Antifungal Activity of Nine Plant Oils Against Local *Rhodotorula* Species and *Scytalidium dimidiatum*

Salah Mahdi Al-Bader, Knowledge University, College of Science, Pathological Analysis Department. Erbil, Iraq.
Email: salahsalim@knowledge.edu.krd; salahalbader@yahoo.com.
Mobile:+9647503992415
ORCID: https://orcid.org/0000-0002-9702-5567

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Abstract

Background: Although there is a development in the production of new anti-microbial agents, however, the new antifungal agents development still limited. One of the approach to overcome such obstacle is to use plants products as antifungal agents.

Aims: The study was conducted to demonstrate the antifungal activity of pharmaceutical crude oils of nine medicinal plants on *Rhodotorula species* and *Scytalidium dimidiatum*.

Materials and methods: Local fungal isolates of *Rhodotorula* species (yeast) and *Scytalidium dimidiatum* (filamentous) were isolated from sheep wool in Erbil city. Pure cultures were prepared on Sabouraud's dextrose agar (SDA). SDA supplemented with 1% (v/v) of plant oils was used for primary screening of oils antifungal activity. Macroscopic characteristics of yeast cultures and radial growth of mold were screened for the primary determination of the oils effectiveness. The tested oils include that of *Zingiber officinale* (Ginger), *Sisymbrium officinale* (Hedge), *Peganum harmala* (Harmel), *Rosmarinus officinalis* (Rosemary), *Pimpinella anisum* (Anise), *Cuminum cyminum* (Cumin), *Salvia officinalis* (Sage), *Linum usitatissimum* (Flax), and *Allium sativum* (Garlic).

Results: Oils of *R. officinalis, A. sativum* and *S. officinale* exhibited the highest antifungal activity on *Rhodotorula species* and *S. dimidiatum*. The rest treatments except *Z. officinalis* showed a moderate antifungal effect. While *Z. officinalis* not demonstrated antifungal activity. The minimum inhibition concentration (MIC test) of garlic, rosemary, and hedge oils showed that garlic was the only treatment that inhibited the growth of tested isolates in all concentrations, while the rosemary and hedge oils showed a diverse activity levels.

Conclusions: The effectiveness of the tested oils on fungal isolated demonstrated variable antifungal activities and the effectiveness was higher on the yeast growth rather than on filamentous fungi. Additionally, *Rodotorula species* was more sensitive to the tested oils *S. dimidiatum*.

Key words: *Rhodotorula, Scytalidium*, plant's oils, antifungal, Iraq.

Introduction

In the recent years, usage of natural products as human's medicines showed a lot of increase, and the trust in using plant extracts as active antimicrobial agents is

widely spread [1-3]. Plants have several properties which encourage peoples to use it in curing infections. Plants extracts are available, easy to use, cheap, and have a few side effects, nowadays about three quarters of world's population use herbal medicines [4-7]. Moreover, in comparison with chemical medicines they are easily biodegraded in living tissues [8], and showed a wide range of activity against pathogenic agents, with a low number of resistant strains [9-11].There is a globally increasing in medicinal plants studies and in Iraq there is a few reported studies [12-16].

Medicinal plants are used traditionally as extracts, juices, and powder. Oils that were used in current study related well known medical plants, they are a source of several bioactive compounds, and their antifungal affect against phytopathogens, animal pathogens, as well as human infectious agents were estimated [17-25]. Rhodotorula the basidiomycetous yeast had three pathogenic species [26]; they were isolated from natural habitats, food, as well as from different human infections [27-29]. *S. dimidiatum* is a dematiaceouscoelomycetes characterize by formation of arthroconidia. It was well known as an etiological agent of numerous human infections particularly onycomycosis [30].

There are a little information about using plant extracts against *Rodotorula* species and *S. dimidiatum*. The current study aimed to determine the antifungal activity of a nine pharmaceutical plant's oils on growth of both isolates, and to determine the minimum inhibition concentration (MIC) for *R. officinalis*, *A. sativum* and *S. officinale* that show the higher growth inhibition.

Materials and Methods

Fungal isolates

In March, 2017, a wool samples were collected from free grazing sheep in Erbil city, a few wool hairs were cultured directly on Soubraud's dextrose agar medium (supplemented with 150 mg/L of chloramphinicol and was sterilized in 121^{o}C for 15 minutes).*Rhodotoula* species were developed adjacent to wool hairs. Identification carried out by direct microscopic examination, the inositol assimilation test was carried out to verify the identification [31, 32]. A pure culture of the yeast on SDA was prepared. *S. dimidiatum* grow rapidly from wool samples on SDA medium at 25^{O}C. The isolate was identified microscopically according to previously reported studies [30, 33], and a pure culture was prepared on SDA.

Plant oils

Plant's oils were brought from a specific pharmacy deal only with traditional medicines and medical plants in Erbil city. These oils were prepared as a medicinal products and were regarded as over counter medicines, they include *Zingiber officinale* (Ginger), *Sisymbrium officinale* (Hedge), *Peganum harmala* (Harmel), *Rosmarinus officinalis* (Rosemary), *Pimpinella anisum* (Anise), *Cuminum cyminum*(Cumin), *Salvia officinalis* (Sage), *Linum usitatissimum* (Flax), and *Allium sativum* (Garlic).

Antifungal activity test

Seven days old cultures were prepared on Sabourad's dextrose agar with 1% (v/v) plant oil to evaluate the primary antifungal activity of the nine oils. The tests had been done under complete aseptic conditions in laminar airflow hood, and were performed in duplicates. A full lops of young *Rhodotorula* culture were added to 10ml of distilled sterilized water in a sterile screw cap test tube. The suspension was shacked thoroughly (without bubbles forming), and then one ml of the suspension was transferred to SDA plates using Gilson micropipette. The drops was disturbed by a sterile glass rod (streaking method),plates were incubated for (7) days in 25^{o}C.The macroscopic characteristics include width of streak, color of growth, thickness of

growth, and continuity of steaks were carefully observed to compare between the nine oils and recognize their primary effectiveness. For *S. dimidiatum*, a disc (0.5mm) from the edge of young colony was transferred to the center of Petri- dish with SDA medium, then was incubated in 25^{o}C.After (7) days, the mean of radial growth in two directions were measured. Decreasing of growth diameter is comparable to antifungal activity. The culture characteristics did not excluded in this test.

Minimum inhibition concentration test

MIC test was carried out by a serial dilutions of the tested oils(v/v) 1%, 0.5%, 0.25% , 0.125%, 0.0625%, 0.031%.The test performed as previously described [34] with a modifications, the dilutions were prepared via direct mixing of sterilized oil with SDA before solidifying. Tween 20 was mixed with SDA (0.5% v/v) to increase homogeneity of culture medium. For *S. dimidiatum* the plates of (SDA+ oil+ tween 20, and SDA+ tween 20 as control) were centrally inoculatedwith5mm disk of fungal isolate, a sterile stainless steel crock borer was used. Plates were incubated in 25^{o}C for seven days, and the lowest concentration that showed decreasing in redial growth was regarded as MIC. For *Rhodotorula* a stock suspension of yeast cell 10^{3}/ml was prepared by hemocytometer, a drop 0.2ml was transferred to triplicate plates per oil, yeast inoculum was spread by sterile glass road. Plates were incubated in 25^{o}C for seven days ,the lowest concentration demonstrated decreasing in mean CFU/plate was determined as MIC.

Results and Discussion

An orange mucoid colonies grow in contact with the wool threads were primarily distinguished as *Rhodotoula sp*ecies. The direct microscopic examination showed a spherical non aggregated yeast cells, without pseudohyhae. The negative result of inositol test (no color change) confirmed the primary recognition and separate *Rhodotorula* from *Creptococcus* with positive result (color change) [31, 32]. *Scytalidium dimidiatum* grew rapidly, it produce a dark grayish colony with abundance aerial mycelia without *Nattrrassia* species as the syanomorph of Scytalidium. A typical arthroconidia were easily identified under the light microscope [31,35].

The antifungal activity of the plant oils (Table.1 and Plate. 1) showed that they have a diverse activity levels against *Rhodotorula species* and *Scytalidium dimidiatum*. Rosemary and garlic oils show the highest effect on both isolates, while hedge oil had a higher effect on *Scytalidium*, and a moderate activity against *Rhodotorula.* Ginger oil was the only one without effect on both isolates. Rosemary oil has a several beneficial uses beside its antifungal activity [36].The hydrochloric extract of its leaves showed a good activity against dermatophytes and was suggested active substance for the formulation of antifungal drugs [37]. Garlic is a popular plant in traditional medicine, oil and extracts of garlic are well known as antimicrobial natural product [38,39].The antifungal activity of garlic mainly related to allicin [40].The activity of Hedge was high against *Rhodotorula*, while it was moderate against *Scytalidium*. Hedge attracted less attention than its predecessors in folk medicine, a number of previous studies aimed to explain the scientific base of its efficacy as antimicrobial and anticancer traditional medicine [41],others reported its activity against bacteria and fungi[18].

Table. 1. Effect of oils on the growth of *Rhodotorula species* and *Scytalidium dimidiatum*.

No.	Plant	Rhodotorula	Scytalidium
1	***Peganum harmala*** **(Harmel)**	++	-
2	***Salvia officinalis*** **(Sage)**	++	-
3	***Rosmarinus officinalis*** **(Rosemary)**	++++	++++
4	***Zingiber officinale*** **(Ginger)**	-	-
5	***Linum usitatissimum*** **(Flax)**	++	+++
6	***Allium sativum*** **(Garlic)**	++++	++++
7	***Pimpinella anisum*** **(Anise)**	++	-
8	***Cuminum cyminum*** **(Cumin)**	+++	+++
9	***Sisymbrium officinale*** **(Hedge)**	++++	+++
10	**Control**	-	-

(++++) high effect; (+++ and ++) moderate effect; (-) no effect

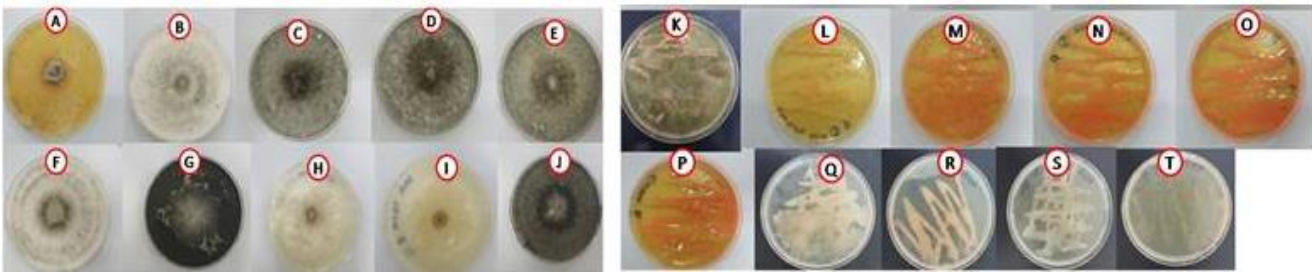

Plate. 1. Effect of oils on the growth of S*cytalidium dimidiatum* (A-J) and *Rhodotorula species* (K-T)

A= Rosemary, B= flax, C= Ginger, D= Harmel, E= Sedge, F= Cumin, G= Anise, H= Hedge, I= Garlic, J= Control, K= Rosemary, L= Cumin, M= Ginger, N= Flax, O= Anise, P= Control, Q= Harmel, R= Sedge, S= Garlic, T= Hedge.

Cumin oil showed a moderate activity on both isolates, a several previous works elucidated its absolute antifungal activity [42]. Oil of cumin was suggested to treat aspergillosis due to its high effect which exceeded that of amphotericin-B [43]. Flax, harmel and sage oils show a moderate activity against *Scytalidium* only and

without any activity against *Rhodotorula*. The activity of flax against *Aspergillus flavus* and *Saccharomyces cereviceae* were reported previously [44]. The flax seed and flax seed extracts showed a significant antifungal inhibition when they were used as food preservative agent [45].The studies about antifungal activity of harmel oil are limited. The plant extracts indicated anti-inhibitory effect on spore germination and mycelia growth of several phytopathogens [46]. The bioassay studies demonstrated that the active ingredient in harmel extract is (β- carboline alkaloids) which may act as antifungal agent [47]. However, these need to be evaluated in vitro and in vivo in a well designed study.

Oil of *S. officinalis*(sage) comprise more than120 components [48]. The studies on sage revealed a wide range of pharmacological activities, but the antifungal activity was reported in few works. Sage antifungal activity was reported against *C. albicans* and *Alternaria alternate* [49, 50]. A moderate activity and inactivity were attributed to anise oil against *Rhodotorula* and *Scytalidium* respectively, Table-1. In contrast a several previous studies confirmed its strong antifungal activity against Candida species and dermatophytes [51]. Antifungal activity of compounds was influenced by the fungal isolate type and/or the concentration of the tested agents and lead to variation in results [52].

The MIC test of garlic, rosemary and hedge (Table-2) showed that garlic was the most effective oil and inhibited the growth of both fungal isolates in all tested concentrations. Garlic MIC may be less than the lowest concentration (0.031%) used in the experiment. Garlic oil caused a complete inhibition of yeast and mold. It antifungal may be exerted to its effects on cellular and organelle membranes which lead cell death [53]. The MIC of Rosemary was higher than that of garlic, colonies disappeared at concentration of (0.062%), while *Scytalidium* shows tiny growth at concentration of (0.125%), which completely inhibited at MIC of 0.25%.Several studies suggested that oil of rosemary contain antifungal and antibacterial component such as cineol, camphor, and verbenon [54]. Hedge oil showed a MIC of 0.25% against both isolates, the sulphated compounds suggested being the main effective constituent that exerts antifungal activity [55].

Table.2. MIC for garlic, rosemary, and hedge oils on *Rhodotorula* and *Scytalidium*

Oil concentration	Fungi	Garlic	Rosemary	Hedge
1%	***Rhodotorula***	**0**	**0**	**0**
	Scytalidium	**0**	**0**	**0**
0.5%	***Rhodotorula***	**0**	**0**	**0**
	Scytalidium	**0**	**0**	**0**
0.25%	***Rhodotorula***	**0**	**0**	**0**
	Scytalidium	**0**	**0**	**0**
0.125%	***Rhodotorula***	**0**	**0**	±
	Scytalidium	**0**	±	±
0.062%	***Rhodotorula***	**0**	**0**	+
	Scytalidium	**0**	+	+
0.031%	***Rhodotorula***	**0**	±	++
	Scytalidium	**0**	+	++

(0) No growth; (±) Tiny growth; (+) Weak growth; (++) Moderate growth

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