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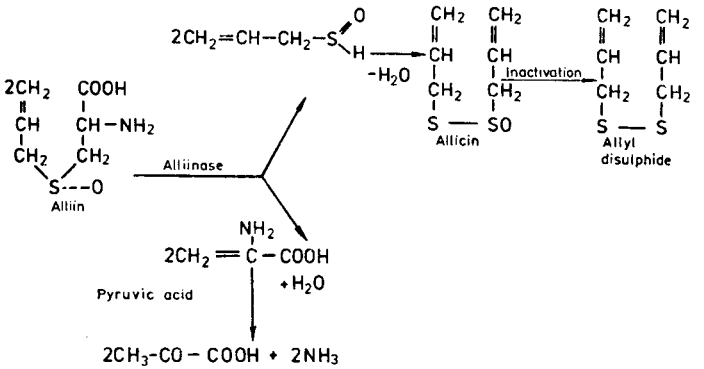
Antimicrobial Action of Garlic (*Allium sativum* L.) and Garlic Preparations Produced in Poland

Przeciwdrobnoustrojowe działanie czosnku (*Allium sativum* L.) i preparatów czosnkowych produkowanych w Polsce

Противмикроорганизмовое действие чеснока (*Allium sativum* L.) и чесночных препаратов вырабатываемых в Польше

The ethereal substances with antimicrobial action produced by higher plants were named phytoncides by Tokin (14). Many of them reveal a broad spectrum of antibiotic activity: they are fungicidal and bactericidal against Gram-positive as well as Gram-negative bacteria and also protozoocidal. At low concentrations phytoncides inhibit the growth of various pathogenic yeasts and moulds. They play a significant role in the antibiotic defence mechanism of higher plants.

The curative action of garlic (*Allium sativum* L.) has been known for a long time in folk medicine, but it was not until 1945 that Cavallito and Bailey (1) described the active substance which they isolated from garlic bulbs and named



Schema 1. Enzymatic conversion of alliin into allicin (4) and its inactivation in the form of diallyldisulphide

it allicin. Allicin proved to be an allyl ester of thiosulphinic acid (2, 9) and it is formed from a biologically inactive precursor of S-allylcysteine sulphoxide named alliin under the influence of alliinase, the enzyme found in garlic tissues (1, 3, 11, 12). This antibiotic is an unstable compound and easily turns into a biologically inactive component, diallyldisulphide (4, 6, 11, 12). The scheme of enzymatic conversion alliin into allicin and the inactivation of the antibiotic in the form of diallyldisulphide is shown in schema 1.

Several authors (13, 17) stated that allicin is an inhibitor of -SH group of respiratory enzymes. Among them xantine oxidase, succinic dehydrogenase and triose phosphate dehydrogenase are most strongly inhibited. The -SO-S-grouping is essential for bactericidal action of allicin (9), and this grouping is also essential for the inhibition of -SH-enzymes, while -S-S-, and -SO-groupings are not effective (17).

It has been reported by several investigators (5, 6, 10, 15, 16) that garlic juice is a very potent antimicrobial agent both against bacteria and *Candida albicans*, and against other pathogenic yeasts. As stated by Tynecka and Skwarek (16), garlic juice did not exhibit any toxic effect against the tissue cultures of chick embryo fibroblasts or monkey kidney cells. At the same concentration it inhibited the growth of the infecting yeasts of *C. albicans*.

Considering the strong antimicrobial action and the broad spectrum of antibiotic activity of allicin, several attempts were made in Poland and abroad to make a medicine out of garlic. At present in Poland, Poznańskie Zakłady Zielarskie Herbapol produce two preparations from this raw material: Alliostabil as an ethanolic extract from fresh garlic bulbs containing about 0.05% of ethereal sulphur compounds, and Alliophil-lyophilizate of garlic prepared in pills.

The authors of this paper estimate critically the biological action of these preparations as compared with the antimicrobial action of fresh garlic bulbs.

MATERIALS AND METHODS

1. Garlic bulbs (*Allium sativum* L.) from 1976 crops.
2. Alliophil (Contents: *Allium sativum lyoph.* 0.20, *Massa tab. et drag.* ad 0.6 g), series 1711175, expiry date 15.II.1978.
3. Alliostabil (ethanolic garlic juice), series 031275, without expiry date.
4. Federative Republic of Germany preparation "Sanhelios 333" containing extracts from *Allium sativum, Viscum album, Crataegus* sp.

Preparation of pulp and active garlic juice. After removing the outer parts, the garlic bulbs were frozen in solid CO_2, then crushed thoroughly and homogenized at 3000 rpm for 5 min. The garlic juice accurately pressed through a sterile gauze was sterilized through a bacteriological funnel Schot G-4, and then the filtrate was preincubated at 37°C for 30 minutes in order to convert alliin into allicin, the antibiotically active substance. Several microbiological methods were used in order to compare the antimicrobial action of garlic, Alliophil and Alliosatbil. Alliophil (*A. sativum lyoph.*) pills without coats were ground into powder and extracted with 96% ethanol or water (100 mg of the preparation per 1 ml) for 2 hrs.

Paper disc agar diffusion method. In order to determine the antimicrobial spectrum of juice and pulp from garlic and preparations, the paper disc agar plate assay was used. Each Petri plate contained 10 ml of optimal agar

medium and 0.025 ml of microbial culture from the exponential growth phase. On each disc (13 mm diameter) 100 mg of pulp was put or 0.05 ml (one drop) of garlic juice, which diffused into agar medium and produced a growth inhibition zone. Approximately 0.05 ml of Alliostabil or ethanolic extract of Alliophil were poured on separate paper discs.

Twofold agar (and broth) dilution methods. The garlic juice or solutions of garlic preparations were diluted in 30% ethanol or water and poured in amounts 1 ml into 9 ml optimal agar or liquid medium. In this way a number of plates were prepared with a decreasing concentration of the antibiotic in the assay medium. The plates were inoculated with 0.5×10^6 CFU of the test organism per 1 ml of the medium and incubated at 35°C. The drop-count test and cup-plate method were also used for these comparisons.

Maximal Inhibitory Dilution (MID) was defined as the amount of antibiotic contained in the greatest dilution, which completely inhibited the growth of the test organism at 35°C for 48 hrs incubation. Maximal Biocidal Dilution (MBD) was defined as a maximal dilution of the antibiotic which completely inhibited the growth of the test organisms for 15 days incubation. After this time the viable cell units of the test organisms usually were not ascertained, as demonstrated by plate count test.

RESULTS AND DISCUSSION

The antimicrobial action of garlic determined by the paper disc agar plate assay was strong and included a wide range of the tested microorganisms, Gram-positive and Gram-negative bacteria, acid-fast bacteria as well as yeasts and moulds. Under the same conditions, the action of Allostabil was light and became visible only in the case of organisms especially sensitive to allicin. The action of Alliophil was similar and in the trace amounts it inhibited *Corynebacterium equi*, *Schizosaccharomyces octosporus* and *Aspergillus niger*, as shown in Table 1.

The MID values of garlic juice and garlic preparations assayed by the twofold agar dilution method for 4 test organisms (*Bacillus subtilis* ATCC 6633, *Sarcina lutea* R-262, *Proteus vulgaris* OX_{19} and *Candida arborea*) are shown in Table 2. In comparison with Alliophil and Alliostabil also in this method garlic juice had a stronger effect against all four organisms tested. From the above data, it is obvious that Alliophil action was two times or even weaker than the Alliostabil action, and MID values determined for garlic juice were in some cases more than eight times higher than those of the Alliostabil action. We have not included in this comparison the antimicrobial action of pulp which contains (as Table 1 indicates) a considerable amount of allicin activity.

The garlic juice had a strong antibiotic action against *S. lutea*, *Pseudomonas aeruginosa* and *Escherichia coli* by the drop-count test in the semi-liquid (0.4%) agar medium and by a cup-plate agar diffusion assay while under the same conditions no trace of the preparations' action was seen.

Table 1. Comparison of antimicrobial action of garlic juice and pulp, and garlic preparations by paper disc agar plate assay procedure

Microorganisms	Medium	Garlic juice	Garlic pulp	Alliophil	Alliostabil
		Diameter inhibition zone in mm			
Bacillus subtilis ATCC 6633	Nutrient agar	43	66	—	Trace
Bacillus cereus ATCC 8145		47	69	—	17
Bacillus lentus IP 5286		40	63	—	Trace
Bacillus brevis IP 5275		42	54	—	Trace
Escherichia coli ROW		32	45	—	—
Proteus vulgaris OX_{19}		30	39	—	—
Pseudomonas aeruginosa H_3		20	26	—	—
Salmonella pullorum		32	43	—	—
Sarcina lutea		36	51	—	—
Micrococcus lysodeikticus		36	52	—	—
Staphylococcus aureus 209-P		43	61	—	—
Staphylococcus albus		48	67	—	—
Coyrnebacterium xerosis	Loeffler agar	60	80	—	—
Corynebacterium equi		52	74	20	20
Mycobacterium sp. ATCC 607	Sauton agar	46	73	—	19
Mycobacterium smegmatis		42	69	—	Trace
Mycobacterium butyricum		40	68	—	Trace
Mycobacterium sp. ATCC 279		40	63	—	17
Saccharomyces cerevisiae	Malt-agar	41	56	—	—
Saccharomyces carlsbergensis		39	48	—	—
Candida arborea		51	82	—	Trace
Rhodotorula rubra		38	57	—	19
Schizosaccharomyces octosporus		53	100	18	20
Aspergillus niger		59	100	20	25

Table 2. Comparative MID's of garlic juice, Alliophil (ethanolic extraxt) and Alliostabil assayed by the twofold agar dilution method

Organism tested	MID, per cent basic solution		
	Garlic juice	Alliophil	Alliostabil
Bacillus subtilis ATCC 6633	0.306	5.0	2.5
Sarcina lutea R-262	0.306—0.612	7.5	2.5
Proteus vulgaris OX_{19}	0.612	7.5	3.75
Candida arborea	0.153	1.25—2.5	1.25

Inoculum size; approx. 0.5×10^6 CFU/ml. The MID values for *C. arborea* determined in malt-agar (7° Blg), other organisms tested in nutrient agar 1.4% at 35° for 24 hours incubation.

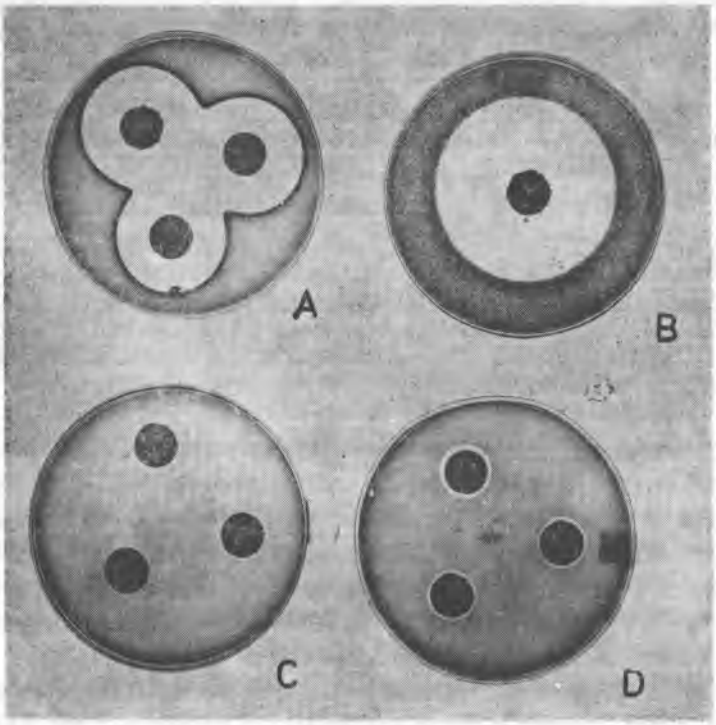


Fig. 1. *Rhodotorula rubra growth* inhibition zones by the paper disc agar diffusion assay procedure; A — garlic juice, B — garlic pulp, C — Alliophil, D — Alliostabil

The results of the assays of the antimicrobial action of garlic juice and garlic preparation using the paper disc agar plate method and the twofold agar dilution method suggested that the diffusive methods are not entirely useful for measuring the antibiotic action of allicin because it seems that this antibiotic after isolation from garlic tissues diffuses weakly in agar media. In the case of the paper disc assay only microorganisms very susceptible to the allicin action were inhibited, mainly yeasts and moulds for which the values of MIC are the lowest, and the amount of antibiotic which diffused from the paper disc to agar medium could reach this level (Fig. 1).

Due to that, the activity of garlic and garlic preparations was assayed by the twofold broth dilution technique against *B. subtilis* ATCC 6633 as a test organism used for the quantitative estimation of alliinase (8), the enzyme responsible for the conversion of the parent form into allicin, a form biologically active. The action of preparations estimated by this method was much higher than those estimated by the diffusive methods and close to action of garlic (strong pulp action was not compared), but transient and bacteriostatic, bactericidal only in higher concentrations, as

Table 3. Biostatic and biocidal action of garlic juice and garlic preparations determined by the twofold broth dilution assay procedure for *Bacillus subtilis* ATCC 6633 as a test organism

Preparation	Incubation period (days)	2	10	15	Ratio of MBD/MID
		MID, per cent basic solution			
Garlic juice		0.153	0.306	0.306	2
Alliostabil		0.306	0.612	1.25	4
Alliophil					
ethanolic solution		1.25	3.75	7.50	6
aqueous solution		10	10	>10	—
Sanhelios 333		10	>10	>10	—

Assay medium and *pH* value; nutrient broth, *pH* 6.5; — not determined. The MBO values (maximal biocidal dilution); lowest allicin concentration in the garlic solution resulting in no visible growth after 15 days incubation.

shown in Table 3. For the garlic juice the ratio of MBD/MID was 2, and for Alliostabil and Alliophil were 4 and 6, respectively, Generally, the antimicrobial action of garlic by the twofold broth dilution method was two times as strong as that of Alliostabil, and eight times as strong as that of ethanolic solutions of Alliophil. However, the antibiotic activity of Sanhelios 333 was very weak, only at 10% concentration the growth of *B. subtilis* was completely inhibited for 3 days.

Allicin is an oil with a characteristic garlic scent, soluble in benzene, ethanol and ether; its solubility in water at 10° comes to 2.5%. In aqueous solutions or in dry base allicin becomes inactive at room temperature after two days, forming a mucilaginous liquid (7). From the above findings and comparisons, it is reasonable to conclude that the comparatively weak action of Alliophil and Alliostabil was connected with the insignificant diffusion of this antibiotic to solid agar media (when they bioactivity were assayed by diffusive methods) as with the inactivation of allicin after isolating this antibiotic from garlic tissues. Similarly, K a b e l i k (6) has been reported that aqueous, ethanolic and acidic extracts from garlic bulbs possessed a weak antimicrobial activity, however, lyophilizate of garlic contains a considerable amount of allicin activity (5). In fresh garlic juice there is both a precursor of this antibiotic and an active enzyme alliinase capable of transforming alliin into allicin and this explains the strong bactericidal action of garlic bulbs.

The authors of this communication do not deny the antimicrobial action of Alliophil and Alliostabil, but they call in question the use of these as remedy. They think garlic bulbs are more powerful. All our experiments were performed *in vitro*.

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STRESZCZENIE

Przy użyciu szeregu metod oznaczania aktywności antybiotyków porównano przeciwdrobnoustrojowe działanie czosnku (soku i miazgi) z aktywnością preparatów czosnkowych produkowanych w Polsce. Działanie Alliofilu (przygotowanego wg podanej w Metodach procedury) i Alliostabilu było przejściowe, biostatyczne, a przede wszystkim słabsze od działania soku czosnkowego, szczególnie przy zastosowaniu dyfuzyjnych metod testowania. Stosunek dawki bójczej do biostatycznej MBC/MIC wyznaczony dla soku z czosnku, Alliostabilu i Alliofilu, wynoszący odpowiednio 2, 4 i 6 wskazuje, że allicyna po wyizolowaniu z tkanek czosnku i odwodnieniu ulega w dużym stopniu inaktywacji oraz słabo dyfunduje do stałych podłoży agarowych. Natomiast sok czosnkowy zawierający alliinę — prekursor allicyny oraz allinazę, enzym odpowiedzialny za enzymatyczną konwersję prekursora do formy aktywnej biologicznie, okazał się silnym, o szerokim spektrum działania, środkiem przeciwdrobnoustrojowym.

РЕЗЮМЕ

При использовании (употреблении) ряда методов определения активности антибиотиков сравнено противмикроорганизмовое действие чеснока (сока и мезги) с активностью чесночных препаратов, вырабатываемых в Польше. Действие

Аллиофила и Аллиостабила было кратковременное, биостатичное, а прежде всего значительно слабее, чем действие чесночного сока, особенно при применении диффузийных методов определения. Отношение убийственной дозы к биостатичной MBC/MIC, назначенное для чесночного сока, Аллиостабила и Аллиофила, составляющее (равняющееся) соответственно 2, 4 и 6 указывает, что аллицина после изолирования из ткани чеснока и водоотлива подвергается в большой степени инактивации, как также слабо дифундует к постоянным агаровым материнским породам. Чесночный сок же, содержащий аллину — прекурсор аллицины, а также аллиназу, фермент ответственный за энзиматичную конверсию прекурсора до биологически активной формы, оказался сильным, в широком спектре действия противмикроорганизмовым средством.