Original Article

# Antifungal Activity of Some Extracts Supplemented with Silver Nanoparticles

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## Abstract

**Background:** Turmeric has long been used in traditional medicine, thanks to its multiple healing properties. **Objectives:** This study seeks to evaluate the effectiveness of various turmeric extracts, including those enhanced with silver nanoparticles (AgNPs), in combating the common skin fungi *Trichophyton mentagrophytes* and *Trichophyton rubrum*. **Materials and Methods:** Nutrient media containing different concentrations (5, 10, 15, and 20 mg/mL) of turmeric extracts were used to evaluate their inhibitory effects on fungal growth. Minimum inhibitory concentrations have been determined for each type of extract. **Results:** Both aqueous and alcoholic turmeric extracts have shown antifungal activity against both fungal species. However, *T. rubrum* has been found to be more sensitive to *T. mentagrophytes*. The addition of AgNPs to the aqueous extract significantly enhanced the antifungal activity. **Conclusion:** The study showed that turmeric extracts have promising antifungal activity, especially against *T. rubrum*. These results encourage further research into developing effective and safe natural medicines for the treatment of fungal infections while understanding the molecular mechanisms behind this effect.

**Keywords:** Antifungal, silver nanoparticles, *Trichophyton mentagrophytes*

## Introduction

Medicinal plants occupy an important place in traditional medicine and herbal treatment in various countries around the world. While most therapeutic plants are safe, a small number are very dangerous to both people and animals.[1]

Only a tiny fraction of the 250,000–500,000 species of medicinal plants that have been identified by scientists is actually utilized for human and animal nutrition, and an even smaller fraction is actually employed for medical treatment.[2]

The active substances used in traditional medical treatment are obtained from whole plants or from their parts such as roots, leaves, bark, or seeds. The extraction of biologically active compounds depends on the extraction solvent used and the temperature of extraction or mixing, with the presence of three classical techniques, namely Soxhlet, soaking, and hydrodistillation.[3,4]

Dermatophytes are fungal pathogens of humans and animals that infect keratinized tissues such as skin, nails, and hair, and are most likely found in warm and humid areas. The infection occurs due to three genera (*Epidermophyton, Microsporum*, and *Tticophyton*). These fungi can readily degrade keratinized tissue by releasing sulfate and secreting enzymes such as endoprotease and exoprotease. Human infections (particularly those affecting the skin) are a serious problem, especially in tropical and subtropical developing countries. Some studies have shown that plant extracts have been used conventionally to treat a number of infectious diseases caused by fungi.[5] Several researchers have contributed to

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the discovery of the antifungal compounds of volatile oils in medicinal plants.[6] The aqueous and alcoholic extracts of thyme, ginger, and cardamom were also shown to inhibit the fungus *Aspergillus flavus* and the efficiency of its production of aflatoxin.

*Trichophyton rubrum* is a dermatophytic fungus in the phylum Ascomycota. It is an exclusively clonal, anthropophilic saprotroph that colonizes the top layers of dead skin, and is the most frequent cause of athlete's foot, fungal infection of nail, jock itch, and ringworm globally. *T. rubrum* was first described by Malmsten [sv] in 1845 and is currently considered to be a complex of species that comprises multiple, geographically patterned morphotypes, several of which have been formally described as distinct taxa, including *Trichophyton raubitschekii, Trichophyton gourvilii, Trichophyton megninii,* and *Trichophyton soudanense*.

They are very minute, measuring between 1 and 100 nm in size. When it comes to developing agricultural operations and food production, nanotechnology is at the forefront of creative technologies. Nanotechnology's use and expansion into new areas, such as genetically modified crops and methods for producing high-quality agricultural chemicals, have been greatly aided by the many different approaches taken to preparing nanoparticles in the fields of materials science, energy, medicine, and bioscience research.[7] Several ways have been documented for creating silver nanoparticles (AgNPs), including physical and chemical procedures, electrochemical reduction, photoreduction, and thermal evaporation production, and demonstrating the rapid acceleration of nanotechnology development in recent years.[8] The field of nanoscience is dedicated to the study of sub-100-nm-sized molecules and chemical substances. Also of interest is the research of phenomena related to the very small scale of these materials, as well as the development of methods for their practical use. Controlling the number of atoms in a particle is very important since it has a profound effect on the substance's qualities, thus it is important to have full and precise control over the manufacturing of the substance.[9] Researchers are looking to find the safest way to prepare AgNPs. One of the safer and faster methods is the use of extracts of leaves and seeds of plants, etc., in the preparation of AgNPs. Several authors have used plant materials to prepare AgNPs.[10]

In the fight against bacteria that have become immune to traditional antimicrobials, nanotechnology has emerged as a promising new tool. Nanoparticles of metals like platinum, copper, silver, and gold are antimicrobial, meaning they may kill the germs that cause fungal infections and other ailments. In particular, AgNPs have been shown to have beneficial effects against bacteria, viruses, and fungi, making them a viable material for use in nanotechnology and medicine. AgNPs have been employed in medicine and industry since the 1880s because of silver's potent antibacterial properties. Since their discovery, AgNPs have garnered extensive study because of their unique properties. These include chemical stability, catalytic activity, excellent conductivity, and most importantly, antimicrobial and antifungal activity against fungal species like *Aspergillus fumigatus*. Silver is also known to be nontoxic and harmless to the human body at low concentrations.[11,12]

AgNPs' great thermal stability also makes them useful as a protective coating for things like stainless steel, textiles, water treatment, and sunscreen.[13]

## Materials and Methods

### Species of fungi examined

In this work, we employed two fungal isolates acquired from the College of Science/Department of Biology at the University of Mosul and cultivated them on sabouraud dextrose agar (SDA) medium: *Trichophyton mentagrophytes* and *T. rubrum* isolate iqt and *Microsporum canis* isolates iqm-no.1 and no.2.[14]

### Preparation of the aqueous extract of turmeric

According to the protocol,[13] the aqueous extract of turmeric was made by combining 40 g of fine powder of the dried medicinal plant with 160 mL of distilled water, that is, in a ratio of 1:4 weight-to-volume, blending the mixture for 5 min at intervals, then stirring the mixture with an electric shaker, type of Gesellschaft fur Labortechnik (GFL), Germany, for 2 h to get a homogenous mixture, and finally storing the mixture. Afterward, the crude aqueous extract of the plant was obtained by repeating the filtration process in a Buchner funnel with filter paper (Whatman No.2) and a Sliding van rotary vacuum pump from VACUUBRAND GMBH Co., Germany. The extracts were then frozen and dried by lyophilization under vacuum pressure and temperature (−50°C) using a lyophilizer device supplied by Edwards High Vacuum, UK.

### Preparation of alcoholic turmeric extract

The method was used in preparing the alcoholic turmeric extract used in the study by dissolving it in alcohol, 20 g of the plant was dissolved in 200 mL of ethyl alcohol at a concentration of 95%, that is, at a ratio of 1:10 weight-to-volume, the mixture was mixed using a blender for 5 min and intermittently, then placed in an airtight container and stirred with an electric shaker, type of (GFL – Germany for 2 h, then kept at 4°C for 24 h for soaking. The mixture was then filtered through several layers of gauze, then centrifuged at 3000 rpm for 10 min, and filtered again by Buchner funnel using filter paper (Whatman No.2) under vacuum. After filtration, the filtrate was placed in a rotary vacuum evaporator supplied by the English company Electrothermal, which works on the basis of evaporation under vacuum

pressure and a temperature of no more than 40°C. After completely evaporating the ethyl alcohol in the mixture, a thick layer was obtained from the extract, which was further dried by a lyophilizer under vacuum pressure and temperature (−50°C). Then, the dried crude extracts were kept in plastic containers with a freeze-tight lid until use.[12]

### Sterilizing the aqueous extract of turmeric

For the sterilization of aqueous turmeric extract, 1 g of dry plant extract was taken and dissolved in 5 mL of sterile distilled water, thus obtaining an extract with a concentration of 200 mg/mL as a standard concentration; then, this extract was sterilized through membrane filters with a diameter of 0.22 microns under vacuum. This standard concentration was considered a source for preparing the dilutions used in the study, according to the dilution equation:

$$N1\ V1 = N2\ V2 \quad (1)$$

### Sterilization of alcoholic turmeric extract

One gram of alcoholic turmeric extract was dissolved in 5 mL of ethylene glycol to obtain a concentration of 200 mg/mL as a standard concentration for preparing the dilutions and then sterilized in a water bath at 50°C for 15 min. This standard concentration was considered a source for preparing the dilutions used in the study, according to the dilution equation:

$$N1\ V1 = N2\ V2 \quad (1)$$

### Test the inhibitory effect of aqueous turmeric extract

The inhibitory activity of aqueous turmeric extract was tested on the fungus *T. mentagrophytes* and *T. rubrum* by adding specific volumes of each sterile standard extract to specific volumes of sterile potato dextrose agar (PDA) before solidification in glass bottles, and after shaking well, the concentrations (5, 10, 15, and 20 mg/mL) were obtained according to the previous equation dilution.

Then, it was poured into three Petri dishes with a diameter of 9 cm, so that each dish contained 15 mL of the culture medium containing the extract, after solidification of the medium, a disc was taken from the edge of the 1-week-old fungal colony by a cork pourer with a diameter of 0.5 cm and placed in the center of the dish under sterile conditions, then the dishes were incubated at a temperature of 27°C for a week in an incubator of the type Memcri, Germany. The process of cultivation and incubation was carried out in an inverted position and under sterile conditions. After a week, the results were taken by calculating the average measurement of two perpendicular diameters for each fungal colony, and each treatment was with three replicates, each replicate being one dish.

### Test the inhibitory effect of aqueous turmeric extract supplemented with AgNPs

The inhibitory activity of aqueous turmeric extract was tested on the fungi *T. mentagrophytes* and *T. rubrum* by adding specified volumes of each sterile standard extract to specified volumes of nutrient medium (PDA) supplemented with 0.1 mg/mL of AgNPs, which were sterilized before solidification in glass bottles, and after shaking it well, the concentrations (5.01, 10.01, 15.01, and 20.01 mg/mL) were obtained, according to the previous dilution equation. Then, it was poured into three Petri dishes with a diameter of 9 cm, each dish containing 15 mL of the culture medium with the extract. After solidification of the medium, a disc was taken from the edge of the 1-week-old fungal colony using a cork pourer with a diameter of 0.5 cm, and it was placed in the center of the dish under sterile conditions; then, the dishes were then incubated at a temperature of 27°C for a week in an incubator of the type Memcri, Germany. The process of cultivation and incubation was performed in an inverted position and under sterile conditions. After a week, the results were taken by calculating the average measurement of two perpendicular diameters for each fungal colony, with each treatment having three replicates, each replicate being one dish.[2]

### Test the inhibitory effect of alcoholic turmeric extract

The inhibitory activity of alcoholic turmeric extract was conducted on *T. mentagrophytes* and *T. rubrum* by adding certain volumes of each sterile extract to specified volumes of sterile PDA before solidification, with shaking to obtain the concentrations (2, 4, 8, and 16 mg/mL), according to the previous dilution equation. Then, it was poured into three Petri dishes with a diameter of 9 cm, each dish contained 15 mL of culture medium, and after solidification of the medium, a disc with a diameter of 0.5 cm was taken from the edge of the 1-week-old fungal colony using a cork borer and placed in the center of the dish. Then, the dishes were incubated in the incubator at a temperature of 27°C for a week. The process of cultivation and incubation was carried out in an inverted position and under sterile conditions; then, the results were taken by calculating the average measurement of each two perpendicular diameters, and each treatment had three replicates, each replicate being one dish.[15]

### Test the inhibitory effect of turmeric extract supplemented with AgNPs

The inhibitory activity of alcoholic turmeric extract was conducted on the fungi *T. mentagrophytes* and *T. rubrum* by adding certain volumes of each sterile extract to specified volumes of food medium (PDA) supplemented with 0.1% AgNPs and sterilized before solidification, with shaking to obtain the concentrations (2.01, 4.01, 8.01, and 16.01 mg/mL) according to the previous dilution equation.

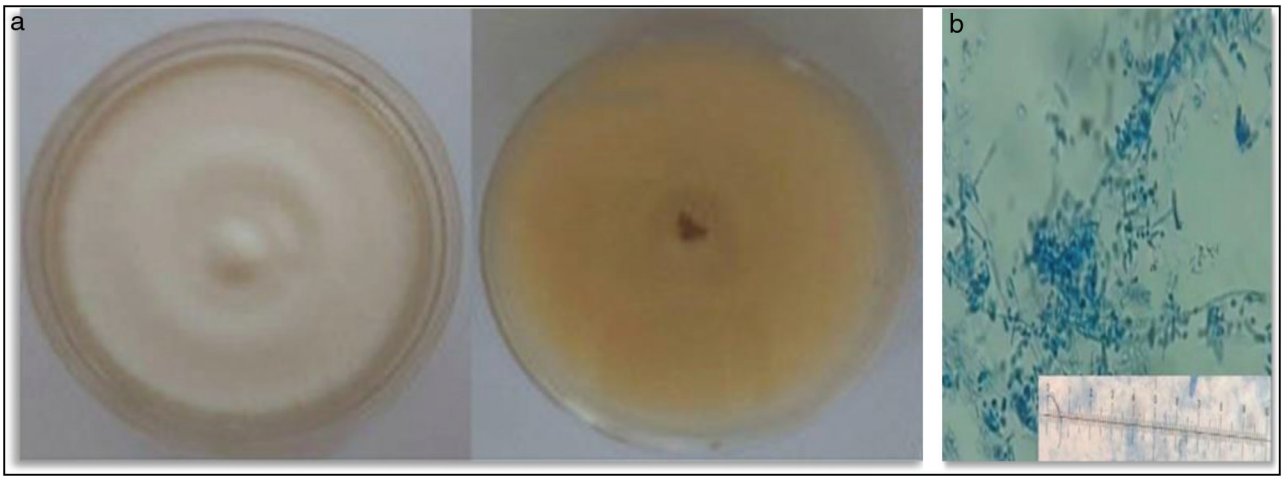


**Figure 1:** (a) The fungus *Trichophyton mentagrophytes* isolate IQT-No.2. (b) Figure caption or Times New Roman font: 9 pt, centered.

Then, it was poured into three Petri dishes with a diameter of 9 cm so that each dish contained 15 mL of culture medium. After the medium solidified, a 0.5 cm diameter disc was taken from the edge of the 1-week-old fungal colony using a cork borer and placed in the center of the dish. Then, the dishes were incubated in the incubator at a temperature of 27°C for a period of 1 week. The process of cultivation and incubation was carried out in an inverted position and under sterile conditions; then, the results were taken by calculating the average measurement of each two perpendicular diameters, and each treatment had three replicates, each replicate being one dish.[16]

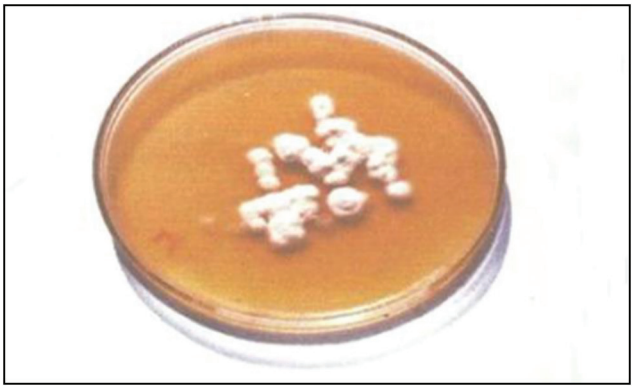

**Figure 2:** The growth of colonies of the fungus *Trichophyton mentagrophytes* is shown on sabouraud dextrose agar medium

## Results

### Phenotypic and microscopic characteristics of the two fungi used in the study

The fungus *T. rubrum* isolates no.1. We note phenotypic characteristics of the fungus, including flat white colonies tending to rise slightly above the surface of the SDA medium with a soft, cottony to fluffy, and loose texture. The back side of the colony also appeared in a yellowish-brown to reddish-brown color. The diameter growth rate of the fungal culture reached 8 cm after 14 days of incubation at 25°C [Figures 1 and 2].

### Phenotypic characteristics of the fungus

After the fungus samples were planted, the colonies appeared on SDA medium, flat and domed in the center, and white in color, as shown in Figure 2. Microscopic examination showed the presence of macroconidia divided by 4–5 thin-walled septa, as shown in Figure 3. Microconidia appeared in clusters, as shown in Figure 4.

### Microscopic characteristics of the fungus

From its septate hyphae, *T. mentagrophytes* sends forth branching conidiophores. Microconidia are generated on the conidiophores in clusters like bunches of grapes. The microconidia are round to pyriform and are between 2 and 4 μm in size. Macroconidia are irregular in form and range from 20–50 nm to 6–8 nm in size. Macroconidia are thin-walled and smooth on the outside, often consisting of 3–8 cells. Macroconidia may be more common in soils with a younger population. The amount of micro and macro conidia produced by an isolate might be different. Hyphae may be coiled or spiral, and in certain strains, you may see structures called nodular bodies or chlamydospores.

Tables 1 and 2 show that there is an effect of the aqueous and alcoholic extract on inhibiting the growth of the fungi included in the study.

## Discussion

*Trichophyton* and *Microsporum* are able to produce enzymes that degrade keratin, fat, and protein, and the high activity of the enzymes is represented by the diameter of the halo. Protease enzyme was the most effective among the enzymes, and the activity of the

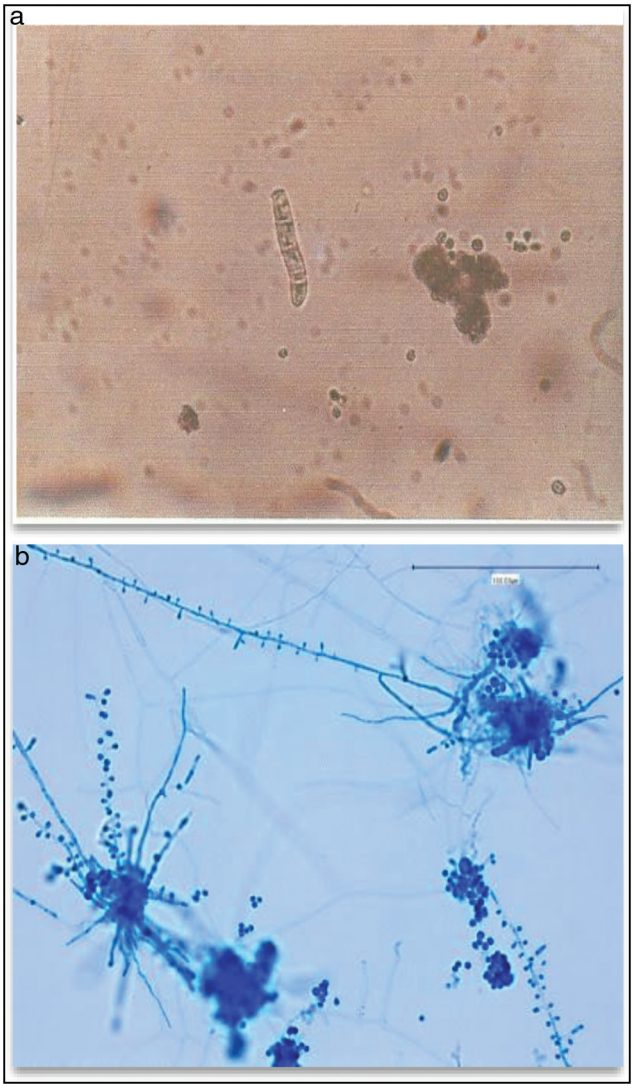


**Figure 3:** (a) The macroconidia of the fungus *Trichophyton mentagrophytes* at a magnification power of 400. (b) *T. mentagrophytes* showing sessile microconidia along septate hyphae. Note 100 $\mu$m bar in the upper right of this and several other photos (400×, lactophenol cotton blue)

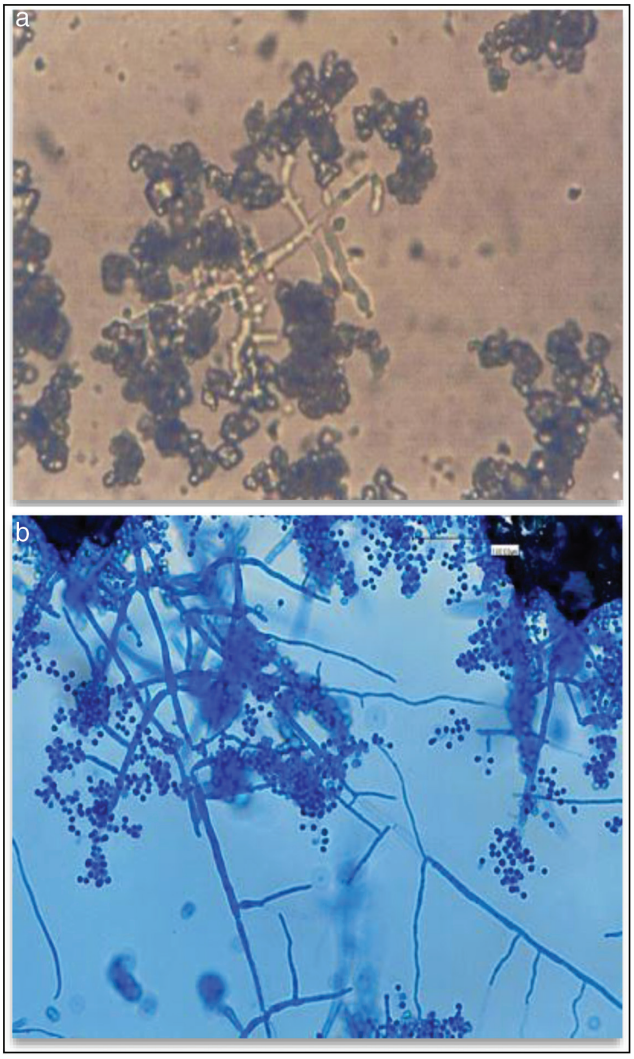


**Figure 4:** (a) The microconidia of *Trichophyton mentagrophytes* are shown at a magnification power of 400. (b) *T. mentagrophytes* – branched conidiophores bearing spherical conidia in clusters seen extending from septate hyphae (400×, lactophenol cotton blue)

enzyme increases with increasing incubation period, as dermal fungi produce and secrete protease enzyme in response to extracellular components such as keratin, as they invade the epidermal layer. Protease may contribute to the ability of dermal cells to degrade components of the deeper layers of the dermis in patients with dermatomycosis.[17]

As a study showed, it presented the ability of the fungus *T. rubrum* to produce protease enzyme, and the gene expression of this enzyme was increased when treated with filters of the fungus *Marasmius palmiyorus*. Dermatophytes have been shown to secrete endopeptidases, exopeptidases, and more than 20 types of proteases when grown in a medium containing nitrogen as a protein source.[18] It is believed that, in addition to the mechanical penetration of fungal elements, proteolytic enzymes degrade components of dermal tissues. The hydrolysis of keratin by proteins is an important aspect of fungal pathogenesis, as it provides a source of nutrition on the keratinocyte layer, which constitutes an obstacle to pathogens.[19] The role of exogenous enzymes as a virulence factor of fungi has been extensively studied. In the case of dermatophytes, their ability to secrete keratolytic activity *in vitro* has drawn the attention of many researchers, primarily to the secretion of the fungal protease enzyme.[20-22] Some studies showed that dermatophytes (*T. mentagrophytes, T. verrucosum, T. tonsurans, T. microsporum canis, T. rubrum, T. violaceum,* and *M. gypseum*) had the ability to produce the enzyme protease but to varying degrees according to its virulence.

*Trichophyton* and *Microsporum* were also able to secrete the enzyme keratinase. Dermal fungi are able to infect

**Table 1: *Trichophyton mentagrophytes***

| *Trichophyton Mentagrophytes* | | | | | |
|---|---|---|---|---|---|
| With aqueous turmeric extract | | | With alcoholic turmeric extract | | |
| Concentrate for aqueous turmeric extract (mg/mL) | Colony diameter with nanoparticles at a concentration of 0.1 mg/mL (mm) | Colony diameter without nanoparticles (mm) | Concentrate for alcoholic turmeric extract (mg/mL) | Colony diameter with nanoparticles at a concentration of 0.1 mg/mL (mm) | Colony diameter without nanoparticles (mm) |
| 5 | 15 | 20 | 2 | 12 | 18 |
| 10 | 12 | 15 | 4 | 10 | 15 |
| 15 | 9 | 11 | 8 | 7 | 10 |
| 20 | 7 | 8 | 16 | 5 | 6 |

**Table 2: *Trichophyton rubrum***

| *Trichophyton rubrum* | | | | | |
|---|---|---|---|---|---|
| With aqueous turmeric extract | | | With alcoholic turmeric extract | | |
| Concentrate for aqueous turmeric extract (mg/mL) | Colony diameter with nanoparticles at a concentration of 0.1 mg/mL (mm) | Colony diameter without nanoparticles (mm) | Concentrate for alcoholic turmeric extract (mg/mL) | Colony diameter with nanoparticles at a concentration of 0.1 mg/mL (mm) | Colony diameter without nanoparticles (mm) |
| 5 | 15 | 20 | 2 | 12 | 18 |
| 10 | 12 | 15 | 4 | 10 | 15 |
| 15 | 9 | 11 | 8 | 7 | 10 |
| 20 | 7 | 8 | 16 | 5 | 6 |

keratin in the skin, nails, or hair and have the ability to break down keratin, which is the main virulence factor. A group of proteolytic enzymes capable of hydrolyzing insoluble keratin more efficiently than other proteases is called keratinase.[23] Keratins are the most abundant proteins in the epithelial cells of vertebrates and represent the main components of the skin and its appendages such as nails, hair, feathers, and wool. Protein chains are tightly linked either in $\alpha$-chain ($\alpha$-keratins) or in $\beta$-keratin structures. Keratin belongs to a family of intermediate filament proteins, and there is a high degree of cross-linking via disulfide bonds, hydrophobic interactions, and hydrogen bonds stabilize the structure of keratin filaments. Therefore, keratinocytes are insoluble in water and highly resistant to degradation by proteolytic enzymes such as pepsin, trypsin, and papain.[24]

The results of the microscopic examinations showed that there are internal abnormalities that can be observed during the microscopic examination, the most prominent of which was the agglomeration of the protoplast inside the fungal cells without affecting the cell wall, and this indicates that the effect on the cell membrane, and another effect that could be observed is the formation of chlamydial spores significantly. The fungal cells suffer from harsh environmental conditions, which led to the formation of chlamydospores in large numbers, and this may explain as a result of physiological changes within the fungus cells and the production of some compounds that act as a kind of protection for the fungus, as well as changes that may lead to the fungus losing its vital processes, protoplasm assembly, and cell contraction and collapse. It may be attributed to the presence of some toxic substances that poison the cell or increase its osmotic pressure, which leads to the destruction of the mycelium. This is reflected in the growth and development of conidia. Some studies have suggested that AgNPs may cause changes in the fungal cell membrane, causing cytoplasmic leakage, and subsequent cell death.[25,26] The plasma membrane was also severely damaged, which led to the leakage of the contents of the cell and the disappearance of the nuclei, which led to the death of the cell. This is in agreement with Targhi *et al*.[15] showed that treatment of *T. rubrum* with TDT 067 containing the antifungal terbinafine led to distortions and ruptures in the mycelium and shrinkage in the protoplasm that led to the formation of void.

The results showed that the secondary silver concentration with the aqueous extract and the alcoholic extract had a significant synergistic effect on the growth rate of the fungi used under study, *T. rubrum* and *T. menticrophyte* by increasing the concentration, the alcoholic extract supplemented with AgNPs was more effective in the growth rate of the fungi than the aqueous extract as well. The fungus is more sensitive as the rate of fixation increases due to the decrease in the rate of the diameter of the fungal colony and its large surface area. The smaller the size, the larger the number of nanoparticles accumulated

on the surface of the cells, which leads to an increase in their toxicity to microorganisms. This leads to affecting the permeability of the plasma membrane of the cell and consequently the death of the cell.[27,28] The mechanism by which nanoparticles interact with microorganisms is that these organisms carry negative charges while nanometals and their oxides carry positive charges, which creates electromagnetic attraction between the cell and the surface of the microorganisms, and the surface of the microorganisms releases ions that interact with the thiol group of proteins that carry nutrients that emerge from the cell membrane which reduces the permeability of the membrane and thus cell death.[29] The nanocomposites have a role in disrupting transport systems, and this is reflected in respiration, cellular metabolism, and interaction between organelles, in addition to the AgNPs known to produce free radicals that act to destroy amino acids, fats, and proteins.[30]

Several mechanisms have been proposed to explain the antifungal activity of AgNPs. One potential mechanism involves the formation of hydrogen peroxide ($H_2O_2$) on the surface of nanoparticles, attributed to hydrogen bonding between the hydroxyl groups of fungal cellulose molecules and the oxygen atoms of AgNPs, resulting in growth inhibition.[31] Alternatively, the release of silver ions ($Ag^{++}$) may disrupt fungal cell membranes and interact with intracellular components.[29] Another mechanism involves the direct interaction of AgNPs with fungal cell membranes, leading to deoxyribonucleic acid replication inhibition, protein and enzyme degradation, cytoplasmic disintegration, and disrupted cell division. Reactive oxygen species generation also plays a role in damaging the fungal cell membrane, causing cellular leakage, plasma membrane contraction, and eventual death.[32,33]

El-Diasty *et al*.[31] observed that treating fungi with 8 mg/mL of AgNPs caused significant damage to fungal membranes, including hole formation in the conidial walls and intracellular leakage. They also reported that inhibition rates increased with nanoparticle concentration, with the highest effect observed at 40 mg/mL. Similar findings were reported by Targhi *et al*.[32] who demonstrated reduced biomass of *Sclerotinia sclerotiorum* with increased AgNP concentrations, with a minimum biomass of 0.132 g in Ag/ZnO nano mixtures.

Saqib *et al*.[33] further revealed that nanomaterials reduced the biomass of *A. flavus* and *A. fumigatus*. At high concentrations, fungal biomass was significantly diminished or completely eliminated. This was attributed to oxidative stress and the formation of free radicals, which disrupted fungal growth.

Resistance to AgNPs and antifungal treatments varies among fungal species. For instance, *T. rubrum* was more resistant than *T. mentagrophytes*, likely due to differences in genetic makeup, cell wall thickness, plasma membrane composition, and hydrophobicity of the cell wall.[34] El-Diasty *et al*.[31] also noted variability in resistance among dermatophytes, such as *Microsporum*, *T. mentagrophytes*, and *Candida albicans*, attributed to differences in genetic and cell wall compositions, which predominantly consist of 1-$\beta$,3-1,6-glucan linked to chitin by a 1-$\beta$,4-linker.

Similar results were reported by Asong *et al*.[35] who found that *T. rubrum* was the most resistant species to phenolic and flavonoid extracts of plants like Elephantorrhiza *Drimia sanguinea* and *Helichrysum paronychioides*. Omer *et al*.[36] demonstrated that fungal resistance to AgNPs varied in terms of colony diameter inhibition rates, emphasizing the diverse resistance mechanisms.

Tables 1 and 2 in the study highlight the inhibitory effects of aqueous and alcoholic plant extracts on fungal growth. Inhibition rates increased with extract concentration, and sensitivity varied between fungal species. Notably, *T. rubrum* was more sensitive than *T. mentagrophytes*, attributed to genetic differences between the species.[35]

## Conclusion

The results of this study showed that turmeric extracts supplemented with AgNPs have antifungal activity, that is, effective against a variety of pathogenic fungi. This activity may be attributed to the synergy between turmeric and silver nanocomposites, resulting in an increased fungus-killing efficiency. These results indicate the possibility of developing new medicinal preparations based on these components for the treatment of fungal diseases. However, more studies are needed to assess the potential toxicity of these compounds and determine safe doses for human use.

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### Conflicts of interest

There are no conflicts of interest.

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