Effect of methanolic extract of *Eucalyptus sp.* leaves in growth of some opportunistic fungi

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Abstract

Objective : to examine the methanolic extract of *Eucalyptus* sp. leaves against some opportunistic fungi.

Methods : leaves of Eucalyptus sp. were collected, dried and then grinded . Powder of leaves were extracted using methanol and then filtered using filter papers. The filtrate was then centrifuged and then the filtrate was concentrated using a rotary evaporator. A stock solution was used to determine the inhibitory activity of the extract against opportunistic fungi.

Results : The results of detecting the active substances in the crude extract showed the presence of the compounds saponins, glycosides, tannins and quinones. The crude extract also showed a strong inhibitory effect against all the fungi used under study, starting from the concentration of 0.5% up to 100% fungal inhibition at the concentration of 8%.

Conclusion : It has been found that the crude aqueous extract of the Eucalyptus plant has antifungal properties used in this study because this extract contains some chemical compounds that, if purified, will be used in the pharmaceutical industry in the future as antifungal agents.

Keywords : *Eucalyptus sp.,* methanolic extract, opportunistic fungi

Introduction

The incidence of fungal infections has increased in the last ten years, with fungi infecting more than a billion people annually, of whom about a million and a half are infected with opportunistic fungi that may sometimes cause death. Fungi usually infect patients with asthma, diabetes, and cancer [1], as well as patients undergoing organ transplantation and those who are treated with corticosteroids [2]. The developing resistance of microorganisms against appealing antimycotic medications which is one of the very important matters that preoccupy researchers and physicians. The treatment of pathogenic microorganisms which include, bacteria , fungi, viruses, and protozoa , is difficult because of the

continuous development of resistance to these organisms [3]. In one of the recent statistics, researchers concluded that human deaths could reach ten million annually by the year 2050 due to the resistance of pathogenic microorganisms to antibiotics, thus exceeding the number of people who die from cancer currently [4]. The antifungals discovered so far are usually partially effective in affecting the pathogenic fungi, and these antifungals also affect the host tissues [5]. The antifungals currently used are divided into four main categories: azoles, polyenes, flucytosines, and echinocandins [6].

There has become an urgent need to discover new medicines that are more effective and have fewer side effects. This has been done by exploiting natural plant products in discovering new medicines [6,7]. Bioactive constituents play an essential role in discover of drugs by provide as constituents of importance for synthetic changes [8]. *Eucalyptus* is a genus of over 700 species of flowering trees, in the myrtle family (Myrtaceae) [9]. *Eucalyptus* is an affluent origin of phytochemical constituents as Tannins, Glycosides, Phenols, Flavonoids, Saponin, Alkaloids, kaempferol, Propanoids extracted from roots, steams, and leaves of the plant [10, 11]. So far, it has been revealed that there are more than 18 chemical compounds in the *Eucalyptus* plant. It was found that the compound Eucalyptol is present in 79.88% of the total chemical compounds found in this plant, which was found to be biologically active against various microorganisms [12].

Materials and Methods

Collection of Plant Material

Eucalyptus leaves were collected during November 2022 from the public gardens of Al-Kadhimiya city . The plant was diagnosed based on available plant diagnosis books. The plant leaves were washed well with tap water and then dried in the oven at a temperature of 50°C. Then the leaves were grinded using a blender to get a powder.

Extraction Method

The method of [13] was used to obtain the plant leaves extract. 200 grams of plant leaf powder put in a 1000 ml glass beaker, then mix up with 800 ml of concentrated methanol (99%), It was mixed well and then placed on a magnetic stirrer at a temperature (70˚C) for (4) hours with slow motion of mixture using magnetic stirrer. The mixture was first filtered using a piece of medical gauze, after which the filtrate was separated using a centrifuge (3000 rpm for 10 min.). The extract was put in rotary evaporator for (1) hour to remove the solvent, so as to obtain a stock solution which used as an antifungal substance .

Chemical Diagnosis of Some Active Ingredients in *Ecalyptus* leaves extract

Saponins detection

1ml of the crude extract of the plant was taken and placed in a graduated cylinder, 20 ml of distilled water was placed on it , then the cylinder was shaken well for 3 minutes, as this led to the emergence of foam with a height of 3 cm , which indicates that the crude extract contains Saponins in composition of the crude extract of the plant (14).

Glycosides Detection

One ml of crude extract of plant was added to 2 ml of Benedict's reagent in a test tube , the mixture was agitated well , the tube was placed in water bath at a temperature of 100°C for 5 minutes , after which the tube was cooled , a red precipitate appeared at the bottom of the tube , indicating the presence of glycosides in the composition of the crude extract (14).

Tannins Detection

1% of $FeCl_3$ was added to 0.5 ml of the crude extract of the plant in a test tube, a bluish-green precipitate appeared, indicating the presence of tannins in the composition of the crude extract (14).

Quinones Detection

Two ml of extract was taken, few drops of concentrated H_2SO_4 were added and appearance of red color indicates the presence of quinones (14).

Fungal isolates

Five genera of fungi were used in this study were *Aspergillus niger*, *Penicillium sp.*, *Rhizopus sp.*, *Alternaria sp.* and *Candida albicans* . They were isolated from plants and soils from Botanical garden of Pharmacognosy department - college of Pharmacy / Al-Nahrain University. All Fungi were isolated and purified using culture medium of Potato Dextrose Agar (PDA). The phenotypic, microscopic and biochemical characteristics were relied upon to diagnose all isolated fungi (15).

Antifungal activity evaluation

The activity of the methanolic extracts of plant leaves was examinated by agar dilution method as declared by (16) to determination of minimum inhibitory concentrations (MICs) for fungi used in this study. A 6mm diameter culture disc from the edge of a newly growing fungal colony was transferred by using Cork Borer from each of the inspected isolates to the center of a plates containing fresh solid culture medium (SDA). After 7 days of incubation at a temperature of 28 °C , influence of various concentrations of the crude extract of plant leaves on the growth of the fungal cultures used in this study was examined by using a measuring ruler to determine the diameters of the fungal colonies for the purpose of calculating the percentage of inhibition using the following equation :

$$\text{Percentage of inhibition} = \frac{C - T}{C} \times 100$$

Where, C = colony diameter (mm) of the control

T = colony diameter (mm) of the test plate.

Results

Chemical diagnosis of some active ingredients in *Eucalyptus* leaves extract

Table (1): Chemical diagnosis of some active ingredients in *Eucalyptus* leaves extract

No.	Detection type	Result
1	Saponins detection	+
2	Glycosides detection	+
3	Tannins detection	+
4	Quinones detection	+

The results showed in table (2) that the methanolic extract of *Eucalyptus sp.* Leaves had a clear effect on the growth of *A. niger*, starting from a concentration of (0.5%) , while it had the strongest effect at a concentration of (8%) Which led to the complete inhibition of fungal growth by 100%.

Table (2) : Inhibitory Activity of methanolic extract of *Eucalyptus sp.* leaves on *Aspergillus niger* after incubation period 7 days , 28˚C

Fungus	Extract concentration (%)	Colony diameter (cm)	inhibition rate (%)
A. niger	control	7.5	0
	0.5	6	20
	1	3	60
	2	1	86.67
	4	0.6	92
	8	0	100

The Results showed in table (3) that the methanolic extract of *Eucalyptus sp.* leaves had a clear effect on the growth of the fungus *Fusarium sp.* starting from a concentration of (0.5%) , while it had the strongest effect at a concentration of (8%) Which led to the complete inhibition of fungal growth by 100%.

Table (3) : Inhibitory Activity of methanolic extract of *Eucalyptus sp.* leaves on *Fusaeium sp*. afer incubation period 7 days , 30˚C

Fungus	Extract concentration (%)	Colony diameter (cm)	inhibition rate (%)
Fusarium sp.	control	6	0
	0.5	5.5	8.34
	1	4	33.34
	2	2	66.67
	4	0.8	86.67
	8	0	100

Results showed in table (4) that the crude methanolic extract of *Eucalyptus sp.* leaves had a clear effect on the growth of the fungus *Alternaria sp.* starting from a concentration of 0.5% , while it had the strongest effect at a concentration of 4% Which led to the complete inhibition of fungal growth by 100%.

Table (4) : Inhibitory Activity of methanolic extract of *Eucalyptus sp.* leaves on *Alternaria sp.* afer incubation period 7 days , 30˚C

Fungus	Extract concentration (%)	Colony diameter (cm)	inhibition rate (%)
Alternaria sp.	control	8	0
	0.5	6	25
	1	3	62.50
	2	1.5	81.25
	4	0	100
	8	0	100

Results in table (5) showed that the crude methanolic extract of *Eucalyptus sp.* leaves had a clear effect on the growth of the fungus *Rhizopus sp.* starting from a concentration of 0.5% , while it had the strongest effect at a concentration of 8% Which led to the complete inhibition of fungal growth by 100%.

Table (5) : Inhibitory Activity of methanolic extract of *Eucalyptus sp.* leaves on *Rhizopus sp.* afer incubation period 7 days , 30˚C

Fungus	Extract concentration (%)	Colony diameter (cm)	inhibition rate (%)
Rhizopus sp.	control	9	0
	0.5	8.5	5.56
	1	7	22.23
	2	3.5	61.12
	4	1	88.89
	8	0	100

Results showed in table (6) that the methanolic extract of *Eucalyptus sp.* leaves had a clear effect on the growth of the fungus *penicillium sp.* starting from a concentration of (0.5%) , while it had the strongest effect at a concentration of (8%) Which led to the complete inhibition of fungal growth by (100%).

Table (6) : Inhibitory Activity of methanolic extract of *Eucalyptus sp.* leaves on *Penicillium sp*. afer incubation period 7 days , 30˚C

Fungus	Extract conc. (%)	Colony diameter (cm)	inhibition rate (%)
Penicillium sp.	control	7	0
	0.5	6	14.29
	1	4	42.86
	2	2.5	64.28
	4	1	85.71
	8	0	100

Discussion

The fungal cell membrane is made up of ergosterol, which vary from the mammalian cell, which essentially contains cholesterol. Ergosterol is the main target when studying antimycotic drugs (17).

It has been found that flavonoids found in many plants are antimicrobial agents for a wide range of pathogenic microorganisms (18,19) through the following mechanisms :

Interruption of cytoplasmic membrane

In general, the mechanism of action of antifungal drugs is to affect the functioning of the cytoplasmic membrane through preventing the biosynthesis of ergosterol in the fungal cell wall, this will lead to the leakage of cell components from the inside to the outside. Excessive production of reactive oxygen will also cause high oxidative stress to the fatty acids, amino

acids, and nucleic acids present inside the fungal cell, and this will lead to an increase in the permeability of the cytoplasmic membrane (19).

Causing a malfunction in the functioning of mitochondria:

Generally, antifungal medications cause a defect in the functioning of mitochondria . This defect may be due to a weakness in the respiratory process, or due to a weakness in the oxidation of fatty acids, or damage to the DNA of the mitochondria, or the reason may be a change in the permeability of the mitochondria. (19).

Disrupting of DNA synthesis, cell division, protein synthesis, and cell wall formation

The antimycotic mechanism act based on distort of cell wall due to inhibition of the synthesis of β-glucans and chitin (19).

Flavonoids affected the fungal filaments growth by reduce the G0/G1 phase and rising the G2/M phase. The inhibition of microtubule polymerization leads to inhibits the mitotic spindle formation and discouragement of cell division (20).

The antimycotic agents enters inside the cell over active transport that arrives at inside the nucleus, hence inhibits protein synthesis , DNA, and RNA. Flavonols inhibit hyphal fungus *Cochliobolus lunatus* over the inhibition of DNA and RNA synthesis. Carvacrol, are disrupts the cellular plasma membrane and impedes synthesis of the nucleic acids (19).

Studies indicate that the volatile oil of the eucalyptus plant affects the physiological activity of fungi by inhibiting glucose breakdown, which in turn affects cell energy metabolism. (21).

E. camaldulensis essential oil perfectly inhibited the mycelium of 5 strains of *Fusarium* spp. at a concentration scope (7 - 8 µL/mL) after incubation for 5 days (22).

In a study , results are revealed that phenol and terpene at (10%) concentration was notably best than others in growth inhibition of *F. oxysporum* which recorded 100 %, while alkaloid at (10 %) was notably less than others registered (63.66%) only (23).

Another study showed that phenol extracted from *Eucalyptus* with a concentration of 10% inhibited the *F.oxysporum* fungus by 33.34%, as it was found that the antifungal properties depend on the lipophilic and hydrophilic properties.(24). These oils led to decay of fungal mycelium that revealed clear of cytoplasmic contents (25). Phenols effect on fungal cells due to these constituents sensitize the phospholipids of the fungal plasma membrane rising permeability of essential vital intracellular constituents. (26).

Conclusion

The crude methanolic extract of *Eucalyptus sp*. leaves was found to be a good source of

antifungal agents. Significant antimycotic activity agents up-against the invesigated fungal isolates were as well noticed which is likely because of the exictence of high bulk of saponins and phenolic compounds in the crude extract. This study highlights the possible utilize of bioactive constituents obtained from *Eucalyptus* extract as antimycotic agents in the industrial production of medicine. in the future.

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