

Antifungal Activity of *Commiphora myrrha* L. Against Some Air Fungi

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To avoid the harmful effects of the chemical fungicides on the human and minimize the environmental pollution, an alternative ecofriendly control strategies should be developed. The extract of *Commiphora myrrha* L. was tested against twenty fungal genera isolated from the indoor air collected from different rooms in King Saud University, kingdom of Saudi Arabia. Disc diffusion test was modified for use in this study and the collected data was statistically analyzed. Variable antifungal efficacy of different *myrrh* extract was recorded against the investigated fungal genera. The efficacy of the extract was increased as the concentration increased. The highest growth inhibition (74.6%) was against *Acremonium strictum* followed by *Trichoderma pseudokoningii* (70.6%). On contrast, the lowest efficacy (12.7%) was against *Ulocladium consortiale*. It could be concluded that *myrrh* extract is promised as a source of substances from which of safer and ecofriendly could be used as antimicrobial agents against number of pathogenic fungi.

Key words: Airborne , *Myrrh*, Antimicrobial, Medicinal plant.

Fungi are among the most widespread and common bio-pollutants all over the world. The variable distribution of those myco-pollutants units in the air over time¹, and the presence of several micro-fungi in the air is associated with risks to human health^{2,3}.

The use of Medicinal plants in the traditional medicines had frequently been documented in many countries^{4,5,6,7}. One of the main sources of active substances used for medicine manufacturing is the medicinal plants that represent a significant part of agriculture and forestry^{8,9}. The medicinal plant products proved to play a major role in the discovery of effective antimicrobial agents^{10,11}.

The use of herbaceous plants which are not risky to the human health and environment, instead of the chemical fungicides is a potentially powerful alternative method^{12,13}. Stem, root, bark, flower and leaves of several plants had been reported to possess antimicrobial properties^{14,15}.

Plant extracts from which represent a rich source of safer and ecofriendly antimicrobial agents had effectively been used against number of pathogenic fungi worldwide^{16,17}.

Myrrh is a traditional drug being used in Arabian and North African regions since ages for successful treatment of many clinical symptoms. It constitutes exudates coming out from stem of plants belonging to different species of genus *Commiphora*, family Burseraceae^{5;18}.

Therefore, the present study aimed to evaluate the potential of *myrrh* extracts against 20 indoor fungal species isolated from different colleges of King Saud University.

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MATERIALS AND METHODS

Plant material

Dried exudates sticking to the bark of a *myrrh* tree growing in the compound of the Directorate of Bait al-Faqih Hodeida, Yemen was collected to be used in this investigation.

Preparation of plant extracts

Gum-resin of guggul were extensively washed under running tap water for removal of dust particles, followed by washing with sterilized distilled water. They were further air-dried on filter paper at room temperature and then powdered with the help of sterilized pestle and mortar under aseptic conditions. Dry powder was further extracted in ethanol. Ten percent (w/v) extract of *myrrh* in ethanol was prepared by shaking 10 g of *myrrh* powder in 100 ml of ethanol for 4 hours. Subsequently, the preparation was centrifuged at 8000 x g for 10 minutes and supernatant was collected as crude extract. Test tubes were cotton plugged and stored in refrigerator at 4°C until use.

Test organisms

Twenty indoor fungal species belonging to 15 different genera; collected from indoor air of different colleges of King Saud University, by the aid of air sampler were tested in this study (Table 1). Fungal identification was carried out based on morphological and microscopic characteristics in the Mycological Center, Assiut University, Egypt.

Antimicrobial assays

In order to determine the susceptibility of the tested fungi to the *myrrh* different concentration, 5, 10, 15, and 20% solutions were prepared separately by dissolving 5, 10, 15, and 20 g of *myrrh* exudates in 100 ml of ethanol respectively.

Antimicrobial activity of *myrrh* in different concentrations was assayed with a modified version of disc diffusion technique (Ref.) as follows :

Discs of 30mm diameter were cut out from No. 1 Whatman filter paper and were washed thoroughly with tap water followed by distilled water. The discs were wrapped in butter paper and were autoclaved at 20 psi for 20 minutes. Sterilized discs in butter paper were dried in hot air oven. Sterilized discs were saturated with 50 µl of the different concentrations of *myrrh* extract brier to place on the surface of PDA plates. The discs were

then inoculated with 6mm diameter plugs cuttings from the margins of fungal cultures. Treated plates were incubated at 25±2 °C for 5 days after which; diameter of the colony was measures. Five plates were maintained as replicates for each treatment and equal number for its corresponding control. The collected data converted into growth inhibition comparing with the control plates.

Inhibition percentage in each treatment was calculated according to the following formula used by Derballah¹⁹:

Inhibition potential = $[(x - y) \div x] \times 100$; where

x = size of the colony on control disc

y = size of the colony on test disc

Statistical analysis

SPSS-16 statistical package was used for ANOVA of the obtained data. The Least Significant Difference (LSD) was used to compare means.

RESULTS

Antifungal efficacy of *myrrh* extract.

The highest efficacy of *myrrh* extract (more than 70% growth inhibition) was against only one fungus representing 5% of the tested fungi. On the other hand, 25% of the tested fungi exhibited growth inhibition ranged from 11% to 40%. Fourteen isolates constituting 70% of the tested genotypes showed amoderate inhibition of 41–70%.

The highest growth inhibition (74.6%) was against *Acremonium strictum* followed by *Trichoderma psuedokoningii* (70.6%). On contrast, the lowest efficacy (12.7%) was against *Ulocladium consortiale* (Table-2)

Effect of *myrrh* concentrations on antifungal efficacy

In order to evaluate the efficacy of different concentrations of *myrrh* extract; six fungal genera were tested. Figure 1 illustrated the effect of different concentrations of *myrrh* extract on the fungal radial growth. Variable antifungal efficacy of different *myrrh* extract was recorded against the investigated fungal genera that could be categorized. The efficacy of the extract was increased as the concentration increased. Representative isolates belonging to high, moderate, and low susceptibility categories behaved differently to increase in the concentration of *myrrh* extract. In high and moderate

susceptibility category, inhibition level declines to half in 20% extract as compared to 5% extract. In the low susceptibility category, however, inhibition level was similar in all treatments (Table 3).

Table 1. List of the tested fungi

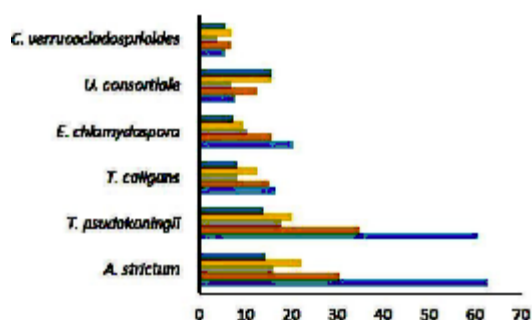
No.	Fungi
1	<i>Acremonium strictum</i>
2	<i>Aspergillus flavus</i>
3	<i>Aspergillus sydowii</i>
4	<i>Chaetomium globosum</i>
5	<i>Cladosporium cladosporioides</i>
6	<i>Cladosporium sphaerospermium</i>
7	<i>Cladosporium verrucocladosporioides</i>
8	<i>Cochliobolus spicifer</i>
9	<i>Drechslera biseptata</i>
10	<i>Embellisia chlamydospora</i>
11	<i>Eurotium amstelodami</i>
12	<i>Fusarium semitectum</i>
13	<i>Myceliophthora lutea</i>
14	<i>Penicillium chrysogenum</i>
15	<i>Penicillium fellutanum</i>
16	<i>Penicillium reticulosum</i>
17	<i>Phoma tropica</i>
18	<i>Torula caligans</i>
19	<i>Trichoderma pseudokoningii</i>
20	<i>Ulocladium consortiale</i>

Table 2. Antifungal efficacy of *myrrha* extract against airborne fungi

Fungi	Inhibition (%)	Inhibition class (%)	Distribution (%)
<i>A. strictum</i>	74.6 ^a	71-80	5
<i>T. pseudokoningii</i>	70.6 ^a	61-70	20
<i>C. spicifer</i>	69.4 ^a		
<i>P. tropica</i>	66.1 ^a		
<i>M. lutea</i>	64.0 ^{ab}		
<i>E. stlodami</i>	53.9 ^b	51-60	20
<i>D. biseptata</i>	53.4 ^b		
<i>C. globosum</i>	52.3 ^b		
<i>P. fellutanum</i>	51.8 ^{bc}		
<i>T. caligans</i>	50.8 ^c	41-50	30
<i>E. chlamydospora</i>	50.0 ^c		
<i>A. sydowii</i>	48.9 ^c		
<i>F. semitectum</i>	46.0 ^c		
<i>C. sphaerospermium</i>	43.3 ^c		
<i>C. cladosporioides</i>	41.3 ^c		
<i>P. reticulosum</i>	40.0 ^{cd}	31-40	10
<i>A. flavus</i>	34.6 ^d		
<i>P. chrysogenum</i>	30.4 ^{de}	21-30	10
<i>C. verrucocladosporioides</i>	26.1 ^e		
<i>U. consortiale</i>	12.7 ^e	11-20	5
LSD t 5% = 12.73, SE ± 6.29			

DISCUSSION

Results of this study proves in vitro antifungal activity of the different concentrations used of *myrrha* extracts against the 20 tested fungi. This finding confirmed the documented antifungal activity of many plant extracts against several fungal genera.^{12,13}. Plant extracts from which represent a rich source of safer and ecofriendly antimicrobial agents had effectively been used against number of pathogenic fungal genera^{16, 17}.



Treatments upward are Control, 5%, 10%, 15% and 20% respectively

Fig. 1. Effect of different concentrations of *myrrha* extract on the fungal radial growth

Table 3. Effect of *myrrh* different concentrations on the growth of tested fungi

Fungi	Growth inhibition (%) / concentrations				Fungal sensitivity
	5%	10%	15%	20%	
<i>A. strictum</i>	51.60	74.56	64.90	77.13	H
<i>T. pseudokoningii</i>	42.85	70.59	67.03	77.47	
<i>T. caligans</i>	10.02	50.83	26.03	52.01	M
<i>E. chlamydospora</i>	22.92	50.03	54.11	63.94	
<i>U. consortiale</i>	-60.76	12.65	-99.87	-104.30	L
<i>C. verrucocladosprioides</i>	-25.14	26.08	-31.33	-6.38	
Mean	6.92	47.45	13.48	26.65	
Standard error	± 9.09	± 7.2	± 9.78	± 7.82	

H= highly sensitive; M= moderately sensitive; L=low sensitive

Myrrh is a traditional drug being used in Arabian and North African regions since ages for successful treatment of many clinical symptoms. It is imported into India since long time and used in perfumery as food additive, fragrance, incense, antiseptic, astringent, stimulant, stomachic, tonic and for embalming²⁰.

This is the first research describing the activity of *C. myrrha* on this species. It constitutes exudates coming out from stem of plants belonging to different species of genus *Commiphora*, family Burseraceae⁵; resins (23-40%), volatile oils (2-8%) and a bitter principle (10-25%); and has a characteristic odor ascribed to the presence of furanosesquiterpenes¹⁸. Numerous researchers have investigated the phytochemistry of *myrrh*, reporting a number of diverse chemical constituents in resin, gum and oil. These reports have been reviewed by Hanus⁵. Ethanol and ether extracts of *Commiphora myrrha* were evaluated for their antimicrobial activity against two Gram-negative organisms (*Escherichia coli* and *Pseudomonas aeruginosa*), two Gram-positive organisms (*Bacillus subtilis* and *Staphylococcus albus*) and fungi represented by *Candida albicans* isolated from gazelles held at King Khalid Wildlife Research Centre, Thumamah²¹.

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