Entomology and Applied Science Letters
Volume 5, Issue 2, Page No: 10-15

Available Online at: www.easletters.com

ISSN No: 2349-2864

Chemical Composition and Antifungal Activity of *Nectaroscordum tripedale* Extract Against Some Pathogenic Dermatophyte Strains

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ABSTRACT

This investigation aims to assess the chemical composition and antidermatophytic properties of Nectaroscordum tripedale against some pathogenic dermatophyte strains. In vitro antidermatophytic effects of N. tripedale extract on Trichophyton mentagrophytes, Microsporum canis, and Microsporum gypseum was determined using the determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) based on the broth microdilution method, according to the protocol M38-A2 of the Clinical and Laboratory Standards Institute (CLSI) for filamentous fungi with some modifications. The components of the N. tripedale extract were identified by gas chromatography/mass spectroscopy (GC/MS) analysis. The results demonstrated that N. tripedale extract had both fungistatic and fungicidal activities with the MIC and MFC ranging from 16.6 to 66 mg/ml. The main compounds are decadienal (11.1%), hexadecanoic acid (10.3%), and heptadecane (9.5%), respectively. Obtained results of this investigation recommend a first step in the search of new antidermatophytic agent and support the use of N. tripedale in the folk medicine for dermatophytic infections.

Keywords: GC/MS, Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes, in vitro

HOW TO CITE THIS ARTICLE: Asghar Sepahvand, Behrouz Ezatpour, Masuumeh Niazi, Mohammad Reza Aflatoonian, Mojtaba Mirshekari, Chemical composition and antifungal activity of Nectaroscordum tripedale extract against some pathogenic dermatophyte strains, Entomol Appl Sci Lett, 2018, 5 (2): 10-15 ,

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Received: 25/12/2017
Accepted: 10/04/2018

INTRODUCTION

In recent years, the occurrence of both community-acquired and nosocomial fungal diseases has raised concerns mainly in individuals with impaired immune system [1]. Diseases caused by fungi belonging to the three main genera of *Microsporum spp*, *Trichophyton spp* and *Epidermophyton floccosum* are called dermatophytosis; which caused significant medical and health problems in all countries around the world [1]. These filamentous fungi are able to infect keratinized tissues, including hair, skin and nails and subsequently cause different types of dermatophytosis for example tinea corporis, tinea pedis, capitis, barbae, cruris, manum and onychomycosis [2, 3].

Currently, the use of chemical treatments available for this disease is limited because of their high toxicity, as well as the occurrence of some drug resistance [4, 5]. Therefore, it is necessary to have more studies to discover a new antifungal agent.

From centuries ago, medicinal herbs are considered as a valuable option for the treatment of a large number of diseases such as infectious diseases [6-8]. One of the most important families of medicinal plants is the Alliaceae family, which has an important genus called *Allium*. *Nectaroscordum tripedale*, from the *Allium* genus, broadly grow in Iran, Iraq, Turkey, etc. [9]. In traditional Iranian medicine,

this plant has many benefits in treating some diseases like rheumatic and joint pains, bladder and kidney stones as well as infectious ones [9, 10].

Additionally, in recent years, reviews have shown the antimicrobial properties of this plant in modern medicine; so that its antibacterial effects against some pathogenic bacterial strains as well as antileishmanial and protoscolicidal activity against some parasites have been proven [11-13]. Since there is no documented study against the antidermatophytic activities of *N. tripedale*; the current investigation aims to evaluate the in vitro antifungal activity of various extracts of *N. tripedale* extract against some pathogenic dermatophyte strains.

MATERIALS AND METHODS

Fungi Strains

Standard strains of *T. mentagrophytes* (PTCC5054), *M. canis* (PTCC 5069) and *M. gypseum* (PTCC 5070) were acquired 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.

Collection of plant materials

The plants materials (aerial parts of wild rising *N. tripedale*) were collected in April 2017 from mountainous areas of Lorestan Province, Iran. After collection, the plant was identified by a botanist in Herbarium of Agriculture and Natural Resource Research Center, Khorramabad, Iran.

Preparation of extract

Two hundred g of air dried plant materials were extracted by percolation technique using methanol (80%) for three days in 21°C. To discard the artifacts, the extract was passed through filter paper (Whatman No.3, Sigma, Germany). After this step, extract was concentrated in vacuum at 50°C by means of a rotary evaporator (Heidolph, Germany) and kept at -20°C, until use [14, 15].

Chemical analysis

Chemical analysis of *N. tripedale* extract was performed by extracting them using a solid phase micro extraction (SPME) technique according to the method described elsewhere [16]. GC/MS (Shimadzu 17A gas chromatograph and QP5050A mass spectrometer, USA) was performed chemical analysis; whereas isolation of structure was carried out in Fused Silica type BP-5 95% polydimethylsiloxane with length of 30m, internal dimensions of 25% mm and film thickness of 25% micrometer. Column temperature increased from 40 to 220°C with speed of 5°C per minute, then the temperature increased to 280°C and kept at 280°C for 2min. Both the injection site and detector (transfer line) temperature was set to 260°C. Helium gas with speed of 0.9 ml/minute with 99/999% purity was used as carrier gas. Spectrometer conditions were exactly in accordance with gas chromatography, just ionization energy of 70 electron volts was used. To determine the spectrum by the retention indices, the injection of normal hydrocarbons (C8-C20) was applied in similar condition with the sample injection.

Antidermatophytic effects

The MIC of extracts against tested dermatophytes was determined by broth microdilution method, according to the protocol M38-A2 of the Clinical and Laboratory Standards Institute (CLSI) for filamentous fungi with some modifications (CLSI, 2002). Before the experiments, dermatophytes were sub-cultured on potato dextrose agar (PDA) slants and incubated at 30˚C for 7 to 10 days. Preparation of inoculum for antifungal susceptibility tests with final concentrations of 1×10^3 to 3×10^3 cfu/mL was performed based on the method described by Mahmoudvand et al [2].

Minimum inhibitory concentration (MIC)

To do this, 0.1 mL of various concentrations *N. tripedale* extract (50, 100, 200, and 400 mg/mL) and 0.1 mL of the final conidia suspension were added to each well of the 96 wells plates. Growth positive control was the well containing 0.1 mL of the inoculum suspension and 0.1 mL of the RPMI only and the negative control was a well containing 0.2 mL of RPMI 1640. The minimum concentrations at which no visible growth was seen were described as the MIC, which were indicated in mg/mL.

Minimum fungicidal concentration (MFC) determination

For calculation of minimum fungicidal concentration (MFC), after interpretation the MIC values, 0.1 mL samples from all optically clear tubes (complete growth inhibition) plus the last tube showing growth were subcultured on SDB Petri dishes. The dishes were incubated at 35˚C for a minimum of 3 days, until growth was clearly visible in the control samples, and MFC values were calculated as the lowest concentration of extract for which there was no visible growth.

Statistical analysis

To analysis of data, SPSS Software ver. 17 (SPSS Inc., Chicago) was employed. The differences among tested groups were assessed using one way analysis of variance (ANOVA) test. P-value of less than 0.05 was considered to be statistically significant.

RESULTS

Chemical composition analysis

Table 1 shows the chemical composition analysis of *N. tripedale* extratct, which presented 24 compounds. The main compounds are decadienal (11.1%), hexadecanoic acid (10.3%), and heptadecane (9.5%), respectively.

In vitro antidermatophytic effects

Table 2 represents the findings of MIC and MFC of *N. tripedale* extracts. These results revealed that this extract had not only fungistatic activity, but also fungicidal effects. The MIC and MFC values for MZ as a control drugs against tested dermatophytes were ranging from 0.0016 to 0.008 mg/ml; while the negative controls did not indicate any inhibitory effects against the tested dermatophyte strains. Our findings also indicated that among the tested dermatophytes, *T. mentagrophytes* and *M. canis* were the most sensitive and resistant strains to the *N. tripedale* extracts, respectively.

Table 1. Chemical composition of *N. tripedale*

No.	Name	KIx	Area%
1.	Tetramethylpyrazyn	1080	1.5
2.	n-Nonanal	1100	4.4
3.	n-Decanal	1195	1.4
4.	Pipertitone Oxide	1237	7.1
5.	2-Decenal	1252	3.7
6.	2,4-Dcadienal	1284	3.8
7.	Disulfide, Dibutyl	1291	3.0
8.	2,4-Decadienal,(E,E)	1307	11.1
9.	1-Undecene,8-methyl	1333	1.4
10.	Trans-2-Undecenal	1354	6.1
11.	4-Heptenal	1370	1.4
12.	Neryl Acetone	1441	2.3
13.	Pentadecane	1487	2.8
14.	Gamma, Cadinene	1498	2.8
15.	2(4H)-Benzofurane,5,6,7,7	1513	1.7
16.	Caryophyllene Oxide	1575	6.0
17.	Hexadecane	1600	4.7
18.	Delta,Cadinol	1635	3.4
19.	Heptadecane	1689	9.5
20.	Octadecane	1788	4.3
21.	Isopropyl Myristate	1816	1.7
22.	2-Undecanone,6,10-dimethyl	1834	2.8
23.	1,2-Benzenedicarboxylic Acid	1953	1.9
24.	Hexadecanoic Acid	1963	10.3

Table 2. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values (mg/ml) of *N. trpedale* extract against *T. mentagrophytes, M. canis* and *M. gypseum*. Data are expressed as the mean (n=3)

Tested samples	Fungal strains					
	T. mentagrophytes		*M. gypseum*		*M. canis*	
	MIC	MFC	MIC	MFC	MIC	MFC
N. tripedale extract	16.6	25	20	41.6	33.3	66
Miconazole	0.0016*	0.004*	0.005*	0.008*	0.006*	0.008*

* The difference is statistically significant (p <0.05).

DISCUSSION

In recent years, with discovering the new synthetic antifungal agents and subsequently the appearance of a number of limitations in the use of these agents has led to a significant increase in people's willingness to use herbs [18-20]. Here we assessed the in vitro antifungal effects of *N. tripedale* extract against some pathogenic dermatophyte strains. The obtained findings demonstrated that *N. tripedale* extract had both fungistatic and fungicidal effects. However, it is necessary to mention that the activity of the plant extract is affected by the plant origin, the weather conditions of plant growth site, part of the plant part which was used, or the applied solvent for extraction, because herbs have various components depending on the factors mentioned [21, 22].

Regarding the antimicrobial activities of *N. tripedale* there are few studies. Ezatpour et al. have reported that *N. tripedale* extract has considerable antibacterial effects on Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, and Pseudomonas aeruginosa [11]. Mahmoudvand et al. have demonstrated that *N. tripedale* extract has remarkable antileishmanial effects against promastigote and amastigote forms of *Leishmania tropica* on in vitro [12]. In the other study conducted by Mahmoudvand et al. *N. tripedale* extract showed high potency against protoscoleces of *Echinococcus granulosus* and may be used in hydatid cyst surgery [13].

The results of the present study showed that the main compounds are decadienal (11.1%), hexadecanoic acid (10.3%), and heptadecane (9.5%), respectively. It is clear that chemical analysis of extracts depends on some factors including species, climate, and time of collection which can the biological activities of plants [23-25]. Reviews have reported the antimicrobial effects of these components (26-28); consequently, these components might be accountable for their antidernatophytic effects but their precise mechanism of action is not clear. Considering the cytotoxicity effects of *N. tripdale* extract, Ezatpour et al. have demonstrated that the extract had no important cytotoxic effect in J774- A1 cells [11].

To conclude, the current investigation showed potency of antidermatophytic activity of *N. tripdale* extract. The results also indicated the scientific suggestions that medicinal herbs might be employed in the folk medicine for the prevention and treatment of dermatophytic diseases.

Conflict of interest

The author declares that there is no conflict of interests in this study.

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