

**THE EFFECTS OF (ETHANOLIC, METHANOLIC, AND AQUEOUS) EXTRACTS
OF CINNAMOMUM ZEYLANICUM AND THYMUS VULGARIS WITH
CLOTRIMAZOL OINTMENT IN PREVENTING THE GROWTH OF CANDIDA
ALBICANS FUNGUS**

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Published online: 15 May 2016

ABSTRACT

Background and Objectives: Considering the increasing demand for applying medicinal plants in the medical treatment, this study aimed to investigate the effect of cinnamon and thyme extracts alone and in comparison with clotrimazole ointment on the *Candida albicans* yeast in laboratory condition.

Materials and Methods: In this study the cinnamon bark available in market was used for providing cinnamon products. The thymus vulgaris was prepared from the medicinal plants farm of Semnan Agricultural Jihad Training Centre. Ethanolic, methanolic and aqueous extracts of cinnamon and thyme powders prepared by maceration method. Then paper discs which coated with herbal products placed in *Candida albicans* medium and their zone diameters of growth inhibition compared with that of clotrimazole ointment.

Findings: The results of observations showed that the following items have the highest inhibition zone respectively: 1) The cinnamon methanolic extract 19.5 mm 2) the cinnamon extract 3) Thyme extract 4) Cinnamon ethanolic extract 5) Thyme ethanolic extract.

Conclusion: The results clearly showed that both cinnamon and thyme extracts have a significant effect in limiting fungal growth.

Key words: Extract; distilled extract; cinnamon; thyme; clotrimazole; *Candida albicans*.

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doi: <http://dx.doi.org/10.4314/jfas.v8i3s.254>



1. INTRODUCTION

In recent years, there has been a growing interest in herbal medicine especially in treatment of infectious diseases. Clinical microbiologists are very inclined to make use of these drugs in treatment of infectious diseases (1). Approximately, 500,000 plant species have been identified in the world (2) which fewer than thousand species are named as medical plants (3). Now, about a third to half of the products in the United States have plant origin (4). Today, the use of herbal products has increased in the United Kingdom (5). Along the beginning of the wide therapeutic developments in medical fields, the need for antifungal drugs with fewer side effects is increased more than ever. In addition, considering the AIDS epidemic, the prevalence of opportunistic fungal infections has increased. Therefore, the need to discover new antifungal drugs is necessary (7,6). One of these opportunistic fungi is *Candida albicans* yeast. Recently, it was reported that the use of some types of plant compounds with other plant compounds reduces their side effects and elevates their beneficial effects (8). Herbal medicine as the independent medicine or along with western medicine could be beneficial for treatment of fungal diseases (9, 10). Some extracts can increase the antifungal effects of macrophages in treatment process (12). Dr. Ezat Nourizadeh (2004), conducted a research entitled, The Antibacterial Effects of Extracts of Cinnamon, Black Pepper and Turmeric on *Helicobacter Pylori* (HP). The results of this study indicated that aqueous and ethanolic extracts of turmeric and cinnamon by agar diffusion on *Helicobacter pylori* have high antibacterial activity. The identification of effective antimicrobial agents of these plants was another aim of this study (19). Plants used in this study called cinnamon and thyme. The scientific name of cinnamon is *Cinnamomum zeylanicum* which due to compounds of tannin extracts has therapeutic properties. Due to having tannin, cinnamon is appropriate for relief of diarrhea, body general weakness. It is also helpful in coagulation and treatment of bleeding in menstrual periods. Cinnamon is added to laxatives that their use is associated with complications to change the flavor. Cinnamon is the aromatic spice and as a food seasoning used daily. It is one of the most commonly used spices in Iran. It has been used in perfumery (13). In this study, Cinnamon essential oil has been studied. Through steam distillation, yellow and fragrant essence with 1% efficiency obtained from Ceylon cinnamon bark. The ingredients of Ceylon cinnamon are amidon, mucilage, Tannin, pigment, calcium oxalate, sugar, many, Cinnamomine, essence, and resin. Among 23 compounds identified in cinnamon essence, trans-cinnamaldehyde (53%), eugenol (7.12%), linalool (9.6%), and beta Caryophyllene (6.6%) had the highest concentration and formed the

mentioned essence. The scientific name of thyme is *Zataria multiflora* and its extract includes Thymol, Seeman, carvacrol, and linalool. In order to obtain definitive results, experiments conducted under laboratory controlled conditions (13). *Candida Albicans* is a part of natural flora of the gastrointestinal tract, mouth and vagina and people often receive it when they pass through the vagina during birth. This fungus under specific conditions (including age, diabetes, taking antibiotics, infectious diseases, cancer, and etc.) overcomes the host natural defense mechanisms and infects him. Sometimes, *Candida albicans* yeast in immunocompromised patients (such as patients infected with cancer and AIDS) associated with fatal septicemia. Additionally, this yeast is one of the important agents of fungal vaginitis among women which can be eradicated with existing drugs (14). According to previous studies (15), we aimed to determine the effect of thyme and cinnamon extracts on *Candida albicans* yeast in laboratory conditions (inhibition zone diameter). We also aimed to compare and investigate the resulting findings and the effect of clotrimazole ointment on *Candida albicans* yeast to detect the pharmaceutical products in treatment above diseases.

2. MATERIALS AND METHODS

A) The preparation of fungal standard samples

Samples of the *Candida albicans stellatoidea* fungus were provided from Imam Ali Hospital laboratory. To identify the *Candida albicans stellatoidea* fungus, germ tube test was used as follow: 1) we used human serum prepared from blood transfer organization. 2) By using a sterile swab, yeast was picked up from pure culture and placed in serum. 3) Suspension was heated for 2-3 hours at 37°C. 4) By using a sterile swab, a drop of the suspension placed in a sterile slide and after covering it by a lamella, we placed it under a microscope and observed the germ tubes. To obtain more complete observation, the suspension containing fungus heated for 8 hours at 37°C.

B) The Preparation of plants extracts

To produce intended extracts, the thyme is washed and then dried in a dark place. Since cinnamon is not cultivated in our country, we used its dried bark. To obtain ethanolic extracts of Cinnamon and thyme, their dried bark is powdered. Then 100g dried powder placed in distillation flask and 1lit water added to the compound and heated on distiller. The resulting distilled extract was used for testing. In this study, cinnamon extract was produced through water distillation. To prepare the alcoholic extracts of cinnamon and thyme, first, their powder was prepared (the initial tests showed that they limit fungal

growth). 10 g cinnamon powder added to ethanol 30 and poured in one flask and 5g thyme (thyme has higher absorption power than cinnamon) added to ethanol 30 and poured in another flask and stored for 48 hours at room temperature. The derived extracts called ethanoic extracts. To prepare metabolic extracts; the same method was exactly used. To prepare aqueous extracts of cinnamon and thyme through ethanolic extraction process, the extraction method was used. In this approach, distilled water was used instead of ethanol and the compound was sterilized in autoclave.

C) The method of using extracts within the medium

The control medium which indicates fungal growth contains distilled water and dextrose agar powder. The plant pure or diluted extracts was used instead of distilled water in samples. After sterilization, the medium was placed in sterile petri dishes as control sample and marked with labels. After a few minutes, the jelly-like medium was ready for fungal culture. Then by using a sterilized swap, a sample of fungi pick up from gelatin medium and cultured in intended medium and stored in incubator for 7 days at 37c and results were investigated. Each test was triplicated. The results showed that, the cinnamon was more effective in limiting fungal growth than thyme.

D) Preparation of sabouraud dextrose agar and potito dextrose agar medium and the use of disc blank.

Sabouraud dextrose agar and potito dextrose agar mediums were prepared and the candida albicans stellatoidea fungus was cultured on their all surface. The disc blanks were separately coated by ethanolic, methanolic, aqueous and distilled extracts of cinnamon and thyme and cultured within the mentioned medium. Then, the alcoholic extracts of discs as control coated by ethanol or methanol and stored in incubated for 24 hours and the zone diameter of growth inhibition was measured by ruler. Tests were conducted by using clotrimazole ointment (one of the most common anti-fungal medications). In this regard, sabouraud dextrose agar was prepared and fungal suspension cultured on all over the medium. A drop of ointment of the approximate size of disc was placed on medium after homogenization. The zone diameter data of growth inhibition analyzed with Minitab software in all samples and control group.

3. FINDINGS

The results of using plant extracts in sabouraud dextrose agar after initial tests and triplicate test indicated that, both cinnamon and thyme inhibit fungigrowth. It also found

that the cinnamon extract was more effective in limiting fungal growth than thyme extract. In other word, cinnamon extract completely inhibits the growth of candida albicans stellatoidea fungus (Table 1 and 2). After homogenization with emulsion capacity, clotrimazole ointment was placed on disc blank in medium. The zone diameter mean of growth inhibition of clotrimazole ointment was 12mm. The zone diameter of growth inhibition was measured by Minitab software. The results indicated that, there is a significant difference between the zone diameter of growth inhibition of thyme ethanolic and methanolic extracts and thyme extract. There was a significant difference between thyme methanolic extract and control sample at the significant level of $p=0.01$. There was no significant difference between thyme ethanolic extract and control sample at the significant level of $p=0.01$ and $p=0.05$. But there was a significant difference at the level of $p=0.1$. At the significant level of $p=0.01$, there was no significant difference between thyme extract and control sample. At the significant level of $p=0.05$, there was a significant difference between thyme extract and control sample. The results of analyzed data indicated that there is a significant difference between the zone diameter of growth inhibition of control sample and both cinnamon and thyme. The antimicrobial effects of 43 herbal extracts of 29 families were investigated at Shahid Beheshti Pharmacy School. It was found that myrtle, has the greatest antimicrobial effects against *Micrococcus Luteus*, *Bordetella Bronchiseptica*, *Bacillus Subtilis* and *Candida Albicans* (18). The antimicrobial effects of traditional medicine herbs were investigated on oral and dental infections. The result indicated that thyme has antifungal effects against *Candida Albicans* and oral bacteria (18). Considering antifungal effects, the herbal extracts classified respectively as follow: Cinnamon methanolic extract, cinnamon extract, thyme extract, cinnamon ethanolic extract, thyme ethanolic extract, thyme methanolic extract. Considering the conducted tests at Baryj Essence Company, it was reported that thyme and myrtle essences have inhibitory effects against candida albicans. It was found that *Agastache rugosa* extract increases the effectiveness of *Ketoconazole* in treatment of *Blastoschizomyces capitatus* (13).

4. CONCLUSION

The results indicated that cinnamon has more antifungal effects and fewer sides Effects than clotrimazole.

Table 1. The results of assessing zone diameter of growth inhibition of disc blank containing extracts in sabouraud dextrose agar medium

Plant	Herbal compounds	Medium of zone diameter of growth inhibition	Control sample disk(ethanol, methanol)	Medium of zone diameter of growth inhibition
Cinnamon	Methanolic extract	19.5	methanol	7.25
	Ethanolic extract	11	ethanol	7.25
	Aqueous extract	-	-	-
	extract	18	-	-
Thyme	Methanolic extract	9.5	methanol	7.25
	Ethanolic extract	10.33	ethanol	8
	Aqueous extract	-	-	-
	extract	12.28	-	-

Table 2. The results of assessing the inhibition zone diameter of disc blank containing extract in potito dextrose agar medium

Plant	Herbal compounds	Medium of zone diameter of growth inhibition	Control sample disk(ethanol, methanol)	Medium of zone diameter of growth inhibition
Cinnamon	Methanolic extract	15.16	methanol	7.25
	Ethanolic extract	11	ethanol	7.5
	Aqueous extract	-	-	-

	Extract	12.8	-	-
Thyme	Methanolic extract	9.83	Methanol	7.25
	Ethanolic extract	10.0	ethanol	7.0
	Aqueous extract	-	-	-
	Extract	11.28	-	-

Table 3. The results of using antifungal medicines (clotrimazole ointment) on the fungus medium

Plant	frequency	The zone diameter of growth inhibition(mm)
Clotrimazole ointment homogenized with emulsion capacity and placed on disc blank in medium	3 0.01	12

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How to cite this article:

Saffarieh E, Pazoki R, Aghaamoo S, and Jahan E. The Effects of (Ethanollic, Methanolic, and Aqueous) Extracts of *Cinnamomum Zeylanicum* and *Thymus Vulgaris* with Clotrimazol Ointment in Preventing the Growth of *Candida Albicans* Fungus. *J. Fundam. Appl. Sci.*, 2016, 8(3S), 668-676.