

Antifungal Activity Of *Rosmarinus officinalis* and *Pelargonium Gravelens* Essential Oils Extracts Against *Aspergillus Flavus*, *Penicillium Brachycaulon*. *Andalternaria alternate*

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Abstract

The present paper aims to describe the antifungal activity of essential oils extracts with three concentrations which are (25, 33 and 50) mg/ml at significant differences ($P \leq 0.05$) of *Rosmarinus officinalis* and *Pelargonium gravelens* essential oils extracts have been projected against some kinds of fungi (*Aspergillus flavus*; *Penicillium brachycaulon* and *Alternaria alternate*). The *Rosmarinus officinalis* essential oils extracts have been found to possess greater inhibition influence of growth for both *Alternaria alternate* and *Penicillium brachycaulon* that reach (100)% in all concentrations respectively, followed by *Aspergillus flavus* at (50) mg/ml that amount to (88.8)%, while *Pelargonium gravelens* essential oils extract effect inhibition of growth *Penicillium brachycaulon* at (50) mg/ml that reaches to (100)%. As well as the same concentrations higher inhibition of percentage to (79.7)% in growth of *Aspergillus flavus*.

Also these extracts that inhibition percentage influence the overlapping types of concentration extract of grow *Alternaria alternate* on SDA reached about (100)%, while the activity of extract against *Penicillium brachycaulon* at concentration (50) mg/ml opposing in control to inhibited amount (100)%. As recorded inhibition ratio at the same concentration above for *Aspergillus flavus* which amounted to (82.4) %.

The highest inhibition ratio of *Alternaria sp.* growth amounts to 100% when it is mixed with *Rosmarinus officinalis* essential oils extracts in all concentration. The proportion of *Aspergillus flavus* has reached (54.1)%, in *Pelargonium gravelens* extract on the same fungi.

Keywords: *Aspergillus flavus*, *Penicillium brachycaulon*, *Alternaria sp.*, Rosemary, Geranium, essential oil.

1. Introduction

Pollution of food and feed by mycotoxin is more deeper than the problems that threaten most of the world especially the developing countries that lack food and healthy storage conditions to achieve the safety and security of food (1). Mycotoxins cause chronic and acute effects such as Cancer of liver. The most common kinds of liver cancer is hepatocellular carcinoma (HCC) which begin in the primary kinds of liver cell (hepatocyte) other types of liver cancer, e.g. (intrahepatic cholangiocarcinoma and hepatoblastoma) are rare. Renal failure, weakness of the immune system and congenital deformities congenital (2). Many types of fungi produced poisoning such as *A. flavus*, *Penicillium* sp. and *A. alternata*. That contribute significantly in fruit spoilage (3).

A. flavus is saprotrophic fungal organism with cosmopolitan distribution. Many strains produce mycotoxins which are toxic to mammals (4, 5). At the same time pathogen opportunistic human and animal causing Aspergillosis (6, 7). *Penicillium* sp. is one of the largest and most important genera that results systemic mycosis named Penicilliosis in immunocompromised patients (8, 9). *Alternaria* sp. is widely distributed in air, soil or indoor environments their spores are among most common, and potent airborne allergens and sensitization to *Alternaria* allergens has been determined as important onset of children asthma in arid regions (10). Therefore the states have developed nation interested much medical plant and how to extract and increased of efforts researchers in isolating plant extracting and classifying of active substrates them and identify installed chemical characteristics therapeutic. The Rosemary (*Rosmarinus officinalis* L.) belongs to Lamiaceae family is a perennial evergreen herb that grows wild in the Mediterranean basin, is used as a spice for flavoring and a medicinal herb against many illnesses (11). Rosemary herbs have many bioactive compounds such as phenolic diterpenes including: carnosic acid, rosmanol or carnosol; flavonoids such as genkwanin, homoplantagin, or cirsimaritin; and triterpenes such as ursolic acid (12, 13). Rosemary essential oil is also important for traditional medicine its used for antibacterial, anti-mutagenic, cytotoxic, anti-inflammatory, antioxidant and chemopreventive properties (14, 15). The major constituents that are founded in essential oil of Rosemary are 1,8-cineol (38.5%), camphor (17.1%), α -pinene (12.3%), limonene (6.23%), camphene (6.00%) and linalool (5.70%) (16). There is no specific data for harvesting Rosemary, as it can be harvested at almost any time although during spring and summer this plant grows quickly, so that the leaves have distinct flavor and strong aroma.

Geranium (*Pelargonium graveolens* L' Herit) belongs to Geraniaceae family that is an aromatic herbaceous shrub, height up to one meter, native to South Africa (Comoros Islands). There are three main regions for the production of geranium oil: Egypt, Reunion, Russia

(17).Essential oil and extracts of geranium have a biological activity against antibacterial, antifungal, antioxidant and others (18,19). Geranium essential oil extract is extensively used in the perfumery, cosmetic industries and aromatherapy (20). The chemical compositions of essential oil are citronellol (24.54%), geraniol (15.33%), citronellyl formate (10.66%) and linalool (9.80%) (21-36).

2.Methdology

2.1. Samples collection

Twenty sample have been collected from three kinds of food substance (corn, rice, peanuts) where one kilo for each sample is taken from the local markets for Al- Najaf Province in order to get the fungi isolated and the samples are transferred to the Laboratory of Advanced Mycology \Faculty of Science \ Kufa\ University for diagnosis and study. The plant materials of *R.officinalis* and *P.graveolens* (fresh leaves) have been collected from a garden in Al-Najaf, Iraq. The identification and authentication of the plants materials are done by the Botany Taxonomy Department, Kufa\University.

2.2.Culture media

Table(1)The following Culture media are used experimental purposes in the current study

Media	Manufacturer
Potato dextrose agar	Fluka
Sabouraud dextrose agar	Hi media \India

These media are prepared in compliance with the recommendation of the Manufacturing company and sterilized by Autoclave at 121°C in 15 minutes for 15psi/inch².cm. This medium is used to isolate and grow the fungi.(22).

2.3.A study of the antifungal efficacy of plant extracts against fungi

The mixing method is used with the SDA to study the antifungal activity of *R. officinalis* and *P.gravelens* essential oils extracts for three concentrations (25;33 and 50)mg\ml of each extract is relocated 0.1 ml from each focus the petry dish, the SDA medium is poured on it, after which the dishes are left to harden. A disc with a diameter of 5 mm, from fungi, is taken by the sterile cork borer from each fungus ,the disc is placed on the surface of the culture medium ,then the petry dish were incubated at a temperature of 25c[±] 2 for 7 days. The antifungal activity of the essential oils extracts were determined by measuring the

diameter of the inhibition zone are measured in millimeters by the ruler(23).Then

percentages of inhibition were calculated according the following equation

The percentage of inhibition (%) = $(R_1 - R_2) / R_1 \times 100$

(R₁ = maximum radial growth of fungus colony (control treatment

R₂ = maximum radial growth of the colony of fungi under study in plates containing essential oils extracts.

2.4.*Extraction of the essential oils

The prepared air-dried plant material(aerial parts:leaves)of Rosemary and Geranium are both separately taken powdered 100g using "Clevenger apparatus" to extract its essential oils (24). The distillation is done for 4 hours and the oil is collected separately in airtight containers that were dried over anhydrous sodium sulphate (25). The oil is stored in a freezer at (- 4C°)for further use. The Dimethyl Sulfoxide (DMSO) is used to prepare different concentrations of the oils, the concentrations of essential oils prepared by mixing a definite amount of the oils with different volumes (ml) of DMSO (1:1, 1:2, 1:3) to get the concentration (25,33 , 50) mg/ml respectively.

3.Results & Discussion

***Isolation and diagnosis of insulated fungi from food (Rice, Maiza,Peanuts)**

The results have revealed that Twenty isolates of fungi genera and species from different food sources, which are isolated and identified as follows: fungus *A.alternate* (5) and *p. brachycaulon*(8) and *A. flavus*(7) isolates. The results revealed the Antifungal activity of Rosemary essential oils extract is found to have actually inhibited influence of growth for both *A.alternate* and *p. brachycaulon* that have reached(100) % in all concentrations(25,33 and 50)mg/ml respectively at significant differences ($P \leq 0.05$) followed by comes after *A.flavus* at (50) mg/ml that amounts to(88.8)%, While appear Geranium essential oils extract influence inhibition of growth of *P. brachycaulon* at(50) mg/ml that reaches to (100)%. Moreover, it's seem that using the same concentration on other fungi shows higher inhibition of percentage of (79.7)% as presented in Table(2).

At the same time the results of study in Table(3) have showed that the inhibition percentage effected overlapping types of concentration extract of grow *A .alternate* on sabouraud dextrose agar has reached about(100)% at the same concentrations which are previously mentioned, while the activity of extract against *P.brachycaulon* at a concentration of (50)mg/ml

has inhibited by (100)% for the inhibition ratio for *A.flavus* in the same concentration mentioned above, it has reached (82.4)% .Figures(3 and 4).

Table(2) The Effect of Rosemary and Geranium Essential Oils Extracts Concentrations in Averages of Inhibition Percentage of Fungi tested by Mixing With the Medium (25±2)C° after seven days.

Fungi isolate	Type of extract	Inhibition of percentage (%)		
		Concentration	25mg/ml	33mg/ml
<i>A . alternate</i>		100	100	100
<i>P.brachycaulon</i>	Rosemary essential oils extract	100	100	100
<i>A.flavus</i>		49.6	81.1	88.8
<i>A . alternate</i>	Geranium essential oils extract	100	100	100
<i>P.brachycaulon</i>		24.1	25	100
<i>A.flavus</i>		41.1	76	79.7
Control		00.00	00.00	00.00
L.S.D(0.05)		0.01		

0*:No Inhibition

Table3: Interaction Effect of Types Concentration Extract *R. officinalis* and *P. graveolens* Essential Oils Extracts in the growth of (*A. alternate* , *P.brachycaulon*, and *Asp.flavus*) on the SDA.

Treatment	Rat of diameter of colony centimeter			Inhibition of percentage (%)		
	25 (mg/ml)	33 (mg/ml)	50 (mg/ml)	25 (mg/ml)	33 (mg/ml)	50 (mg/ml)
<i>A . alternate</i>	0	0	0	100	100	100
<i>P.brachycaulon</i>	3.42	4.5	0	62	50	100
<i>A.flavus</i>	4.03	1.77	1.58	55.2	80.3	82.4

L.S.D (0.05)	0.4			2		

The results illustrated in table (4) indicate that Rosemary extract has reflected an inhibitory effective towards the fungi studied and showed that the highest inhibition ratio of the growth of *Alternaria* sp. Each (100)% and the proportion has lesser in *A. flavus* that has reached (54.8)%. While in *P. graveolens* extract on the same fungi mentioned above has reach (75,54.1)% respectively. and that lowest than grew on *P. brachycaulon* about (31)%. Figures(1 and 2). This study revealed that impacted of different concentration from essential oil extract on fungi growth of (*A. alternate*, *P. brachycaulon*, and *A. flavus*) and they are positive results increasing of the rate area diameter of inhibition zone. This might be the reason to the biological activity of essential oils has been shown to be dependent on their basic chemical component such as (limonene, cineole and cymene).

In addition the mechanism of action of extract essential oils is relating to changes in cell membrane permeability. The lipid soluble nature of extracts and their facilitates the overlapping with cellular structures, that have lipid constituents. due to increased permeability of cell membrane which due to electrolyte nonbalance and cell lysis then death. They are resulting agreement with (26). As well agree with Phongpaichit et al., (27) the studied was appeared the fungal colonies suffered cellular collapse and denatured after exposure to the extract of natural production.

Table(4) Antifungal activity of types extract in growth of fungi on the SDA.

Type of Extract						
	A.alternate	P.brachycaul on	A.flavu s	A. alternate	P. brachycaulon	A fla vus
Rosemary Extract	1.5	2.25	4.06	100	75	54.8
Geranium Extract	2.25	6.21	4.13	75	31	54.1
Control	SDA	00.00	00.00	00.00	00.00	00.0
L.S.D (0.05)	0.29			0.6		

0*:No Inhibition



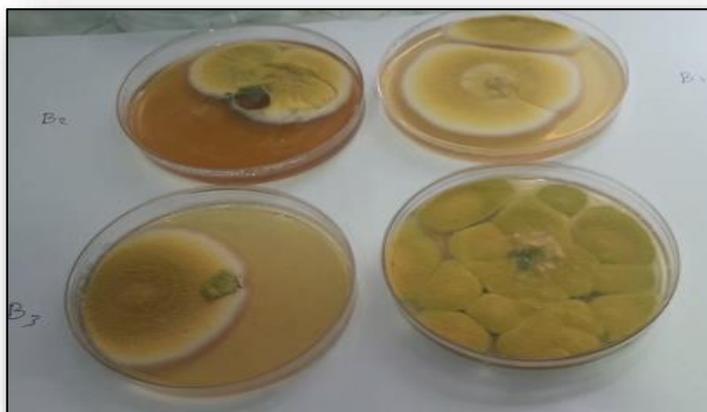
Figure(1) Shows the Rosemary Oli Extract Inhibition the Grow of P. brachycaulon; Concentration: A1=25cm; A2=33cm ; A3=50 cm; CO=Control



Figure(2) Shows the Geranium Oli Extract Inhibition the Growth of P. brachycaulon; Concentration: B1=25cm; B2=33cm ; B3=50 cm; CO=Control



Figure(3) Shows the Rosemary Oli Extract Inhibition the Growth of A.flavas;Concentration:
A1=25cm;A2=33cm;A3=50cm and CO=Control



Figure(4) Shows the Geranium Oli Extract Inhibition the grow of A.flavus;Concentration
B1=25cm;B2=33cm ;B3=50 cm;CO=Control

4.Conclusion

These results suggest that essential oils studied can be real alternatives in the control of fungi . We identified concentrations of R. officinalis essential oils extracts to possess greater inhibition influence of growth for both Alternaria alternate and Penicillium brachycaulon that reach to (100)% in all concentrations respectively .whileP. gravelens essential oils able inhibition of growth Penicillium brachycaulon at (50) mg\ml to (100)%.

Conflict of interest statement

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership,or other equity interest; and expert testimony or patent-licensing arrangements),or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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