



***In vitro* Antifungal Activity of Musk**

Kamil M. M. AL-Jobori¹, Aseel I. AL- Ameen², Noor M. Witwit¹

¹Institute of Genetic Engineering and Biotechnology for Post Graduate Studies, University of Baghdad - Iraq

²College of Veterinary Medicine, University of Baghdad -Iraq

ABSTRACT

Fungi are everywhere. Fungi play a great role in causing some of the dangerous diseases affecting human, animal and plant. This study was carried out to evaluate in vitro effects of different concentrations of musk (25, 50, 75 or 100 %) and amounts (1, 2 or 4 ml) on five fungi include Aspergillus fumigates , Aspergillus niger, alternaria Spp., Trichomyphyton mentagrophytes, and Fusarium Spp. Results indicated that all concentrations and amounts of musk had inhibitory effects on the growth of studied pathogenic fungi and eliminated completely. The results revealed that musk has inhibitory and killer effect at the low concentration 25 % and small amount 1 ml. Also showed that the musk was more effective than the antibiotic Clotrimazole . These results indicated that musk can be used as safe natural product in management and control of pathogenic fungi, so it provides a promising source for new drugs development.

Keywords: Musk, Pathogens, Fungi, inhibition, Antibiotics

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INTRODUCTION

Musk is known to have been used in medicine and as a fragrance since 3500 BC. The musk scent was thought to have been used in the early civilizations of ancient China and ancient India in ritual purposes [1]. Musk is currently used for expensive perfume all over the world and for traditional medicine in oriental countries . Musk is formed of several compounds, the main compound which causes the odour is muscone(3- methylcyclopentadecan-one -1) the active ingredient of musk [2], has medicinal properties, Other compounds present in musk include steroids, paraffins, triglycerides, waxes, muco pyridine, other nitrogenous substances and fatty acids [3, 4].It has been long used in traditional medicine a sedative and stimulant of the heart, nerves, breathing ,sexual [4,5, 6], in resuscitation and refreshment, promoting blood flow and clearing channels, detumescence and alleviating pain [7]. It is also thought to be effective against snake venom and as an anti-inflammatory agent and to treat a variety of ailments [8, 9].

Fungi are everywhere. Fungi that are pathogens are usually plant pathogenic, there are approximately 1.5 million different species of fungi on earth; fungal diseases are often caused by fungi that are common in the environment. Fungi live outdoors in soil and on plants as well as on many indoor surfaces and on human skin. Most fungi are not dangerous, but some types can be harmful to health [10 , 11] .Fungi According to Hawksworth [12], there are a little more than 400 of these species are known to cause disease in animals, and far fewer of these species will specifically cause disease in people.

Fungi can cause Aspergillosis, pneumomycosis or bronchomycosis. The most common fungus causing diseases is *Aspergillus fumigatus*, however, other species can cause diseases such as *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus terreus*. Clinical signs of *Aspergillus* infection can be classify into three types: Allergic Aspergillosis, with similar symptoms to bronchial asthma disease, and the third is the infection with the invasive Aspergillosis [13]. *Fusarium* is one of the opportunistic fungi, its toxicity is known by Fusariotoxicosis caused by mold corn toxicosis in many animals. Besides the harm occurs due to *Fusarium* infection that can cause stem rotting of *Zea mays* and necrosis, as well as scab of barley and wheat. Makun *et al.* [14] found that among 49 millet there were 12 of them infected by Aflatoxin B1 and 35 out of 55 of isolated fungi to study their toxin production are considered a rat killer were *Fusarium*, *Aspergillus*, *Penicillium*, *Mucor*, and *Rhizopus*.

Due to the widespread and often indiscriminate use of antimicrobial drugs, many microorganisms have acquired resistance to specific antibiotic treatments and these strains are particularly evident in the

hospital environment [15]. This has created immense clinical problems in the treatment of infectious diseases [16]. In addition to this problem, antibiotics are sometimes associated with adverse effects on host, which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppression and allergic reactions [17]. Repeated consumption of antibiotics lead to development of more resistant fungi and increased damages of great amount of disease spread with side effects. Hsueh *et al.* [18] mentioned that among 59 isolated spore species from *C. glabrata* about 16 appeared isolated (27%), and were not affected by the antifungal fluconazole. Because of the side effects and the resistance that pathogenic microorganisms build against the antibiotics, therefore, it is worthwhile to look for an alternative cure such as extract biological active compounds from plants species that used in herbal medicine [19] or Musk [20, 21, 22]. Most researches were directed and dedicated to study and discover new natural sources that can suppress pathogenic fungi and replace chemical use of the antifungal drug. One of those sources was the musk, many investigations were carried out to study the use of musk to inhibit the growth of many pathogenic microorganisms for human, animals and plants [23]. Saddiq [24] mentioned that 25% of musk gave the highest percentage of suppression of biomass for each of *A. niger*, *F. oxysporum* and *C. albicans*. Saddiq and Al-Elyani [25] mentioned the high potency of both musk and sider in limiting liver toxicity in rats treated with *Aspergillus flavus* and Aflatoxin. Saddiq [20] reported the ability of musk to inhibit the growth of *Penicillium puberulum* fungus. Saddiq and Kalifa [26] proved the effectiveness of musk and sider extract in treating renal mycotoxicity Al-Jobori *et al.* [22] reported that musk has inhibitory effects on the growth of *Cryptococcus neoformans*, *Candida albicans* and *Saccharomyces cerevisiae*, results also showed that the musk was more effective than antibiotics. Badawy *et al.*[21] mentioned that Musk is a safe natural product having the privilege of being anti *Trichomonas vaginalis* as well as antifungal. This study was carried out to evaluate *in vitro* effect of different concentrations and amounts of Musk on five types of pathogenic opportunistic fungi. The experimental fungi are *Aspergillus fumigates*, *Aspergillus niger*, *alternaria Spp.*, *Trichomphyton mentagrophytes*, and *Fusarium Spp.*

METHODOLOGY

Musk

Synthetic musk (a Pakistani product) was purchased from local Iraqi markets. The various concentrations (25, 50, 75 or 100 %) and amounts (1, 2 or 4ml) of musk were tested for their inhibitory potency were and alcohol as control.

Microorganism Strains

A total of five isolates of fungi namely are *Aspergillus fumigates*, *Aspergillus niger*, *alternaria Spp.*, *Trichomphyton mentagrophytes*, and *Fusarium Spp* were isolated and diagnosis from Zoontic Disease Unit, College of Veterinary Medicine, Baghdad University. They were maintained on sabaroud dextrose agar.

Screening of Antibacterial and Antiyeast Activity

Sabouraud dextrose Agar (SDA) (Merck Company) was used as base medium for screening of antifungal activity.

Preparation and Standardization of Inoculums

Four to Five colonies from pure growth of each test organism were transferred to 5 ml. of broth (SDB). The broth was incubated at 25 °C for three days. The turbidity of the culture was compared to 0.5 Mcfarland Nephelometer Standard which contains 1.5*10⁸ cell ml⁻¹, the standardized inoculums suspension was inoculated within 15 – 20 minutes.

Experimental Study *In vitro*

The experimental study *in vitro* for screening fungal activity was carried out according to [27]. 19, 18 or 16 ml of agar were sterilized at 121°C for 20 min in the autoclave, and then mixed with the amounts 1, 2 or 4 ml from each concentration 25, 50, 75 or 100 % of musk. The agar-musk mixture was then poured into 75 mm Petri dishes and was allowed to cool and set. The SDA plates were seeded with 0.1 ml of standardized inoculums of each test organism (*Aspergillus fumigates*, *Aspergillus niger*, *alternaria Spp.*, *Trichomphyton mentagrophytes*, and *Fusarium*). The inoculums were spread evenly over plate with loop or sterile glass spreader or cotton swab, and ethanol 80% was used as control. The experiment was performed five times.

Incubation

The inoculated plates were incubated at 25 °C for 7 days, and the activity of musk was determined by measuring the diameter of inhibition zone(mm).

Sensitivity Test for Antibiotic

Discs of antibiotics were used to comparative between sensitivity of fungi for musk activity and drugs of antibiotics. Clotrimazole 0.01g/ml (1% dilution) was used as the control. The Petri dishes were left at

room temperature for 2 hours to allow the extract diffuse into the medium after which it was incubated at room temperature for 7 days [28,29].

Statistical study

The experiment was conducted and analyzed as a factorial experiment with five replication in a Completely Randomized Design (CRD). Statistical analysis was performed using Statistical Analysis System- SAS -computer package program [30]. The means were separated following least significance deference (LSD) test.

RESULTS

Effect of musk concentrations was significant on fungi in all treated types at the end of incubation period (Table 1). All concentrations (25 , 50 ,75 or 100 %) inhibited the growth of fungi and eliminated completely, and gave the inhibition zone of 75 mm at all concentrations (figure 1) , with the exception of the fungus *T. mentagrophytes* , who has exhibited weak resistance and showed growth 5.33 % at 25% and 6.23% at 100% , and the fungus *A. niger* 6.67% at 100% . Whilst control treatment showed intensive growth of fungus (figure 2). There were no significant differences between musk concentrations; also fungi did not differ significantly in their response to musk treatments.

Table 1. Effect of musk concentrations on fungal types growth

Fungal types	Diameters of inhibition zones (mm)#					Mean of fungal types
	Musk concentrations (%)					
	0	25	50	75	100	
<i>Aspergillus fumigatus</i>	0.00	75.00	75.00	75.00	75.00	75.00
<i>Aspergillus niger</i>	0.00	75.00	75.00	70.00	70.00	72.50
<i>Trichophyton mentagrophytes</i>	0.00	71.00	75.00	75.00	70.33	72.83
<i>Alternaria Spp.</i>	0.00	75.00	75.00	75.00	75.00	75.00
<i>Fusarium Spp.</i>	0.00	75.00	75.00	75.00	75.00	75.00
Mean of musk concentrations	0.00	74.20	75.00	74.00	73.00	

Inhibition zones (75mm)diameter

L.S.D.0.05 (conc .25% =3.09 , conc. 50% = N.S , conc. 75% = 3.11 , conc. 100% = 3.19)

L.S.D.0.05 (fungal types = N.S , conc. = N.S , fungal types *conc.= N.S)



Figur 1.effect of musk treatment at concentration of 100% in amounts of 1,2 and 4 ml. on *A. fumigatus*.



Figur 2. Control treatment (with out musk).

Table 2 shows that all musk amounts (1, 2 or 4 ml) used in this experiment inhibited fungi growth and eliminated completely, the inhibition zone was 75 mm at all amounts. With the exception of *A. niger* fungus, which showed growth slightly 10% when using the amount 1 ml , and the fungus *T. mentagrophytes* 4.67 and 4.0 % when using the amounts of musk 1 and 2 ml , respectively. There were no significant differences between musk amounts; also fungi did not differ significantly in their response to musk amounts. With the exception of at 1ml where fungi differed significantly in their response, *A. fumigates*, *Alternaria Spp.* and *Fusarium Spp.* were eliminated completely, whilst *A. niger* and *T. mentagrophytes* showed weak resistance to musk.

Table 2. Effect of musk amounts on fungal types growth

Fungal types	Diameters of inhibition zones (mm)#			Mean of fungal types
	Musk amounts (ml)			
	1	2	4	
<i>Aspergillus fumigatus</i>	75.00	75.00	75.00	75.00
<i>Aspergillus niger</i>	67.5	75.00	75.00	72.50
<i>Trichophyton mentagrophytes</i>	71.5	72.00	75.00	72.83
<i>Alternaria Spp.</i>	75.00	75.00	75.00	75.00
<i>Fusarium Spp.</i>	75.00	75.00	75.00	75.00
Mean of musk quantities	72.8	74.4	75.00	

Inhibition zones (75mm)diameter

L.S.D.0.05 (amount 1 ml =3.23 , amount 2 ml = N.S, amount 4 ml = N.S)

L.S.D.0.05 (fungal types = N.S , amount = N.S , fungal types *amount = N.S)

The most activity was found in the interaction between the amounts 2 and 4ml with all concentrations (25 , 50 , 75 or 100%) , whilst the interaction between the amount 1 ml with concentrations showed a degree of a antifungal activity (Table 3).

Table 3. Effect of musk concentration and musk quantities on inhibition zone.

Musk concentrations (%)	Diameters of inhibition zones (mm)#			Mean of musk conc.
	Musk amounts (ml)			
	1	2	4	
0	0.00	0.00	0.00	0.00
25	72.60	75.00	75.00	74.20
50	75.00	75.00	75.00	75.00
75	72.00	75.00	75.00	74.00
100	71.6	72.60	75.00	73.07
Mean of musk quantities	58.24	59.52	60.00	

Inhibition zones (75mm)diameter

L.S.D.0.05 (conc .25% =N.S, conc. 50% = N.S, conc. 75% = N.S, conc. 100% = N.S)

L.S.D.0.05 (amount 1 ml =N.S, amount 2 ml = N.S, amount 4 ml = N.S)

L.S.D.0.05(conc.= N.S , amount= N.S , conc* amount= N.S.)

The interaction of amounts (1, 2 or 4 ml) and concentrations (25 , 50 , 75 or 100 %) of musk with the types of fungi (*Aspergillus fumigates* , *Aspergillus niger* , *alternaria Spp.*, *Trichomphyton mentagrophytes*, and *Fusarium*) did not show significant differences in the effectiveness of inhibitory (Table 4).

Table 4. Effect of musk concentration and musk quantities on fungal types growth

Musk conc. (%)	Diameters of inhibition zones (mm)#														
	0			25			50			75			100		
Musk amounts (ml)	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4
Fungal types															
<i>Aspergillus fumigatus</i>	0	0	0	75	75	75	75	75	75	75	75	75	75	75	75
<i>Aspergillus niger</i>	0	0	0	75	75	75	75	75	75	60	75	75	60	75	75
<i>Trichophyton mentagrophytes</i>	0	0	0	63	75	75	75	75	75	75	75	75	73	63	75
<i>Alternaria Spp.</i>	0	0	0	75	75	75	75	75	75	75	75	75	75	75	75
<i>Fusarium Spp.</i>	0	0	0	75	75	75	75	75	75	75	75	75	75	75	75

Inhibition zones (75mm)diameter

L.S.D.0.05(fungal types *conc. * amount =N.S)

The results from the bioassay are tabulated in Table 5. *T. mentagrophytes* showed resistance for antibiotic Clotrimazole with inhibitory zone 5 mm. Higher inhibitory effect showed on *A. fumigatus* and *A. niger* with zone diameter 34.0 and 36.0 mm, respectively (Table 5). Also *Alternaria Spp* and *Fusarium Spp* were susceptible to the antibiotic than *T. mentagrophytes*. In all, musk exhibited more pronounced inhibitory effect on fungi compared to antibiotic.

Table 5. Antibiotics sensitivity of fungi

Fungal types	Diameters of inhibition zones (mm)#
	Clotrimazole antibiotic
<i>Aspergillus fumigatus</i>	34
<i>Aspergillus niger</i>	36
<i>Trichophyton mentagrophytes</i>	5
<i>Alternaria Spp.</i>	23
<i>Fusarium Spp.</i>	21

Inhibition zones (75mm)diameter

L.S.D.0.05 = 3.329

DISCUSSION

We have studied the influence of musk and pharmaceutical form on clotrimazole activity against 5 fungi isolate. The standardized method for the susceptibility testing of antifungal is the broth dilution method [31], we have used a method derived from the agar diffusion method of susceptibility testing to antimicrobial musk and drugs, in order to test the ability to diffuse from different concentrations. Many investigations were carried out to study the use of musk to inhibit the growth of many pathogenic microorganisms for human, animals and plants [12,20, 21,22].

Table 1 shows the inhibitory effect of Musk extract on the growth of *Aspergillus fumigates*, *Aspergillus niger*, *alternaria Spp.*, *Trichomphyton mentagrophytes*, and *Fusarium Spp.* pathogens. Results indicated that Musk extract is more effective on the tested fungi, All concentrations (25, 50, 75 or 100%) and amounts (1, 2, 4 ml) inhibited the growth of fungi and eliminated completely, and gave the inhibition zone of 75 mm at all concentrations, with the exception of the fungus *T. mentagrophytes*, who has exhibited weak resistance and showed growth 5.33% at 25% and 6.23% at 100%, and the fungus *A. niger* 6.67% at 100% (Tables 1, 2). The results revealed that musk has inhibitory effect at the low concentration 25% and small amount 1 ml. Our results were in agreement with [24] who mentioned that 25% of musk gave the highest percentage of suppression of biomass for each of *A. niger*, *F. oxysporum* and *C. albicans*. Other authors [13,21,22] reported that musk has inhibitory effects on the growth of fungi.

Musk had great role in suppression of the opportunistic fungal growth. Musk action can be caused by chemical structure of musk as it contained muscone the active ingredient of musk (2), other compounds and metabolic products such as alkaloids, flavonoids, sterols and antibiotics which have great effect as antimicrobial agents [32]. Highly volatile oils percentage and contain sterol hormones in which the most important was muskopyridine besides some enzymes that can elongate lag phase or affect mitotic divisions and elongate fungal cells acids [3,4]. These compounds may affect fungi cells through disrupting their membranes, thereby depriving the substrate or inactivating the enzymes. This leads to cell lysis and death. Cowan [33] suggested that polyphenols act on the microbes by disrupting their membranes, depriving the substrate or inactivating the enzymes. Also, Musk extract compounds may inhibit the

microorganisms through inhibiting the synthesis of nucleic acids resulting in formation of abnormal proteins [34]. However, its inhibitory effect may be due to the presence of volatile oils [35].

There were no statistically significant effect of the interaction between musk concentrations with musk amounts or (fungi x concentrations x amounts) (Tables 3, 4). Results presented in these tables indicate the inhibitory and lethal effectiveness of musk at the low concentrations and small amounts on all types of fungi studied in this experiment. Saddiq (36) indicated that treatment with Musk extract and Seder is highly effective in growth inhibition and reducing the biomass of *Aspergillus flavus* pathogenic fungus, that produce Aflatoxin resulting various hazards for bio tissues as liver toxicity. Jan and Agar [37] mentioned that musk caused inhibition in spore germination of five otomycotic pathogens *Aspergillus niger*, *Aspergillus flavus*, *Absidia corymbifera*, *Penicillium nigericans* and *Candida albicans*. Musk can also decrease growth due to suppression of spores or due to formation of complex toxic substance formed after joining the protein with musk inside the cells and enzyme activity suppression can affect negatively the metabolic processes of the pathogenic fungus during the growth period, that is similar to the role of fungicidal substances that cause suppression [38, 39].

Occasionally, in some cases, antifungal therapy is a failure because of resistance to the antifungal drugs by the fungi. Table 5 shows affection of Clotrimazole drug. The fungus was *T. mentagrophytes* show more resistance compared with other fungi. Musk also proved more effective against the tested fungi more than Clotrimazole antibiotic (Tables 1-5). Clotrimazole (1-o-chloro- α,α -diphenylbenzyl) imidazole is a synthetic imidazole, having a broad spectrum of fungicidal activity, being effective against both dermatophytes and yeast-like fungi. The mechanism may involve an action on the fungal cell membrane whereby the uptake of essential nutrients is inhibited (28). Previous studies indicated that musk was more effective than Nystatin antibiotic [25], and Clotrimazole antibiotic [22]. It was discovered that the high concentration of musk 100% was less effective compared with other concentrations as shown in Tables 1, 2 and 4. In previous study, AL-Jobori *et al.* [22] attributed the reason of this probably due to the fact that musk with high concentration had high viscosity and caused cracks in the media, which impeded its spread through the media.

CONCLUSION

This study suggested that musk have an efficient role in suppression and eliminated of pathogenic fungi. In comparison with antibiotics, the results showed that the musk was more effective than Clotrimazole antibiotic. low concentrations and small amounts of musk is hereby recommended for use.

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