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Antifungal Activity of *Solanum nigrum* Extract Against *Microsporum canis,* The Causative Agent of Ring Worm Disease

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Abstract

The present study was performed to evaluate the anti-fungal effect of alcoholic extract of *Solanum nigrum* (AESn) on the growth of *Microsporum canis*, the causes agent of ring worm. The results of this work referred to the inhibitory effect of the studied extract on the growth of tested fungi. The percentages of inhibition were (7.88 %, 19.88%, 23.41%, 57.65%), in comparison to the control, when (2%, 4%, 6%,8%) of tested extract were used, respectively. The data illustrated that the higher concentrations of the extract are applied, the more inhibition of fungal growth is produced.

Key words: *Solanum niger* extract, ring worm, inhibition, *Microsporum canis*

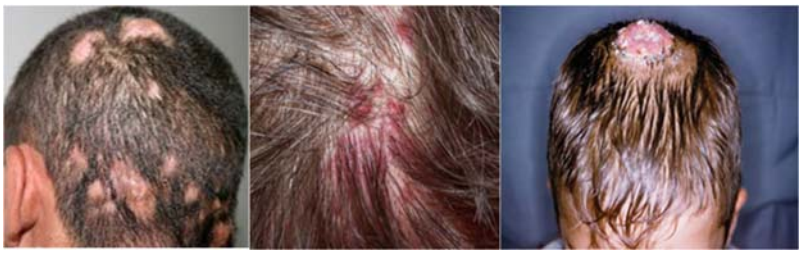
1. Introduction

Dermatophytes are group of pathogenic fungi attacking the keratinized tissues such as hair, skin, nails of human and animals causing dermatophytosis [1]. Historically, the term "dermatophytes" referred to the dermal plant and defined as a parasitic fungi infect the keratinized tissues of skin causing the Ring Worm or Tinea [2]. The hot and moist environments are required as the best growth conditions for dermatophytes, so they are widespread in the tropical and subtropical areas [3]. These fungi include three geneses; *Microsporum, Trichophyton, Epidermophyton* [1]. *Microsporum canis* is globally distributed species [4] infecting the dead superficial layer of skin [5]. At the site of infection, these fungi are loose, white and scattered with the hair tissue [6]. *M. canis* was isolated from infected scalp and various body sites causing tissue destruction and hair loss [4]. In mild cases of infection, localized anti-fungal treatment is usually applied at the site of infection. While in severe cases, a combination of anti-fungals is required, such as griseofulvin combined with itraconazole or terbinafine) for treatment of infection [7, 8].

The commonly used anti-fungal drugs play an important role in the treatment of fungal infections. However, many of these antimicrobials possess a harmful side effects on human and animals health if they would be administrated incorrectly. In addition, these antimicrobials characterized by the low efficacy due to their poor tissue penetration [9]. The abuse and overuse of these antimicrobials lead to the emergence of antifungal resistance [10]. Finding an alternative medication is attracting the scientists' attention to avoid fungal resistance.

Plants are reported as a good source of antimicrobials [11]. The demand on using some plant extracts as antibacterial and antifungal substances has risen recently. These extracts are produced as secondary metabolites which could be used in pharmaceutical applications [12, 13]. Norikura et al. [14] confirmed that the mode of plant extracts action depends on their chemical composition.

Solanum nigrum is herbesiaus plant come from solonales order, solonaceae family, under the plantae kingdom. This plant was world-widely used as a traditional treatment for several disorders and chronic skin infections, such as ring worm, tinea, diarrhea, eye diseases, etc. The most active compounds of *Solanum niger* are alkloids and saponins. Furthermore, *Solanum niger* is composed of glycosides, carbohydrates and proteins which have the anti-clumping and antitumor activity [15, 16,17, 18] . The aim of this study is to determine the effective concentrations of AESn against *M. canis*, the pathogenic fungi of ring worm infection.



These pictures illustrated the early and late stages of infection by *M. canis*

2. Materials and Methods

Preparation of plant extract

The alcoholic extract of *Solanum's* fruit (AESn) was prepared according to [19] with minor modifications. After removing their seeds, the fruits were dried then crashed. The powdered fruits were weighted (30 gm) then mixed with 70% of ethanol and incubated with shaking and at darkness for three days at 35 °C. After incubation, the mixture was filtered by gauzes then by filter paper. Filtration process was repeated to get rid of contaminants and ensure getting a purified solution. Then, the solution was poured into sterile glass-made Petri dishes and transferred into the oven at 45 °C for ethanol evaporation and extract production. The extract was collected in sterilized glass-made tubes which were closed tightly and then kept at 8 °C until they were used

Culture media and growth conditions

M. canis was isolated and diagnosed at Veterinary College/ Diyala University. The fungi were inoculated and grown on sabouraud sucrose agar containing chloramphenicol 250 µg\ml to avoid bacterial growth and contamination. The inoculated agar plates were incubated at 37 °C for 6 days.

Preparation of the extract concentrations

Each 1 gm of extract was dissolved in 2 ml of phosphate buffer saline (pH=7). The mixture was strongly mixed by vortex to dissolve it completely. A stock solution (100%) of extract was prepared then diluted by agar dilution method in a suitable culture medium following [20], with some modifications. Briefly, several dilutions of AESn (2%, 4%, 6%, 8%) were prepared and added into sabouraud sucrose agar at 45 °C and immediately poured into sterilized Petri dishes. The prepared agar plates were left to solidify for 10 minutes inside the biosafety hood and then inoculated with 10^6 spores/ml of *M. canis* which were spread homogenously over the agar. All the agar plates were incubated at 37 °C for 6 days. To measure the inhibition percentages of AESn against M. *canis*, the following equation was used:

$$\text{Inhibition\%} = \frac{\text{diameter of control} - \text{diameter of treatment}}{\text{diameter of control}} \text{x}100$$

Statistical analysis

Each experiment was performed at least three times. The collected values were then analyzed mathematically using SPSS system.

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Tables and Figures legends:

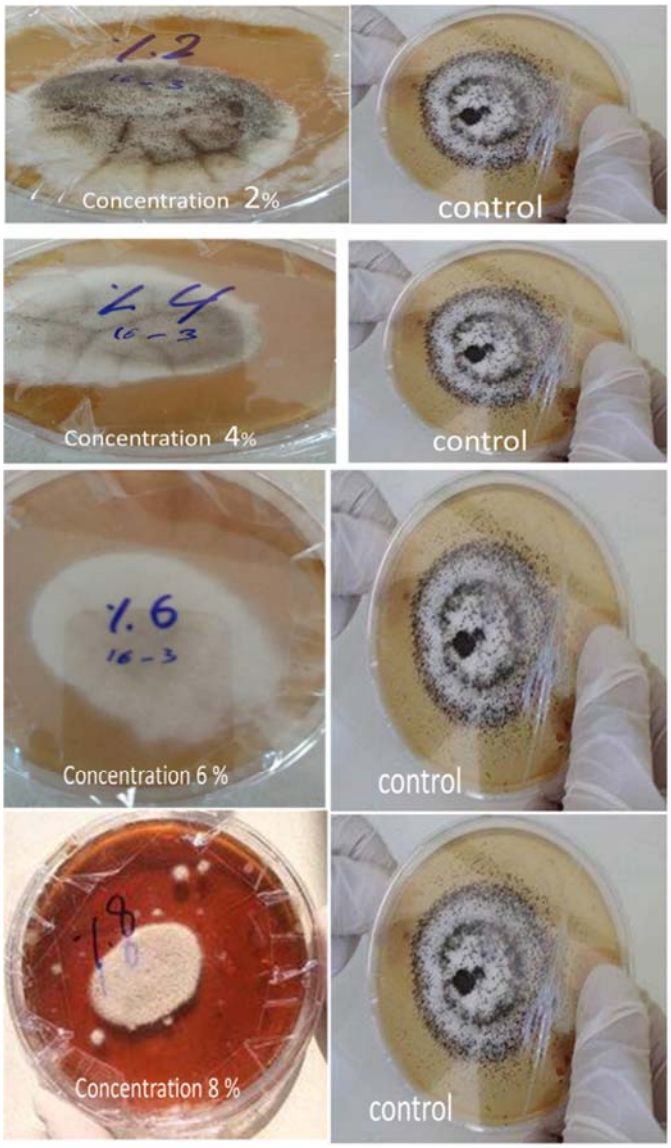

Figure (1): The growth of M. canis treated with various concentrations of AESn

Table (1): Percentages of fungal growth inhibition treated with AESn

Concentrations of extract	**0%**	**2%**	**4%**	**6%**	**8%**
Diameter of treated colonies Average (mm)	8.5	7.8	6.8	6.5	3.6
% inhibition	0%	7.88%	19.88%	23.41%	57.65%

Table (2): Statistical analysis of *M. canis* treated with AESn

Concentrations	Replications	Diameter (mm) of fungal colonies after treatment
2%	R1	8.50
	R2	7.40
	R3	7.60
Means	**7.83a**	
4%	R1	6.85
	R2	7.00
	R3	6.60
Means	**6.81a**	
6%	R1	7.25
	R2	6.10
	R3	6.20
Means	**6.51a**	
8%	R1	4.00
	R2	3.10
	R3	3.70
Means	**3.60b**	
0%	R1	8.50
	R2	8.50
	R3	8.50
Means	**8.50c**	
LSD	**NS**	

3. Results & Discussion

In this study, no difference in fungal growth inhibition was noticed when 2% (T2) and 4% (T3) of AESn were used. In comparison to the control 0% (T1), a slight inhibitory effect on *M. canis* growth was reported when fungi treated with 6% (T4) of the extract. While the fungi growth inhibition by 8% (T5) of AESn was significantly different ($P < 0.05$) when compared to control Tables (1 and 2). We concluded that the higher concentrations of AESn are used, the more *M. canis* growth inhibition is produced. Our findings were in agreement with Saleem *et al*. [21] who observed an increasing in the zone of fungi inhibition when the concentration of their tested extract was increased. An association between antimicrobial activity of plant extract and its composition was published. The AESn is basically composed of alkaloid, saponins, glycosids and antitumor molecules. These substances disrupt and inhibit the biological activities of succinate dehydrogenase, which is an important enzyme in metabolic processes, oxidation phsphorolation and electron transport mechanisms during fungi respiration and eventually, lead to cell death [17, 18].

4. Conclusion

Several studies were reported refer to resistance of *M. canis* to many of commonly prescribed anti-fungal substances. This study is suggested AESn as an alternative antimicrobial for treatment of ring worm infection in which M. *canis* is the main pathogenic cause.

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