



**EVALUATION EFFECT OF ARTEMISIA SIEBRI ESSENCE ON SOME  
DERMATOPHYTES FUNGI IN VITRO COMPARE TO CLOTRIMAZOLE**

**GHARACHORLOU AA<sup>1\*</sup>, ALIZADEH J<sup>2</sup>, BEHMARD F<sup>2</sup> AND MOROVVATI B<sup>2</sup>**

**1:** Department of Mycology, College of Veterinary Medicine, Tabriz branch, Islamic Azad University, Tabriz, Iran.

**2:** Department of Veterinary Medicine, College of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

**\*Corresponding Author's E Mail: [Gharachorlou@iaut.ac.ir](mailto:Gharachorlou@iaut.ac.ir)**

**ABSTRACT**

Dermatophytosis is one of the dermal mycosis that results from the group of fungus actions in the keratinized tissue (such as hair, nail, and skin keratinized tissue) that called dermatophytes. Nowadays 41 species of dermatophytes were identified that totally divided into three geniuses (with notice to the asexual phase) with names *Microsporium*, *Trichophyton*, *Epidermophyton*. Mycotic infection of the scalp, tinea capitis, is a common disease in developing countries. The use of medicinal herbs in the treatment of skin diseases including mycotic infections is an age-old practice in many parts of the world; *A. sieberi* has forage value for animals and also medicinal properties for humans. Some pharmacological effects of *A. sieberi* plant such as spasmolytic, vermifugal, insecticidal, anticandidal and asexual reproduction inhibition of some filamentous fungi were confirmed. Clotrimazole is used to treat yeast infections of the vagina, mouth, and skin such as athlete's foot, jock itch, and body ringworm. The aim of current study is evaluation effect of *Artemisia sieberi* essence on some dermatophytes like *Microsporium canis*, *Trichophyton rubrum* and *Epidermophyton floccosum* fungi In vitro condition compare to clotrimazol. Among the species tested, *Epidermophyton floccosum* most sensitive species fungal than the three combined. *Trichophyton rubrum* the most resistant species to the antifungal effects of alcoholic and aquatic extracts and *Trichophyton rubrum* and *Microsporium canis* is the most

resistant to the effects of Antifungal miconazole were found. Among the species tested, *Epidermophyton floccosum* most sensitive species fungal than the three combined. *Trichophyton rubrum* and *Microsporum canis* is the most resistant to the effects of Antifungal miconazole and *Artemisia sieberi* were found. Our results demonstrate that *A. sieberi* extract have good effect on saprophyte.

**Keywords:** *Artemisia sieberi*, Dermatophytes, Clotrimazole

## INTRODUCTION

Dermatophytosis is one of the dermal mycosis that results from the group of fungus actions in the keratinized tissue (such as hair, nail, and skin keratinized tissue) that called dermatophytes. Dermatophytes is a group of keratinophilic fungus that known from many years ago. Nowadays 41 species of dermatophytes were identified that totally divided into three genres (with notice to the asexual phase) with names *Microsporum*, *Trichophyton*, *Epidermophyton*. Mycotic infection of the scalp, tinea capitis, is a common disease in developing countries [1, 2]. The use of medicinal herbs in the treatment of skin diseases including mycotic infections is an age-old practice in many parts of the world [3]. This use has been supported by the isolation of active antifungal compounds from plant extracts [4]. These compounds represent secondary metabolites that serve as defense agents against invading micro-organisms.

The genus *Artemisia* is one of the largest and widely distributed genres of the *Asteraceae*.

*Artemisia siberi* besser (*Artemisia herba Alba* Asso Var. *laxifolia* Boiss) is named locally “Dermaneh” and is widely distributed in the desert area of Iran. This plant has forage value for animals and also medicinal properties for humans. Some pharmacological effects of *A. sieberi* plant such as spasmolytic, vermifugal, insecticidal, anticandidal and asexual reproduction inhibition of some filamentous fungi were confirmed. There are some investigations on chemical composition and antimicrobial activity of *A. sieberi* essential oil. Analysis of *A. sieberi* essential oil ingredients has shown differences in its constituents depending on the region of cultivation even within one country. It has been found that *A. sieberi* oil from north of Iran (Tehran and Semnan Province) contain camphor, 1, 8- cineole, from northeast of Iran (Khorasan Province) contain;  $\alpha$ -thujone,  $\beta$ -thujone and camphor with antifungal activity against soil born bacteria. Clotrimazole is used to treat yeast infections of the vagina, mouth, and skin such as athlete's foot, jock

itch, and body ringworm. It can also be used to prevent oral thrush in certain patients. The aim of current study is evaluation effect of *Artemisia siebri* essence on some dermatophytes like *Microsporum canis*, *Trichophyton rubrum* and *Epidermophyton floccosum* fungi In vitro condition compare to clotrimazol.

## MATERIALS AND METHODS

*Microsporum canis* (PTCC 5069), *Trichophyton rubrum* (PTCC 5143) and *Epidermophyton floccosum* (PTCC 52063) provided from fungus collections and industrial and infectious bacterial dependent on Iran scientific and industrial researches organization. Then, a uniform suspension of *Microsporum canis*, *Trichophyton rubrum* and *Epidermophyton floccosum* 14-day medium cultures grown on sabouraud dextrose agar produced by germany merck factory, using sterile saline containing, 0.5 Percent Tween 80 was obtained. In order to eliminate excess particles and fragments of hyphae, the resulting suspension was passed through several sterile layers. Thus, fungal spores and isolation were collected [5]. Suspension containing spores collected in sterile tubes and the number of spores per ml was counted using neobar slide and finally concentration of 10<sup>3</sup> cfu /ml to transfer a certain amount of suspension of the fungal

culture on sabouraud dextrose agar and colony counts were made.

For the preparation of Clotrimazol solution, One mg of Clotrimazol dissolved in one ml dimethyl sulfide (DMSO) and was maintained for 30 min in Laboratory temperature. Drug solution divided in 100 µl sterile vials and until use at - 70 ° C was maintained. To preparation of *Artemisia siebri*, the plant materials were washed with water and 2% sodium hypochlorite for 30 minutes was disinfected, then to remove residual hypochlorite, washed with sterile distilled water and dried in the shade and the dried plant material was powdered. 50 g of dried powder was soaked in 500 ml of methanol and 48 hours was shaken by shaker. Then by two layers of sterile linen filtered after that centrifuged for 10 min at 9000 rpm and filtered whatman paper number 41 again. The extract of Plant filled in separate vials in concentrations of 0.5, 0.75, 1 and 1.5 mg/ml [6]. By adding the yeast suspension to serial dilution tubes and pipes incubation for 48 hours at concentrations every drug on the MIC inhibited the growth of fungi, MFC was determined visually. Finally the data collected according to the

## RESULTS

The minimum inhibitory concentration MIC of aqueous and alcoholic extracts of *Artemisia siebri* and Clotrimazole and in liquid medium

for fungal species tested, and was shown at **Table 1**.

Among the species tested, *Epidermophyton floccosum* most sensitive species fungal than the three combined. *Trichophyton rubrum* the most resistant species to the antifungal effects of alcoholic and aquatic extracts and *Trichophyton rubrum* and *Microsporum canis* is the most resistant to the effects of Antifungal miconazole were found.

The minimum concentration fatality (MFC) of aquatic and alcoholic extract of *Artemisia siebri* and Clotrimazole and in liquid medium for fungal species tested, and was shown at **Table 2**.

Among the species tested, *Epidermophyton floccosum* most sensitive species fungal than the three combined. *Trichophyton rubrum* and *Microsporum canis* is the most resistant to the effects of Antifungal miconazole and *Artemisia siebri* were found.

**Table 1: Minimum Inhibitory Concentration of *Artemisia siebri* and Miconazole in Fungal Species**

Fungal species	Alcoholic extracts	Aqueous extracts	Clotrimazole
<i>Microsporum canis</i>	0.1	1.6	0.00094
<i>Trichophyton rubrum</i>	0.4	1.6	0.00323
<i>Epidermophyton floccosum</i>	0.047	0.5	0.00019

**Table 2: Minimum Fatality Concentration of *Artemisia siebri* and Miconazole in Fungal Species**

Fungal species	Alcoholic extracts	Aqueous extracts	Clotrimazole
<i>Microsporum canis</i>	0	0	0.00499
<i>Trichophyton rubrum</i>	0.28	0	0.00566
<i>Epidermophyton floccosum</i>	0.56	1.13	0.00377

## DISCUSSION

The emergence of different species of fungi resistant to antifungal compounds Including *Candida albicans*, *Cryptococcus neoformans* and dermatophytes, scientists to develop new therapies with minimal toxicity effects the man makes the fight fungus [7]. In general, screening of medicinal plants for antimicrobial activities is important for

finding potential new compounds for medicinal and industrial purposes. In many cultures, some infectious diseases are known to have been treated with herbal remedies and also some pathogenic bacteria are commonly resistant to many antibiotics. So, interests at antimicrobial properties of extracts from aromatic plants particularly essential oils are very important. The genus of *Artemisia*

belongs to the family *Asteraceae* is presented by 34 species in Iran that are found wild all over Iran [8]. Our survey exhibited the main components of *A. sieberi* oil were very variable from different place of Iran [8, 9, 10]. Bagheri *et al.*, [11] showed that grazing affects on essential oil compositions of *A. sieberi*. The essential oil of *A. sieberi* from our study was rich in  $\beta$ -thujone,  $\alpha$ -thujone, camphor and its oil compositions were similar to the oil compositions of the Khorasan type [8]. Negahban *et al.*, [9] suggested that *A. sieberi* oil containing camphor (54.7%), camphone (11.7%), 1,8-cineole (9.9%) may have potential as a fumigant agent against *Callosobruchus maculatus*, *Sitophylus oryzae* and *Tribolium castaneum*. Farzaneh *et al.*, [8] demonstrated that *A. sieberi* oil with  $\beta$ -thujone (19.8%),  $\alpha$ -thujone (19.5%), and camphor (19.5%) being its major components was slightly effective against *Tiarosporella phaseolina*, *Fusarium moniliforme* and *Fusarium solani* but had high antifungal activity against *Rhizoctonia solani*. Also antifungal and antibacterial activity of  $\beta$ -thujone,  $\alpha$ -thujone and camphor were reported in different studies [12, 13]. Our results demonstrate that *A. sieberi* extract have good effect on saprophyte.

## REFERENCE

- [1] Ali-Shtayeh MS & Arda HM, A study of *Tinea capitis* in Jordan (West Bank), J. Trop. Med. Hygiene, 89, 1986, 137-141.
- [2] Gruseck E, Splanemann V, Bleck O, Ring J & Abeck D, Oral terbinafine in tinea capitis in children. Mycoses 39, 1996, 237-240.
- [3] Irobi ON & Daramola SO, Antifungal activities of crude extracts of *Mitracarpus villosus* (Rubiaceae), J. Ethnopharmacol., 40, 1993, 137-140.
- [4] Fabry W, Okemo P & Ansorg R, Fungistatic and fungicidal activity of East African medicinal plants, Mycoses 39, 1996, 67-70.
- [5] Fernandez-Torres B, Vazquez-veiga H, Liovo X and Pereiro MJ, In vitro susceptibility to itraconazole clotrimazole ketoconazole and terbinafine of 100 isolates of *Trichophyton rubrum*, Chemotherapy, 46, 2000, 390-394.
- [6] Irobi ON & Daramola SO, Bactericidal properties of crude extracts of *Mitracarpus villosus*, J. Ethnopharmacol., 42, 1994, 39-43.
- [7] Squeo RF, Beer R, Silvers D, Weitzman I, Grossman M. Invasive *Trichophyton Rubrum* Resembling Blastomycosis Infection in the

- Immunocompromised Host, *J. Am. Acad. Dermatol.*, 39, 1998, 379-380.
- [8] Farzaneh M, Ghorbani-Ghouzhdhi H, Ghorbani M and Hadian J, Composition and antifungal activity of essential oil of *Artemisia sieberi* Besser on soil-borne pathogens, *Pak. J. Biol. Sci.*, 9, 2006, 1979-1982.
- [9] Negahban M, Moharramipour S and Sefidkon F, Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored product insects, *J. Stored Product Res.*, 43, 2007, 123-128.
- [10] Weyerstahl S, Schneider S, Marshall H and Rustaiyan A, The essential oil of *Artemisia sieberi* Besser, *Flavor Fragr. J.*, 8, 1993, 139-45.
- [11] Bagheri R, Chaichi MR, Mohseni Saravi M, Amin GR and Zahedi G, Grazing affects essential oil compositions of *Artemisia sieberi* Besser, *Pak. J. Biol. Sci.*, 10 (5), 2007, 810-813.
- [12] Carson CF, Riley TV. Antimicrobial activity of the major components of the essential oil of *M. alternifolia*. *J Applied Bacteriol* 1995; 78: 264-269.
- [13] Pattnail S, Subramanyan VR, Bpaji M, Kole CR. Antibacterial and antifungal activity of aromatic constituents of essential oils. *Microbios* 1997; 29:39-46.