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Evaluation The Antifungal Activity of Black Pepper and Cumin Alcoholic Extracts

Manar Kareem Alquraishy[1], and Ahmed Adnan AL-Khafagi[2]

1 College of Health and Medical Techniques/Kufa, Al-Furat Al-Awsat Technical University, 31003 Al-Kufa, Iraq.

2 Anesthesia Techniques Department, College of Health and Medical Techniques, Al-Mustaqbal University, 51001, Babylon, Iraq

Corresponding author: Manar Kareem Alquraishy

E-mail: manar.fadelckm@atu.edu.iq

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ABSTRACT

Background: Plants containing spices can produce secondary metabolites described as biologically active constituents that play important roles, such as antimicrobials, anti-cancer, anti-inflammatory, antioxidants, and other important biological activities. **Objectives:** Evaluate the activity of black pepper and cumin alcoholic extract against *T. mentagrophytes* and *T. rubrum*. **Methodology:** The study involved preparation an alcoholic extract from black pepper and cumin at concentrations (25,50,75 and100 mg/ml), to evaluate the antifungal activity against the growth of T. *mentagrophytes* and *T. rubrum* comparing its antifungal activity with the antifungal Clotrimazole at 2µg/ml by agar well diffusion method to detect the inhibition zone. The Minimum Inhibitory Concentration was also determined for the black pepper and cumin extracts. **Results:** The results exhibit that the alcoholic extract has a higher level of inhibition compared with Clotrimazole, T. rubrum's inhibitory zone was achieved at 28,32.5,35 and 40 mm at the concentrations of black pepper extract 25,50,75 and 100 mg/ml respectively, while the effect of extract against the inhibition zone of *T. mentagrophytes* reached to (20, 30.5, 34 and 38.5 mm) at the same concentrations. The MIC of black pepper extract reached 14 and 12mg/ml against *T. mentagrophytes* and *T. rubrum*. The results of cumin alcoholic extract inhibition zone reached to 27.5,33.5, 35 and 37mm at 25,50,75 and 100mg/ml respectively against *T.rubrum* and 24,30.5,33 and 36mm against *T.mentagrophytes*. The MIC of cumin extract was 14mg/ml for *T. mentagrophytes* and *T. rubrum*. **Conclusion:** black pepper extract has stronger antifungal properties than cumin extract.

Keywords: Plant extracts, Antifungal activity, Cumin, Black pepper, Dermatophytes.

INTRODUCTION

In recent years, there has been a significant rise in research on natural medicines, as plants have been recognized for providing compounds that promote human health. This is in response to the challenges of antibiotic toxicity and multidrug resistant pathogens in the medical field(1).

Spices not only provide important nutrients but can also serve as remedies for various health issues and enhance the taste of dishes (2). Spices are produced using different parts of plants, including seeds, fruits, leaves, bark, and roots. Spices have various uses beyond just cooking, including medicine, food preservation, religious rituals, fragrances, cosmetics, and vegetables (3-5). It is believed that plants possess antibacterial properties due to their ability to produce complex secondary metabolites that exhibit antimicrobial activity. (6).

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Black pepper (*Piper nigrum* L.), also known as "The King of Spices", is a highly potent medicinal plant (7). Black pepper is found in a variety of dishes worldwide. Black pepper has been utilized for many years in the treatment of different health conditions. Black pepper is appropriate for medicinal use and is utilized as a preservative and flavoring in the cosmetic and perfume sectors (8), as well as in pharmaceutical products either on its own or combined with other ingredients. Black pepper is known for its active components like saponins, flavonoids, essential oils, chavisin, resins, egg white, starch, piperine, piperylline, piperoleine, piperanine, dihydrocarveol, karyo fillene oxide, cariptone, tran piocarrol, and pepper oil, making it useful for antibacterial, antifungal, antihypertensive, antiplasma cell, anti-inflammatory, hepatoprotective, and antioxidant properties (9).

Cumin (*Cuminum cyminum*) is the second most common type of seed after black pepper (10). Cumin seeds are sometimes used for digestive issues, as they are known for their carminative properties, and also for addressing nervous infections and bronchopulmonary disorders, as well as for coughs and toothaches (11). It is additionally utilized as an antioxidant, antibacterial, antifungal, anti-inflammatory, antidiabetic, and insecticide. Rephrase the text while maintaining the input language and word count (12). Cumin seeds have fatty oils, essential oils, acids, proteins, and other active substances like pinene, cymene, terpinene, cumene, oleoresin, thymol, and additional components (13).

The rising antibiotic resistance in medically important microorganisms necessitates the continuous development of new and efficient treatment options. Nevertheless, in the last three decades, there has been a significant amount of joy in isolating natural chemical compounds from healing plants to find new antioxidants and antimicrobial substances. As a result of the importance and frequent use of black pepper and cumin in people's lives, the current research is directed at investigating the antifungal effects of black pepper and cumin methanolic extracts on *T. rubrum* and *T. mentagrophytes* strains isolated from tinea capitis.

METHODOLOGY:

Fungi Isolates used in this study:

Fungal isolates include *T. rubrum* and *T. mentagrophytes* as dermatophytes, which were isolated from tinea capitis and identified for previous studies from the Microbiology Laboratory of the University of Karbala. It was activated by culturing in a medium of Potato Dextrose Agar and incubating it at 25 ° C for 5-7 days.

Collection of plant specimens:

Black pepper and cumin seeds were typically sourced from popular markets. To ensure their cleanliness, the seeds were thoroughly rinsed with tap water to remove dirt and contaminants, followed by an air-drying period. After confirming that the seeds were completely dry, they were ground into a fine powder using an electric grinder and subsequently stored in sterile containers until use.

Preparation of plant extracts:

After preparing and weighing 200 grams of the plant using a balance, 200-gram portions of the dried powdered plant were soaked in 600 milliliters of 99% ethanol using the maceration technique, following the guidelines from Singh (14). The mixture was left to stand at room temperature for 7 days with occasional shaking. The samples were filtered through double coatings of muslin cloth and filter paper. Then, the solvent was evaporated at room temperature to obtain a dry extract. The final dry products were stored in sterile containers at 4 °C under refrigerated conditions up to testing.

Preparation of different concentrations of alcoholic extracts:

Prepare a stock solution for each type of extract by dissolving 1000 mg of extract in 10 ml of distilled water to become a final concentration of 100 mg/ml,

from which concentrations of 25, 50,75, and 100 mg/ml were introduced.

The concentrations were then calculated according to the following equation:

"C1V1 = C2V2"

Test the effectiveness of plant extracts in the growth of fungi:

The diffusion method of Agar well was used (15). Each test plate was filled with 20 ml of PDA. After solidification, 100 µL of each fungi suspension of $0.5–2.5\times10^3$ concentration was added and spread over plates with an L-shaped glass rod (at three replicates). Wells with a diameter of 6 mm were prepared by borehole prepared to assay the well diffusion experiment, and 40 µL of the plant extract at several concentrations were added. 2 µL of chlotrimazole (an antifungal) as a standard (control +ve) and as a blank, 20% ethanol was loaded. Following a loading procedure, the plates were maintained in aseptic conditions to absorption of the plant extracts. Plates were incubated for 3-5 days at 25 ° C. After the incubation, the ruler was used to measure the inhibition zone's diameter (two orthogonal diameters) for each concentration, and then the inhibition rate was calculated for the same concentration in the prepared replicates.

Minimal inhibitory concentration (MIC):

The Agar dilution method (MIC) was used to mix 2 ml of each concentration from plant extract from 18 ml of dissolved and cooled PDA to 50 ° C in addition to the control dish, which does not contain the plant extract, and then inoculate the dishes with fungi in the form of a spot. Then incubate the dishes in the incubator at 25-28 ° C for 3-5 days. Results were recorded based on the presence of growth (+) or absence of growth (-), and at the lowest concentration where growth was not shown to be the minimal inhibitory concentration (16).

RESULTS:

The antifungal activity of Black pepper extract

The table and figure (1) show that the *T.rubrum* is the most sensitive with the effect of extract, the inhibition zone reached to 28,32.5,35 and 40 mm at the concentrations of extract (25,50,75 and 100 mg/ml respectively), at the concentration 100 mg/ml of alcoholic extract the antifungal inhibition percent reached to 100% compared with the antifungal drug followed by the inhibition growth of *T. mentagrophytes* which the inhibition zone was reached to 20, 30.5, 34 and 38.5 mm.

The results indicate that there is a relationship between the percentage of fungal growth inhibition used and the concentration of the extract, as when the concentration of the alcoholic extract increases, the inhibition zone increases compared to the antifungal effect.

Table (2) observed the result of the minimum inhibitory concentration of plant extracts. The MIC of black pepper was 14mg/ml for growth of *T. mentagrophytes* and 12mg/ml for the growth of *T. rubrum*.

The antifungal activity of Cumin extract

The table (3) and figure (2) show that the *T. rubrum* is the most sensitive with the effect of extract, the inhibition zone reached to 27.5, 33.5, 35 and 37 mm at 25,50,75 and 100mg/ml of alcoholic Cumin extract respectively, followed by the inhibition of *T. mentagrophytes* when the inhibition zone reached to 24,30.5,33 and 36mm compared with the effect of Clotrimazole (2µg/ml). The MIC of Cumin extract (4) was observed at 14 mg/ml for the growth of *T. mentagrophytes* and *T. rubrum.*

Table 1: The antifungal activity of Black Pepper extracts

Concentration mg/ml	Inhibition zone (mm)	
	T. mentagrophytes	*T. rubrum*
25	20	28
50	30.5	32.5
75	34	35
100	38.5	40
Clotrimazole 2µg/ml	40	40
Control (-ve)	0	0

The results in the table above represent the average of three replicates

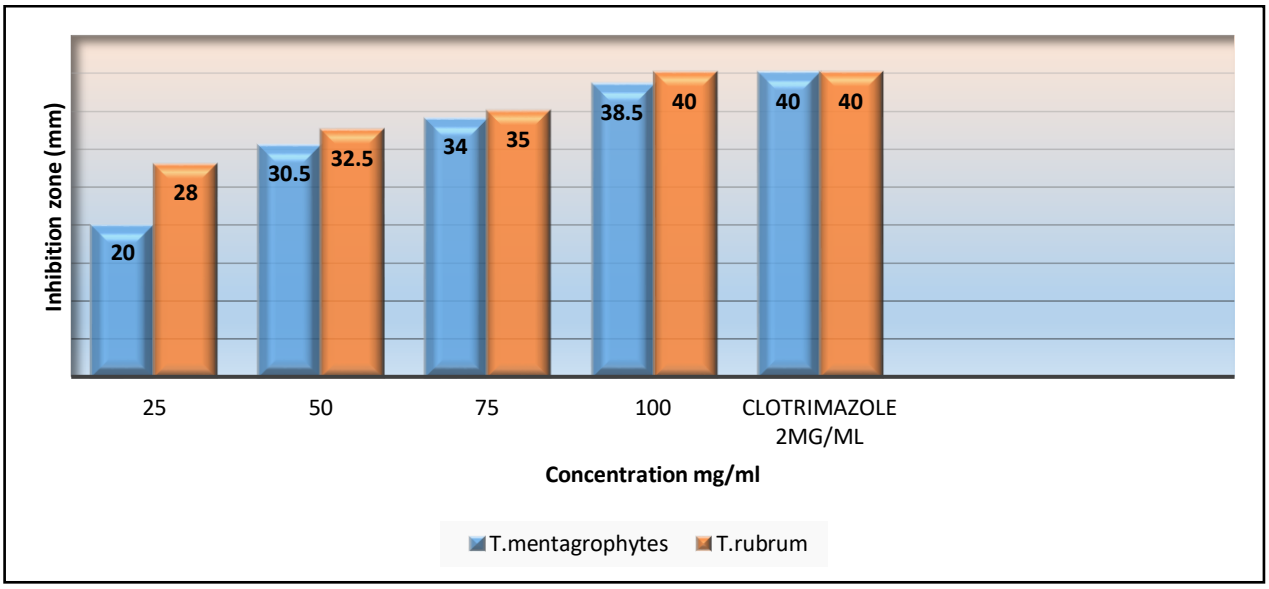

Figure 1: The antifungal activity of Black Pepper extract

Table 2: The MIC of Black pepper extract

Type of extract	Type of fungi	Concentration of extract mg/ml						
		10	12	14	16	18	20	22
Black Pepper extract	*T.mentagrophytes*	+	+	–	–	–	–	–
	T.rubrum	+	–	–	–	–	–	–

Table 3: The antifungal activity of Cumin extract

Concentration mg/ml	Inhibition zone (mm)	
	T. mentagrophytes	*T. rubrum*
25	24	27.5
50	30.5	33.5
75	33	35
100	36	37
Clotrimazole 2µg/ml	40	40
Control (-ve)	0	0

The results in the table above represent the average of three replicates.

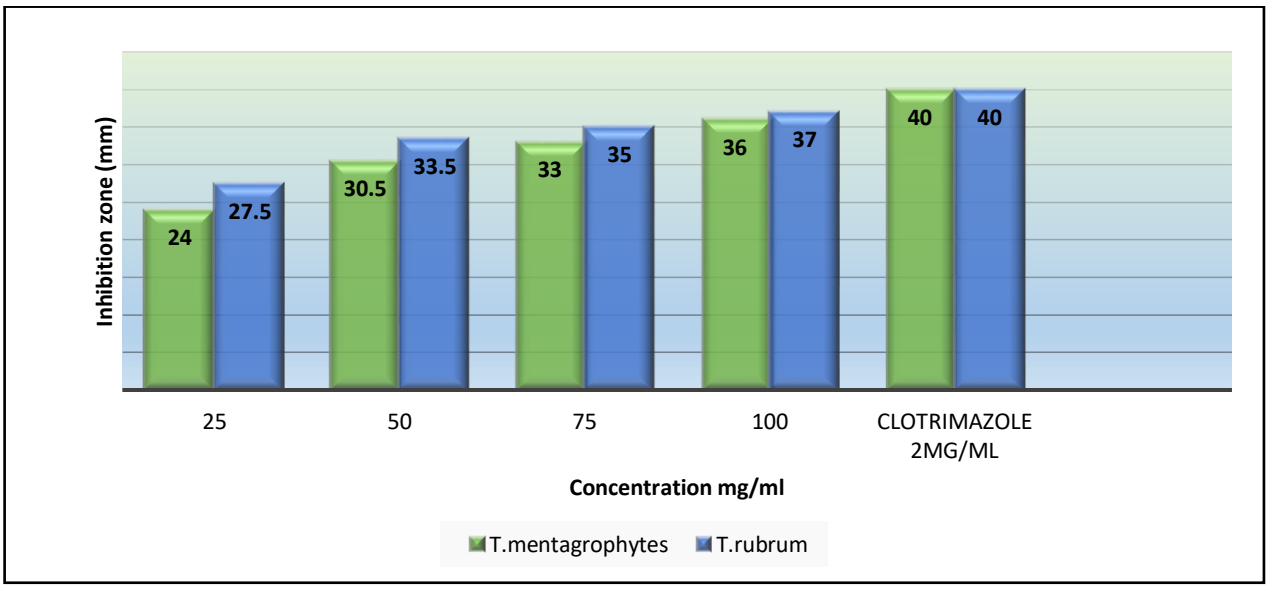

Figure 2: The antifungal activity of Cumin extract

Table 4: The MIC of Cumin extracts

Type of extract	Type of fungi	Concentration of extract mg/ml						
		10	12	14	16	18	20	22
Cumin extract	*T. mentagrophytes*	+	+	–	–	–	–	–
	T.rubrum	+	+	–	–	–	–	–

DISCUSSION:

This study demonstrates the black pepper ethanol extract's antifungal properties against *T. mentagrophytes* and *T. rubrum* growth. Numerous research, including Aalaa *et al*., (17), have demonstrated the efficacy of the alcoholic extract of black pepper or its active ingredients as antifungals and have recommended the use of pepper extracts as antimicrobials. These findings are consistent with the conclusions of the present examination. which demonstrated the alcoholic and aqueous extracts' biological efficacy against *Aspergillus species*. It has been proven that black pepper has antibacterial properties against a variety of bacteria and fungi, including *F. oxysporum, A. niger,* and *Candida* spp. (18). Aqueous seed extract was found to inhibit *Penicillium citrinum* growth and to have modest action in suppressing the mycelial development of *Aspergillus niger* and *Aspergillus flavus* by Ghadir and Amira (19).

The activity of piperine, which was purified from black pepper and assessed against the growth of various types of bacteria and fungi, including *Alternaria alternata, Aspergillus niger, Aspergillus flavus,* and *Fusarium oxysporum,* was studied by Shiva Rani *et al.* (20) and Abraham *et al*. (21), the results demonstrated the significant activity of piperine against the fungi. According to Banso *et al*., (22). Plants that are extracted by ethanol have their organic compounds dissolved, releasing the antimicrobial components, which break up the fungal cell wall and cytoplasmic membrane.

Numerous investigations have been carried out to evaluate the impact of spice extracts, their active components, or the oil included with Cumin to evaluate the efficacy against fungus and bacteria. This investigation exhibits the efficacy of Cumin ethanolic extract against dermatophytes and shows that at larger doses, the ethanol extract's inhibitory zone also increases. This result is consistent with anterior studies such as (23), which settled that the ethanolic extract of cumin seeds revealed the antibacterial and antifungal possessions versus of *Bacillus subtilis* and *Candida albicans*. Bameri *et al*., (24) described the antibacterial activity of ethanol extract of cumin seeds, which displayed a range of antimicrobial properties against the biofilm *E. coli*. This has to do with the active ingredients in cumin seed extract—thymoquinone, thymol, and carvacrol—which have been exhibited to decrease the antimicrobial activity of bacteria and yeast as *Candida albicans* (25). In addition to a study by Abdul Jabbar (26) established that cumin oil has potent antibacterial and antifungal effects on *Aspergillus flavus, Candida albicans, and Cryptococcussp*.

CONCLUSION:

The findings of this study exhibit the antifungal effect of alcoholic extract of black pepper and cumin against two types of dermatophytes (*T. mentagrophytes* and *T. rubrum)* compared with Clotrimazole as medication. The inhibition activity is increasing directly with the concentration of extracts. The most sensitive fungi was *T. rubrum,* than *T. mentagrophytes.*

Conflict of interest: NIL

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