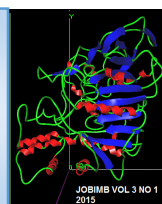


# JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

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## Antimicrobial Activity of Bergamot Oil against *Trichophyton verrucosum* Isolated from Local Cows

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### HISTORY

Received: 9<sup>th</sup> February 2015  
Received in revised form: 10<sup>th</sup> of April 2015  
Accepted: 11<sup>th</sup> of July 2015

### KEYWORDS

Bergamot oil  
*Trichophyton verrucosum*  
antifungal activity  
dermatophytes  
ringworm

### ABSTRACT

The fungal infection is zoonotic infectious disease, caused by different species of dermatophytes. In this study, skin scrapings and hair samples were collected from cattle with clinical symptoms of dermatophytosis. The collected samples were directly examined for fungal elements by direct microscopy. Bergamot oil was shown to be a potent antimicrobial agent *in vitro* against clinically important *trichophyton verrucosum*. In this study, the activities of bergamot natural essence on *trichophyton verrucosum* were investigated. The results indicated that the MICs (v/v) of the bergamot oil against the fungi *Trichophyton verrucosum* was 0.125.

### INTRODUCTION

Fungal infection is a zoonotic infectious disease caused by different species of dermatophytes. These microorganisms are a group of closely related fungi, which utilize keratin and tend to be confined to the superficial integument, including skin, nails, claws, and hair of both animals and humans [1]. Although dermatophytes can be divided into several groups, cattle is mainly affected by *Trichophyton verrucosum*.

Dermatophytosis can be transmitted from animal to animal and from human to animals. Zoophilic fungi prefer animals as hosts but they often cause acute inflammatory reactions when they invade humans [2]. The disease is responsible for great economic losses due to skin injuries and many casualties in animal products (wool, meat, etc.) [3]. Sanguinetti et al. [4] stated that bergamot oil is active *in vitro* and *in vivo* topical treatment for dermatophytoses. Finally, *Trichophyton verrucosum* is the most common etiologic agent of cattle [5]. The scope of this work is determination of bergamot MIC for *Trichophyton verrucosum*-infected calves in El-Giza governorates.

### MATERIALS AND METHODS

#### Samples collection

The samples of skin scraping and hair were collected from cattle farm located in El-Giza governorate. The site of lesion

after cleaning by cotton soaked in 70% alcohol was from infected buffaloes and cows, which have clinical manifestations of dermatophytosis (Fig. 1).

#### Direct microscopical examination using KOH (20%)

One or two drops of 20% KOH (potassium hydroxide) was placed on a microscopic slide, a small amount of the specimen was added, and then, the slide was gently passed through a low flame and covered by a cover slip. After 2 h, the specimen must be examined for the presence of arthrospores and hyphae for *Trichophyton verrucosum* under a light microscope [6].

#### Isolation and identification of dermatophytes from cattle samples

##### Skin scrapings

Hairs were reduced in size to pieces approximately 1 mm across and the hair roots were cut into similarly sized fragments. Each sample was cultured on the surface of Sabourad Dextrose Agar (SDA) containing chloramphenicol and cycloheximide and without cycloheximide to isolate nondermatophytes. The media incubated at 25°C and 30°C for up to 5 and 21 days. After isolation the cultures were transferred to SDA media to purify cultures [7] and [8].

##### Identification of dermatophytes

Based on colony appearance, production of pigment and the micro morphology of the spores produced. Cultures were

observed at 4 or 5 days intervals from the onset. Some characteristics were also noted on the texture, color and shape of the upper thallus [6,9,10].

#### **In vitro anti-fungal assays**

The following procedure was used according to Magaldi et al. [11]. Harvesting of Fungal spores after 7 days old. Culture washed with 10 ml normal saline in 2% Tween80 with the help of glass beads for the dispersion of the spores' aid. The spore suspensions were standardized to  $10^5$  spores/ml. SDA supplemented with 0.05% chloramphenicol and dispensed into Petri dishes of 11 cm diameter. One ml of each standardized spore suspension was spread on the surface of the SDA plates evenly. A sterile cork borer (6 mm in diameter) was utilized to create wells on the surface of SDA plates. Vernier calipers was utilized for measuring zone of inhibition.

#### **Determination of minimum inhibitory concentrations (MIC)**

According to El-Diasty et. al, [5] MICs for the bergamot oil were established using a special instrument known as sterile cork borer (6 mm in diameter), wells were made on the surface of plates.

**Mold inoculum preparation:** All molds were subcultured from stock cultures onto plates of either Sabauroud dextrose.

These plates were then incubated at 30°C until a lawn had developed over the entire plate. The inoculums were prepared by aseptically cutting the lawn growth into 10 x 10 mm squares.

**Plate preparation and analysis:** The agar dilution method described above was modified for filamentous fungi by substituting either malt extract or Sabauroud dextrose agar as the test medium and incubating cultures at 30°C until growth of the positive control covered approximately 90% of the agar plate.

The MIC was recorded as the lowest concentration of test material where at least five out of the six readings showed no growth. Growth of less than 5 mm around the inoculums was considered a negative result. Growth of greater than 5 mm around the lawn was considered a positive result.

#### **Test compounds and susceptibility testing assays**

Natural essence of bergamot was produced by Consorzio del Bergamotto of Reggio Calabria, Italy. The chemical composition was determined by GC mass spectroscopy analyses according to established method [4].

## **RESULTS AND DISCUSSION**

#### **Bergamot oil Antifungal Activity**

The bergamot oil has antifungal activity against a *Trichophyton verrucosum* in oil concentration at 5% as presented in Fig. (2). Bergamot oil has antagonistic activity against pathogenic fungi and the concentration and type of active compounds are important factors to determine their potential antifungal activity [12]. In addition, the topical agents applied to the surface of the skin penetrate into the stratum corneum as a mode of action to kill the fungi or render them unable to grow or divide. This agrees with previous results [4]. Uncomplicated lesions are treated with topical agents to cure or to speed up treatment [13]. However, use of itraconazole and terbinafine in treatment of fungal infection for long period requires periodic laboratory monitoring for liver function tests [14]. Moreover, these antifungal agents may have drug interactions with other medications [15]. Griseofulvin for many years is considered the only antifungal available for the treatment of dermatophytoses as the systemic therapy which should be given when lesions involving a large infected area with aid of topical preparation but toxicities with this agent must be considered [16]. On the

other hand, the safety profile of bergamot oil is well known because of its citrus-like flavor, (+)- limonene is employed as a safe flavoring agent in perfumes, creams, soaps, and in some food products such as ice creams and fruit beverages [17].

The MIC of bergamot oil against *Trichophyton verrucosum* isolate was 0.125 (v/v). In this respect, it was stated that the natural essence of a bergamot oil is a clear yellow green liquid and extracted from the peel of the fruit by a cold-pressing procedure [14]. It consists of a volatile fraction (93– 96%), whose main components are in approximate percentage, limonene (40%), linalool (8%) and linalyl acetate (28%), and a non-volatile fraction (4–7%) formed essentially by coumarins and psoralens (i.e. bergamottin, citroptene, bergaptene, etc.) (Fig. 3). In addition, it is well known that limonene is considered as antifungal [18]. Moreover, Sikkema et al., mentioned that that Limonene antifungal mode of action is it accumulates in the microbial plasma membrane and thus causes a loss of membrane integrity and dissipation of the proton motive force [19]. Finally, it was found that limonene causes damage to the outer membrane in order to gain access to the periplasmic space and cytoplasmic membrane and inactivate the bacterial cell [20]. Once the outer membrane permeability to (+) - limonene is increased, there would be an enhanced interaction of (+) - limonene molecules at pH 4.0 with the components in the cytoplasmic membrane. Limonene affects cytoplasmic membrane and causes the inactivation of these sub-lethally damaged cells.

Treatment for fungal infection of cattle is cumbersome and expensive especially on a herd level, this may be due to difficult application of most antifungal drugs also they are expensive. In this study, we evaluate the antifungal activity of Bergamot oil (*Citrus bergamia*) against *Trichophyton verrucosum* in order to provide an effective cheap antifungal drug easy for application. In addition, we use different concentrations of *Bergamot oil* 1.25%, 2.5% and 5% use it alone and in combination with salicylic acid. The study reported that *Bergamot oil* has antifungal activity against *Trichophyton verrucosum* at all concentrations (1.25, 2.5 and 5%) with and without combination with salicylic acid.

Therefore, we recommend treating skin with Bergamot oil. In addition, and we recommend the application *in vivo* and determine the antifungal activity and the best method for application. These results give substantial support to popular or anecdotal beliefs in the effectiveness of treating skin with bergamot oil.



**Fig. 1.** *Trichophyton verrucosum* lesions in a 4 month old calf. Where there are thick, grayish-white and crusty lesions on neck, ear, face and around the eye.

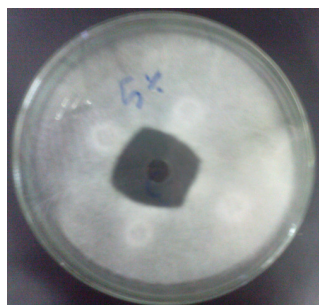


Fig. 2. *Trichophyton verrucosum* grow on potato dextrose agar and shown antifungal activity bergamot 5% with zone of inhibition 40 mm.

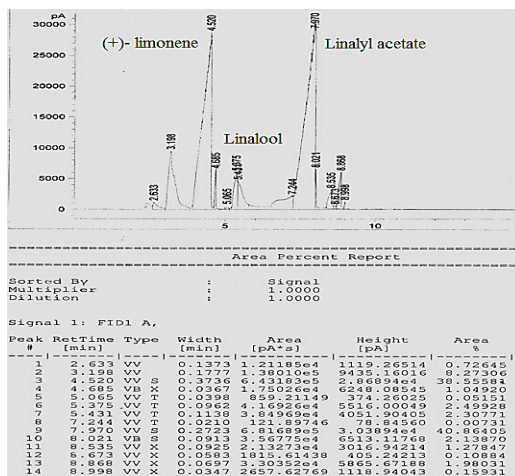


Fig. 3 GC-MS analysis of bergamot oil showing (+) – limonene percentage about 38.5%.

## CONCLUSIONS

The results of the present study highlight the importance of cattle ringworm as an economically important zoonotic infection. According to findings of this study, bergamot oil showed significant antifungal properties. Consequently, to eliminate ringworm infection, bergamot oil can be a proper candidate as a disinfectant agent and can be used as an active ingredient for dermatological applications.

## ACKNOWLEDGEMENT

We would like to thank Dr. ShehabEl-din talat at the microbiology department of NODCAR for his assistance in editing this research.

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