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ORIGINAL ARTICLE

Evaluation of The Activity of Alcoholic Extract of Frankinens (*Boswellia spp.*) Against Some Dermatophytes and Pathogenic Fungi of Plant Crops

Anteithar Jabar Mohammad Al-Edani [1] and **Sarab Fathel Hussein** [2]

[1,2] Department of Biology, College of Education for Pure Science, University of Kerbala, Karbala, Iraq.

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Corresponding author:
Anteithar Jabar Mohammad Al-Edani
Email antethar.j@uokerbala.edu.iq
Department of Biology
College of Education for Pure Science
University of Kerbala
Karbala
Iraq

ABSTRACT

Objectives: The current study aimed at isolating dermatophytes from patients, as well as isolating pathogenic fungi of plant crops and testing the alcoholic extract of the frankincense against them.

Methods: Skin fungi were isolated from patients with skin fungal infections who visited Al-Hussein Hospital in Karbala governorate, which has a total of 90 samples, as they were diagnosed based on sources. Then samples of onions, garlic, pomegranate, orange and apple infected with fungi were taken, and they were purified and diagnosed. After that, an alcoholic extract of frankincense was prepared in three concentrations of 5, 10, and 15 mg / ml, and its efficacy was tested against the isolated fungi by mixing with the medium , The diameter of the developing colony was measured (average of two perpendicular diagonals), and the results were recorded.

Results: During the study, four dermatophytes were isolated: *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum gypseum and Microsporum canis*.The study showed that the alcoholic extract of Frankenins had an inhibitory ability on the skin of isolated fungi, and that the highest rate of effect of the extract was at a concentration of 15 mg / ml on *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Microsporum gypseum*, while the lowest effect of the extract at a concentration of 5 mg/ml was on *Microsporum canis*.

The results also showed that the alcoholic frankincense extract inhibited the growth of pathogenic fungi of plant crops: - *Aspergillus*, *Aspergillus*, *Penecillum digitatum*, *Alternaria sp.* , *Penecillum expasum* by 100% at 30 mg / ml, while the lowest effect of the extract at a concentration of 10 mg/ml was on *P. expasum*.

Conclusion: Most of the isolated dermatophytes from the skin, hair, and nails, as well as the isolated pathogenic fungi of plant crops, were inhibited in varying proportions when the alcoholic frankincense extract was added to the culture medium.

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INTRODUCTION

Dermatophytes are a pathogenic microorganism that cause many skin infections in humans and animals [1]. Dermatophytes, *Trichophyton*, *Microsporum* and *Epidermophyton* are keratin-loving as they are used as a foodstuff because they contain the keratinase enzyme that analyze keratin [2]. Dermatological injuries have become widespread all over the world, especially in hot regions and areas with overcrowded populations, as well as in areas that lack health care [3] and that it is difficult to control it, as its treatment requires the use of special antifungals such as Ketoconazole and Griseofulvin [4]. However, the use of these antagonists on a continuous basis for long periods of time leads to genetic mutations in the fungal species, which makes them more resistant to the action of these antigens [5]. At the same time, some antagonists have side effects on humans, such as itching, redness, and local irritation [6]. For the purpose of avoiding the complications of chemical drugs and to overcome the non-emergence of strains resistant to life antibiotics, attention was drawn to the use of medicinal plants as alternatives in treating fungal infections because of their medicinal value in inhibiting the growth of neighborhoods[7].

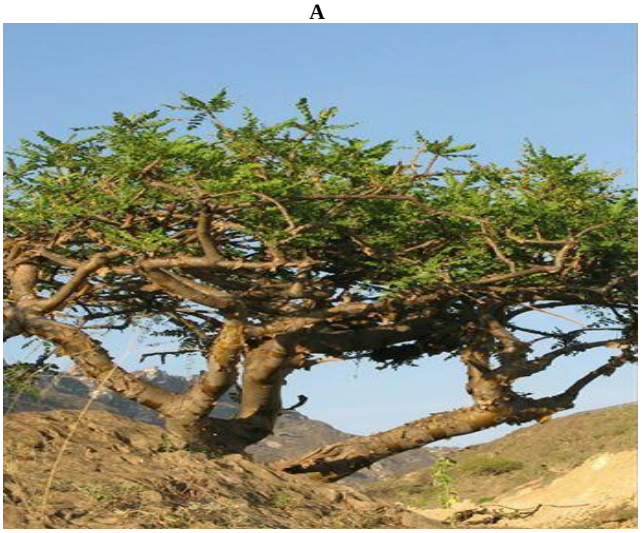
The pollution of the environment with fungi is one of the problems facing humans at present. Where the destructive effect of fungus is not limited to the destruction of agricultural crops, but there are many fungi that multiply on crops during storage or even while there is a crop is in the field [8]. When eating such foods contaminated by humans and animals, a series of health problems will begin, such as hepatotoxic, nephrotoxic poisoning, immunotoxic immunization, teratogenic mutagenic and mutagenic mutagenicization, leading to severe and chronic effects of human and animal damage to the Central nervous system, blood vessels, respiratory tract and gastrointestinal tract to death [9]. The methods used in the control of pests, including agricultural pesticides, are no longer effective in reducing the damage of these pests due to the emergence of resistant strains in many agricultural pests, as well as the increased costs of using agricultural pesticides as well as the source of environment polluting Therefore,alternative methods to reduce the damage of these pests.

The frankincense is one of the most famous materials sold in most Iraqi perfumery and medicinal herbs. It has been used since ancient times in folk medicine. It has many names, including bitter gourd, spaghetti, kandar, Boswellia, which belongs to the family Burseraceae spread in some countries of the Arabian Peninsula as well as African countries such as Somalia, Sudan and southern Egypt and extract frankincense through the wounds on the legs of this tree and then dry the resulting material to become small pieces of different shapes [10,11].

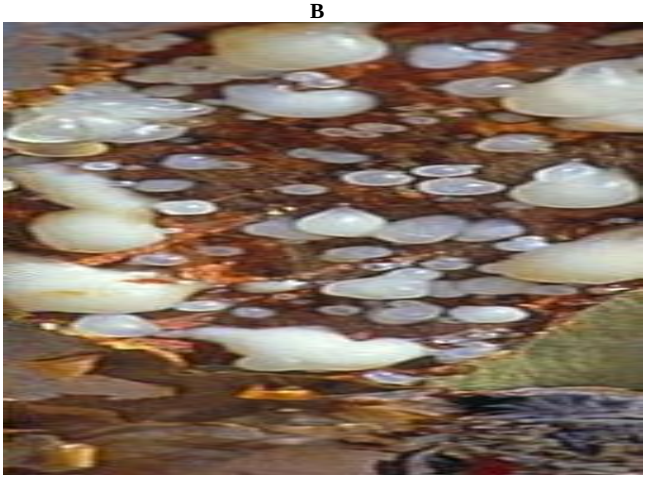
The frankincense is used in the treatment of many diseases, including alleviating the pain of rheumatism, arthritis and anti-inflammatory wounds and helps to heal quickly and treat diseases such as cough and asthma, which helps to breathe deeply, which leads to relaxation and strengthening of the heart and also used in the manufacture of incense [12]. The frankincense contains many chemical contents, including glutamic acids, emulsifiers and volatile oils [13,14] (Fig. 1).

Due to the therapeutic importance of fungal skin infections and to identify the impact of some local plants in it, and due to the economic importance of foodstuffs, as the main source of food for humans, and the possibility of causing harmful effects of human consumption of food contaminated with some fungal species Therefore, the present study aimed at the effect of the use of alcohol extract of frankincense plants against some dermatophytes and fungi that cause food damage.

A



B



C

Figure 1. **A- *Boswellia spp* tree B-Extract frankincense from the tree c- Pure frankincense**

MATERIALS AND METHODS

Collection frankincense: The frankincense material was obtained from the local markets of Karbala Governorate .

Collection of Sample of Dermatophytes: Dermatophytes were isolated from patients with dermatophytes who visited the Al-Hussein Hospital in Karbala governorate, which was 90 samples. The samples were clinically diagnosed by dermatologists from the hospital and samples were collected from skin, head hair and nails.

Direct microscopic examination: The samples were examined using a method [15] by cleaning the area with a cotton swab saturated with alcohol 70% to get rid of external bacteria and fungi Saprophytes, and then take a scrape from the affected skin, head hair or nails infected by a tool Loop pollination and then placed On a clean glass slide with a drop of 0% potassium hydroxide and then put the glass slide cover and warm the sample on the flame of a benzene lamp to melt the host cells and examined by microscope for the presence of spores or dermatophytes hypha, and for large nails parts was taken by using sterile forceps flat end and also took pieces Of infected nails after sterilization and put the infected parts taken in a small amount of potassium hydroxide 10% and left at room temperature for a whole night. Fungi were diagnosed based on the following sources: [16,17,18]. The microscopic properties and phenotypic characteristics of spores and fungal colonies were adopted by identifying the forms of hypha and spores, as well as the shape of the farm and the appearance and color of the colony from the bottom of the dish.

Collection of Sample of pathogenic fungi of plant crops: Pathogenic fungi of plant crops were taken from onions, garlic, pomegranate, oranges and apples that were infected with fungi. These samples were immersed in KoH solution at aconcentration of 10% for 1-2 minutes to purify the external surface of the samples from pollution, after the samples were taken out,They were placed on filter paper to get rid of the excess solution and washed samples with sterile distilled water. The samples were placed on the surface of the culture medium and with three replications of the sample. Then the dishes were incubated at atemperature of 25 °C. for 5 days and after the incubation period ended the isolation of the fungus was purified by taking a disk of each dish and planting it in a dish containing the Agar dextrose potato (PDA) and repeated the process several times until obtaining isolates for the fungus in a completely pure and was diagnosed The fungus is based on the taxonomic characteristics mentioned by the sources [19,20].

Preparation of alcohol extract of frankincense plant: The method [21] was followed in the extraction process, mixing 20 g of frankincense with 100 ml of 70% ethanol alcohol and leaving the solution with continuous stirring by Shaker and 37 °C for 24 hours, the filter was then filtered using several layers of medical gauze and then using Whitman No.1 filtration paper. After that, the filtration was introduced to the effect of 2500 cycles/minute and 10 minutes to the centrifuge. Then put the leachate in pure and sterile glass Petri dishes and put in the incubator at 40°C and for (2-3) days until dry the extract, then dry the extract with a clean, sterile knife and dry powder after weighing in clean plastic containers until use. This was called alcohol extract.

Effect of alcoholic extract of frankincense plant on dermatophytes growth: Method [22] were followed, The alcoholic extract of frankincense was mixed with cultivated media Sabroid Dextrose Agar (SDA) before solidification, and with three concentrations 5, 10, 15mg/ml with an average of three replicates for each concentration. After a hardening of the medium, a hole was made in the center of each dish by a cork borer piercing 5 mm in diameter. A comparison was used as no material was added to the media. The dishes were inoculated with the fungus vaccine studied and grown on the medium of SGA and at the age of 3 weeks each by planting a disk with a diameter of 5 mm each in the hole that worked in the center of the dish. All dishes were incubated at a temperature of 25 °C and for two weeks, the diameter of the developing colony was measured (the average of two perpendicular diameters). Results were recorded, and the inhibition ratio was calculated by using the following equation :

Inhibition ratio =

$$\frac{\textit{Average diameter of fungus in control dish} - \textit{Average diameter of fungus in tretment dish}}{\textit{Average diameter of fungus in control dish}} \times 100$$

Effect of alcoholic extract of frankincense plant on pathogenic fungi of plant crops: The method [23] was followed in Preparation of different concentration s of extracts , dried extracts of Frankinens were mixed with the sterile Potato Dextrose Agar medium at 250 mm befor solidification and in the three concentrations of 10, 20 and 30 mg/ml at a rate of three replication, After the hardening of the medium , a disk of ammeter 5 mm transferred from the fungus farm and at the age of 7 days to the center of the dish and incubation at 25±2°C for 7 days. The results were recorded and the assessment ratio was calculated by using the following equation:

RESULTS

Effect of frankincense plant extracts on the growth of dermatophytes growth: The results showed that the alcohol extract of the frankincense plant was highly efficient in inhibiting the studied fungi at concentrations 15, 10, 5 mg/ml. The colony had a diameter of 0 mm where the colony appeared as a small, white isolation of all concentrations for *Trichophyton mentagrophytes*. while *Trichophyton rubrum* The colony diameter was 0.00 , 0.50 , 1.75 cm at concentrations 15, 10, 5 mg/ml respectively. As for *Microsporum gypseum* ,it was The colony diameter 0.00 cm at concentrations 15, 10 mg/ml and 1.00 cm at concentration 5 mg/ml. *Microsporum canis* was colony diameter 0.75 cm at concentration 15mg/ml and 1.25 cm at concentration 10 mg/ml and 2.00 cm at concentration 5 mg/ml (Table 1).

Table 1: **Effect of alcoholic extract of frankincense plant against dermatophytes (inhibition ratio cm).**

Dermatophytes	Control	Concentration of Alcoholic Extract (mg/ml)		
		5	**10**	**15**
Trichophyton mentagrophytes	9.00	0.00	0.00	0.00
Trichophyton rubrum	9.00	1.75	0.50	0.00
Microsporum gypseum	9.00	1.00	0.00	0.00
Microsporum canis	9.00	2.00	1.25	0.75

Effect of frankincense plant extracts on the growth of pathogenic fungi of plant crops: The results showed in the Table 2 that the alcoholic extract of frankincense was highly efficient in inhibition of *A. niger* at concentration 30 mg/ml. The colony diameter was 0 cm, while the concentrations (20 and 10) mg/ml showed colony growth of 2.13 and 2.88 cm respectively.

As for the *A. terrus*, the alcohol extract showed efficiency in inhibition of the fungus at a concentration of 30 mg / ml. The diameter of the colony was 0 cm, while the concentrations (20 and 10) mg/ml showed colony growth of 1.75 and 2.00 cm respectively. In the case of *Penecillum expasum* , the alcohol extract showed high efficiency in inhibiting it. In the (30, 20 and 10) mg/ml concentrations, the diameter of the colony was 0 cm .

The results showed that the alcoholic extract of frankincense has a high efficiency in inhibiting of *P. digitatum*. The fungus diameter was 0 cm at 30 mg/ml and 0.83 cm at the concentration of 20 mg/ml and 5.1 cm at the concentration of 10 mg/ml. *Alternaria* sp was colony diameter 0cm at concentration 30, 20 and 10 mg/ml Table 2.

Table 2: **Effect of alcoholic extract of frankincense against pathogenic fungi of plant crops (inhibition ratio cm).**

Dermatophytes	Control	Concentration of alcoholic Extract (mg/ml)		
		10	**20**	**30**
A. niger	9.00	2.88	2.13	0.00
A. terrus	9.00	2.00	1.75	0.00
P. expasum	9.00	3.04	1.50	0.00
P. digitatum	9.00	5.1	0.83	0.00
Alternaria sp.	9.00	0.00	0.00	0.00

DISCUSSION

Many The frankincense plant is considered one of the medicinal plants as it has a high efficiency in inhibiting dermatophytes. These results were consistent with the findings of several studies where [24] found that the water and alcohol extracts of Frankinens had a inhibitory effect against the S.aureus bacteria and the coagulase negative Staphylococci (CoNS) *S. epidermis* (S). As study [25] showed, extracts from Boswellia serrata and Boswellia carterii reduce inflammatory conditions in the course of rheumatism by inhibiting leukocyte elastase and degrading glycosaminoglycans. It is believed that the important activity of this substance is due to the presence of Boswellic acides and that works in addition to being anti-inflammatory as anti-cancer, allergic and others [26].

The study also showed that the alcoholic extract of the frankincense plant has a high efficiency in the growth of pathogenic fungi of plant crops, as the results indicate that the higher the concentration, the greater the inhibitory activity, and this paves the possibility of using higher concentrations of frankincense extract, especially as some studies confirm the lack of toxicity of this extract when used in laboratory animal experiments [27].

The results also agree with a study [28] that found the essential oils extracted from *Boswellia serrata* had a inhibitory effect on the field fungus and storage of *Aspergillus flavus* and *Fusarum verticillioides* where the inhibition ratio was 62.6% and 75.5%. % Respectively. The antifungal efficacy of the frankincense plant may be attributed to the content of monoterpene, dyterpene and cisotropine [25]. Also, the frankincense plant contains in its composition Chemical substances are phenolic and these substances are known for their antibacterial efficacy [29].

CONCLUSIONS

The current study was seen that the alcoholic extract of Frankinens has inhibitory activity against the growth of some fungi isolated from the skin, head hair and nails. and the highest rate of extracted effect was on *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Microsporum gypseum*, while the lowest rate of extracted effect was on *Microsporum canis*. Also study showed that, the alcoholic extracts of Frankinens inhibited the growth of pathogenic fungi of plant, He found that inhibited the growth all studied fungi by 100%, while the lowest rate of effect extracted on *P. expasum*.

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