The effect of garlic extract and volatile oil on growth of Trichophyton mentagrophytes

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Abstract:
This study was conducted in the College of Medicine-laboratory of microbiology to assess the effect of crude garlic extract and essential volatile oil on growth of Trichophyton mentagrophytes. Antidermatophytic activity of different concentrations of volatile oil, aqueous extract of garlic were investigated in vitro against T. mentagrophytes by using agar dilution technique. The results showed that minimal inhibitory concentration (MIC) of aqueous garlic extract (crude) was 0.5 mg/ml, while the MIC of essential oil was 0.25 mg/ml.
Introduction
The dermatophytes superficial fungal infections usually involve the skin, hair, and nail. The groups of fungi most commonly responsible for causing infection of these sites are known as dermatophytes, include the genera *Trichophyton*, *Microsporum* and *Epidermophyton* (1). Antifungal agents work by exploiting differences between mammalian and fungal cells to kill the fungal organism without significantly harming the host (2). From the time of Egyptian pharaohs and the earlier Chinese dynasties, garlic (*Allium sativum*) has been widely consumed as both food and medicine (3). Garlic was first recorded use by the Sumerians of Mesopotamia, in the now Mid-East regions of the Tigris and Euphrates rivers (4). Dozens of compounds have been identified from garlic and the volatile oil include thirty three sulfur compounds, seventeen amino acids germanium, calcium, copper, iron, potassium, magnesium, selenium, zinc, vitamin A, B1 and C (5). The main components of the volatile oil are sulfur compounds, especially allicin, diallyl disulfide, diallyl trisulfide. These compounds are considered to be the primary active components of garlic (6).

Materials and Methods
Isolates of *Trichophyton mentagrophytes* were obtained from the laboratory of College of Medicine/ Tikrit University. Garlic extract was prepared as follows.

Aqueous extract
Take crude garlic, crushing or brushing to small piece then weight 50 g of garlic, add 1000 ml of D.W., mixing by shaker for 30 minutes, left for 1 hour. First filtration by gauze, second by filter paper. Distributed the filter solution into glass plate put in oven under 45°C for 5 days then use in culture (7).

Effect of garlic extract on growth of *Trichophyton mentagrophytes*
Examination effect of garlic extract was occur by method of measurement colony diameter, it uses method (Le kady et al) mix the media Sabouraud Dextrose Agar (S.D.A.) with aqueous extract in concentration 10, 5, 2, 1, 0.5, and 0.25 mg/ml, then the media was solid. The center of plate inoculated with tested fungi. The form of disk 3mm cutting by cork purer from colony growth of *T. mentagrophytes* on the media. Put the disk form in the same well diameter in media. Incubate culture in cooled incubator at 25 °C for 7 days, and then measure diameter of colony growth. It uses three replication for each concentration (8).

Effect of garlic (essential oil) on growth of *T. mentagrophytes*
Take capsule of garlic (essential oil) (provided by company, India) dissolve in alcohol. Mix the oil with media in concentration 10, 5, 2, 1, 0.5, and 0.25 mg/ml, then the media was solid. The center of plate inoculated with tested fungi. The form of disk 3mm cutting by cork purer from colony growth of *T. mentagrophytes* on the media. Put the disk form in the same well diameter in media. Incubate culture in cooled incubator at 25 °C for 7 days, and then measure diameter of colony growth. It uses three replication for each concentration (8, 9).

Effect of griseofulvin on growth of *T. mentagrophytes*
Take capsule of griseofulvin (provided by company, India). Broke capsules to powder, weight 10, 5, 2, 1, 0.5, and 0.25 mg/ml, added to the media. After mix wait to solid,
the center of plate inoculated with tested fungi. The form of disk 3mm cutting by cork purer from colony growth of T. mentagrophytes on the media. Put the disk form in the same well diameter in media. Incubate culture in cooled incubator at 25 °C for 7 days, and then measure diameter of colony growth. It uses three replication for each concentration. An infected area was not affected by garlic extract because essential oils work as rubbish collectors, attaching themselves to toxins, free- radicals, cell debris, heavy metals, renegade cells fungi bacteria viruses or other debris and taking them to the exits for disposal.

**Results**

**Inhibitory effect of garlic extracts (crude and essential oil) on growth of T. mentagrophytes.**

The results refer that the growth of *T. mentagrophytes* was inhibited when use garlic extract (crude and essential oil), MIC of crude oil was 0.5 mg/ ml, MIC of essential oil was 0.25 mg/ ml when compared with control group as show in table 1 and 2.

**Table (1) :- Effect of garlic extract (crude oil) on the growth of *T. mentagrophytes*.**

<table>
<thead>
<tr>
<th>Concentration (mg/ ml)</th>
<th>Percentage growth in mm</th>
<th>-ve control</th>
<th>Mean</th>
<th>S.D.</th>
<th>P value</th>
</tr>
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<td>10.087</td>
<td>±5.314</td>
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<td>1</td>
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<td></td>
<td></td>
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<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>0.5</td>
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</tbody>
</table>

-ve control: Growth rate of fungi on SDA (without any addition).

**Table (2):- Effect of garlic extract (essential oil) on the growth of *T. mentagrophytes*.**

<table>
<thead>
<tr>
<th>Concentration (mg/ ml)</th>
<th>Percentage growth in mm</th>
<th>-ve control</th>
<th>Mean</th>
<th>S.D.</th>
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<tr>
<td>0.25</td>
<td>0.01</td>
<td></td>
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</tr>
</tbody>
</table>

-ve control: Growth rate of fungi on SDA (without any addition).

**Discussion**

The importance of garlic extract is containing of volatile oils. It called volatile
oils because evaporate and volatile without analysis when exposure to air in normal temperature. These oils regarded very active in treatment such as allicin founded in garlic (6). Many studies done about the effectiveness of garlic extract and its contain active material in the inhibitory effect on different dermatophytes. In a study done by Yamada and Azuma (10) to test the effective ness of allicin in dermatophyte T. mentagrophytes, T. rubrum, E. floccosum, Microsporum and C. albicans. Garlic extract is antimicrobial against many Gram positive and Gram negative organisms including Escherichia coli, Salmonella typhi, Bacillus subtilis, Brucella abortus, Pseudomonas pyocyaneus, Staphylococcus aureus, Streptococcus viridians, Klebsiella pneumoniae and Proteus vulgaris (11). This show limited effect of this mixture in the inhibitory germination and spindle fiber growth. Venugopal and Venugopal uses aqueous garlic extract eighty eight colony form different dermatophyte and compare results using this extract with result using ketoconazole against fungi itself by uses agar dilution method (12). They show that aqueous garlic extract has more activity than ketoconazole on inhibition of dermatophyte growth. This study shows that garlic extract has inhibitory effect on T. mentagrophytes growth, MIC of crude oil was 0.5 mg/ ml. The concentrations 10 and 5 mg/ml kill this fungus completely, but the concentrations 2, 1, 0.5 mg/ml inhibit the growth. When essential oil is used the MIC was 0.25 mg/ml. the concentrations 10 and 5, 2 and 1 mg/ml kill this fungus completely, but the concentrations 0.5 and 0.25 mg/ml inhibit the growth (12). It was thought that fungus was killed by preventing protein synthesis or DNA synthesis or fat forming of cell membrane or preventing swollen and germination spores. Differences in MIC because of long period time were shown. We use aqueous garlic extract after preparation directly in the testes process, because maturity of allicin mixture in garlic extract (8). This mixture analysis into other compounds that has smaller activity after forming it a period of five hours. This mixture loss his activity when store it for a long time or stored in refrigerator. This results agreed with Yamada and Azuma (10) notes that garlic extract has antidermatophytic activity of T. mentagrophytes and Microsporum because of containing active allicin in this extract. Sensitivity of dermatophytes different from aqueous garlic extract because fungi nature from the structure of cell membrane rapidly growth and the working method of allicin mixture. These results agreed with the study of Yoshida et al (13) and Singh et al (14) that show the differences in the structure of cell membrane from fungi to another that effect in sensitivity of fungi toward garlic extract. Wills (15) show that allicin compound react with (S-H) of protein and amino acid in cell membrane and oxides. Allicin compound effect on fat metabolism from effectiveness on enzyme activity 3-hydroxyl- 3- methyl-glulase responsible for forming mevolanic acid that star forming sterols and inhibitory enzyme acetyl-CoA-synthetase, preventing forming fatty acid and sterols (11, 16). It effect is directed on forming fat that cause destroyed cell membrane of fungi and then inhibitory growth of fungi (17). Inhibition happen by allicin compound that prevent swollen and germination spores. It regarded radiation growth of colony fungi that cause production not normally mycelia (10).

References


