* particularly aqueous extract had no significant cytotoxic effect, whereas, thymoquinone in comparison with essential oil and various extracts of *N. sativa* revealed higher cytotoxicity on murine macrophage cells. However, these different cytotoxic effects between extracts might be due to the type of solvent which has been used for extraction and also the number of compounds, which are greater and together in some extracts such as EO.

Similar to these results, some studies indicated that the administration of *N. sativa* seed extract by oral or intraperitoneal route has a low level of cytotoxicity in rat and mice [11,15,26]. Moreover, Badary et al. indicated that inclusion of thymoquinone as main component of the *N. sativa* seed in the drinking water of mice at concentrations of up to 0.03% for 3 months led to no sign of toxicity, except for a significant decrease in fasting plasma glucose concentration [6]. Therefore, we can conclude that the *N. sativa* seeds and their derivatives appear to have a low level of toxicity and could be considered safe at low concentrations in the host cells.

To conclude, the results of this study suggest a first step in the search of new antidermatophytic drugs and aid the use of *N. sativa* seeds in the traditional medicine for dermatophytic infections, particularly in the treatment of onychomycosis.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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