in Vitro Antimycotic Activity of Eucalyptus Camaldulensis Extract Against Trichophyton Rubrum

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ABSTRACT
Surface mycosis of the nails, hair, and skin ia a prevalent ailment worldwide. They are caused by several yeast and molds.The primary cause of these illnesses is the dermatophytes, which are filamentous fungi that rely on the keratin in the skin, the corneal layer, and the superficial growths on both human and animal skin. The distribution of these fungi varies between country and within a single country depending on the climate and other variables that promote their growth. the dermatophytes are a major health risk. The current study aim to assess the antifungal activity of Eucalyptus camaldulensis extracts against dermatophytosis-causing Trichophyton rubrum. Three species of dermatophyte (Trichophyton rubrum, Trichophyton mentagrophyte and Microsporum canis) were isolated from patients; Trichophyton rubrum was the most common type of fungus. The acquired results demonstrated that, in comparison to the controls, Trichophyton rubrumś mycelium growth was impacted by both aqueous and ethanolic extract. Additionally, it was discovered that when tested against Trichophyton rubrum, ethanolic extracts exhibited the greatest growth suppression.

1. Introduction

Human infections, especially those that effect skin and mucosal surface, are a major issue, particular in developing nations that are tropical and subtropical; the most common pathogens are *dermatophytes* and *Candida* spp. many organism, mostly fungus called dermatophytes, can infect human and animal Skin, hair and subcutaneous tissues .(Ali and Majid, 2008). Molds that thrive on the keratin in the skinś cornea layer and the superficial body growths on the human and animals. (Venturini *et al*., 2012, Ndiaye, 2013). According to reports, *T. rubrum* is the primary source of chronic infections associated with dermatophytosis (Sousa, *et al*., 2015).

Unchecked use of antifungal drugs may be the cause of this, as it can provide a selection pressure that makes it possible for a resistant strain to proliferate in a population.Longterm drug exposure to flucon azole and itraconazole was proven to cause T. rubrum to develop resistance to the medications.T. rub rum has a tendency to develop resistance to fluconazole when compared to itraconazole, as evidenced by analysis of minimum inhibitory concentration (MIC) values.Interaction patterns between these tw o azole antifungals were also identified in this investigation.Improved drug efflux, reduced drug abso rption, structural target site alterations, and biofilm formation are some of the underlying processes th at may lead to T. rubrum resistance. (Hryncewicz-Gwozdz, et al., 2013). Antifungal medications have been utilized to treat contagious contaminations everywhere, except the greater part of them have wellbeing, adequacy, and cost issues. Furthermore, the normal utilization of these specialists has caused the development of safe types of the growth. Thusly, there is a tremendous interest for novel antifungal drugs that specifically follow up on original focuses while making the least conceivable side impacts and have a place with an expansive scope of underlying classes. This accentuates the need to explore novel antimicrobial medications and approaches for treating sicknesses welcomed on by the previously mentioned microbes (Sati and Joshi, 2010).

Like this, growths have created protection from azoles, poliens, and echinocandins; as a matter of fact, drug-safe kinds of all fungal species have been recorded (Robbins *et al.*, 2017).

Developing nations have relied heavily on herbal Herbal drugs to treat common infection, such as

fungus-related illnesses. Studies have shown that Eucalyptus species oil and leaves have antifungal and repellent properties. (Pattnaik, *et al*. 1996).

It has been tracked down that *E. Camaldulensis* unrefined concentrate restrains the development of *Candida albicans*. Moreover, studies directed by Babayi *et al*., (2004) and Essien and Akpan, (2004) have exhibited a prominent fungicidal viability of an ethanolic leaf concentrate of *Eucalyptus camaldulensis* against clinical dermatophytic contagious disengages, explicitly *Microsporium gypseum* and *Trichophyton mentagrophytes*. To evaluate *Eucalyptus Camaldulensis'* antifungal adequacy against *Trichophyton* spp., this study was done in Iraq.

2. Material and Methods

Source of plants

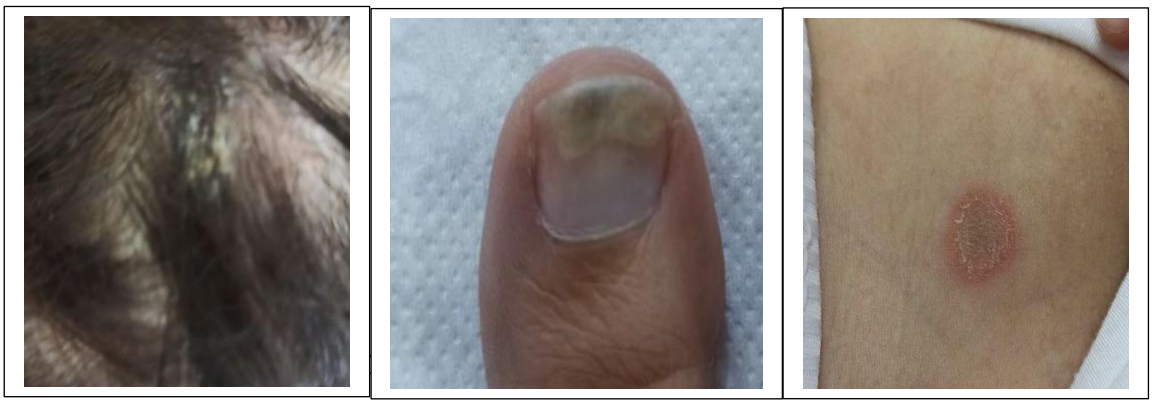
Prof. Dr. Majid Hanoon Sharhan in wasit university- college of Science-department of biology, perceived the *Eucalyptus* plant (*Eucalyptus camaldulensis*) after it was taken from a garden in Wasit province

Source of antifungal drug

Clotrimazole, itraconazole, and griseofulvin, the antifungal drug utilized in this review, were bought as a standard arrangement from a drug store in Wasit.

Sample collection

Patients at the medical clinic and Alkarama Hospital in Wasit Region gave 80 clinical examples. The patient's skin scrapings, hair, and nails are among the examples Figure (1). In the wake of disinfecting the impacted region by washing it with 70% alcohol, skin scrapings were gotten from the middle or edge of the sore utilizing a clean new edge and sterile spread paper. In a sterile Petri plate, hair tests were taken from the foundation of the hair. Test of nails: Subsequent to utilizing 70% ethanol to clean the nail site, the scrapings were accumulated. The examples are fastidiously moved to Petri dishes loaded up with SDA (Sabouraud Dextrose Agar) medium, including 0.5 milliliters of cycloheximide and 0.05 grams of chloramphenicol per liter. Subcultures were prepared for identification. The pathogenic contagious separate become on SDA medium was recognized morphologically utilizing the Immediate assessment (KOH-test), and unadulterated provinces were ready for the ensuing test in light of the qualities of the settlements, including size, surface, development rate, and pigmentation created on the front-side and opposite sides of culture on SDA medium.(Jartarkar *et al*., 2021).



Prepare of *Eucalyptus* extract

The leaves of the *Eucalyptus camaldulensis* plant were accumulated, cleaned, and heated for two hours at 60 °C. The accompanying stage was mixing these dried leaves to make a powder. Eventually, until their utilization, these powders were kept in plastic compartments Figure (2) (In

1995, Toma and Sada).

Figure (2) Dried powder of plant material

Preparation of Aqueous Extracts

In flask Figure (3), 500ml of distilled water and 100g of drying powder were blended to make a fluid concentrate of *Eucalyptus* spp. The cream colored liquid was made in the wake of heating up the watery mix over low intensity for 20 minutes. The supernatant was separated utilizing 0.45µ Whatman filter paper, and dried in a revolving evaporator set at 50°C. The weight of extract were measured and put away for additional utilization. (Ahmad and Beg, 2001).

Preparation of Ethanolic Extracts

To make an ethanolic extract of *Eucalyptus* spp., 500ml of 70% ethanol was blended in with 100g of drying powder Figure (3). Utilizing a glass pole, the ethanolic remove blend was twirled at regular intervals while it was kept at lab temperature for an entire day. Following filtration through 0.45 µ Whatman filter paper, and then dried at room temperature. The weight of extract were measured was then evaluated and put away for additional utilization (Ahmad and Beg, 2001).

Figure (3) aqueous and ethanolic extract of *Eucalyptus*

Effect of *Eucalyptus* extract on growth of *Trichophyton rubrum*

to survey utilizing the disc diffusion technique the effect of ethanolic and aqueous *Eucalyptus* extracts on *Trichophyton rubrum* growth. To make the stock solution (S) 100 percent of *Eucalyptus* extract, two grams of dried extract were blended in with 80 milliliters of distilled water on account of aqueous extract and Dimethyl Sulfoxide (DMSO) on account of an ethanolic extract. The stock solution was then diluted to make concentration of 50% and 25%. Subsequent to being autoclave-

disinfected, 5 mm-width paper circles were absorbed solutions containing 100, 50, and 25% centralizations of plant extracts (ethanolic and aqueous),and then positioned in a petri dish containing SDA that had proactively been immunized with the objective growth. To permit the solution to diffuse, the agar plate was left in the room temperature for two hours. Following that, each plate was incubated for a foreordained measure of time at 28 °C. The inhibitory zones were then measured in millimeters after that. (Muthomi *et al*., 2008).

3. Results and Discussion

Sample Isolation and identification

As per the review's discoveries, which are displayed in table (1), of 80 clinical samples, 19 samples (or 23.75%) were negative and 61 samples (or 76.25%) were positive. From the 61 positive clinical samples, 46 (75.41%) were *dermatophyte* growth, while 15 (24.59%) were non-*dermatophyte* fungi, which included other fungi, for example, *Candida albicans, Aspergillus niger*, and *Aspergillus flavus* Figures (4, 5, and 6). Of the *dermatophyte* fungi, *T. rubrum* represented 24 (39.34%), *T. mentagrophytes* for 13, (21.32%), and *M. canis* for nine (14.75%). *Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans* are among the non-*dermatophyte* fungi that were separated from 15 samples. Six specimens (9.83%) included *C. albicans*, five specimens (8.19%) contained *Aspergillus niger*, and four specimens (6.56%) contained *Aspergillus flavus*. As indicated by the review's discoveries, *Aspergillus flavus* was the least normal organism, while *T. rubrum* and *T. mentagrophyte* were the most widely recognized.

Table 1: Prevalence of dermatophyte fungi in tested specimens

Dermatophyte fungi			Non dermatophyte fungi		
Fungi	Number	Percentage	Fungi	Number	Percentage
Tricophyton rubrum	24	39.34	*Candida albicans*	6	9.83
T. mentagrophytes	13	21.32	*Aspergillus niger*	5	8.19
M. canis	9	14.76	*Aspergillus Flavus*	4	6.56
Total	46	75.42		15	24.58

However numerous as 69.5% of human diseases seem to be brought about by *dermatophytes*, which are normal, turning out to be more normal around the world, and viewed as a serious general wellbeing worry in different locales of the world (Chen and Friedlander, 2001). Concerning connection between climatic variables and the pervasiveness of dermatophytoses, it is for the most part acknowledged that nations in tropical or subtropical locales with humid, warm environments might have higher prevalences of dermatophytoses in light of the fact that *dermatophytes* flourish in warm, muggy conditions (Havlickova *et al*., 2008; Coulibaly *et al*., 2016).

The discoveries of this study show that *Trichophyton rubrum* was the most normally tracked down fungi. These discoveries are reliable with those of other investigations that have created comparable discoveries, like Kadhim's, (2018) study, which found that *Trichophyton rubrum* had the most noteworthy recurrence of *dermatophytes* separates, 29 (17.68%), *Trichophyton mentagrophytes*, 26 (15.85%), and *Microsporum canis*, 24 (14.63%).

Sakkas *et al*., (2020) directed a concentrate on onychomycosis in North West Greece and tracked down that *T. rubrum* (74.4%) and *T. mentagrophytes* (21.4%) were the most regularly secluded

dermatophytes.*T. rubrum* has been the most frequently detached *dermatophyte* growth, as indicated by various examinations that have been finished to survey the commonness of *dermatophyte* fungi (Bhagra *et al*., 2014 ; Lakshmanan *et al*., 2015).

The review's outcomes likewise uncovered an adverse outcome (20%), which is predictable with those of Liu *et al*., (2000), who found an adverse outcome (19%). This could be because of an example ailing in fungi components or a lacking measure of material, as well with respect to an ill-advised temperature, a deficient incubation period, or the presence of "impurities" that can prevent the microbe from creating.

A review was directed in Baghdad to find out the rate and causative specialists of dermatophytosis in a gathering of Iraqi patients. The outcomes showed that *Trichophyton rubrum* 20 (half) and *Trichophyton mentagrophytes* 13 (32.5%) were the essential etiological specialists (Mohammed *et al*.,2015).

As indicated by a few examinations, *Trichophyton* species are the primary drivers of dermatophytosis, with commonness paces of 53-86% for other fungus diseases and 70-90% for onychomycosis. *Trichophyton rubrum*, *T. mentagrophytes* complex, *Microsporum canis*, and *M. gypseum* are the other primary etiological specialists among them (Yadav *et al*., 2015; Balakumar *et al*., 2012).

A review that investigated the clinical and epidemiological elements of *T. rubrum* diseases was done. It saw that as, of 131,122 patients with dermatophytosis, 115,846 patients (88.35%) had *T. rubrum* infection, and that the yearly rate of patients with *T. rubrum* infection had been ascending during the tried period (Lee *et al*., 2015).

Nanoty *et al*., (2023) analyzed 60 clinically analyzed instances of dermatophytosis in patients who went to the dermatology open air patient division. The outcomes showed that fungus corporis, which is brought about by the *dermatophyte* fungus *Trichophyton rubrum*, was the most regular clinical show in patients.

Effect of *Eucalyptus* extracts on *T. rubrum* growth

The study's findings, which are shown in Table (2) and Figure (2) demonstrated that the growth of *T. rubrum* was affected by the two extracts (aqueous and ethanolic) in fluctuating degrees. The ethanolic extract outperformed the aqueous one in terms of effectiveness, and the impact of the extract developed with focus.

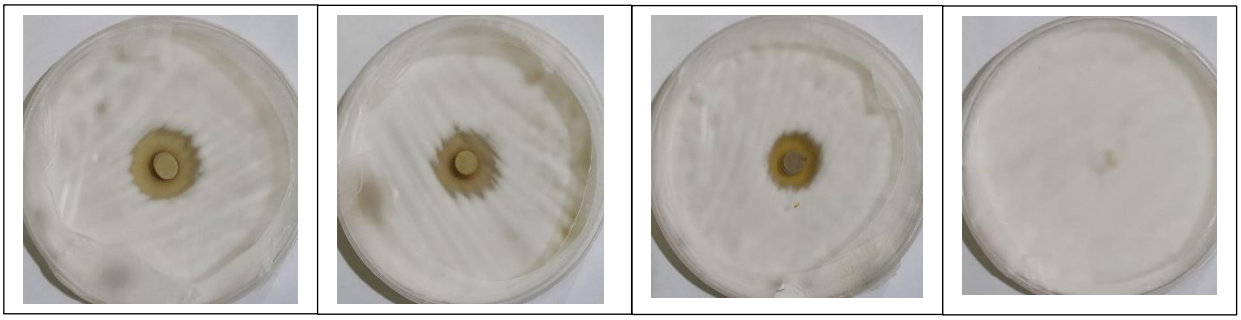
The ethanolic extract at 100 percent concentration had the biggest inhibition zone, measuring 25.30 mm, whereas the aqueous extract at 25% percent had the lowest inhibition zone, measuring 6.6 mm.

Table (2) Effect of *Eucalyptus* extracts on *T. rubrum*

Treatment		Inhibition zone (mm)
Extract	Concentrations (%)	*T. rubrum*
Ethanolic extract	100%	25.30
	50%	12.82
	25%	7.1
	100%	22.53

Aqueous extract	50%	11.37
	25%	6.6
Control		0
LSD (0.05)		0.558

Each value is a mean of three replicates



A b c d

Figure (4) Effect of *Ethanolic* extracts on *T. rubrum* by disc diffusion method

(a- 25mgm/ml , b- 12.5mgm/ml , c- 6.25 mg/ml , d- control)

Based on the findings, the objective microorganism in this study was significantly repressed by the aqueous and ethanolic leaf extract of *Eucalyptus camaldulensis*. Contingent upon the sort and convergence of the extract, the antifungal effect fluctuated. Also, the ethanol extracting more strong antifungal minerals might be the reason why the alcoholic extract outperformed the aqueous extract in terms of effectiveness.

The findings showed that the impact of the extract developed as the extract concentration increased, and other authors had previously affirmed this conclusion (Samuel *et al*., 2000; Shams-Ghahfarokhi *et al*., 2006; Gaston *et al*., 2014).

Furthermore, the result is consistent with that of Alizadeh *et al*., (2013) who assessed the antimicrobial properties of alcoholic and aqueous extract of *Eucalyptus camaldulensis* leaves against various significant food pathogens and discovered that the alcoholic extract was superior to the aqueous.

As indicated by Singh *et al*., (1988) *Eucalyptus rostrata* leaves have antidermatophytic action against four *Trichophyton mentagrophytes*, including *Epidermophyton floccosum*, *Microsporum gypseum*, and *M. Canis*.

Falahati *et al*., (2005) analyzed the antifungal properties of *Eucalyptus camaldulensis* methanolic leaf extracts against *Tricophyton rubrum*, *Tricophyton schoenleinii*, *Tricophyton mentagrophytes*, and *Epedermophyton floccosum* in vitro. They discovered that the extract repressed *T. rubrum* development, with the impact developing with concentration and the inhibition zones going from 2-35 mm.

Using an in vitro weakening methodology, the hydro-alcohlic extract of *Eucalyptus camaldulensis* was considered in contrast to *dermatophytes* and demonstrated highest inhibitory adequacy against the *dermatophytes* (Moghimipour *et al*., 2009).

Similar research was finished in (2010) by Aly and Bafeel, to assess the antifungal properties of *Eucalyptus globules* water and organic crude extracts against *Trichphyton rubrum, T. mentagrophyte*,

Candida tropicals, and *C. albicans*. The results showed that both the water and organic crude extracts demonstrated strong antifungal action against the fungi under test, with inhibition zones going from 11 to 14 mm.

4. Conclusion and future scope

Using the agar well diffusion technique, the antifungal action of unrefined aqueous, ethanolic, and methanolic extracts of Eucalyptus microtheca leaves was assessed in vitro against Penicillium digitatum and Aspergillus niger. More so than water extracts, alcoholic extracts emphatically suppressed P. digitatum and A. niger's mycelial growth (Mahmoud, 2012). Wong et al., (2015) gave proof of the antifungal activity of Eucalyptus on dermatophytes by showing that E. globulus inhibits the development of T. mentagrophytes. Therefore, Elaissi et al., (2012) work has demonstrated the antifungal, antibacterial, and antiviral activity of various Eucalyptus species. The essential oils of these species were tested against five fungal strains, which included shape (Scopulariopsis brevicaulis), yeast (C. albicans), and three dermatophytic species (T. rubrum, T. sudanense, and M. canis). Ennaghra et al., (2019) did a study to assess the inhibitory impact of three plant aqueous extracts against three dermatophytic species (Trichophyton rubrum, Trichophyton verrucosum, and Microsporum ferrugeneum). The findings demonstrated that all extracts inhibited mycelium development in comparison to the controls, with the Eucalyptus extract showing the highest level of growth inhibition (60-80%). The presence of anthraquinones, flavonoids, saponins, and terpenoids in the rough methanolic leaves extract of E. camaldulensis is responsible for its antifungal activity (Singh and Thakur, 2016). As per Sánchez-Loredo et al., (2024) bioactive substances such ellagitannins, tellimagrandin, and pedunculagin are responsible for Eucalyptus camaldulensis' antibacterial activity.

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