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CHARACTERISTICS OF BIOCIDE PROPERTIES OF GARLIC AND ITS PRODUCTS

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Key words: garlic, bacteria, yeast fungi, biocide properties

The research has aimed to estimate biocide properties (antibacterial and antifungal) of selected preparations of fresh garlic and garlic processed in household conditions (thermal processing) as well as commercially processed garlic. Fresh garlic was characterised by the strongest inhibiting activity in relation to the growth of all examined microorganism strains. It inhibited the growth of pathogenic colonies of coagulase positive strains of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* serotype A. Lyophilized garlic lost partially its bactericidal properties. It did not inhibit the growth of *Pseudomonas aeruginosa* nor *Staphylococcus aureus* ATTC 25923 strains, however, it showed a high ability to inhibit the growth of *Staphylococcus aureus ATTC 33591*, *Escherichia coli* ATTC 35218 and *Candida albicans* B serotype strains. Garlic after short-term thermal treatment (frying) as well as aging garlic extract (AGE) did not show its bactericidal properties, however, they had the inhibiting activity of *Candida albicans* A and B serotypes.

INTRODUCTION

Garlic (*Allium sativum*) belongs to the group of plants of medicinal, pro-health and nutritious activity. Garlic health benefits result from the content of over 200 biologically-active substances [Lawson & Wang, 2001]. Biologically-active substances contain alliin and its alkyl derivatives (methyl alliin and propyloalliin), alliin and methyl di-and trisulphides, numerous flavonoids as well as fitosteroles [Kim *et al.*, 2005]. During crushing or mechanical damage of a garlic bulb, under the influence of alliinase, alliin is disintegrated into allicin, the substance of strong irritating odour able to bond free radicals, limit the growth of viruses, bacteria and fungi [Freeman & Kodera, 1995]. Allinase, as an enzyme, remains active at pH ranging from 4 to 5.8, but its activity is inhibited at pH <3.5 and after boiling. Warming up in a microwave inactivates the activity of this enzyme within 1 min [Lawson & Wang, 2001]. Alliicin and diallyl trisulphide, which is also present in garlic, retain bacteriostatic properties even in strongly dilute solutions (1:25000) [Lawson, 1996].

Allicin is highly unstable and reactive. It disintegrates into, among others, ajoene, allixin, diallyl sulfides (DAS, DADS, DAT), vinyldithiin, which show antibacterial and antimutagenic properties [Cutler & Wilson, 2004]. Alliicin in the presence of cysteine combines with it to create S-allylmercaptocysteine (SAMC), which is present in the aging garlic extract [Krest & Keusgen, 1999].

Garlic is characterised by complex and multidirectional pharmacological activity: it lowers cholesterol and triglycerol concentration in blood serum, regulates and improves humoral and cell response of the immune system and lowers platelet aggregation [Miron *et al.*, 2000]. It also shows antioxidant and anitmutagenic properties [Borek, 2006; Yizhong *et al.*, 2004]. More and more attention is paid to garlic antibacterial, antifungal and antiviral activities with simultaneous excitation of body immunity [Niedworok, 2001]. Commonly used garlic preparations undergo technological treatment (among others, crushing, warming, drying, extraction), which can lead to the decrease or loss of these properties.

MATERIALS AND METHODS

Research material constituted of four selected products: fresh raw garlic (*Allium sativum*), garlic after thermal treatment (fat-free fried for 2–3 min) as well as commercially produced garlic: aging garlic extract (AGE of FINZELBERG GMBH & Co. Kg) and lyophilized garlic of WPPH "ELENA".

Before conducting the research, selected garlic products underwent microbiological control with the use of agar enriched with ram blood in the conditions of aerobic and anaerobic cultures. Antibiotic properties of sterile products were examined with the use of model strains: *Escherichia coli ATTC 35218*, *E. coli 25992*, *Pseudomonas aeruginosa ATCC* 27853, *Staphylococcus aureus ATCC 25923*, *Staphylococcus aureus ATCC 33591* and fungi *Candida albicans*, obtained from the collection of strains from the Immunology Clinic of Mother and Child Institute in Warsaw. In a physiological buffered saline solution (PBS), the suspensions of microbe cells of the above-mentioned strains of proper density (Mc Farland scale) were prepared, which were spread on the Muller-Hinton solid medium (Difco Laboratories, Detroit, MI).

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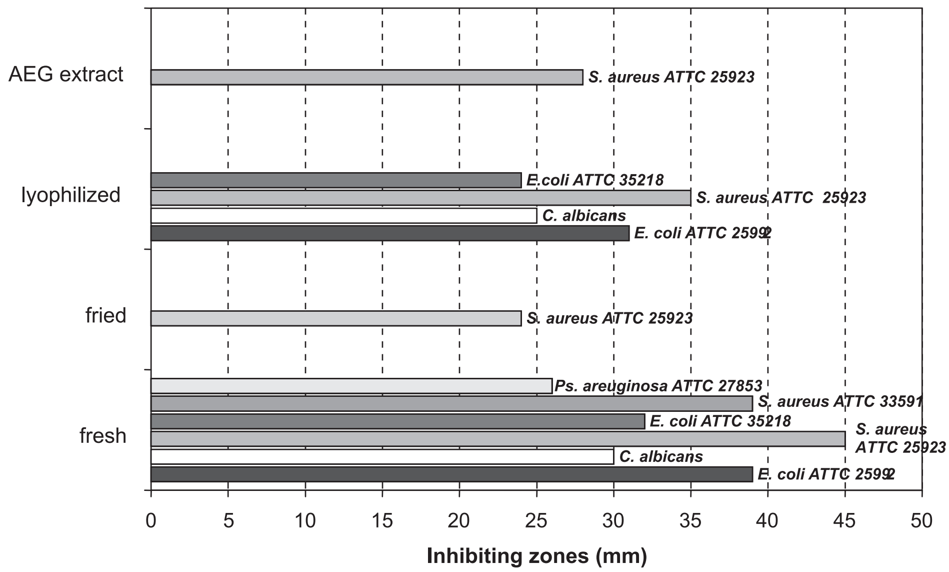

FIGURE 1. The comparison of microorganism inhibiting zones by garlic and its products on a Muller-Hinton medium.

The susceptibility of bacteria and fungi strains to the activity of products containing garlic and its products was examined with the method of disc diffusion on the Muller-Hinton medium (Difco Laboratories, Detroit, MI) [Benkeblia, 2004]. The examined garlic preparations were placed on sterilized filter paper discs put on the surface of agar with the suspension of a particular strain. The susceptibility of the examined strain was measured with the diameter of the inhibition zone around the disc, after a 24-h incubation at 37°C. Microorganism susceptibility to different garlic preparations was confirmed with the serial dilution method. The smallest preparation dilution, which inhibited the growth of bacteria and fungi strains, was considered Minimal Inhibitory Concentration.

RESULTS AND DISCUSSION

The results of conducted microbiological research point at the wide bactericidal and antifungal activity of raw fresh garlic. Antibiotic activity on the growth of *in vitro* colony was shown both in relation to the strains of Gram-negative bacteria: *Escherichia coli* and the strains of Gram-positive bacteria *Staphylococcus aureus* as well as the strain of *Candida albicans* (Figure 1). The most efficient inhibiting activity of fresh garlic was shown in relation to the growth of coagulase-positive, pathogenic strain of *Staphylococcus aureus ATTC 33591* colony (growth inhibition zone = 35 mm). Inhibiting activity on the strain's growth was presented in Figure 2. *Pseudomonas aeruginosa* ATTC 27853 (growth inhibition zone – 12 mm) proved to be the least susceptible to fresh garlic. The four remaining strains turned out to be of medium susceptibility to this preparation (Figure 1). The results obtained are consistent with findings of Iwalokun *et al.* [2004], who also observed similar, significant differences in the inhibition of the growth of pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli.* Moreover, considerable inhibition of *Candida albicans* colony growth as well as *Escherichia coli* and *Staphylococcus aureus* bacteria was observed in the research conducted on fresh garlic by Arora & Kaur [1999], however, growth inhibition zones were smaller than the ones obtained in this work. Strong inhibiting activity of garlic water solutions on the growth of *Candida albicans* isolated from patients in Nigeria was earlier described in the work of Ghannoum [1988]. The differences in microorganisms' susceptibility seem to result both from the kinds of microorganism strains used and various methods of solution preparation or garlic varieties grown in different geographical zones. Cutler & Wilson [2004], while conducting the research on the susceptibility of *Staphylococcus aureus* on alliicin activity in liquid form and as cream, obtained different values of bacteria growth inhibition. At the 0.5 μg/mL of alliicin concentration, growth inhibition zone

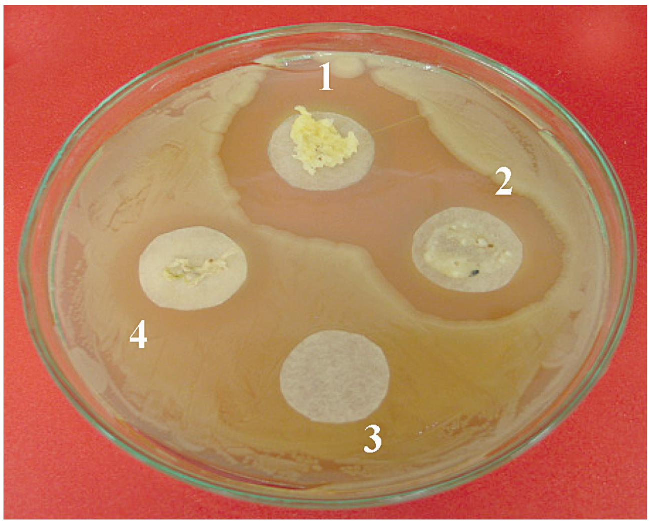

FIGURE 2. The image of *Staphylococcus aureus* ATTC 33591 growth inhibiting zones by examined garlic and its products on a Muller-Hinton medium (1 – fresh garlic, 2 – lyophilized garlic 3 – fried garlic, 4 – aging garlic extract).

TABLE 1. Minimal inhibitory concentration (MIC) influencing the growth of selected strains of bacteria and fungi by the examined garlic preparations.

Strain	Garlic form			
	fresh (mg/mL)	fried (mg/mL)	lyophilized (mg/mL)	AEG extract (mg/mL)
Escherichia coli ATTC 25992	3.12		25.0	
Escherichia coli ATTC 35218	12.5*		3.12	
Candida albicans A	1.56*	25		6.25
Candida albicans B	3.12	12.5	3.12	12.5
Staphylococcus aureus ATTC 33591	6.25		6.25	
Staphylococcus aureus ATTC 25923	3.12			
Pseudomonas aeruginosa ATTC 27853	25			

*$p < 0.05$

amounted to 23–28 mm, whilst at 1 μg/mL of concentration it totaled 31–44 mm. Below 0.062 μg/mL dilution, growth inhibition of *Staphylococcus aureus* was not observed. Although alliicin is the most active antibacterial garlic component, its instability limits its use as bactericidal means. Alliicin is only present in fresh crushed garlic and only such a form shows strong bactericidal properties.

Lyophilized garlic examined in this work only partially lost its bactericidal properties. It did not inhibit the growth of the examined strains of *Pseudomonas aeruginosa* i *Staphylococcus aureus* ATTC 25923, however, it showed a high inhibiting ability of *Escherichia coli, Staphylococcus aureus* ATTC 33591 and *Candida albicans.* Garlic after thermal treatment used frequently in food preparation (frying) as well as extract (AGE) lost its bactericidal properties completely, but it retained fungicidal properties in relation to *Candida albicans* (Figure 1).

Garlic oil research conducted by Benkeblia [2004] showed an inhibiting influence of the examined concentrations (50–500 mg/L) on *Staphylococcus aureus* ATCC 11522. Growth inhibition zone for the lowest and highest concentration amounted respectively to 6.3 mm and 9.3 mm.

The minimal inhibiting concentration (MIC) of the examined fresh and lyophilized garlic preparations determined for bacteria and fungi strains was presented in Table 1. Fresh garlic showed the strongest antibiotic properties. Even little concentration of fresh garlic water concentration inhibited the growth of *Candida albicans* A serotype (1.56 mg/mL), *Escherichia coli* ATTC 25992 and *Staphylococcus aureus* ATTC 25923 (3.12 mg/mL). Similarly, in the research conducted by Bakri & Douglas [2005], fresh garlic solutions in the concentrations of 35.7–1.1 mg/mL inhibited the growth of G(-) bacteria, and in the concentrations of 17.8–1.1 mg/mL inhibited the growth of yeast fungi isolated from patients' mouth. Also the research by Rees *et al.* [1993] proved that the water extract of lyophilized garlic *(Allium sativum)* inhibited the growth of many bacteria, fungi and virus strains. Minimal inhibiting concentration (MIC) for bacteria and fungi ranged from 0.8 to 40.0 mg/mL. Fried garlic and aging garlic extract preparations examined in this work showed weak inhibiting activity on the growth of yeast fungi strains, whilst they did not show the inhibiting influence on the bacterial growth. The smallest concentration inhibiting the growth of *Candida albicans* for garlic extract was 4 times higher in comparison with the lyophilized garlic (Table 1).

CONCLUSIONS

1. Fresh and lyophilized garlic showed efficient *in vitro* activity inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus* as well as *Candida albicans* pathogenic microorganisms, which enables their use in the prophylaxis and treatment of diseases induced by these microorganisms.
2. High antibacterial and antifungal activity of lyophilized garlic, similar to that of fresh garlic, points at the purposefulness of using this preparation in the food and gastronomical industry not only as a flavor additive, but as a natural additive with health-promoting properties.
3. Garlic after short-term thermal treatment (frying) and aging garlic extract, used frequently in diet supplements, in *in vitro* research, showed weak fungicidal and bactericidal activity.

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CHARAKTERYSTYKA WŁAŚCIWOŚCI BIOBÓJCZYCH CZOSNKU I JEGO PRZETWORÓW

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Celem badań było oszacowanie właściwości biobójczych (przeciwbakteryjnych i przeciwgrzybicznych) wybranych preparatów czosnku świeżego i przetworzonego w warunkach domowych (obróbka termiczna) i przemysłowych. Czosnek świeży charakteryzował się najsilniejszym działaniem inhibicyjnym w odniesieniu do wzrostu wszystkich badanych szczepów mikroorganizmów. Hamował wzrost kolonii patogennych szczepów koagulazo-dodatnich szczepów *Stapyloccocus aureus*, *Escherichia coli* oraz grzybów drożdżoidalnych *Candida albicans* serotypu A. Czosnek liofilizowany utracił częściowo swoje właściwości bakteriobójcze. Nie hamował wzrostu szczepów *Pseudomonas. aeruginosa* i *Staphylococcus aureus* ATTC 25923, posiadał jednak wysoką zdolność hamowania wzrostu szczepu *Staphylococcus aureus ATTC 33591*, szczepów *Escherichia coli* ATTC 35218 i grzybów drożdżoidalnych *Candida albicans* serotypu B. Czosnek poddany obróbce termicznej (smażenie), oraz ekstrakt czosnku (AGE) nie wykazywały właściwości bakteriobójczych, posiadały jednak działanie hamujące wzrost szczepów grzybów drożdzoidalnych *Candida albicans* serotypów A i B.