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Antifungal Activities of Methanolic Extract of Plants

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Research Article

Abstract

Using modern medicine is seriously proposed as a worldwide public health concern due to its adverse side effect problems, whereas the use of herbal drugs in fungal infections claimed to be not only effective but also safe and out of danger.Herbal medicine as an independent treatment way or alongside western medicine can be helpful for in treating fungal infections. *Curcuma longa* L. (Zingiberaceae family) and *Quercus sessilifolia* (Fagaceae family) have been noticed in a variety of antifungal investigations due to extensive traditional uses and low side effects.

This paper attempts to show that for the first time the effect of two plant extracts against fungi was studied. Antifungal activities of methanolic extract of plants were evaluated against pathogens (*Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Microsporum canis*) fungi with pourplate method (50, 25, 12.5, 6.2, 3.1, 1.5 mg ml^{-1} extract in medium). Concentration of clotrimazol as a control was 10 $\mu g.ml^{-1}$. The results indicated that plant extracts prevent the growth of dermatophytes fungi. The foremost inhibitory effect of extracts in dermatophytes fungi cultures with minimum dose (6.25 $mg.ml^{-1}$) was comparable to a concentration of 10 $\mu g.ml^{-1}$ clotrimazole.

The findings indicate that the methanolic extract of plants have antifungal effects on the growth of dermatophytes.

Keywords: *Curcuma longa* L.; *Quercus sessilifolia*; Antifungal; Clotrimazol.

1. Introduction

One of the major public health problems is Dermatophytosis. Dermatophytes include three types of fungus such as Microsporum, Trichophyton and Epidermophyton which commonly causes skin diseases in animals and humans. Dermatophytosis is an infectious disease of skin, hair and nailswhich attack on the keratinized tissue. The soil is inhabited by majority of these fungus involved in decomposition, thus dermatophytes can infect the living hosts. Members of Microsporum and Trichophyton cause illness in both humans and animals but *E. floccosum*, an anthropophilic species are the only species of Epidermophyton known to cause disease solely on humans [1,2]. The prevalence of dermatophytoses varies in different geographical locations. Many epidemiological studies have investigated the prevalence of fungi which was responsible for superficial mycoses in different regions of the world and many parts of Iran [3,4]. The immigration of labour, troop movements, emigrations and other travelling ways played key role in spreading of these fungi [5].There are three primary aims of this study: 1) to investigate new herbal medicine 2) to exhibiting their minimum toxicity properties for human 3) applying them in treatment of fungal diseases.

Various medicinal herbs have been used for years in daily life to treat disease all over the world. Herbs have a wide range of usage in folk medicine, food flavoring, also in food industries. In recent years there has been an increasing interest in the use of natural substances due to concern about the safety of some synthetic compounds, which have encouraged more detailed studies on originated substances. The extracts and essential oils of many plants have been shown to exert biological activity, which leads to traditional medicine researchers focused on the characterization of antimicrobial activity of these plants [6,7]. *Curcuma longa* L., which belongs to the Zingiberaceae family, is a perennial herb that grows up to 1m height with a short stem, distributed throughout tropical and subtropical regions of the world, and cultivated in Asiatic countries, mainly India and China. Its remedial and effective properties include anti-inflammatory, anti-human immunodeficiency virus, anti-bacteria, antioxidant effects and nematocidal activities [8].

Quercus sessilifolia (Fagaceae family) is a small tree found in Greece, Asia Minor and Iran. It is a tree up to 25 m tall. Twigs are waxy, Leaves can be as much as 15 cm long, thick and leathery [9]. The extract of Quercus is effective for control of bacteria strains, especially S. *aureus,* so it could be used as a natural antimicrobial agent [9] and, moreover, the antifungal effects of plant were reported [10].

2. Methods

2.1. Plant material and extraction

The rhizomes of *Curcuma longa* L. (from India)

Table 1: The effects of *Quercus sessilifolia*, *Curcuma longa L.* and Clotrimazol on growth of dermatophytes fungi.

Fungi	Clotrimazol (10 µg ml^{-1})	Without extract	Concentration (mg ml^{-1})					
			50	25	12.5	6.2	3.1	1.5
Epidermophyton floccosum	+++	-	+++	+++	+++	+++	++	+
Microsporum canis	+++	-	+++	+++	+++	+++	++	+
Trichophyton mentagrophytes	+++	-	+++	+++	+++	+++	++	+

+++ No growth of fungi; - growth of fungi

and *Quercus sessilifolia* (from Kermanshah) were collected. A voucher specimen of plants was deposited at the Herbarium of the Faculty of Sciences, Payame Noor University, Tehran, Iran.

The plants were separated, dried in shade and crushed into powder using a blender. For extraction, 100 g of plant powder were macerated for 3 days with 1000 ml methanol, then filtrated. Extraction was performed three times.

2.2. Medium

The media used for fungi testing were the Dermatophytes Selective Agar (DTM) and Sabouraud Dextrose Agar (SDA) (Merck, Germany).

2.3. Preparation of fungi strains

Fungus strains used for the test were *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Microsporum canis*. The human fungi pathogens were clinical isolates obtained from the mycology laboratory, the school of public health, Tehran University of medical science. After microscopic direct checking by KOH 15%, colonies in dextrose agar and kermyl agar were purified and identified.

2.4. Determination of antifungal activities

Antifungal activities of methanolic extract of plants were evaluated against fungal pathogens with pour plate method. For this purpose, we were diluted the sample, then placed an aliquot of the prepared sample (0.5 mL) in a labeled empty sterile plate and poured 20 mL of melted agar (SDA,DTM), cooled to 45°C, Mixed thoroughly by tilting and swirling the dish. And let it became cool to solidify (without disturbing) then inverted and incubated to develop colonies (7-14 days at 25°C).

For each fungus as a control, a plate containing methanol without extract and also one plate containing of clotrimazol were cultured.

3. Results and Discussion

Plant extracts prevent the growth of all three dermatophytes fungi at all doses especially at 6.2 mg.ml^{-1} concentration of extracts (Table 1).

The methanol extracts of Q. *sessilifolia* and C. *longa* showed MIC and MFC at 6.2 mg/mL concentration for all tested strains of dermatophytes. The MIC and MFC of the extracts were similar, which shows that MIC is sufficient for measuring fungicide activity. In recent years, there has been an increasing amount of literature on the role of plant extracts in treating variety of diseases and their biological activities like antimicrobial, antibacterial, antiviral and antifungal activities [11,12]. The most striking result to emerge from the data of this study is that methanol extracts of two plants have antidermatophytic properties.

Many compounds of *Quercus sessilifolia* and *Curcuma longa* L were oil, sugar, tannin, nitozan, amidan and oil, Curcumin, demethoxycurcumin, bis-demethoxycurcumin, respectively [9]. A *Curcuma longa* rhizome has been traditionally used as antimicrobial agent [13,14]. The major constituent of *C. longa* L. is curcumin (diferuloylmethane), widely used as antibacterial, antiviral, antifungal and antimalarial activities. Curcumin assessed by clinical trials in human and shows antimicrobial activity also safety property even at high doses (12 g/day). It was used as a structural sample to design the new modified antimicrobial agents and increased antimicrobial activities through the synthesis of various derivatives related to curcumin [15,16].

Therefore, observed antifungal effect is likely related to one of the compounds. However, using these compounds as antifungal material needs further investigations.

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