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The Fungistatic Activity of Garlic (*Allium sativum* L.) *in vitro*

Fungistatyczne działanie czosnku (*Allium sativum* L.) *in vitro*

Грибостатическое действие чеснока (*Allium sativum* L.) в опытах *in vitro*

In recent years an increase in mycosis in man has been observed. This can be ascribed to the wide application of antibacterial antibiotics (13, 16), steroid hormones (10, 11), immunosuppressive agents (5), cytostatics and radiation (2), which by means of various mechanisms favour the occurrence of fungal diseases in man. The situation is complicated by a small number of antifungal antibiotics available, the majority of which are not absorbed from the intestinal tract (nystatin, candidin, candicidin, trichomycin, pimaricin); others have a narrow antifungal spectrum (griseofulvin, variotin), or are very toxic, like amphotericin B or filipin (4, 6, 9, 14, 15). Taking this into consideration, the search for a new antifungal agent seems to be justified.

Recently some reports appeared in literature concerning the inhibitory activity of garlic against bacteria and *Candida albicans* (7, 8, 19, 20). The aim of this paper was to study the effect of garlic against other species of pathogenic fungi. The results were compared with the sensitivity of strains to nystatin, griseofulvin and amphotericin B.

MATERIALS AND METHODS

Strains.

17 strains of pathogenic fungi were examined. *Epidermophyton floccosum* and *Trichophyton mentagrophytes* were isolated from the patients at the Dermatologic Clinic of Medical Academy in Lublin. *Microsporum gypseum* and and *Scopulariopsis brevicaulis* were obtained from the Department of General Microbiology UMCS in Lublin. The remaining strains were obtained from the Infective and Invasive Clinic of the Veterinary Faculty of Agricultural Academy in Lublin. All strains were grown on a solid and liquid Sabouraud media.

Preparation of garlic juice.

Garlic was frozen by means of solid CO_2, which was crushed and the juice pressed out, filtered through a gauze and then incubated for 1 hr. at 37° to convert alliin into allicin, the antibiotically active substance. Then the juice was kept in the frozen state at —10°. Under these conditions it did not loose the antimicrobial activity, when tested against *Staphylococcus aureus* Oxford and *Candida albicans* after one year of storage. The content of allicin in the juice was estimated according to the method described in another paper (19).

Estimation of the sensitivity of fungi to antibiotics and garlic juice.

Two methods were employed for testing the sensitivity of fungi to antibiotics and garlic juice: growth on plates and in broth in the presence of decreasing concentrations of the inhibitors. 0.1 ml of twofold decreasing concentrations of antibiotics was put into a well (9 mm diameter) cut out in the middle of agar plates. Similarly 0.1 ml of the juice — undiluted or diluted 1:5, 1 : 10 and 1 : 20 with distilled water, was applied to the plates. As an inoculum, homogenized broth cultures of fungi were used — O.D. 0.3 in case of *Candida albicans* and 0.6 of the other strain suspensions. The homogenized cultures were then diluted 1 : 100 or 1 : 50 with saline. 2 ml of the diluted suspensions were spread over agar plates, the excess removed and the plates were dried, then the wells were cut out. The cultures were incubated for 1—9 days, depending on the strain, in wet chamber, at 25—30°.

The sensitivity of strains in tubes was estimated by growing them in twofold decreasing concentrations of antibiotics in broth and in twofold increasing dilutions of the juice (1 : 2 to 1 : 10,240). The inoculum was the same as above, but only diluted 1 : 10 or 1 : 5. To each tube 0.02 ml of fungal suspension was added. Incubation was continued for 1—5 days, depending on the strain, at 25—30°.

Concentrations of antibiotics.

Nystatin — (pro suspensione, Polfa, Kraków) was suspended in propylene glicol according to Wawrzkiewicz (21) and added: a) into a well on plates: 1,600 μ — 3,12 μ, b) into broth: 160 μ/ml — 1.25 μ/ml.

Amphotericin B — (Fungizone, E. R. Squibbs, Great Brittain, containing 50,000 u in phosphate buffer with sodium deoxycholate) was dissolved in distilled water and added: a) into a well on plates: 800 μ — 3.12 μ, b) into broth: 10 μ/ml — 0.018 μ/ml.

RESULTS

The content of allicin in the juice was the same as reported by us earlier (19). Table 1 presents the sensitivity of fungi to nystatin and griseofulvin estimated by the plate method. The application of propylene glicol in which nystatin dissolves well, eliminated the possibility of its poor diffusion into agar. The most sensitive to nystatin were yeastlike organisms, less sensitive were *Aspergillus, Penicillium* and *Dermatophytes*. The diameter of the inhibition zones were below 46 mm, even in the presence of the highest concentration 1,600 μ. Griseofulvin was dissolved in DMSO to obtain its diffusion into agar. In accordance with the reports in literature (6), only dermatophytes were sensitive to griseofulvin. The inhibition zones with 400 μg were large (46 to 80 mm), though dermatophytes were also fairly sensitive even to smaller concentrations of the antibiotic.

Table 2 shows the sensitivity of fungi to amphotericin B and to garlic juice. Compared with nystatin, amphotericin B had a weaker mycostatic activity against all fungi tested. It is interesting to note that the largest inhibitioin zones were obtained with 0.1 ml of the undiluted garlic juice — 70 to 90 mm. Even the diluted garlic juice 1 : 20 caused a more intense inhibition of growth of fungi than nystatin, griseofulvin and amphotericin B, when used in large

Table 1. The sensitivity of fungi to nystatin and griseofulvin, estimated by the plate method

Strains	Nystatin (μ)										Griseofulvin (μ)									
	3.12	6.25	12.5	25	50	100	200	400	800	1600	0.78	1.56	3.12	6.25	12.5	25	50	100	200	400
	Inhibition zone (mm)																			
Aspergillus flavus	—	—	20	21	22	26	27	28	29	32	—	—	—	—	—	—	—	—	—	—
Aspergillus fumigatus	22	25	30	31	33	34	38	38	38	39	—	—	—	—	—	—	—	—	—	—
Aspergillus niger	18	22	26	27	30	34	35	37	38	38	—	—	—	—	—	—	—	—	—	—
Candida albicans	23	25	28	30	32	36	36	36	36	36	—	—	—	—	—	—	—	—	—	—
Candida tropicalis	29	30	30	36	36	36	36	37	38	39	—	—	—	—	—	—	—	—	—	—
Cryptococcus neoformans	24	26	28	32	33	36	40	40	40	41	—	—	—	—	—	—	—	—	—	—
Penicillium sp.	—	—	16	17	18	20	25	27	28	29	—	—	—	—	—	—	—	—	—	—
Penicillium sp.	—	—	—	—	—	—	24	27	28	32	—	—	—	—	—	—	—	—	—	—
Geotrichum candidum	23	25	28	30	32	34	35	36	38	40	—	—	—	—	—	—	—	—	—	—
Scopulariopsis brevicaulis	18	20	21	22	30	35	39	40	40	40	—	—	—	—	—	—	—	—	—	—
Epidermophyton floccosum	24	24	28	32	36	40	44	44	45	46	26	38	42	48	54	60	64	66	68	70
Microsporum gypseum	17	17	18	20	21	25	27	28	29	30	—	—	26	34	47	54	55	57	59	59
Trichophyton mentagrophytes	—	—	16	17	18	20	25	27	27	29	—	—	21	25	37	42	44	45	46	46
Trichophyton mentagrophytes var. interdigitalis	15	16	17	18	19	22	23	23	25	25	—	—	20	28	33	40	48	56	58	60
Trichophyton rubrum	—	16	18	20	22	26	30	35	36	36	—	30	38	45	58	60	64	68	70	74
Trichophyton schoenleini	15	17	17	19	22	25	30	31	31	31	—	23	38	45	53	57	63	70	71	74
Trichophyton verrucosum	—	—	—	16	19	19	20	21	21	22	35	41	50	58	63	65	74	76	78	80

Table 2. The sensitivity of fungi to amphotericin B and garlic juice, estimated by plate the method

Strains	Amphotericin B (μ)									0.1 ml of garlic juice			
										diluted			
	3.12	6.24	12.5	25	50	100	200	400	800	1 : 20	1 : 10	1 : 5	undiluted
	inhibition zone (mm)												
Aspergillus flavus	—	—	—	—	—	—	—	16	20	—	25	30	60
Aspergillus fumigatus	23	25	26	26	26	26	26	27	27	47	53	57	78
Aspergillus niger	20	22	22	22	22	22	23	24	25	37	47	57	79
Candida albicans	26	26	26	26	27	27	27	28	28	38	45	56	70
Candida tropicalis	27	28	28	29	29	30	30	30	30	31	36	46	72
Cryptococcus neoformans	20	22	23	24	25	25	26	27	28	45	50	60	80
Geotrichum candidum	20	21	22	22	22	22	23	24	25	37	47	57	70
Scopulariopsis brevicaulis	—	17	17	17	17	17	17	17	17	51	72	80	90
Penicillium sp.	16	16	17	17	17	17	18	18	18	17	20	25	30
Penicillium sp.	24	26	26	27	27	27	27	27	27	40	58	90	90
Epidermophyton floccosum	26	26	27	27	28	28	28	29	30	50	64	74	90
Microsporum gypseum	16	20	20	20	20	21	21	21	21	40	50	65	90
Trichophyton mentagrophytes	—	—	—	—	—	—	17	25	27	50	58	70	90
Trichophyton mentagrophytes var. interdigitalis	13	15	15	15	15	16	17	17	17	43	51	62	90
Trichophyton rubrum	—	14	16	16	17	18	19	19	19	45	50	65	90
Trichophyton schoenleini	14	16	17	18	19	19	20	21	21	44	54	68	90
Trichophyton verrucosum	—	—	—	—	—	—	—	—	—	40	45	62	78

concentrations. All strains, but one representative of the *Penicillium genus*, were higly sensitive to the juice, which is an indication of its broad antifungal spectrum of activity. The most sensitive were dermatophytes, inhibited also strongly by griseofulvin, but the inhibition zones obtained with 400 μg of this antibiotic were smaller than those obtained with 0.1 ml of undiluted juice.

The mycostatic properties of the juice are also illustrated on Fig. 1, 2, 3 and 4.

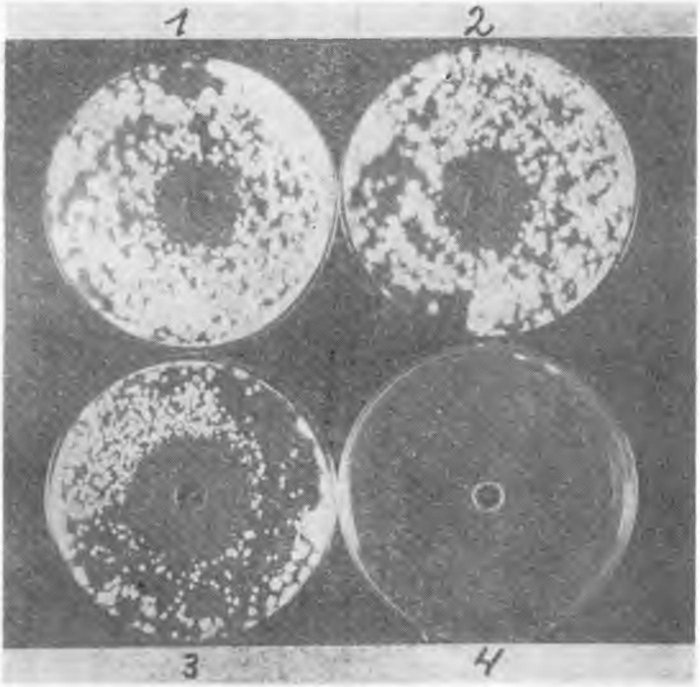

Fig. 1. The effect of garlic juice and antibiotics on growth of *Trichophyton mentagrophytes*; 1 — Amphotericin B (800 μ), 2 — Nystatin (1600 μg), 3 — Griseofulvin (400 μg), 4 — Garlice juice (0.1 ml)

Table 3. The sensitivity of some fungi to antibiotics and garlic juice, estimated by the tube method

Strains	Minimal inhibitory concentration of antibiotics			Maximal inhibitory dilutation of garlic juice
	Griseofulvin μg/ml	Nystatin μ/ml	Amphotericin B μ/ml	
Candida albicans	n.t.	5.0	0.07	1:1280
Cryptococcus neoformans	n.t.	5.0	0.15	1:2560
Geotrichum candidum	n.t.	5.0	1.20	1:1280
Aspergillus fumigatus	n.t.	10.0	0.30	1:1280
Epidermophyton floccosum	0.8	5.0	0.30	1:2560
Microsporum gypseum	0.8	40.0	0.15	1:5120
Scopulariopsis brevicaulis	n.t.	80.0	2.50	1:2560
Trichophyton mentagrophytes	3.12	80.0	0.6	1:2560

n.t. — not tested.

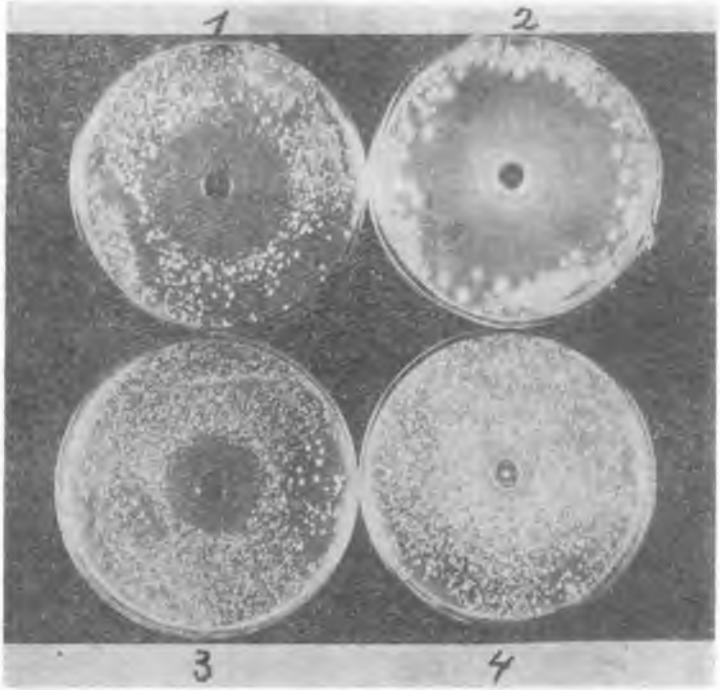

Fig. 2. The effect of garlic juice and antibiotics on growth of *Aspergillus niger;* 1 — Griseofulvin (400 μg), 2 — Amphotericin B (800 μ), 3 — Nystatin (1600 μ), 4 — Garlic juice (0.1 ml)

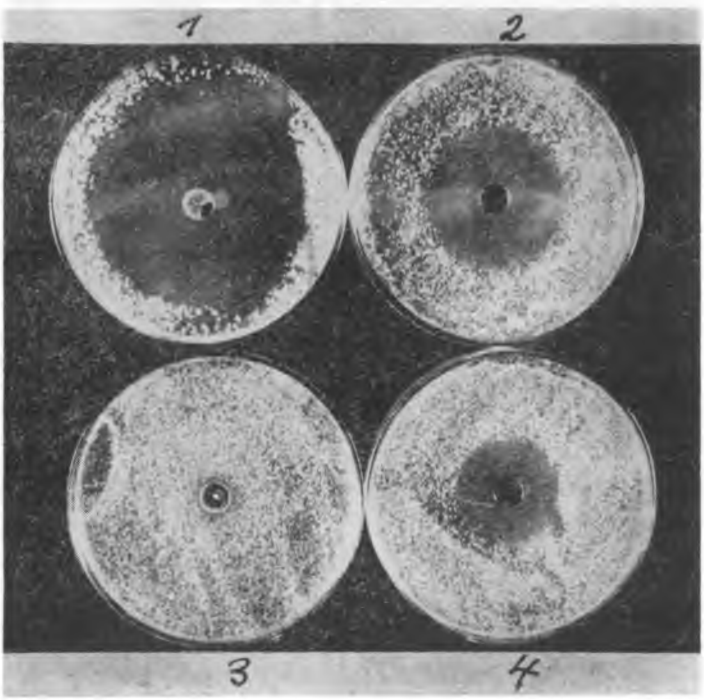

Fig. 3. The effect of garlic juice and antibiotics on growth of *Candida albicans;* 1 — Garlic juice (0.1 ml), 2 — Nystatin (1600 μ), 3 — Griseofulvin (400 μg), 4 — Amphotericin B (800 μ)

Table 3 shows the sensitivity of some strains to antibiotics and garlic juice estimated by the twofold dilutions in broth. These results confirmed the high sensitivity of fungi to the juice.

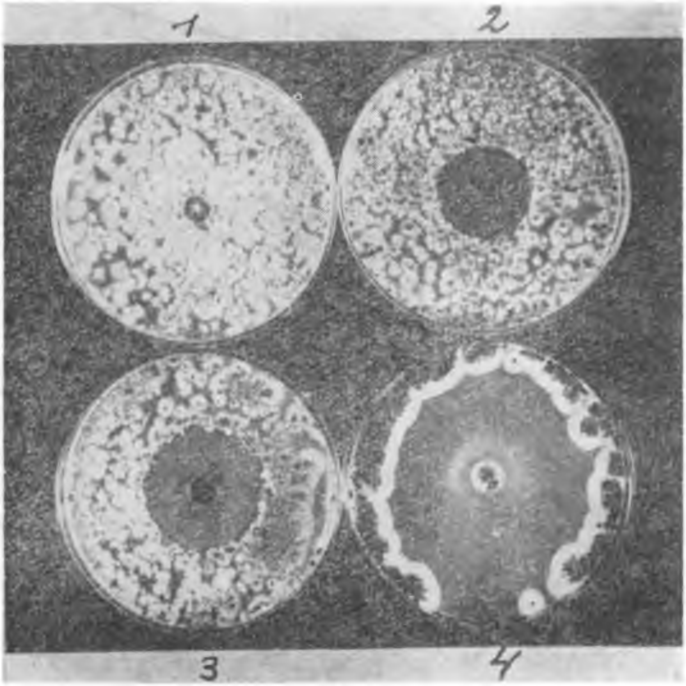

Fig. 4. The effect of garlic juice and antibiotics on growth of *Geotrichum candidum*; 1 — Nystatin (1600 μ), 2 — Garlic juice (0.1 ml), 3 — Amphotericin B (800 μ), 4 — Griseofulvin (400 μg)

DISCUSSION

Garlic, a known and cultivated vegetable since centuries, has attracted much attention recently, because of its multiple beneficial influence on macroorganism and also because of its marked antimicrobial activity. The active principle of garlic is diallyl disulphide, the main compound of the volatile oil, called allicin (3). It is present in intact bulbs of garlic in the inactive form as alliin, which on crushing undergoes a spontaneous conversion to allicin by an enzyme alliinase (17) present in the juice (Fig. 5).

According to literature, garlic inhibits the growth of several bacterial species and of *Candida albicans* (7, 8, 18, 19, 20). The mechanism of the antimicrobial action of garlic is based on the inhibition of the activity of microbial sulphydryl enzymes, mainly the respiratory ones, by allicin (18, 19, 22). Most probably the labile atom of oxygen in allicin molecule oxidises SH groups, converting them into SS, which leads to the inactivation of enzymes. (22).

The results of this of this paper show that garlic possesses a broad antifungal activity both on agar plates and in broth. Neither nystatin, nor amphotericin B and griseofulvin displayed such a high activity as garlic juice on agar plates. Also large dilutions of the juice in broth strongly inhibited growth of the fungi.

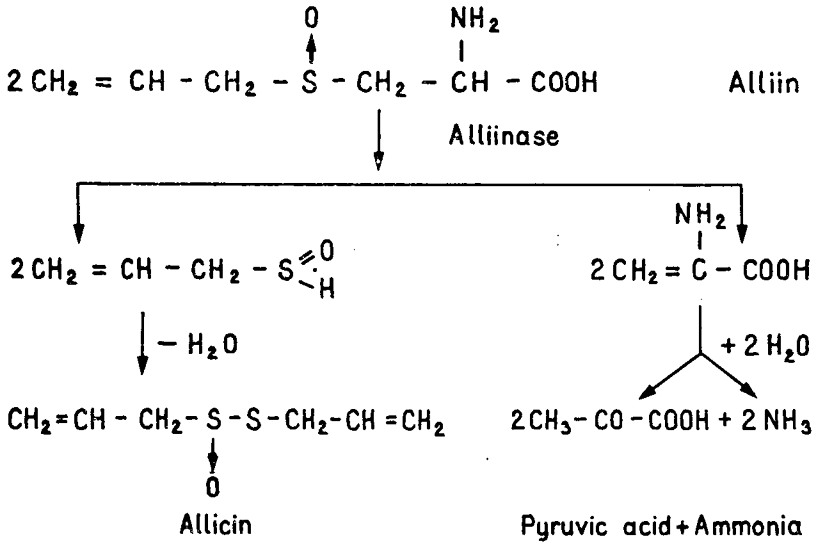

Fig. 5. Enzymatic conversion of allicin into allicin (17)

In Fig 2 and 4 one can see an abundant growth around the inhibition zones caused by 0.1 ml of undiluted garlic juice. A similar stimulation of bacterial growth by garlic was observed by Kabelik (8). This is in accordance with our earlier observation (19) that endogenous respiration and glucose oxidation in *Escherichia coli* and *Staphylococcus aureus* were higly stimulated by small amounts of the juice in the Warburg apparatus. The observed lack of stimulation of oxygen uptake in *Candida albicans* under the same conditions can be explained by the high sensitivity of respiratory enzymes of this organism to the same amount of the juice. Even those minute amounts of the juice were too toxic for *Candida albicans* to stimulate respiration and caused only inhibition. This observation indicates that respiratory enzymes of this organism to the same amount of the juice. The strong influence of garlic on the growth of pathogenic fungi and the lack of toxicity for macroorganism suggest that it deserves attention as a possible mycostatic agent of broad spectrum activity.

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STRESZCZENIE

Określono wrażliwość kilkunastu gatunków grzybów chorobotwórczych na sok czosnkowy oraz antybiotyki — nystatynę, gryzeofulwinę i amfoterycynę B. Wszystkie badane szczepy były w wysokim stopniu wrażliwe na sok, co świadczy o jego szerokim zakresie działania mykostatycznego. Wzrost grzybów w bulionie był hamowany przez duże rozcieńczenia soku, rzędu 1 : 1,280—1 : 5,120. Żaden z antybiotyków, użyty nawet w dużych stężeniach, nie dorównywał aktywności przeciwgrzybiczej 0.1 ml nierozcieńczonego soku na płytkach agarowych.

Wydaje się, że z uwagi na swe silne działanie na drożdżaki, pleśnie i dermatofity czosnek zasługuje na dalsze opracowanie jako ewentualny lek przeciwgrzybiczy o szerokim spektrum działania.

РЕЗЮМЕ

Определено чувствительность некоторых сортов болезнетворных грибов к соку чеснока и к антибиотикам: ныстатин, гризеофульвин и амфотерицин В. Все исследованные штаты были в большой степени чувствительны к соку, что свидетельствует о его широком диапазоне действия на болезнетворные грибы. Большие разбавления сока ряда 1:1280—1:5120 тормозяще действовали на прорастание грибов в бульоне.

Ни один антибиотик из исследованных, выступающий даже в большой концентрации не сравнялся с противогрибичей активностью 0,1 мл неразбавленного сока.

Учитывая его сильное действие на дрожжи, плесени и дерматофиты, чеснок заслуживает дальнейшего разрабатывания как противгрибичий медикамент о широком спектре действия.