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Original Research

In Vitro Effects of Natural Garlic Juice on Some Fungal Strains

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ABSTRACT

Objective: Garlic (Allium sativum) is a commonly available plant that is presumed to have antimicrobial properties. This study aimed to investigate the antifungal effects of natural garlic juice on different fungal isolates.

Methods: Garlic bulbs obtained from the market were aseptically cut cross-sectionally into slices, and the juice was collected via a sterilized electric mincer. First, a susceptibility screening test was performed using the slices and juice for *Candida, Epicoccum, Fusarium, Epidermophyton, Trichophyton, Aspergillus,* and *Penicillium* genera. Sabouraud dextrose agar (SDA) medium was inoculated with fungal isolates. Circular wells were drilled in the center of the SDA, and 270 µl of garlic juice were added to these wells. After incubation, the inhibition zones were evaluated, and a dilution test was performed for the most susceptible isolate. Increasing dilutions of garlic juice from 1/2 to 1/1024 were added to the wells that were drilled on Mueller Hinton agar, and the inhibition zone diameters were measured after incubation.

Results: All isolates were inhibited by the garlic juice. In the dilution test for Candida albicans, an inhibition zone larger than 25 mm was observed in up to the 1/128 dilution. After the 1/256 dilution, the inhibition zone gradually got smaller and no inhibition was observed at the 1/1024 dilution.

Conclusion: Natural garlic juice was effective in all the isolates. The most significant antifungal effect was observed against the Candida albicans isolate. In underdeveloped areas where access to healthcare and medicine is difficult, garlic juice may be an inexpensive and easily accessible alternative to classical antifungal drugs.

Keywords: Garlic, antifungal, fungi, Candida

INTRODUCTION

Garlic (*Allium sativum*) is a species in the onion-like genus—*Allium* (the genus name *Allium* is Latin name for garlic); the genus includes many other plants that are consumed by humans like onion, scallion, shallot, leek, and chives (1). Garlic is originally native to Central and South Asia, but China is still the largest producer of garlic with approximately 80% of the total global production (2). However, garlic has been widely consumed from ancient times all over the world for culinary and medicinal purposes (3-5). The historically appealing culinary and medicinal uses of garlic are well attributed to the fact that its cloves have a pungent smell and taste, caused by the numerous sulfur compounds present in garlic (6). Several organosulfur compounds have been identified in garlic, many of which had been studied for their potential medical benefits. The following are examples of such sulfur-containing compounds: three γ-glutamyl peptides, including γ-L-glutamyl-S-(2-propenyl)-L-cysteine (GSAC), γ-L-glutamyl-S-(trans-1-propenyl)-L-cysteine (GSPC), and γ-L-glutamyl-S-methyl-L-cysteine (GSMC); their three corresponding sulfoxide derivatives, including (+)-S-(2- propenyl)-L-cysteine sulfoxide (alliin), (+)-S-(trans-1-propenyl)- L-cysteine sulfoxide (isoalliin), and (+)-S-methyl-L-cysteine sulfoxide (methiin), respectively; and a seventh compound, (1S,3R,5S)-5-methyl-1,4- thiazane-3-carboxylic acid 1-oxide (cycloalliin) had also been investigated (7). The latter sulfoxides of garlic have been reported to have some medicinal properties; for example, alliin has anticancer effects (8) and also lipid-lowering effects (9, 10). On the other hand, the former γ-glutamyl peptides have been reported to lower blood pressure (11) and to have cholesterol-lowering and antioxidant effects (12). However, when garlic bulbs are crushed, these organosulfur compounds are transformed into other compounds such as allicin, ajoene, dithiins, and diallylpolysulfides (13).

This is mainly due to the release of enzymes such as alliinase, which act on the already present organosulfur compounds in garlic tissues, thereby transforming them into novel forms as mentioned above (13, 14). One of these compounds of interest is allicin. Upon garlic crushing or chopping, the enzyme alliin lyase (alliinase) catalyzes the cleavage of alliin into allicin, which reacts further to form ajoene (14). Allicin (allylthiosulfinate, diallyl disulfide-S-monoxide) is a molecule with strong aroma and is probably involved in the defense mechanism by the garlic plant against pests (15).

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Allicin material was first isolated and studied in the laboratory by Chester J. Cavallito and John Hays Bailey in 1944 (16), and in that early study, it was shown that allicin has antibiotic activity in the form of an antibacterial effect. Thereafter, the antibiotic activity of allicin and its derivative, ajoene, against various microorganisms, including bacteria, fungi, viruses, and even parasites, was well studied (17-20). Regarding studies that specifically target the antifungal activity of garlic products, the available scientific literature suggests that the antifungal activity of garlic was first established in 1936 by Schmidt and Marquardt whilst working with epidermophyte cultures (21). Since then, several studies have tested different components of garlic against different species of medically important fungi (22, 23). To this end, the present study aimed to investigate the antifungal effects of natural garlic juice on several fungal genera and *Candida albicans* species.

METHODS

Garlic bulbs were obtained from over-the-shelf grocery markets, and was first peeled and aseptically cut cross-sectionally into slices to be placed directly into solid agars plates for the first step. For the second step, the garlic juice was obtained by mincing the peeled garlic bulbs in a sterilized electric mincer. The bulb solid remnants were discarded and only the homogenous watery part of the juice was collected and kept in sterile bottles at +4°C for one week. One week later, the clear supernatant fluid was taken and used for the study, while the sediment was discarded.

The Screening Step

A total of seven isolates from different fungal genera, including *Candida, Epicoccum, Fusarium, Epidermophyton, Trichophyton, Aspergillus,* and *Penicillium,* were investigated. The isolates were obtained from the samples of patients in Gaziantep University Hospital. The isolates were previously identified via the routine work flow in the medical microbiology laboratory of Gaziantep University Hospital at the genus level (except *Candida albicans* species) and selected randomly for the study. The isolates were stored at −80°C before the study. In the screening step, we used a freshly cut slice from a garlic bulb to evaluate the antifungal activity of fresh garlic as a whole plant on several fungi. Then, we used the undiluted fresh garlic juice to screen and detect the most susceptible fungal genus to the garlic juice as described below.

First, pure colonies of the fungal strains were transferred into normal saline solutions (0.85% NaCl) until a fungal suspension of 0.5 McFarland turbidity is obtained. Sabouraud dextrose agar (SDA) plates were inoculated with 100 microlitres of 0.5 McFarland fungal suspensions and kept at room temperature for 5 minutes. Two millimeters thick slices of garlic were placed on the surface of each SDA just at the center to determine the inhibitory effect of the whole plant. For the other set, a single circular well was drilled in the center of the SDA, which was already inoculated with fungal suspensions, and 270 µl of fresh garlic juice was added to the well on the SDA without diluting. This procedure was repeated for all the seven isolates to obtain more homogenous and clearer inhibition zones.

Finally, the 14 plates were incubated at 37°C for 24 hours for *Candida* and 4 weeks for the other fungi. At the end of the incubation, the inhibition zones were noted for each isolate, and the isolate with the most significant inhibition zone was tested with natural garlic juice in the second dilution step.

Main Points:

- Natural garlic juice has antifungal effect on various fungal genera.
- Natural garlic juice inhibited growing of *Candida albicans* even at 1/128 dilution.
- Natural garlic juice has a potential to be used as antifungal agent.

The Dilution Step

For the dilution test using the natural garlic juice, serial saline dilutions of the garlic juice were prepared at 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, and 1/1024 concentrations. A single circular well was opened in the center of each Mueller Hinton agar (MHA), which was already inoculated with 100 µl of 0.5 McFarland suspension of the selected fungal isolate (*Candida albicans*), and 270 µl of fluid at increasing garlic juice concentrations were added to the wells on the MHA plates. A total of 10 plates with increasing concentrations of the garlic juice were incubated at 37°C for 24 hours. As mentioned above, the inhibition zone diameters of each media were measured using a compass and noted after the incubation.

RESULTS

In the screening step, with the cross-sectional slices of the garlic bulb, we could not observe a regularly shaped inhibition zone even if there was a visible inhibition.

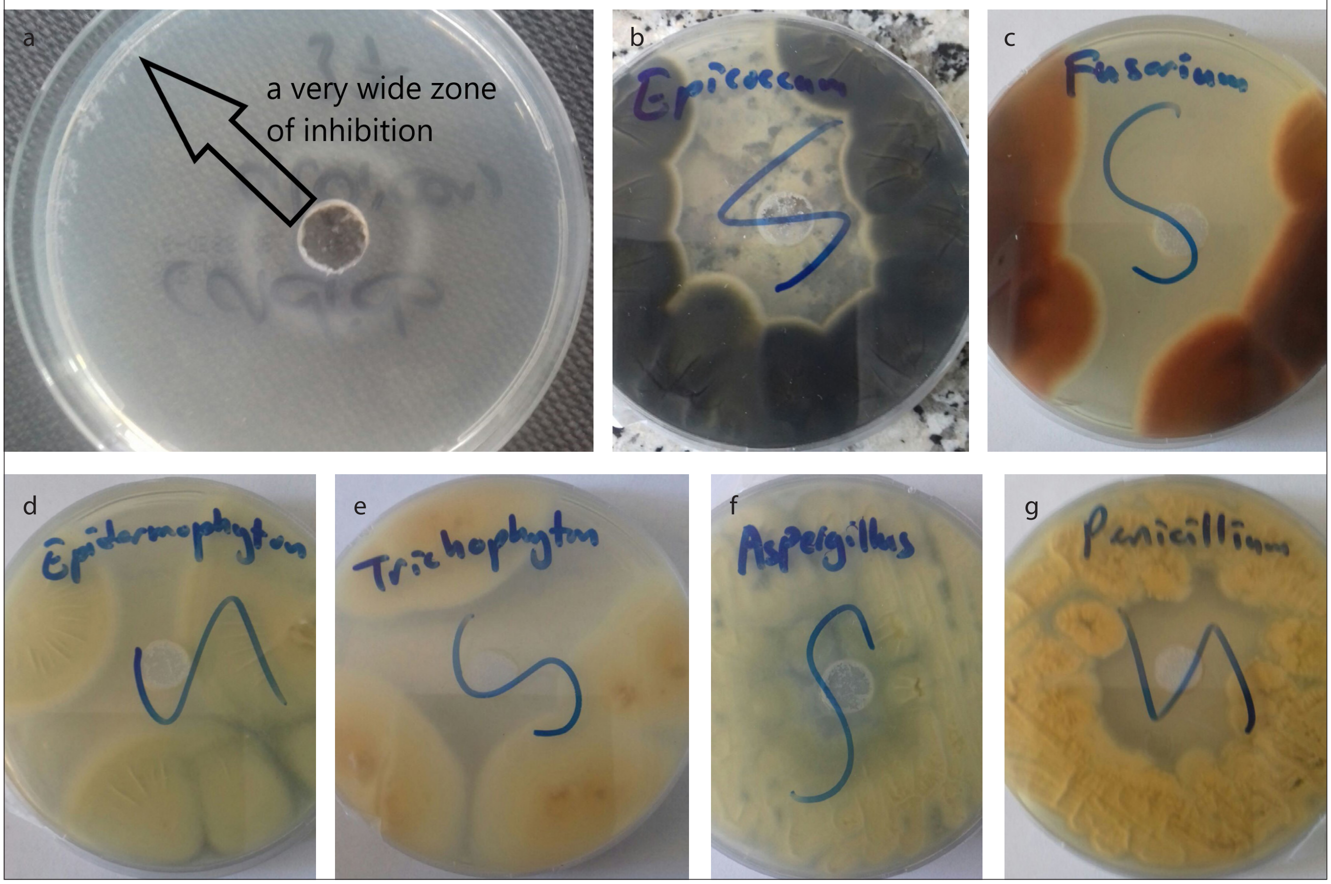
In the screening step, using the fresh garlic juice, the inhibition zones were clearer, especially for the *Candida albicans* isolate (Figure 1a). For all the isolates, including *Aspergillus, Epicoccum, Fusarium, Epidermophyton, Trichophyton,* and *Penicillium*, full concentration of the garlic juice showed an inhibitory effect but with a variable and an irregular extent (Figure 1. b-g). In addition, the inhibition zone was very small for the *Aspergillus* genus (Figure 1F). The largest and the clearest inhibition zone was detected in plate of the *Candida albicans* isolate (Figure 1a); therefore, this isolate was selected for the dilution test step.

In the dilution step, the *Candida albicans* isolate was subjected to a full serial dilution susceptibility test using different concentrations of garlic juice. In this step, we observed the largest inhibition zone at the 1/2 dilution of the garlic juice (almost all plates) (Figure 2A). At up to the 1/128 dilution, we observed significant inhibition zones greater than 25 mm in diameter (Figure 2. b-e). A small inhibition zone diameter was observed at the 1/256 dilution and then, the widths of the inhibition zones gradually decreased with higher dilutions (Figure 2. f, g). The inhibition zone did not appear at the 1/1024 dilution (Figure 2h).

DISCUSSION

The mechanism by which allicin and its derivatives function as an antimicrobial agent may involve several biological effects, in-

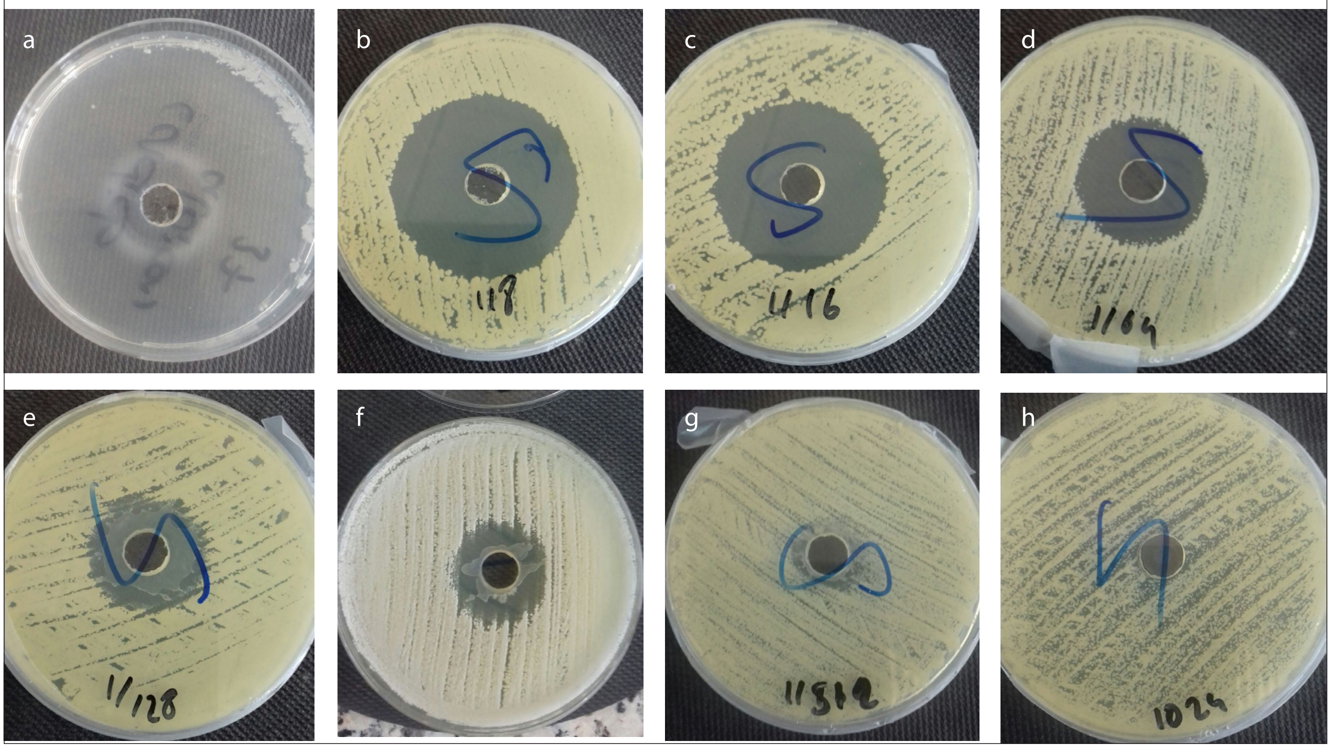
Figure 1. a-g. a) Effect of full concentration of garlic juice on *Candida albicans* b) Effect of the full concentration of garlic juice on *Epicoccum* c) Effect of the full concentration of garlic juice on *Fusarium* d) Effect of the full concentration of garlic juice on Epidermophyton e) Effect of the full concentration of garlic juice on Trichophyton f) Effect of the full concentration of garlic juice on *Aspergillus* g) Effect of the full concentration of garlic juice on *Penicillium*



cluding its permeability through cell membranes and its reactivity with various biomolecules within the target cells, regulation of enzymatic activity, protein synthesis, and nucleic acid synthesis (15, 24). Most previously cited studies used methods involving steam distillation or solvent extraction (such as those using alcoholic compounds) to obtain the essential oils from garlic plants that contain allicin and ajoene prior to testing these compounds or even used synthetic preparations of such compounds (16-20). However, we used natural garlic juice instead of chemically or physically processed garlic in our study. We opted to test the *in vitro* effect of garlic on clinically relevant fungal species, especially the *Candida* isolate (*Candida albicans*), from patients using freshly cut garlic bulb slices or mechanically obtained garlic juice from locally sold culinary garlic that is available at grocery markets, as this provides the easiest and cheapest means of obtaining garlic's active molecules (allicin and ajoene). In our study, garlic juice was found to be more effective than garlic slices, most probably due to the formation of garlic's active molecules. When the results were analyzed, garlic juice was found to be extremely effective on *Candida albicans*; moreover, garlic juice was effective on all fungal genera except *Aspergillus*. Fungal infections are a major public health concern, with an increasing demand for treatment, especially for topical infections caused by various fungi including dermatophytes and *Candida* species (25). *Candida albicans* is one of the most widespread causes of superficial cutaneous fungal infection, and when the immune system is weakened, it can spread to deeper tissues and even into the blood, where it causes systemic candidiasis (26). Therefore, natural garlic juice might be an alternative for the treatment of fungal infections caused by *Candida albicans.*

The limitation of our study is that it was only an *in vitro* study, which may not reflect the *in vivo* effect of garlic juice against human infections. Secondly, we only tested a limited number of fungal agents, which may not present all the members of the genus. However, in most of the fungi tested, natural garlic juice was shown to have significant inhibitory effects. Another limitation is that we performed the diffusion tests using different dilutions instead of the broth microdilution test, which can determine a minimum inhibitory concentration (MIC). Since our study was about the potential effect of natural garlic juice for topical use in fungal infections and there is no standard MIC for garlic juice, we preferred using the diffusion method, which was more practical. In a similar study that tested the antibacterial effect of fresh garlic juice using a similar well diffusion method, Yadav et al. (27) also found an efficiency of fresh garlic juice against the tested strains of bacteria. In a study designed to investigate the antifungal activity of garlic against *Candida albicans* at molecular levels,

Figure 2. a-h. a) Effect of the 1/2 dilution of garlic juice on *Candida albicans* b) Effect of the 1/8 dilution of garlic juice on *Candida albicans* c) Effect of the 1/16 dilution of garlic juice on *Candida albicans* d) Effect of the 1/64 dilution of garlic juice on *Candida albicans* e) Effect of the 1/128 dilution of garlic juice on *Candida albicans* f) Effect of the 1/256 dilution of garlic juice on *Candida albicans* g) Effect of the 1/512 dilution of garlic juice on *Candida albicans* h) Effect of the 1/1024 dilution of garlic juice on *Candida albicans*



Li et al. (28) found that garlic products have a more significant efficiency against Candida species than for other fungi; similar to our findings that garlic juice has a potential fungotoxic activity against Candida albicans specifically. We suggest that new *in vivo* and *in vitro* studies evaluating the effects of natural garlic juice should be performed with much more strains and various other fungal species in the future.

CONCLUSION

In our study, natural garlic juice was found to be effective against *Candida albicans* even at very low concentrations such as 1/128 dilution. Since garlic juice was evaluated as an effective antifungal agent in our study and it is a naturally occurring, easily accessible, and cheaply available product, natural garlic juice might be recommended as an alternative agent for the treatment of several fungal infections, especially for the *Candida albicans*, in the countries with a poor healthcare system and where accessing the classical antifungal drugs is difficult. However, more *in vivo* and *in vitro* studies are necessary to confirm the antifungal effects of natural garlic juice.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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