Activity of Myrrh (*Commiphora molmol*)
Essential Oil on Growth of *Candida Albicans*

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Abstract
The study was conducted to evaluate the effect of Myrrh essential oil on growth of three isolates of *Candida albicans*. Results showed inhibitory effect of Myrrh essential oil on growth of the tested isolates. MIC for vaginal and nail isolates was 5% where is for the standard isolate it was 10%. Additionally, Essential oil affect morphology types of *Candida albicans* cells, resulting double increase in pseudohyphae percentage and double decrease in budding cell percentage compared to the control types which may indicate possible effect on division and reproduction.

Activity of essential oil of Myrrh using gaseous contact method showed inhibitory effect of the oil for the three isolates with MIC more than (75) µl.

Introduction
Myrrh is an oleo gum resin obtained from the stem of *Commiphora molmol* (Family: Burseraceae). Trees of Myrrh are native to desert areas of northern Africa and the Middle East (1).

Traditionally, Myrrh has been used by Sumerians and Greeks to treat "Worms" (2), by Chinese to relieve pain and swelling due traumatic injury (3). In modern times, tincture of Myrrh is used for therapy of aphthous ulcer and for reduction of cholesterol and triglycerides (4), it has also cytotoxic and anticarcinogenic potentials (5), and proved to be effective in the treatment of fascioliasis (1). Studies have began to confirm that Myrrh has antibacterial, antifungal and anti-inflammatory properties (6,7,8). Various publications have documented the antimicrobial effect of essential oils produced by
plants, and since there is very little reports in the literature concerning the essential oil of Myrrh, therefore the aim of the present study was to assess the anticandidal activity of essential oil produced by Myrrh using two different methods.

**Material and Methods**

- **Organism and inoculum preparation:**
  Two pathogenic isolates as well as a standard isolate (ATCC 102301) of *C. albicans* were obtained from the culture collections of the Department of Biology, College of Education, Ibn Al-Haithm, Baghdad University. Cultures were maintained on SDA. Inoculum for each isolate was prepared by taking a single isolated colony grown on SDA for (24-48) hr., dispensed in 5ml sterilized distilled water, the inoculum then adjusted to approximately $10^7$ (CFU/ml). The suspension was further diluted with distilled water as required.

- **Essential oil**
  Extraction of Myrrh oil from resin was done in our laboratory using a "Quick-Fit" essential oil Distillation apparatus.

- **Test for anticandidal study**
  Two test methods for anticandidal activity of Myrrh oil were used:

  A- Broth dilution methods: a series of two fold dilution of oil ranging from 0.25-10% (v/v) was prepared in SD broth and a final concentration of (0.05%) tween-80 was incorporated into the broth medium to enhance oil solubility. Inoculum of (0.1) ml was added to each tube. Tubes were incubated for (24) hr at (30) °C. A drop from each tube was taken and put on clean slide, adrop of lactophenol cotton blue was added to each slide and covered with cover slip, slides were examined to assess the effect of different oil concentrate on types of cell growth. To determine the MIC of the oil, serial dilution from the cultured medium was made and (0.1)ml from each dilution was put in a petridish (three replications for each dilution) in addition to the control treatment without oil, SDA medium was poured in each plate, plates were incubated at (30)°C for (48)hr. Minimum inhibitory concentrations (MIC) was determined as the lowest concentration of oil inhibiting the visible growth on the agar plate.

  B- **Effect of vapour of essential oil:**
  The technique used in this study is derived from the microatmosphere method of kellner and kober and modified by (9)
which carried out as the following: Pyrex glass petridishes were used, with exactly the same shape (9 cm diameter, 1.5 cm high). An exact volume of (22) ml of SDA medium was poured into each dish, leading to an identical internal atmosphere volume in all the dishes. Each dish was inoculated with (0.2) ml of inoculum prepared previously as radial lines (3 isolates per dish) (Fig. 1). The petridishes were turned upside down and a 2-cm diameter filter paper was put in the middle of the cover and soaked with variable amount of essential oil ranged from (50-150) μl.

Control Petri dishes were prepared under the same condition except soaking the filter paper with (50) μl ethanol. The Petri dishes were incubated in reversed position for 2-3 days at (30) °C.

Results and Discussion

Anticandidal activity of Myrrh essential oil using broth dilution method and MIC determination shown in the table (1), the essential oil was inhibitory to the growth of the tested Candida isolates. MIC for vaginal and nail isolates was 5% where is for the standard isolate it was 10%, indicating that vaginal and nail isolates were more susceptible to the oil than the standard isolate, this result agrees with (7) who found that the MIC of Myrrh essential oil using agar dilution method against C. albicans was >2% (v/v). This effect may be due to the active components the oil contains. In this respect (8) extracted, purified and characterized 8 sesquiterpene fractions from Commiphora molmol and focused their attention on amixture of furanoguai-9-ene-8-one which showed antibacterial and antifungal activity with MIC ranging from 0.18-2.8 microgram/ml.

Effect of concentrations (1.25)% and (2.5)% of oil on morphology types (Fig. 2A,B) showed double increase in pseudohyphae percentage and double decrease in budding cell percentage compared to the control types for both isolates tested (Nail and Vaginal) especially at concentration 2.5%. This result indicate possible effect of the oil on division and reproduction of the yeast cells.

Since there has been very little recognized measure to express the antimicrobial activity by gaseous contact, and because essential oils are highly volatile at room temp. they used now adays as ethical medicines for cold (10, 11) and inhalation therapy of essential oils has been used to treat acute and chronic bronchitis and acute sinusitis,
additionally evaluation of cinnamon bark oil against respiratory tract mycoses has been reported (12), therefore the vapour effect of Myrrh essential oil was introduced in this investigation using the technique of (9) which is modified from the original method of Kellner and Kober.

The technique used is inexpensive since it allows the study of several isolates using the same Petri dish, and the radial inoculation offers the advantage of easily estimating the inhibition effect by measuring the extent of growth and thus to compare several isolates under the same conditions for their sensitivity. Comparing the activity of different concentrations of Myrrh essential oil (50, 75, 100, 125, 150) μl (Fig. 1) it can be observed that the inhibition of growth started at conc. (75) μl with different degrees of inhibition, vaginal isolates showed more resistance to this concentration compared to the nail and standard isolates which showed same degree of sensitivity and reduced growth compared to (50) μl treatment, higher concentration (100, 125, 150) μl showed complete inhibition of the three isolates which may indicate that the MIC using gaseous contact is more than (75) μl.

References

Table (1) Anticandidal properties of myrrh essential oil (inhibition index as a percentage relative to control CFU).

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Myrrh essential oil (%)</th>
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<tbody>
<tr>
<td></td>
<td>1.25</td>
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<tr>
<td>Standard</td>
<td>42.02</td>
</tr>
<tr>
<td>Vaginal</td>
<td>51.3</td>
</tr>
<tr>
<td>Nail</td>
<td>25.4</td>
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</tbody>
</table>
Fig. (1): Effect of vapour of Myrrh essential oil on growth of three isolate of C. albicans.
S= Standard isolate.
N= Nail isolate.
V= Vaginal isolate.
Fig. (2): Effect of Myrrh essential oil (percentage) on morphology types of two C. albicans isolates
تأثير الزيت الأساسي للمر (Commiphora molmol) في نمو خميرة Candida albicans

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الخلاصة

استهدفت الدراسة الحالية تقييم فعالية الزيت الأساسي للمر تجاه ثلاث عزلات من خميرة C. albicans. أظهرت النتائج فعالية تثبيطية للزيت تجاه العزلات الثلاثة، وأظهرت العزلتان (المهل والأطام) تأثيراً مماثلاً، إذ كان MIC للزيت 5% بينما كان MIC للزيت تجاه العزلة القياسية 10%. تم كذلك دراسة تأثير الزيت الأساسي في الأنماط الشكلية لخميرة C. albicans الذي أظهر تأثيراً واضحاً في ذلك، إذ أدت التراكيز الأقل من MIC إلى زيادة مضادة في تكوين البلازما. وقصص مضاد في الخلايا المترعبة مما قد يدل في التأثير على إفسام الخلايا.

تعد كذلك دراسة تأثير بخار الزيت الأساسي للمر الذي أظهر تأثيراً مضاداً للعزلات الثلاثة وكان MIC أعلى من (75) ميكروليلتر.