

Antimicrobial effects of garlic (*Allium sativum* L.)

Strika, I.^a, Bašić, A.^b, Halilović, N.^b

^aUniversity of Sarajevo, Faculty of Science, Department of Biology, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina

^aUniversity of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina

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*Corresponding author:

E-mail: ilma.strika2512@gmail.com

Phone: 00-387-62-182670

Abstract: Garlic has been used as a source of food and medicine for thousands of years. Given that the garlic contains different biologically active materials and acts as an antibiotic and a fungicide, the purpose of this research was to estimate the degree of sensitivity of three different Gram-positive bacteria: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis*; two types of Gram-negative bacteria: *Escherichia coli* and *Salmonella enteritidis*; as well as the fungus *Candida albicans*. The degree of sensitivity of tested microbes with regards to the agency of fresh and thermally processed local and imported garlic was determined using the disc-diffusion method. Examined antimicrobial-test substances exhibited antibacterial effect on all tested gram-positive bacteria and gram-negative bacteria, as well as the fungistatic agency upon fungus *C. albicans*. The strongest antimicrobial effect on all tested species was exhibited by fresh local garlic. Preparates based on *A. sativum* could be introduced in clinical practice for the treatment of infections caused by *C. albicans*.

INTRODUCTION

Garlic (*Allium sativum* L.) is a plant from the family of arcs (Aliaceae). It is a herbaceous plant with height of 20-40 cm, a bulb of strong odour and pungent taste. Sulphur compounds in garlic are responsible both for its strong smell, and for its medicinal properties. The undamaged plant contains alliin (S-allyl-L-cysteine sulfoxide). Alliin is a soluble, crystal, odourless compound. It is a cysteine derivative and has antimicrobial properties. Standardized garlic powder contains 1.3% of alliin. Slight damage causes changes in alliin, which is broken down under the influence of enzymes allinase into lactic acid and 2-propenyl-sulfonic acid. This acid instantly dimerizes and builds allicin - diallyl sulfate or diallyl disulfide. Allicin was first isolated in 1940 and has been shown to have antimicrobial activity against many viruses, bacteria, fungi and parasites. Allicin produces diallyl sulfide, the most important volatile compound of garlic and gives it its characteristic smell. Garlic acts as an antibiotic, antiseptic, antitoxic, antiviral, bactericide, carminative, hipoholesterolemik, depurative, diuretic, expectorant, fungicide, hypoglycemic, hipotensiv, and stomachic. It is

used in the food and pharmaceutical industries (Agarwall, 1996). Given that garlic contains a variety of biologically active substances and at the same time acts as an antibiotic and fungicide, objectives of this study were to examine the *in vitro* antimicrobial activity of both fresh and thermally processed domestic and imported garlic onto selected representatives of Gram-positive and Gram-negative bacteria and fungus *Candida albicans*, compare the antimicrobial effectiveness of heat treated and fresh domestic and imported garlic and assess the degree of sensitivity of selected microorganisms to the effect of garlic.

MATERIALS AND METHODS

This study examined the antimicrobial activity of both fresh, and heat treated *Allium sativum* as well as the garlic of domestic origin (Kakanj) and the imported (China) garlic. The study used three Gram-positive bacteria: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Bacillus subtilis*, two Gram-negative bacteria: *Escherichia coli*, *Salmonella*

enteritidis and the fungus *Candida albicans*. The practical part of this work performed in the microbiology laboratory of Faculty of Sciences in Sarajevo. The aseptic technique applied during the research.

All experiments performed using sterile dishes and utensils. Culture medium Mueller-Hinton agar prepared for the growth of bacterial cultures used in the study, (Mueller & Hinton, 1941). For the cultivation of *Candida albicans* Sabouraud Dextrose agar was used (Sabouraud, 1892). The juice of fresh domestic and imported garlic prepared by crushing small pieces of garlic in a mortar until a thick mass. The content sieved through sterile gauze in order to obtain the juice. Juice of the heat treated domestic and imported garlic was obtained by a number of cleaned pieces of garlic placed in separate beakers and cooked at 100 °C for 5 minutes. Once the cooking was completed, such garlic was shredded in a mortar and sieved through sterile gauze. The study used method seeding on the agar plate. The degree of sensitivity of the tested bacteria and fungi *Candida albicans* activity of the garlic was determined by the disk diffusion method (Bauer et al., 1966). The sterile filter paper discs of 8 mm in diameter used and soaked in prepared juices.

After completing the procedure, the dishes carefully closed with the parafilm and left in the incubator (manufacturer Sutjeska) for 24 hours at 37 °C, after which the results recorded. After the incubation period, the zones of inhibition measured in all of the Petri dishes by using graph paper.

RESULTS AND DISCUSSION

In this research based on the measured zones of inhibition, it determined that the fresh homemade garlic manifests the best antimicrobial effect. It had the highest level of inhibition on the fungus *Candida albicans* (62 mm), while the lowest level of antimicrobial activity recorded in *Bacillus subtilis* (13mm) (Table 1). Heat-treated domestic garlic demonstrated the weakest inhibitory effect in MRSA and the fungus *Candida albicans*, wherein the zone of inhibition was 0 mm, while the other type of bacteria, both Gram-positive and Gram-negative show slightly better anti-microbial activity (Table 1.). The highest inhibitory effect recorded for *Salmonella enteritidis* (11mm).

Table 1. Results of the study on the antimicrobial effects of fresh and thermally processed domestic and imported garlic

SPECIES	Zone of inhibition (mm)			
	Fresh domestic garlic	Heat treated domestic garlic	Fresh imported garlic	Heat treated imported garlic
<i>Staphylococcus aureus</i>	31	9	9	9
MRSA	29	0	27	0
<i>Bacillus subtilis</i>	13	8	12	9
<i>Escherichia coli</i>	19	8	13	8
<i>Salmonella enteritidis</i>	15	11	11	9
<i>Candida albicans</i>	62	0	54	0

The largest zone of inhibition of 54 mm measured for *Candida albicans*, while the least antimicrobial activity demonstrated by the imported fresh garlic against the *Staphylococcus aureus* where the zone of inhibition was 9 mm (Table 1). Heat-treated imported garlic shows little or no antimicrobial effects. On MRSA and *Candida albicans*, heat-treated imported garlic showed no antimicrobial effect while the effects on the other type of bacteria, indicate a very weak activity (Table 1). In addition to the bactericidal and fungicidal activity on selected types of microbes, fresh local and imported *A. sativum* showed bacteriostatic activity, or affect their reproduction that it ends their metabolic activity. Bacteriostatic effect observed for MRSA with the best performance shown by the fresh homegrown *A. sativum*. Zones of inhibition measured, and the results presented in Table 2.

Table 2. Bacteriostatic agency of *A. sativum* on MRSA

Type of Garlic Juice	Zone of inhibition (mm)
Fresh Domestic	5
Boiled Domestic	0
Fresh Chinese	4
Boiled Chinese	0

Comparative analysis showed better effects of fresh garlic in relation to heat treatment. In comparing the zones of inhibition of fresh domestic and imported fresh, it is important to emphasize that the strongest antimicrobial effects showed fresh garlic of domestic origin. The biggest difference in the measured zones of inhibition that emerged because of the activity of domestic and imported *Allium sativum L.*, was observed on the tested bacteria *Staphylococcus aureus*, where the difference between fresh local garlic, which showed the strongest antimicrobial effects, and imported fresh garlic, which showed weak antimicrobial effect on the bacteria was even 22 mm (Table 1). From the research conducted that the domestic fresh garlic had the best effects to the type of fungus *Candida albicans*.

Numerous research has demonstrated that allicin, one of the active ingredients of fresh crushed garlic exhibits different antimicrobial activity (Ankri & Mirelman, 1999; Goncagul, 2010; Ross et al., 2001).

Allicin has been shown that in pure form it displays: antibacterial activity against a broad spectrum of Gram-positive and Gram-negative bacteria, particularly anti-fungal activity against *Candida albicans*, anti-parasitic activity and antiviral activity (Ankri & Mirelman, 1999). Allicin and its derivatives inhibit the cysteine protease, thereby acting antiparasitic on the human and animal pathogenic protozoa (Waag et al., 2010).

From the research conducted that the domestic fresh garlic had the best effects to the type of fungus *Candida albicans*.

Studies have shown that scanning electron microscope and the failure of cells that were treated with garlic, affects the structure and integrity of the outer surface of fungi cells (Ghannoum, 1988).

Since the cells of Gram-negative bacteria have beside a peptidoglycan layer also an outer lipid membrane, for a substance to exhibit any antibacterial activity on these bacteria, the lipid membrane must at least be partially

dissolved or pores created in it to act on the permeability of the membrane, leading to molecules and ions from bacterial cells leaking, and at the end, cracking the bacteria (Zaika, 1988).

Results of this research correlated with the reference literature (Gongacul, 2010; Ghannoum, 1988; Lemar, 2002; Shuford, et al., 2005; Zaika, 1988) where in both domestic and imported *Allium sativum* L. showed weaker antimicrobial activity against Gram-negative bacteria *Escherichia coli* and *Salmonella enteritidis* than the tested Gram - positive bacteria (*Staphylococcus aureus*, MRSA and *Bacillus subtilis*).

CONCLUSIONS

Tested domestic and imported garlic showed antimicrobial effect against all tested of the Gram-positive (*Staphylococcus aureus*, MRSA and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*), and the fungus *Candida albicans*. The investigated fresh domestic and imported garlic showed bacteriostatic activity against the methicillin-resistant *Staphylococcus aureus* (MRSA). Fresh domestic and imported garlic showed better antimicrobial effect compared to heat treated domestic and imported garlic. The strongest antimicrobial activity against all species tested was found in fresh homemade garlic. Fresh domestic and imported garlic showed the strongest antimicrobial activity against *Candida albicans*. Following these findings, particularly the intense antimicrobial effect of *Allium sativum* L. against *Candida albicans*, we believe that the preparations based on *A. sativum* L. could be introduced into clinical practice in the treatment of infections caused by *C. albicans*.

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Summary/Sažetak

Bijeli luk već hiljadama godina se koristi kao hrana i lijek. Budući da bijeli luk sadrži različite biološki aktivne materije i pri tome djeluje kao antibiotik i fungicid, svrha ovog istraživanja je ispitati osjetljivost tri vrste Gram-pozitivnih bakterija: *Staphylococcus aureus*, meticilin-rezistentni *Staphylococcus aureus* (MRSA) i *Bacillus subtilis*; dvije vrste Gram-negativnih bakterija: *Escherichia coli* i *Salmonella enteritidis*, te gljivicu *Candida albicans*. Step en osjetljivosti ispitivanih mikroorganizama na djelovanje svježeg i termički obrađenog domaćeg i uvoznog bijelog luka određivan je disk difuzionom metodom. Testirana antimikrobna-test supstanca pokazala je antimikrobni efekat na sve ispitivane vrste Gram-pozitivnih i Gram-negativnih bakterija kao i fungicidno djelovanje na gljivicu *Candida albicans*. Najjače antimikrobno djelovanje na sve testirane vrste pokazao je svježi domaći bijeli luk. Preparati na bazi *A. sativum* mogli bi se uvesti u kliničku praksu kod liječenja infekcija uzrokovanih *C. albicans*.