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In vitro Investigation of the Effects of Various Media and *Eucalyotus Camaldulensis* Essential oil on *Dermatophyte* spp. Growth

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ABSTRACT:
The study was finished in the biology division lab of the College of Science, University of Wasit, between January 9, 2023, and January 9, 2024. Purpose of the research the effects of various media and *Eucalyptus camaldulensis* essential oil on *dermatophyte* spp growth. The experimental factors: Seasons and geographic area affect the creation example of essential oil. A Significant essential oil is *Eucalyptus* oil ($C_{10}H_{18}O$). Fresh and dried leaves, as well as branch tips, are used to extract the oil. Bioactive properties, and a length history of usage against many disease make Eucalyptus oil a valuable medicinal tool. The results showed that the essential oil considerably affected *Trichophyton rubrum's* growth in liquid medium (SDB), and that impact developed as concentration increased. In comparison to the control, which weighed 4.87 gm, the weight of the mycelium mat was 1.17, 2.83, 3.57 gm at concentrations of 100, 50, and 25%, respectively. The lowest weight was found at concentration 25%, which was 3.57 gm. The concentration of 100 percent yielded the extreme level of inhibition (76.13%), while the concentration of 25% yielded the lowest level of inhibition (26.54%). The medium SDA showed the most noteworthy growth, with radial growth of 76.67 mm following 7 days, trailed by the medium PDA at 70.33 mm. The case of the medium YEA 20.33 mm showed the most minimal growth rate. A statistical analysis uncovered that there is no significant difference ($P < 0.05$) between the medium MEA and YEA, but there is one among SDA and every single tried medium. Subsequently the accompanying tests can be performed utilizing the medium SDA.

Keywords: *culture media, Dermatophyte, Eucalyptus, essential oil*

1. INTRODUCTION

To evaluated the spread of dermatophyte in Wasit Province and how to reduce the infection by using Eucalyptus essential oil. Dermatophytes is equipped for attacking human and animal keratinized tissue (skin, hair, and nails) and causing Dermatophytosis, frequently known as ringworm. Since the growth can't infiltrate the more deeply tissues or organs of immunocompetent hosts, disease is many times cutaneous and restricted to the nonliving cornified layers [1].

The accompanying genera of dermatophyte growth are remembered for the phylum Deutromycota: Microsporum, Trichophyton, and Epidermophyton. While the other two genera, Microsporum and Trichophyton, are comprised of a few animal types, the family Epidermophyton just has one pathogenic animal types [2]. Some dermatophytes are adjusted to people (anthropophilic species), and are typically spread from one individual to another. A few living beings, known

as zoophilic species, are animal adjusted. A couple of animal types are by and large natural (geophilic), despite the fact that they can likewise at times capability as parasites. Because of their adequacy in penetrating keratinous tissues, dermatophyte growths are among the best human parasites, as per the proof [3], [4].

Pathogenic dermatophyte growths like Microsporum, Trichophyton, and Epidermophyton are the wellspring of dermatophytosis. Trichophyton and Microsporum species can contaminate people and animals. The main known types of Epidermophyton that causes infection is E. floccosum, and it commonly just influences people. Around the world, the most well-known reason for dermatophytosis was the family Trichophyton, especially T. rubrum [5].

As per [6] Eucalyptus oil is viewed as quite possibly of the strongest disinfectant in its group. It has strong disinfectant characteristics.

As indicated by [7] Eucalyptus oil might have hepatoprotective characteristics. This suggests that it very well may have the option to safeguard the liver from hurt welcomed on by unambiguous poisons. It is believed to be connected with ursolic acid, a substance present in Eucalyptus cross breed E. tereticomis leaves. Because of its notable calming and cancer prevention agent characteristics, ursolic acid might assist with protecting liver cells from hurt and empower their recovery.Coronary illness, dementia, and disease can be in every way battled by eats less high in these flavonoids. As indicated by research, Eucalyptus can help the lungs and reduce mucous. The essential part of this plant's calming characteristics is eucalyptol, otherwise called cineole, which can be found in Eucalyptus oil or concentrate [8].

Eucalyptus oil breathed in can lighten side effects. Cineole and limonene, two mitigating substances tracked down in Eucalyptus, have pain-relieving properties. As indicated by [9], this plant can diminish the movement of the thoughtful sensory system and the pressure reaction framework while expanding the action of the parasympathetic sensory system, which advances unwinding.

Thus, the purpose of this study was evaluate how various media and Eucalyptus essential oil affected the growth dermatophytes.

2. MATARALS AND METHODS

In vitro investigation was conducted in the laboratory of biology Division College of Science, University of Wasit to study the effect of different media and essential oil on the growth of fungi *dermatophyte*

2.1 Source of culture media

The study's culture media (Sabouraud Dextrose Agar, Potato Dextrose Agar, Malt Extract Agar and Yeast Extract Agar) were acquired from a medical equipment store in the province of Wasit.

2.2 Source of essential oil

Agrarian Research Directorate of the Ministry of Science and Technology provided the essential oil, These incorporate components 1,8-eucalyptus (72.71%), α-terpine (2.54%), Terpiene-4-old((0.34%), Linalool (0.24%), α-eudesmol (0.39%), (-)- globulol (2.77%), Epilobulol (0.44%), α-terpineol acetic acid derivation (3.1%), Geranyl acetic acid derivation (0.71%), L-pinocarveol (0.36%), β-sabinene (0.25%), terpinolene (0.19%), and an expected 0.26% of *Eucalyptus* oil's items are obscure.

3. INFLUENCE OF CULTURE MEDIA ON TRICHOPHYTON SPP GROWTH

3.2 Cultural media

This study utilized four different culture media, which were delivered and dissected as per maker directions: potato dextrose agar (PDA), yeast extract agar (YEA), malt extract agar (MEA), and sabouraud dextrose agar (SDA). In the wake of dissolving every one of these media in distilled water, they were totally autoclaved at 121°C for 15 minutes at a strain of under 15 psi and put away at 4°C for future exploration.

3.3 Influence of cultural media on growth of *Trichophyton* rubrum

Different media, including SDA, PDA, MEA, and YEA, were utilized to evaluate the effect of cultural media on Trichophyton growth. Each tried medium was made autonomously, and it was autoclaved for 15 minutes at 121°C and 15 psi of pressure. Then, sterile Petri dishes measuring 9 cm were loaded up with 20ml of each example. For roughly an hour, the media were left to solidify at room temperature. In the wake of being exclusively infused in the tried media, a disc (5 mm) containing a created pure culture of Trichophyton rubrum was incudated at 28 ± 2°C. The province distances across of Trichophyton rubrum in all media were measured seven days after the inoculation. There were three replications of every treatment in a completely randomized arrangement. [10].

3.5 Effect of essential oil on mycelial mat of *T. rubrum* in liquid Media

Reasons for choosing liquid media for growing fungi, 1. Enhance growth: compared to solid media, liquid media offer fungus agreater surface area on which to grow. Batter aeration and nutrient absorption are made possible by this, which encourages faster and more prolific fungal growth. 2. Handling ease: working with fungi in a liquid environment it more comfortable for researches since liquid media are simpler to handle amd work with. Transferring fungus for sub-culturing or experimentation is made easier using this procedure. 3. Scale-Up: Large-scale fungal cultures can be readily established using liquid media. This is especially helpful in industrial situations where huge amounts of fungi are required for a variety of uses, including the manufacture of food, medicines, and biotechnology. 4. Extraction of Metabolites: for the extraction of metabolites made by fungi, liqud medium work best. It is simpler to gather and extract bioactive chemical, enzymes, and other substances released by fungi for additional examination and study when the fungi are grown in liquid media. To survey the effect of *Eucalyptus* essential oil on *T. rubrum* growth, 225 ml of melted SDB medium, 250 ml of melted SDB medium as a control, and 25 ml of every concentration of essential oil — 100, 50, and 25% — were consolidated independently. Each medium was added to a 300 ml flask. Mycelial disc with a width of 10 mm from an old *T. rubrum* culture were utilized to inoculated the flask, and they were all then, at that point, incubated at 28 °C for the given measure of time. Following 21 days, the mycelial mats were gathered by going them through a solitary piece of Whatman No. 1 filter paper. Then, at that point, utilizing the equation, the real weight of the mycelium was determined:

Mycelium Weight = (Mycelium weight +filter paper weight) - (filter paper weight) [11].

The Percent inhibition (%) = (C - T)/C * 100.Where:

C= Mycelium weight in control

T= Mycelium weight in treatment [12].

4. RESULT AND DISCUSSION

4.1 Influence of cultural media on growth of *Trichophyton rubrum*

The media were all observed to be reasonable for the growth of the *dermatophyte* fungus, as indicated by the after effects of the investigation of the impact of various media (SDA, PDA, MEA, and YEA) on the development pace of *Trichophyton rubrum*. Figure (1). Nonetheless, the medium SDA showed the most noteworthy growth, with radial growth of 76.67 mm following 7 days, trailed by the medium PDA at 70.33 mm. The case of the medium YEA 20.33 mm showed the most minimal growth rate. A statistical analysis uncovered that there is no significant difference ($P < 0.05$) between the medium MEA and YEA, but there is one among SDA and every single tried medium. subsequently the accompanying tests can be performed utilizing the medium SDA.

Table 1. Effect of different media on growth of *Trichophyton rubrum*

Type of Fungi	**Colony diameter in (mm) Type of media**			
	SDA	**PDA**	**MEA**	**YEA**
Trichophyton rubrum	76.67	70.33	35.33	20.33
LSD(0.05)	1.087			

Each value was a mean of three replicates.

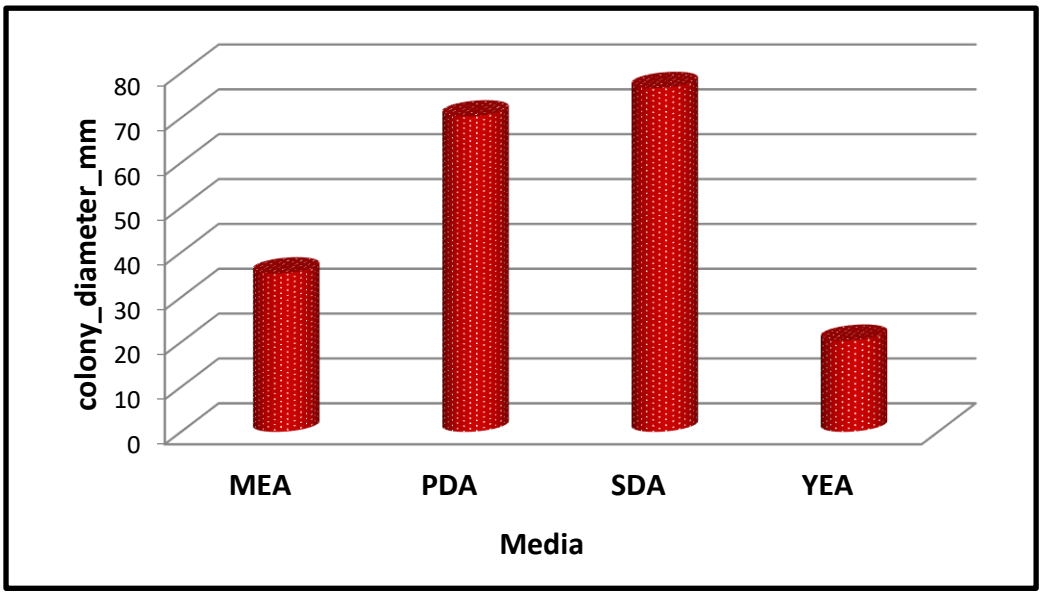

FIGURE 1. Radial growth of *T. rubrum* in different media (a- SDA, b-PDA c- MEA d- YEA)

The findings of this investigation demonstrated that *T. rubrum* developed to its most extreme outspread length in the SDA medium. This may be credited to the medium's capacity to meet the healthful needs of the test fungus as well as its high grouping of carbon and nitrogen supplies as dextrose and peptone.The findings of this investigation were in accordance with those of [13] who led a connected investigation and covered the effect of culture media on the mycelial growth and sporulation of some *dermatophytes* fungus. They also discovered that the ideal medium was Sabouraud's dextrose ager (SDA).The findings of this investigation were consistent with those of who discovered that culture media significantly affected any microorganism's capacity to multiply, sporulate, and discharge conidially. further demonstrated that Sabouraud's dextrose medium (SDM), trailed by potato dextrose medium (PDM), gave the best conditions to the colony radial growth and sporulation of *dermatophyte* colonies.
[14], investigated the way of culture conditions for *Trichophyton rubrum* and *mentagrophytes*, two significant infections associated with Dermatophytosis, and observed that Sabouraud's dextrose broth was a decentgrowth medium for them.
As indicated by [15] research, which analyzed the effects of four fluid media on the development of keratinophilic and dermatophytic fungus, SDA medium took into account the greatest fungi development and sporulation.
Eight isolates of the *dermatophyte* fungus *Trichophyton rubrum* were studied by [16] to decide the effect of culture media (SDA, PDA, CMA, and YEA) on growth rate during various incubation periods. The study uncovered that SDA created the best development for all tested isolates contrasted with other media used.
Similar research was finished in [17], to assess the effect of several ecological conditions on *T. rubrum* growth. They discovered that the SDA and SDB were the most helpful for *T. rubrum* development.
As per a study by [18] the SDA was a superior medium for the development of *dermatophyte* fungi because it contains nutrients that are necessary for the development of fungi in that medium, such as 10 g of peptone and 40 g of dextrose. In the last option, the level of nitrogen was achieved at 13%. Furthermore, proof suggests that glucose is the essential component impacting sufficient fungi turn of events.
The findings of this investigation lined up with the research directed by [19], [20], demonstrating that the media has an effect on the advancement of *dermatophyte* fungus. The SDA medium has demonstrated to be outstandingly suitable for the growth of *dermatophyte*.

4.2 Effect of essential oil on mycelial mat of *T. rubrum* in liquid media

That's what the study's findings showed, when contrasted with the control, the essential oil considerably affected *Trichophyton rubrum's* growth in liquid medium (SDB), and that impact developed as concentration increased. In comparison to the control, which weighed 4.87 gm, the weight of the mycelium mat was 1.17, 2.83, 3.57 gm at concentrations of 100, 50, and 25%, respectively. The lowest weight was found at concentration 25%, which was 3.57 gm.The concentration of 100 percent yielded the most extreme level of inhibition (76.13%), while the concentration of 25% yielded the lowest level of inhibition (26.54%).

Table 2. The effect of essential oil on *T. rubrum* growth in liquid medium

Treatment		Myceliumwet weight (gm)	Percentage of inhibition
Essential oil	Concentrations (%)		
	100	1.17	76.13
	50	2.83	41.76
	25	3.57	26.54
Control		4.87	
LSD (0.05)		0.775	

Each value is a mean of three replicates

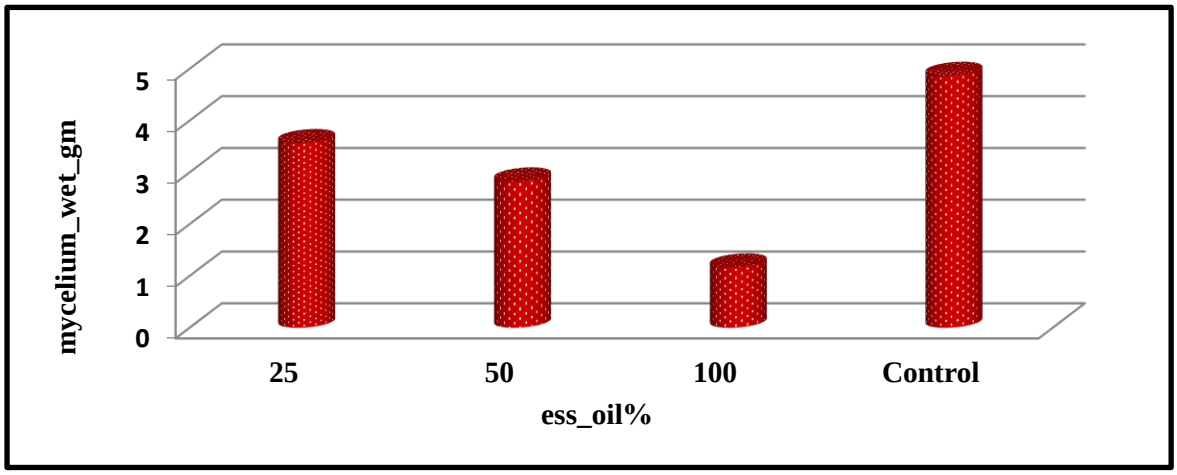

FIGURE 2. The effect of essential oil on *T. rubrum* growth in liquid medium (a- 100, b- 50, c- 25%, d- control)

Our study's findings showed that essential oils inhibited mycelium development at all concentrations, and it's possible that these effects were caused by the presence of active component. This likely relationship between the suppression of ergosterol and the inhibition of fungal growth could make sense of the last option. Ergosterol is a novel biomarker that is a decent objective for most of synthetic antifungal medications. It is specific to the fungal cell layer and keeps the cells liquid and unblemished in various ecological circumstances [21].

Numerous researchers have as of late analyzed the mechanism of activity of EOs and suggested that the disruption of plasma layer guideline by ergosterol inhibition, spillage of significant cell contents, and collapse in membrane potential and ATP pools could be the possible cause of EOs' inhibitory impact [22],[23].

Similar research was finished to investigate the antifungal action of *Cymbopogon winterianus* essential oil against the *dermatophyte T. rubrum*. The results showed that the essential oil decreased *T. rubrum's* mycelial dry weight and that it also changed the morphology of the contagious mycelium when inspected under a light microscope [24].

When contrasted with essential oils from other species of the genus *Eucalyptus*, those from *E. camaldulensis* had the highest degree of antifungal action against *Candida albicans*, as per a study done by [25].

[26], announced that the essential constituents in the essential oil (EO) got from *E. camaldulensis* were spathulenol (20.84%), eucalyptol (12.01%), and sabinene (9.73%); in C. aurantium, the compounds included linalyl acetic acid derivation (42.29%) and linalool (29.76%); in C. sinensis, the compounds included D-limonene (73.4%) and γ-terpinene (22.6%), which demonstrated compelling contagious mycilial development inhibition against *A. flavus and A. niger*.

In their investigation of the antifungal movement of essential oils from *E. citriodora*, *E. camaldulensis*, and *E. grandis* against the fungus *Hemileia vastatrix*, [27] tracked down that at the highest concentration, *E. camaldulensis* showed 100 percent in vitro antifungal action against *H. vastatrix.*

5. CONCLUSIONS

The media were all observed to be reasonable for the growth of the dermatophyte fungus, as indicated by the after effects of the investigation of the impact of various media (SDA, PDA, MEA, and YEA) on the growth of Trichophyton. Our study's findings showed that essential oils inhibited mycelium growth at all concentrations, and it's possible that these effects were caused by the presence of active component.

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