Inhibitory Effects of Aqueous Extract of Garlic on Growth and Keratinase Activity of Trichophyton Mentagrophytes

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Abstract

Background: The effect of garlic extracton fungal growth and keratinolytic activity was studied in Trichophytonmentagrophytes as one of the major etiologic agents of human and animal dermatophytosis in Baghdad and other parts of the World.

Objective: To investigated an alternative antidermatophyte with minimum side effects which is plant based and biodegradable natural product

Methods: Culture conditions for 30 isolates of T. mentagrophytes isolated from human dermatophytosis from both sexes with ages of 5-63 years in Central Medical city for the period July 2009 to October 2009 were cultured on specific solid medium.

Results: The aqueous extract of garlic at various concentrations inhibited the growth of T. mentagrophytes. This inhibition reached to a maximum of 100٪ for extract at 10٪concentration. Keratinase synthesis was also inhibited by the extract about 91٪ at 8 ٪ concentration.

Conclusion: Fungal growth and keratinolytic activity are important factors in pathogenesis of the dermatophytes, their inhibition by garlic indicate that this substance may have potential values for treatment of human and animal dermatophytosis.

Key words: Dermatophytosis, Garlic, Keratinase , Tricophytonmentagrophytes

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Introduction:

Dermatophytes are fungi capable of invading keratinized tissues of human and animals because of their abilityto synthesize an extracellular enzymes called keratinases, causing dermatophytosis[1,2,3] . Keratinases are proteolytic enzymes which play very important role in the invasion of keratinized tissues of skin , hair and nails and have mostly studied in the dermatophyte*Tricophytonmentagrophytes* ,the world wide fungi in distribution and have both anthropophilic and zoophilic forms [4,5,6] . Antifungal drugs of azole are often used in the treatment of dermatophytes and because of the increased use of these medications , azoles are known to cause drug resistance , hence this study investigated an alternative antidermatophyte with minimum side effects which is plant based and biodegradable natural product

Discovery of antimicrobial activities of garlic (Allium sativum) has a long history and it is reported on different microorganisms such as fungi, bacteria and viruses [7]. It is also strength the immune system at the same time[8].

Garlic present alliin and any damage to the plant results in the breakdown of alliin by alliinase enzyme to produce allicin which is the active component of garlic and have a broad – spectrum antimicrobial, capable of warding off different types of infection [9,10].

Methods:

Dermatophytes: 30 isolates of T.mentagrophytes were used in this research which isolated from patients with dermatophytosis by making scraping during routine diagnostic works in Central Medical City during July 2009 until October 2009 from both sexes their ages range from 5-63 years. These isolates were identified based on colony and microscopic morphology, urease test, hair perforation test and the test of growing in temperature at 37 °C [11].

Screening of keratinolytic activity

On agar plates: The isolateswere screened for keratinase production according to Wawrzkiewiczet *al* [12,13] using solid mineral medium, and adding keratin powder as a keratin source with concentration of 0.06%, added to the sterile agar medium. The agar plates were inoculated with 20 µL of fungal suspensions. Keratinolytic activity of the isolates was detected as aclear zone arownd the colony after incubation at 30°C for 10 days. The diameter of the clear area was measured to quantify enzyme activity.

Preparation of aqueous garlic extract: By using Freezing Thawing Method [14].One hundred gram of fresh garlic washed with distilled water and covered with poly ethylene bag and put at - 4°C for 72 hours, then put at room temperature for thawing. The active material obtained after one day by making a hole in the bag. The obtained fluid were sterilized by passing through 0.22 µm Millipore filters. The D.W added at different volumes to make different concentrations from the extract and use.

Keratinase activity assay: A keratinolytic proteinase with enzyme activity at acidic pH was isolated from culture filtrates of Trichophyton mentagrophytes, a major pathogenitic fungus of dermatophytosis. The keratinolytic activity of culture filtrates was measured spectrophotometrically [15, 16,17] with using keratin Powder as a keratin source. Keratin powder (20 mg), 3.0 ml phosphate buffer (28 mm, pH 7.8) and 2.0 ml culture filtrates were incubated in a shaker water bath at 150 rpm at 37°C for 1 hour. After the addition of 10% trichloroacetic acid (TCA) and centrifugation at 10000 rpm for 15 min, then the optical absorption of the supernatant was measured at 280 nm wavelength. Fungal dry weight was determined after the complete drying of a known amount of the w et mycelium at 80°C and considered as growth index.

Results:

Dermatophyte. *T. mentagrophytes* isolates were separated from skin scales of dermatophytic patients after culturing of the specimens on Mycobiotic agar plates. The isolates were identified based on the production of powdery or cottony white-cream colonies, microscopic features as spiral hyphae and grape-like globose microconidia, positive results in urease , hair perforation tests and the ability of growing at 37 °C (11).

Agar plate screening. Screening of thirty *T. mentagrophytes* isolates for keratinase production on solid mineral medium showed that all of the examined isolates were able to produce extracellular keratinase at different levels. Keratinolytic activity was assessed based on the observation of a clear zone around the fungal colony on the plate. There was a significant difference in keratinolytic activity on solid medium among some isolates ($P<0.05$).

Effects of aqueous garlic extract on fungal growth and keratinase production. The results showed that fungal growth was significantly inhibited by garlic extract in all concentrations as compared with control (Table 1). Minimum inhibition for garlic extract was measured as 12٪ at 0.50٪ concentration . The growth was completely inhibited in the presence of maximum concentration of the extract 10 ٪. The keratinase activity was also inhibited by this extract (Table 1) at the lowest concentration 0.50 ٪ caused 40 ٪inhibition in enzyme activity by the fungus. The maximum inhibition of keratinolytic activity of 8 ٪concentration of garlic extract was 91٪ (Table 1). These inhibitions were significant as compared with the controls ($P<0.05$).

Discussion

The dermatophytes divided into three mainly genus which are Microsporum ,Epidermophyton

and Trichophyton. The fungus T. mentagrophytes considered the most common world wide in the infection of dermatophytosis. These fungi produce different types of proteolytic enzymes specially keratinases that have key roles in fungal invasion of stratum corneum ,and pathogenesis in human and animal dermatophytosis.[1,2,3,18] In present study, the inhibitory effects of aqueous garlic extract on growth and keratinolytic activity of a selected high keratinase producer isolate of *T.* mentagrophytes were established. Thirty isolates of T. mentagrophytes were first screened for the selection of best keratinase producer for further analysis using solid mineral medium. The obtained results were suitably correlated with results from other workers on keratinolytic activity of different dermatophytes[8,9,10]. After preliminary studies on optimization of keratinolytic activity in *T.* mentagrophytes, the effect of garlic extract on growth pattern and keratinase synthesis by this fungus was subjected. The growth was completely inhibited for the extract at the highest concentration of 10 %. Several reports shown the antifungal propreties of garlic against dermatophytes and other fungi because of its components, and the most important one is allicin which have broad-spectrum antimicrobial ([8, 9, 10]. It is interesting to note that the keratinolytic activity of T. mentagrophyteswas also inhibited by garlic extract. This inhibition was significant for the garlic extract concentrations as compared with the controls ($P<0.05$). Our work on fungal structure indicate that aqueous garlic extract disrupts hypha cell wall and causes massive necrosis and disarrangement in some cellular compartments specially nucleus and mitochondria in T. mentagrophytes. Thus, changes in hyphal structure may be responsible for inhibitory effects of garlic extract on growth of this important dermatophyte. Further results are needed for confirming this hypothesis and also finding actual mechanism of garlic extract mediated keratinase inhibition.

The study concluded that garlic extract can be used as potential candidates for preparation of anti-dermatophytic drug formulations and may be useful in the treatment of different kinds of dermatophytosis in human and animals.

Table 1. Effect of aqueous garlic extract on Trichophytonmentagrophytes growth and extracellular keratinase activity.

Consentration Of Garlic Extract(%)	Mycelia dry weight (mg)	Growth inhibition(mm)	Keratinase activity (%)
0.00	10.0 ± 0.70	0.00	100.00
0.50	8.50 ± 0.65	12.00	60.00
.001	7.25 ± 0.60	20.00	55.00
.002	6.00 ± 0.52	32.00	51.00
.003	5.50 ± 0.47	45 .00	47.00
.004	4.75 ± 0.35	50.00	42.00
5.00	4.10 ± 0.45	62.00	35.00
6.00	3.20 ± 0.30	70.00	27.00
7.00	2.95± 0.30	77.00	21.00
8.00	1.75± 0.20	80.50	9.00
9.00	1.00± 0.10	90.50	9.00
10.00	0.00	100.00	Not Determined

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