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ANTIMICROBIAL EFFECT OF RAW GARLIC (*Allium sativum L.*) EXTRACTS, GARLIC POWDER AND OIL AND COMMERCIAL ANTIBIOTICS ON PATHOGEN MICROORGANISMS

Garlic powder, essential oil and aqueous extracts from raw garlic and garlic powder prepared in accordance with the corresponding procedures were tested for antimicrobial activity on Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 25922, Bacillus subtilis 6633, Sarcina lutea ATCC 4391, Pseudomonas aeruginosa ATCC 9027, Salmonella enteritidis, Klebsiella pneumoniae, Proteus vulgaris, Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404; and, three pathogen microorganisms isolated from human material were also used: Staphylococcus aureus, Escherichia coli and Candida albicans. The antimicrobial activities of the preparations were compared with that of commercial antibiotics and four commercial preparation based on garlic powder and essential oil. Aqueous extracts from raw garlic, from freshly prepared powder and powder stored for 18 months at +4°C had the greatest antibacterial and antimycotic activities on all the microorganisms tested. The essential oil obtained by hydrodistillation and commercial preparations based on garlic essential oil showed no antimicrobial activity on the microorganisms tested. Thermal oils showed a bacteriostatic or very weak bacteriostatic effect on all the microorganisms except Pseudomonas aeruginosa. The garlic powder and commercial preparations based on the powder have a strong antimicrobial effect on all the microbes except on Pseudomonas aeruginosa.

Garlic (*Allium sativum* L.) components have a wide range of antimicrobial effects on bacteria, viruses, fungi and some enteric parasites. In 1858 L. Pasteur determined out that garlic had a bactericidal effect, and in 1944 it was ascertained that garlic juice and allicin in small concentrations inhibited the growth of *Staphylococcus, Streptococcus, Bacillus, Brucella* and *Vibrio* species [1,2]. They were found to inhibit the occurrence of gum diseases, caries, intestinal decay processes, dysentery, and colitis. The inhalation of volatile garlic components aids in the healing of upper respiratory organs. Garlic components have a bactericidal effect on germs causing tuberculosis, cholera, typhoid and paratyphoid fever [3–5]. Their most important clinical use is the treatment of infections and the prevention of cancer and cardiovascular diseases. Clinical trials of *Candida albicans* inhibition on animals and *in vitro* showed that garlic had greater inhibitory capacity than commercial antibiotics such as Nistatin [3, 6–11]. The virucidal effect of raw garlic, allicin and other garlic thio–components was demonstrated with respect to *Herpes simplex* type 1 and 2, type 3 *Parainfluenza virus, Vaccinia virus, Vesicular stomatitis virus* and *Human rhinovirus* type 2. Alliin, deoxyalliin, diallyl disulfide and diallyl trisulfide showed no antiviral effect [12]. The most pharmacologically active component, allicin, is not present in garlic cloves, it is produced by an enzymatic reaction from alliin with alliinase in aqueous solution [13].

Antimicrobial effects were tested on some pathogen microorganisms for four commercial antibiotics, four commercial preparations based on garlic powder and essential oil, aqueous extracts of raw garlic bulbs and bulb powder, and garlic essential and thermal oils obtained by original procedures.

EXPERIMENTAL

Materials

a) Garlic bulbs obtained at the local market;

b) Garlic powder [14];

c) Garlic essential oil [15];

d) Garlic thermal oils [16];

e) Raw bulbs and powder aqueous extracts [14];

f) Antibiogram tablets of commercial antibiotics: Chloramphenicol, Gentamicin, Tolicar, Erithromicin and Bactrim (Torlak Immunology and Virology Institute, Belgrade)

g) "Cirkulin" pearls (Zdravlje, Leskovac) commercial preparation based on garlic powder;

h) "Bio–slim®" pearls (Bozen Cosmetics, Belgrade) commercial preparation based on garlic oil macerate;

i) "Sanatogen®" capsules (ICN Galenika, Belgrade) commercial preparation based on garlic essential oil.

Microorganisms

Staphylococcus aureus ATCC 6538, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Sarcina lutea* ATCC 4391, *Pseudomonas aeruginosa* ATCC 9027,

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Salmonella enteritidis, Klebsiella pneumoniae, Candida albicans ATCC 10231 and *Aspergillus niger* ATCC 16404; as well as, three pathogen microorganisms isolated from human material were used: *Staphylococcus aureus, Escherichia coli* and *Candida albicans*.

Media

1) Mueller–Hinton agar (Torlak Immunology and Virology Institute, Belgrade) was used for growth of the pathogen bacteria.

2) Mueller–Hinton agar with 7% sheep blood (Torlak Immunology and Virology Institute, Belgrade) was used for growth of the pathogen fungi.

3) Bacto antibiotic medium 1 dehydrated (Difco Laboratories, Detroit, USA B–1) was used for growth of the non–pathogen bacteria.

4) Tripton soya–agar (Torlak Immunology and Virology Institute, Belgrade) was used for growth of the non–pathogen fungi.

Methods

Antimicrobial activity

The antimicrobial activity was tested by the disk method, i.e. the diffusion method. Sterile paper disks (12,7 mm in diameter), previously soaked in the corresponding samples (50 μl), were inserted in Petri dishes with microorganism cultures inoculated on corresponding media. The samples were incubated at 37°C (bacteria) and 25°C (fungi) for 24 hours.

Preparation of samples for microbiological analysis: The oily content from 'Bio–slim' capsules and 'Sanatogen' pearls was used directly without additional preparation. Essential and thermal oils were also used without additional preparation for the microbiological analysis. Supernatants were prepared from plant material, garlic powder and 'Cirkulin' pearls as follows:

– The plant material was homogenized with distilled water (1/10 m/V) and filtered after 10 minutes of intensive stirring at room temperature.

– The garlic powder was re–suspended in distilled water (1/10 m/V), stirred vigorously for 30 minutes at room temperature and filtered.

– 'Cirkulin' pearls were crushed and treated as garlic powder.

Spectrophotometry (VIS)

The total thiosulfinate content calculated as allicin was determined by using a Perkin–Elmer lambda 15 UV/VIS spectrophotometer. N–Ethyl–maleimide in alkaline medium was used as the colouring agent and the maximal absorbance was read at 515 nm [14, 17, 18].

RESULTS AND DISCUSSION

The results of the study of the bactericidal and fungicidal effect of the aqueous extracts from fresh bulb immediately after preparation and after aging for 4 days at room temperature, from freshly made powder and powder stored at +4°C for 18 months are presented in Table 1.

Aqueous extracts from fresh bulbs and from freshly prepared powder showed the greatest antimicrobial activity, while the powder aqueous extract stored at +4°C for 18 months showed a slightly weaker antimicrobial effect on all the microorganisms tested. The aqueous extract of fresh bulbs aged 4 days at room temperature almost did not contain any antimicrobial substance (negligible inhibition of microbe growth). This indicated the instability of thiosulfinates and their rapid decomposition in aqueous extract at room temperature.

Table 1. Results of the microbiological activity of the bulb and garlic powder aqueous extracts

Microorganism species	Sample							
	1		**2**		**3**		**4**	
	effect	inh. z.	effect	inh. z.	effect	inh. z.	effect	inh. z.
Candida albicans	+++	30.08	+++	30.01	+	17.80	–	–
Aspergillus niger	+++	27.05	+++	27.00	+	20.60	–	–
Escherichia coli	+++	30.20	+++	30.09	+	22.20	–	–
Staphylococcus aureus	+++	30.90	+++	30,85	+	23.50	–	–
Bacillus subtilis	+++	33.40	+++	33.40	+	20.95	–	–
Pseudomonas aeruginosa	+	14.95	+	14.90	+	13.00	–	–
Sarcina lutea	+++	25.50	+++	23.50	+	20.90	–	–
Klebsiella pneumoniae	+++	27.00	+++	24.00	+	21.90	–	–
Proteus vulgaris	+++	24.50	+++	23.85	+	23.01	–	–
Salmonella enteritidis	+++	24.00	+++	23.30	+	23.00	–	–

inh. z. inhibition zone; diameter, mm;
effect: +++ strong bactericidal effect; ++ bactericidal effect; + mild bactericidal effect; – no microbiological activity detected.
1. aqueous extract from fresh garlic immediately after preparation; **2**. aqueous extract from fresh powder immediately after preparation; **3**. aqueous extract from powder stored for 18 months at +4°C; **4**. aqueous extract from fresh bulbs aged 4 days at room temperature.

Table 2. Results of the essential oil and thermal oil antimicrobial effect

Microorganism species	Sample					
	1		2	3	4	5
	effect	inh. z.	effect	effect	effect	effect
Candida albicans	+	13.02	±	±	±	–
Aspergillus niger	+	12.90	±	±	±	–
Escherichia coli	+	12.95	±	±	±	–
Staphylococcus aureus	+	12.87	±	±	±	–
Bacillus subtilis	+	12.80	±	±	±	–
Pseudomonas aeruginosa	–	–	–	–	–	–
Sarcina lutea	+	13.00	±	±	±	–
Klebsiella pneumoniae	+	13.05	±	±	±	–
Proteus vulgaris	+	12.90	±	±	±	–
Salmonella enteritidis	+	12.90	±	±	±	–

inh. z. inhibition zone, diameter, mm
effect: + mild bactericidal effect; ± bactericidal effect; – no microbiological activity detected.
1. oil obtained during 1 hour processing period; **2**. oil obtained during 2 hour processing period; **3**. oil obtained during 5 hour processing period; **4**. oil obtained during 10 hour processing period; **5**. essential oil obtained by hydrodistillation.

Pseudomonas aeruginosa was the least susceptible to aqueous extracts having an antimicrobial effect, while all other bacteria and fungi were very susceptible to their effect.

The results of the study of the bactericidal and fungicidal effect of essential oil and oil obtained by thermal processing of the plant material are shown in Table 2. The essential oil obtained by hydro distillation showed no antimicrobial effect on any of the cultures tested. The oils obtained by thermal processing of the bulb showed only a bateriostatic effect on all the microbes tested, except the oil obtained after a 1 hour processing period, which had a weak bateriostatic effect.

The different antimicrobial effect of the garlic preparations was due to their different chemical structure depending on the method used for their production.

The results of the comparative study of the fungicidal and bactericidal effect of garlic powder stored at +4°C for 24 months and commercial preparations based on garlic ("Cirkulin" pearls by Zdravlje, Leskovac; "Sanatogen®" pearls by ICN Galenika, Belgrade, and "Bio–slim®" capsules by Bozen Cosmetics, Belgrade) are presented in Table 3.

"Cirkulin" pearls and garlic powder (aged 2 years at +4°C) showed similar microbiological activity with all the microbes tested. The least susceptible microbes were *Pseudomonas aeruginosa*, while the other microbes were very susceptible to "Cirkulin" pearls and garlic powder. "Bio–slim®" capsules showed weak microbiological activity, and "Sanatogen®" pearls based on essential oil, showed no microbiological activity on the microorganisms tested, in accordance with the investigation results for the essential oil.

The results obtained showed that para–pharmaceutical preparations based on garlic powder with a 24 month life time can be used for the treatment of various infections.

Table 3. Results of the comparative study of the antimicrobial activity of garlic powder and commercial preparations based on garlic

Microorganism species	Sample							
	1		2		3		4	
	effect	inh. z.	effect	inh. z.	effect	inh. z.	effect	inh. z.
Candida albicans	+	18.4	+	18.1	+	16.5	–	–
Aspergillus niger	+++	24.4	+++	22.6	+	17.3	–	–
Escherichia coli	+	20.0	+++	24.6	+	18.2	–	–
Staphylococcus aureus	+++	26.2	+++	25.4	+	17.1	–	–
Bacillus subtilis	+++	29.3	+++	24.3	+	17.8	–	–
Pseudomonas aeruginosa	+	12.9	+	12.8	+	12.8	–	–

inh. z. inhibition zone, diameter, mm
effect: +++ strong bactericidal effect; ++ bactericidal effect; + mild bactericidal effect; – no microbiological activity detected.
1. "Cirkulin" pearls by Zdravlje, Leskovac; **2**. garlic powder stored for 24 months at +4°C; **3**. "Bio–slim®" capsules by Bozen Cosmetics, Belgrade; **4**. "Sanatogen®" pearls by ICN Galenika, Belgrade

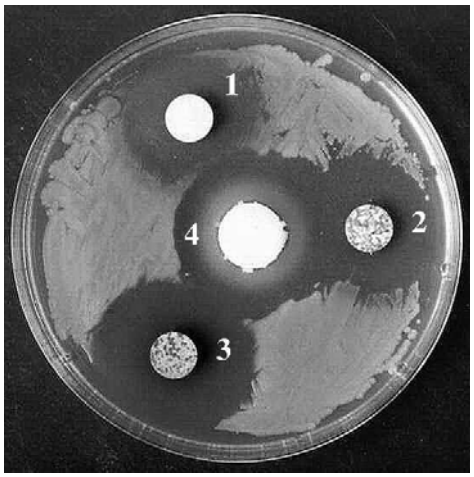

Figure 1. Microbiological study of commercial antibiotics and the aqueous extract of garlic powder on Escherichia coli isolated from human material originating from patients. 1) Chloramphenicol 30 μg, 2) Gentamycin 30 μg, 3) Tolicar 30 μg 4) aqueous extract of garlic powder (30 μg of allicin in 50 μl)

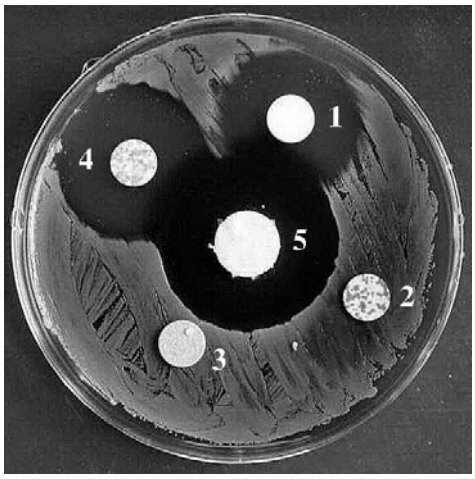

Figure 2. Microbiological study of commercial antibiotics and the aqueous extract of garlic powder on Staphylococcus aureus isolated from human material originating from patients. 1) Chloramphenicol 30 μg, 2) Tolicar 30 μg, 3) Erythromycin 15 μg, 4) Bactrim 25 μg, 5) aqueous extract of garlic powder (30 μg of allicin in 50 μl)

The data on the microbiological activity of garlic powder and the commercial antibiotics are presented in Figures 1 - 3.

Figure 1 shows the susceptibility of *Escherichia coli* to Chloramphenicol (tablet 1), Gentamicin (tablet 2), Tolikar (tablet 3) and the aqueous extract of garlic powder stored at +4°C for 18 months (disk 4). It can be seen that *Escherichia coli* is susceptible to all the antibiotics tested, Tolikar and the aqueous extract of garlic powder having the greatest inhibition zones (31 mm), while Chloramphenicol and Gentamycin have the same activity (inhibition zone 28 mm).

The effect of Chloramphenicol (tablet 1), Tolikar (tablet 2), Eritromicin (tablet 3), Bactrim (tablet 4) and

Figure 3. Microbiological study of commercial antibiotics and the aqueous extract of garlic powder on Candida albicans isolated from human material originating from patients, aqueous extract of garlic powder (30 μg of allicin in 50 μl)

Table 4. The content of allyl thiosulfinate (allicin) in garlic and garlic preparations

	Sample	Concentration (mg/g)
1	Aqueous extract of fresh garlic	1.9
2	Aqueous extract of fresh garlic powder	2.22
3	Aqueous extract of garlic powder kept for 18 months	1.8
4	"Cirkulin" pearls	1.1
5	"Bio–slim®" capsules	0
6	"Sanatogen®" pearls	0

the aqueous extract of garlic powder (disk 5) on the growth of pathogen *Staphylococcus aureus* is shown in Figure 2. The aqueous extract of garlic powder (36 mm) had the greatest inhibition zone, followed by Bactrim (31 mm) and Chloramphenicol (28 mm), while the microorganism was resistant to Tolicar and Erytromycin.

Among the pathogen fungi, *Candida albicans* was tested (Figure 3) and showed very high susceptibility to the aqueous extract of garlic powder with the growth inhibition zone up to 40 mm.

The content of total thiosulfinates taken as allicin in the aqueous extract of fresh garlic, powder, and in garlic oils, as well as in preparations based on garlic was determined spectrophotometrically [14,17] (Table 4).

The microbiological activity of the aqueous extract of garlic powder existing after a storage period of two years indicates that it can be applied both internally and externally for the treatment of various infections. "Cirkulin" pearls exhibit similar microbiological activity, while the thermal oils, essential oil, "Bio–slim®" pearls and "Sanatogen®" capsules do not.

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IZVOD

ANTIMIKROBNO DEJSTVO EKSTRAKATA SVEŽEG, PRAHA, ULJA BELOG LUKA (*Allium sativum L.*) I KOMERCIJALNIH ANTIBIOTIKA NA PATOGENE MIKROORGANIZME

(Naučni rad)

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Ispitana je antimikrobna aktivnost praha, etarskog ulja, termalnog ulja i vodenih ekstrakata sveže lukovice i praha belog luka, pripremljenih po odgovarajućim procedurama, na mikrorganizme *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Bacillus subtilis* 6633, *Sarcina lutea* ATCC 4391, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enteritidis, Klebsiella pneumoniae, Proteus vulgaris, Candida albicans* ATCC 10231 i *Aspergillus niger* ATCC 16404; korišćena su i tri patogena mikroorganizma izolovana iz ljudskog materijala: *Staphylococcus aureus, Escherichia coli* i *Candida albicans*. Upoređena je antimikrobna aktivnost naših preparata sa aktivnošću komercijalnih antibiotika i četiri komercijalna preparata na bazi praha i etarskog ulja belog luka. Vodeni ekstrakti iz sveže lukovice, iz sveže pripremljenog praha i praha posle 18 meseci čuvanja na + 4°C imaju najveću antimikrobnu i antimikotičnu aktivnost na sve ispitivane mikrobe. Etarsko ulje dobijeno hidrodestilacijom i komercijalni preparati na bazi etarskog ulja belog luka, ne pokazuju antimikrobnu aktivnost na ispitivane mikrobe. Termalna ulja pokazuju bakteriostatičko ili slabo bakteriostatičko dejstvo na sve mikrobe osim na *Pseudomonas aeruginosa*. Prah belog luka i komercijalni preparati na bazi praha imaju visoku antimikrobnu aktivnost na sve mikobe osim na *Pseudomonas aeruginosa* na koju slabo deluju.

Ključne reči: Beli luk • Antimikrobno dejstvo • Antimikotično dejstvo • Ekstrakt • Etarsko ulje • Termalno ulje • Antibiotik • Mikroorganizam • Hidrodestilacija • Tiosulfinati •
Key words: Garlic • Antimicrobial activity • Antimycotic activity • Extract • Essential oil • Thermal oil • Antibiotics • Microorganism • Hydrodistillation • Thiosulfinates •