

Effect of aqueous root extract chicory (*Cichorium intybus* L.)

plant on growth of some dermatophytes

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Abstract:

Chicory (*Cichorium intybus* L.) is a plant contains number of medicinally important compounds such as inulin,esculin, volatile compounds , coumarins, flavonoids and vitamins. In the present study, antifungal activity of the root extracts of chicory against pathogenic fungi (Dermatophytes) like *Tricophytone tonsurans*, *Tricophytone verrucosum* and *Microsporum canis* by in vitro agar well diffusion method was don at two different concentration (100mg/ml ,200mg/ml) . The aqueous root extracts of chicory showed inhibition effect on dermatophytes . Root extracts showed more inhibitory action on *T. verrucosum* with diameter of inhibition zone 3.50 cm while less inhibition zone appeared for *M.canis* 1.25cm .

There observed significant ($p>0.05$) effects of concentration of root extract on fungal growth and there was significant differences ($p>0.05$) between fungal species in response to root extract

Key words : Dermatophyte , *Cichorium intybus* L., Antifungal activity

**دراسة تأثير المستخلص المائي لجذور نبات الهندباء *Cichorium intybus L.*
على نمو بعض أنواع الفطريات الجلدية**

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الخلاصة :-

يحتوي نبات الهندباء على عدد من المركبات المهمة طبييا مثل الانيسولين والاسكيولين وغيرها من المركبات ، ففي الدراسة الحالية تم دراسة الفعالية التثبيطية للمستخلص المائي لجذور نبات الهندباء على بعض Tricophytone *Microsporum canis*, *verrucosum* أنواع الفطريات الجلدية مثل باستخدام طريقة الانتشار من الحفر مع استخدام التراكيز Tricophytone tonsurans, (200mg/ml, 100mg/ml) . أظهرت النتائج ان للمستخلص المائي لجذور نبات الهندباء فعالية تثبيطية على الفطريات الجلدية المدروسة اذ بلغ اعلى نسبة تثبيط للمستخلص عند التركيز 200mg/ml للفطر *Tricophytone verrucosum* وبلغ قطر التثبيط 3.50 cm بينما اقل منطقه تثبيط ظهرت تجاه الفطر *Microsporum canis* وعند التركيز 100mg/ml وبلغ قطر التثبيط 1.25 cm وأظهرت نتائج التحليل الإحصائي وجود تأثيرا لتركيز المستخلص على نمو الفطر كما وجدت الدراسة أيضا وجود فرق معنوي عند مستوى احتمالية $p > 0.05$ بين الأنواع الفطرية في الاستجابة للمستخلص .

1- Introduction

Dermatophytosis is one of the most important superficial infections worldwide which refers to three main genera of *Epidermophyton*, *Microsporum* and *Trichophyton* (Behzadi *et al.*, 2014). Dermatophytes are filamentous fungi which commonly infects skin, hair and nails or dead cornified layer of epidermis as they are unable to penetrate living tissue of the host. (Garg *et al.*, 2009). Typical infections caused by dermatophytes are popularly known as ringworm infections (Bhatia *et al.*, 2014). Dermatophytes are divided according to ecologic habitat into three groups of anthropophilic microorganisms (from person to person), zoophilic microorganisms (from animal to either animal or human), and geophilic microorganisms (transmitted from soil to animals or humans) (Baldo *et al.*, 2012). Zoophilic dermatophytes such as *T. verrucosum*, *M. canis*, *T. mentagrophytes*, *M. gypseum*, and *T. equinum* have been reported as important causes of human tinea capitis and tinea corporis in many areas of the world. (Cafarchia *et al.*, 2012). Although dermatophytes are not life threatening microbial agents but they are distributed around the world and cause mycotic infections with high morbidity (Kelly, 2012). *Cichorium intybus* is used in traditional system of medicine since many years, even though it has many medicinal values and industrial uses. Chicory has necessary to scientifically validate with experimental and clinical study.

The whole plant contains a number of medicinally important compounds such as inulin, esculin, volatile compounds (monoterpenes and sesquiterpenes), coumarins, flavonoids and vitamins (Chandra *et al.*, 2016). The carbohydrates are the main groups of compounds of the chicory root, including saccharose, glucose and fructose, fructooligosaccharides and inulin, (Gałazka, 2002). Root chicory, is a source of sesquiterpenes and polyphenols (Mares *et al.*, 2005; Hoste *et al.*, 2006). Polyphenolic acids of chicory root express a wide range of health-promoting activities such as antiviral, anticarcinogenic, antibacterial, anti-inflammatory, antifungal, antimutagenic, immunostimulating and antioxidant. Moreover, they can act against HIV virus, they can protect the alimentary tract and influence the reduction of cholesterol level in blood (Innocenti *et al.*, 2005; Mares *et al.*, 2005). Therefore, preparations rich in prebiotic saccharides and polyphenols produced from chicory can be used as supplements promoting healthy properties of a diet. Chicory root extract could delay or prevent the early onset of diabetes mellitus and improve bowel movements. (Nishimura *et al.*, 2015). This study aimed to study the effect of root chicory extract on fungal growth such as dermatophytes

2- Materials and Methods

Preparation of root extracts plant roots were collected from local market , plant roots have been grinding using electric mill and make it powder and then washed thoroughly in tap water to remove dust and dried in room temperature for 15 days. The dried 25 g root was added to 100 ml of sterilized distal water and mixing by keeping it in a shaker for 3 days .The extracts were filtered through chces cloth and then took the filtrate and put in centrifuge at 4000 rpm for 30 minutes then extracts were filtered by using filter paper (whatmam No.1) The extracts were sterilized by autoclave . The extracts were reduced to 10% of its original volume. aqueous extract was dried using water bath.(Satish *et al.*, 2007)

Phytochemical screening of root extracts: The phytochemical components of the chicory roots such as alkaloids, volatile oils, fatty acids, flavonoids, anthracene glycosides, tannins, phenolics and saponins were screened by using the methods of Brindha *et al.*, 1977 and Harbone ,1988.

Inoculums: The test of microorganisms, *Tricophyton* , *Microsporium* dermatophyte which isolated from patients suffering from skin infection were inoculated into Sabroud dextrose broth medium, and incubated at 27°C for 3 days. The fungal cells were harvested by centrifuging at 5000g for 15 min. The pellet formed was washed twice with PBS (Phosphate BufferSaline), (10 mM Sodium Chloride, pH 7.4) and the cells were counted by hemocytometer. The fungal cells were diluted to approximately 10^5 CFU (Colony forming unit)ml⁻¹ before use (Owais *et al.*, 2005).

Determination of antifungal activity

The antifungal activity of the root extracts was determined using agar well diffusion method by following the published procedure with slight modification(Perez *et al.*, 1990). Sabroud dextrose agar was inoculated with fungal cells by spreading fungal inoculums on the media. Wells (6mm diameter) were punched in the agar and filled with plant extracts. Control wells containing (DMSO) dimethyle sulfixid the plates were incubated at 27°C for 7-10 days and the antifungal activity was assessed by measuring the diameter of the zone of inhibition.

Statistical analysis: The data were expressed as the means of three independent experiments statistical comparisons of the results were performed by two –way ANOVA using SPSS ver 19. Significant differences (P<0.05) between the fungus and concentration were analyzed by L.S.D triplicates range test Bryman and Cramer (2012).

3- Results

Phytochemical screening of root extracts: The phytochemical screening of the root extracts using water was reported (Table 1). The aqueous extracts showed the presence of alkaloids ,tannis , Flavonoids , Phenolics and saponins while glycosides and fatty acids were absent. The presence of flavonoids and Tannins in *Cichorium intybus* extract are in agreement with previously reported results of Nandagopal and Ranjitha (2007) and Muthusamy *et al.* (2008).

Table 1: Preliminary phytochemical analysis of chicory root extracted with water

Compound	Results
Alkaloids	+
Volatile oils	-
Fatty acids	-
Anthracene glycosides	-
Tannins	+
Flavonoids	+
Phenolics	+
Saponins	+

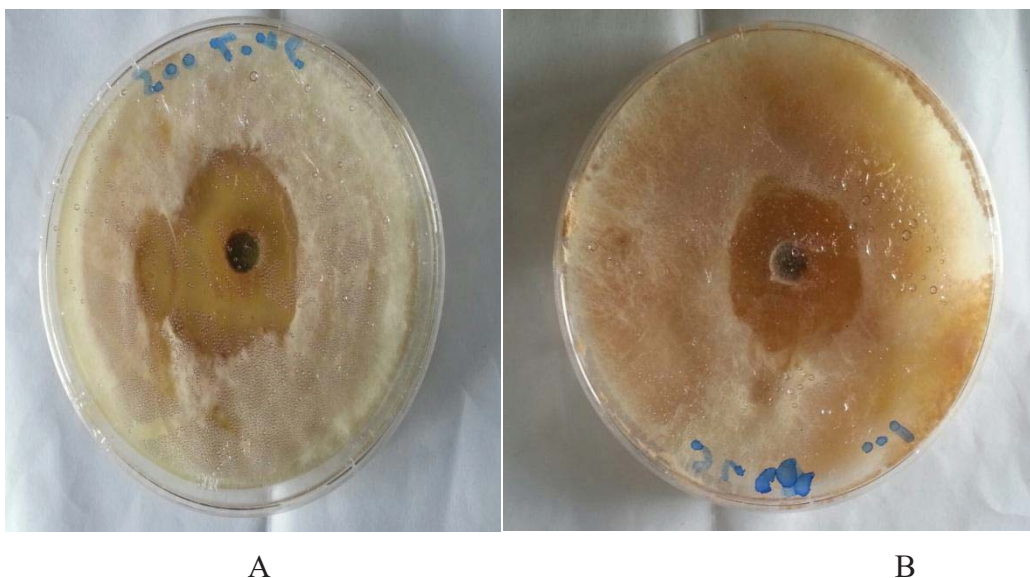
+ Present, -Absent

Antifungal activity of chicory Aqueous extract

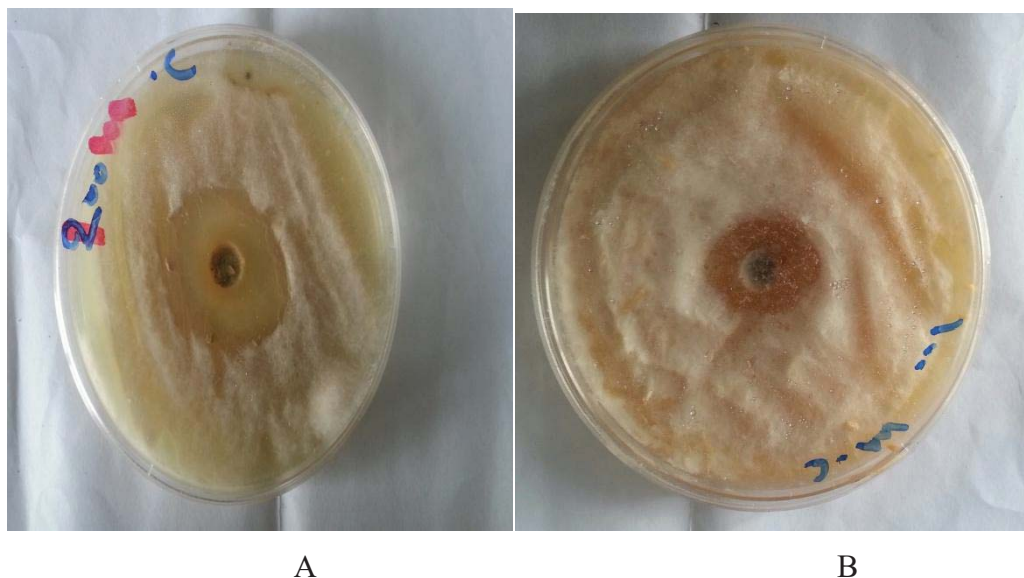
The antifungal activity of the chicory roots was assessed using the agar well diffusion method by measuring the diameter of growth inhibition zones with 100 and 200µl of chicory root extracts (Table 2). The results showed that chicory water extract possesses antifungal activity against the tested dermatophyte (*Microsporium canis* , *Trycophyton tonsurans* and *T.verrucosum*) the maximum inhibition was observed at 200 µl concentration against the fungus *T.verrucosum* with diameter of growth inhibition zones reached to 3.50 cm while less inhibition zone appeared for *M.cans* at concentration 100 mg/ml with diameter of growth inhibition zones 1.25cm (figure 1,2,3) . Biological activity of chicory root extracts on fungi from a variety of ecological environments were studied by Mares *et al.* (2005) and they found that chicory root extracts inhibited the growth of zoophilic and anthropophilic dermatophytes , in particular *Trichophyton tonsurans* var. *sulfureum* .

Antifungal activity by using chicory root extract was also observed by Koner *et al.*, 2011, they showed that the ethyl acetate extract of chicory root had also antifungal effect on *Aspergillus niger*

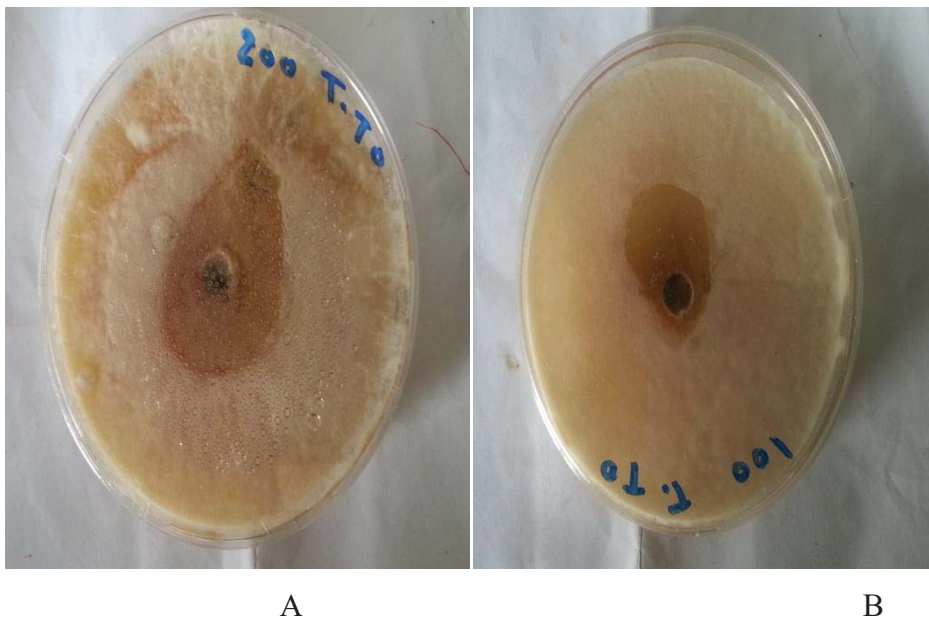
and *Sachharomyces cerevisiae*, while the Hexane extract of chicory root had no effect on the growth of *Aspergillus niger* and *Sachharomyces cerevisiae*, even with higher concentration. Eslami ,2015 found that extracts from the leaves of chicory had no antibacterial effect, but most of the seed extracts of this herb had Inhibitory and bactericidal effects in first to third dilutions and also had no Antifungal activity in any of the chicory plant extracts.



Figure(1): Effect of aqueous root chicory extract on *T. verrucosum* (A: at 100mg/ml , B: at 200mg/ml concentration)



Figure(2): Effect of aqueous root chicory extract on *M.canis* (A: at 100mg/ml , B: at 200mg/ml concentration)



Figure(3): Effect of aqueous root chicory extract on *T. tonsurans* (A: at 100mg/ml , B: at 200mg/ml concentration)

Table 2: Antifungal activity of root extracts of chicory at 100 and 200µl concentration

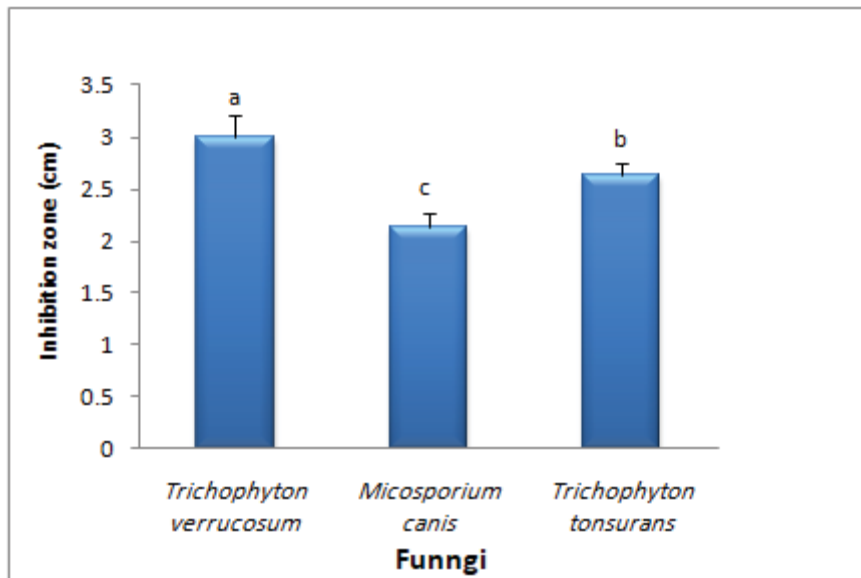
Fungi	Concentration mg/mL	Inhibition zone (cm)
Trichophyton verrucosum	100	2.51 ± 0.24
	200	3.50 ± 0.43
Micosporiumc anis	100	1.25 ± 0.05
	200	3.00 ± 0.14
Trichophyton tonsurans	100	2.00 ± 0.15
	200	3.25 ± 0.02

L.S.D = 0.38

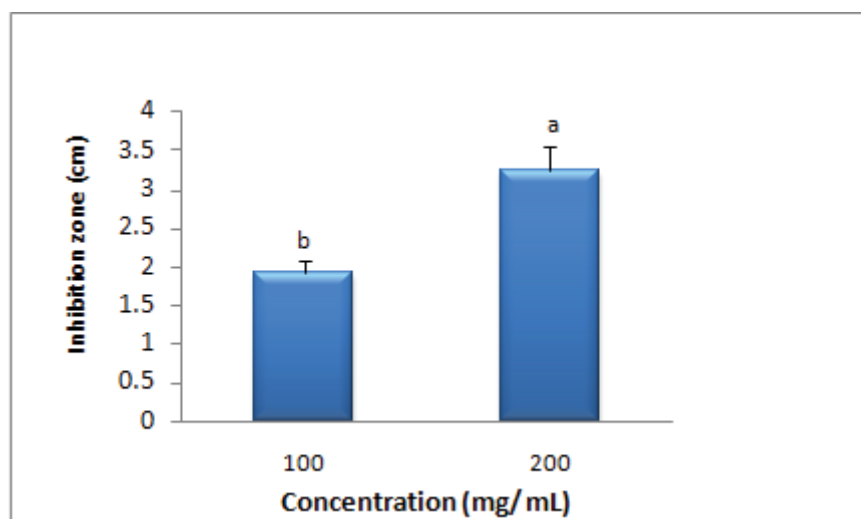
Each value represents the mean ± standard error (SE) of three replicates

The Results of statistical analysis and test least significant difference between the averages below the level of the probability ($p \leq 0.05$) has indicated to the existence of significant differences between concentrations, as well as the presence of significant difference between the fungal species (Table 2, Figure 4, 5) The difference in the inhibition rats due to the nature of the fungal cells because of the disparity in the composition of cell membranes and the thickness and size of the fungal cells

and the variation in the speed of growth of fungus , which has thick walls are more resistant to do active compounds in the extracts because it impedes the entry into force these compounds into cells to affect them (Ian and Ian , 1976).



Figure(4): Effect of chicory extract on three dermatophytes species



Figure(5): Effect of chicory extract concentration on fungi growth

4- Discussion

Dermatophytes are fungi causing dermatomycosis in humans and animals by invading keratinized tissues . Azoles antifungal drugs are often used in the treatment of dermatomycosis but azoles are expensive, toxic, and faced resistance by dermatophytes (Kabbashi and Omer , 2016). Chicory extract proved its efficiency in treatment of skin diseases, and play important role in

treatment bacterial and fungal infection. The antimicrobial activity of Chicory root was reported earlier but study the effect of this plant on growth of dermatophyte was few so this study it is demonstrated that effect of chicory root on growth of sum dermatophyte such as *Trichophyton verrucosum* *Micosporiumc anis* *Trichophyton tonsurans* and the active compounds that responsible for this antimicrobial action.

In this study aqueous chicory extract was used as crude because crude extracts are more effective toward studied organisms compared to isolating the one active compound from the same plant as there is a mixture of active compounds that cooperate with each other in the crude extracts to inhibit microorganisms growth (Cowan, 1999) This is due to the synergism phenomenon that mean union more than a compound in effect rather than its use alone (Mansour, 2005) . We found that aqueous extract of chicory roots were successful in inhibiting the dermatophyte that may be due to the presence of many potent compounds such as alkaloids ,tannis , Flavonoids and saponins which have antimicrobial activity. Many previously study showed that Chicory plant was active against Gram positive and Gram negative bacteria, yeast and filamentous fungi.(Aqil and Ahmed , 2003). This study clear showed that root extract of chicory plant have antifungal activity therefore it recommended for use in treatment of skin infections causing by dermatophytes.

5- References

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