Full Length Research Paper

Cytotoxicity and antimicrobial activity of allicin and its transformation products

Dušica Ilić[1], Vesna Nikolić[1]*, Ana Ćirić[2], Marina Soković[2], Tatjana Stanojković[3], Tatjana Kundaković[4], Mihajlo Stanković[1] and Ljubiša Nikolić[1]

[1]Department of Organic Chemical Technology, Faculty of Technology Leskovac, University of Niš, Serbia.
[2]Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia.
[3]Institute for Oncology and Radiology of Serbia, Beograd, Serbia.
[4]Department of Pharmacognosy, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia.

Cytotoxicity and antimicrobial activities of allicin and related organo-sulphur products obtained by microwave-assisted transformation of allicin in methanol were studied. The mixture of transformation products of allicin was analysed using liquid chromatography-mass spectrometry (LC-MS) and represents the mixture of ajoenes, vinyldithiins and diallyl disulfide. Cytotoxicity of allicin and its transformation products of allicin were studied against human melanoma FemX and human embryonic lung fibroblast MRC-5 cell lines using MTT test. Allicin and the transformation products of allicin exhibited high selectivity against FemX (IC_{50} = 0.71 ± 0.20 and IC50 = 0.64 ± 0.52 µg/ml, respectively), while the activity against healthy MRC-5 cells was lower (IC_{50} = 66.48 ± 3.75 and IC_{50} = 64.5 ± 1.50 µg/ml, respectively). Allicin has shown strong activity against tested Gram-positive and Gram-negative bacteria and very powerful antifungal activity with minimum inhibitory concentration (MIC) at 0.001 to 0.008 mg × 10^{-1}/ml and MFC at 0.004 to 0.03 mg × 10^{-1}/ml. The transformation products of allicin possessed also very strong antibacterial and antifungal activity, but lower than allicin.

Key words: Cytotoxicity, allicin, organo-sulphur products, antimicrobial activities, liquid chromatography-mass spectrometry (LC-MS).
.

INTRODUCTION

Garlic is a food, as well a medicine. Many reports concerning its beneficial effects on human health were published, but the researcher's interest never decreased. The medicinal properties of garlic could be due to the presence of sulphur-containing compounds as alkyl- and alkenyl-sulphides, thiosulphinate, vinyldithiins (2-ethenyl-4H-1,3-dithiins and 3-ethenyl-4H-1,3-dithiins) and ajoenes (4,5,9-trithiadodeca-1,6,11-trien-9-oxydes) (Kritchevsky et al., 1991; Jung et al., 1991; Ghannoum 1988; Davis et al., 1994; Prasad et al., 1995; Han et al., 1995). Under the influence of heat or organic solvents spontaneously or depending on chemical reaction, allicin could be decomposed in secondary products with specific pharmacological activity (Cruz-Villalon, 2001; Block et al., 1986). The use of garlic in the treatment of tumours dates all the way back to 1550 BC when ancient Egyptians administered it orally and topically; the modern era, however, begins in the 1950s when Weisberg and Pensky (1958) demonstrated *in vitro* and *in vivo* that thiosulfinate extracts of garlic inhibited the growth of malignant cells and prevented growth of sarcoma 180 ascites tumour. Since that time, garlic has been demonstrated in epidemiologic studies to be associated with a reduced risk of stomach cancer and in animal models, to have antitumour activity in sarcoma, mammary carcinoma, hepatoma, colon cancer and

---
*Corresponding author. E-mail: nvesna@yahoo.com.

squamous cell carcinoma of skin and oesophagus (Wu et al., 2005). Allicin inhibits *in vitro* the invasion and metastasis of human colon carcinoma cell SW480, human gastric cancer SCG-7901 cells, L-929 murine fibrosarcoma, SiHa cells of human cervical cancer, HeLa cells human cervical cancer and two human leukemia cells (U937 and HL-60) (Wu et al., 2005; Gao et al., 2009; Miron et al., 2008). Data show that allicin is an efficient immunomodulator of macrophage secretory activity (Kang et al., 2001).

There are various literature reports on the anti-cancer activity of Z-ajoene (Terrasson et al., 2007; Hassan, 2004; Dirsch et al., 2002; Li et al., 2002; Ledezma et al., 2004), but no general data on the activity of the E-isomer. Ajoene has shown antiproliferative activity and apoptotic ability against HL-60, U937, HEL and OCIM-1 (Sigounas et al., 1997; Li et al., 2002; Dirsch et al., 1998). Also, with different mechanisms of action, ajoene induced apoptosis of B16F10 murine melanoma cells and TE354T basal cell carcinoma cells (Wu et al., 2005). Very important characteristic of ajoenes is to have synergistic effects with chemoterapeutics citarabin and fludarabin (Ahamed et al., 2001).

Allicin manifests a wide spectrum of antibacterial activity against numerous Gram positive and Gram negative bacteria (Ilić et al., 2010; Amagase et al., 2001; Ross et al., 2001; Chen et al., 2009; Ankri and Mirelman, 1999), but also against an acid-resistant bacterium *Mycobacterium tuberculosis* (Hasan et al. 2007). Fungi susceptible to allicin are *Candida albicans*, *Aspergillus niger*, and *Penicillium* sp. (Khodavandi et al., 2011; Nikolić et al., 2005; Cavagnaro et al., 2005). Allicin has antiviral activity both *in vitro* and *in vivo*. Among the viruses susceptible to allicin are *Herpes simplex* type 1 and 2, *Parainfluenza* virus type 3, human *Cytomegalo* virus, *Influenza B, Vaccinia* virus, *Vesicular stomatitis* virus and *Human rhinovirus* type 2 (Ankri and Mirelman, 1999).

Ajoenes, also, have antimicrobial effect (they affect a great number of bacteria, viruses, fungi and other parasites) (Canizares et al., 2002; Turos et al., 2008). They participate in the inhibition of *in vitro* growth of *Helicobacter pylori*, a bacterium responsible for gastric diseases such as gastric ulcer and stomach cancer (Sigounas et al., 1997). The data about antimicrobial and antitumour activity of 2-vynil-4H-1,3-dithiine and 3-vynil-4H-1,2-dithiine in the literature are not known. In this research we are presenting the results of cytotoxicity against melanoma cell line (FemX) and healthy human embryonic lung fibroblast (MRC-5), as well antimicrobial activity against broad range of bacteria and fungi, of allicin obtained by synthesis, and a mixture of more stable sulphur products obtain after microwave's transformation of allicin.

MATERIALS AND METHODS

Reagents

Allyl disulfide, 80%, hydrogen peroxide, 30%, diethyl ether and acetone were bought from the Sigma-Aldrich Chemie GmbH Company (Germany). Fetal bovine serum (FBS) was obtained from Gibco BRL (USA). l-Glutamine, streptomycin, penicillin, MTT (3-(4,5-dimethylthiazoyl)-2,5-diphenyltetrazolium bromide) and RPMI 1640 were obtained from Sigma (USA). Other solvents and reagents used were p.a. and HPLC purity.

Allicin synthesis

Allicin was synthesized from allyl disulfide according to the procedure developed by Nikolić et al. (2004). Allicin was synthesized by oxidation of allyl disulfide with acid hydrogen peroxide (molar ratio was 1:1) at room temperature for 4 h. Reaction mixture was neutralized by sodium hydroxide solution (85 g NaOH in 150 ml water) with vigorously stirring and cooling. During stirring, reaction mixture was separated into the two layers and allicin was obtained by liquid extraction with diethyl ether. By the evaporation of ether, allicin with 73% purity was obtained.

Transformation of allicin

Obtained allicin was treated by microwave in "Discover" focus microwave reactor, CEM Corporation, Matthews, NC, USA. The frequency and the power applied were 2.45 GHz and 150 W, respectively. The temperature regulation was carried out by infrared mass measuring system and maintained at 100°C. Transf ormation reaction was carried out in acetone with 1:10 allicin to methanol volume ratio. Reaction was performed at 55°C for 2 mi n.

Liquid chromatography-mass spectrometry (LC-MS)

The identification of allicin and the components obtained in the mixture after transformation reaction was done using liquid chromatography-mass spectrometry (LCQ Fleet Ion Trap LC/MS[n] Thermo Scientific) with UV detector set at 205 nm, in the positive ion mode, using an extractor voltage of 4.5 kV for the analysis. The column used was a Zorbax Eclipse XDB-C18 (4.6 × 250 mm; 5 μm) operated at a temperature of 25°C. Acetonitrile/water (80/20, V/V) were used as a mobile phase with flow 1 ml/min. Injected volume was 20 μl. The identification of compounds in the mixture was based on the comparison of their retention times and mass spectra with authentic samples and literature data.

Cytotoxicity

Cell lines

The human melanoma FemX and human embryonic lung fibroblast MRC-5 cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). Both cancer cell lines were maintained in the recommended RPMI-1640 medium supplemented with 10% heat-inactivated (56°C) fetal bovine serum, l- glutamine (3 mM), streptomycin (100 mg/ml), penicillin (100 IU/ml) and 25 mM HEPES and adjusted to pH 7.2 by bicarbonate solution. Cells were grown in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.

Treatment of cell lines

Stock solutions (100 mg/ml) of compounds, made in dimethylsulfoxide (DMSO), were dissolved in corresponding medium to the required working concentrations. Neoplastic FemX cells (3000 cells per well) and human embryonic lung fibroblast MRC-5 cells (5000 cells per well) were seeded into 96-well microtiter plates and 24 h later, after the cell adherence, five different, double diluted, concentrations of investigated compounds, were added to the wells except for the control cells to which a nutrient medium only was added. Nutrient medium was RPMI 1640 medium, supplemented with l-glutamine (3 mM), streptomycin (100 µg/ml), and penicillin (100 IU/ml), 10% heat inactivated (56°C) FBS and 25 mM Hepes and was adjusted to pH 7.2 by bicarbonate solution. The cultures were incubated for 72 h.

Determination of cell survival

The effects of allicin and its transformation products of allicin on cancer cell survival were determined by MTT test, according to Mosmann (1983) with modification by Ohno and Abe (1991), 72 h upon addition of the compounds, as it was described earlier. Briefly, 20 µl of MTT solution (5 mg/ml PBS) were added to each well. Samples were incubated for further 4 h at 37°C in 5% CO_2 and humidified air atmosphere. Then, 100 µl of 10% SDS were added to extract the insoluble product formazan, resulting from the conversion of the MTT dye by viable cells. The number of viable cells in each well was proportional to the intensity of the absorbance of light, which was then read in an ELISA plate reader at 570 nm.

Absorbance (A) at 570 nm was measured 24 h later. To get cell survival (%), A of a sample with cells grown in the presence of various concentrations of allicin and mixture of transformation products of allicin was divided with control optical density (the A of control cells grown only in nutrient medium) and multiplied by 100. It was implied that A of the blank was always subtracted from A of the corresponding sample with target cells. IC50 concentration was defined as the concentration of an agent inhibiting cell survival by 50%, compared with a vehicle-treated control. All experiments were done in triplicate.

Antimicrobial activity

Microorganisms and culture conditions

For the bioassays we used eight bacteria: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Enterococcus faecalis* (human isolate), and Gram-positive bacteria: *Listeria monocytogenes* (NCTC 7973), *Bacillus cereus* (human isolate), *Micrococcus flavus* (ATCC 10240), and *Staphylococcus aureus* (ATCC 6538). Eight fungi were used for antifungal activity: *Aspergillus flavus* (ATCC 9643), *Aspergillus niger* (ATCC 6275), *Aspergillus fumigatus* (ATCC 9142), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061), *Candida albicans* (human isolate), *Candida kruzei* (human isolate).

All of the organisms tested were from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research „Siniša Stanković", Belgrade, Serbia. The micromycetes were maintained on malt agar (MA), bacteria on Mueller-Hinton agar (MH) and cultures were stored at +4°C and subcultured once a month (Booth, 1971).

Microdilution method

In order to investigate the antimicrobial activity of allicin and the transformation products of allicin, the modified microdilution technique was used (Hanel and Raether, 1998; Daouk et al., 1995). Bacterial species were cultured overnight at 37°C in Luria-broth (LB) medium. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (V/V). The fungal and bacterial cell suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 CFU/ml in a final volume of 100 µl per well. The inocula were stored at +4°C for further use. Dilutions of the inocula were cultured on solid MH for bacteria and solid MA for fungi to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentrations (MICs) determination was performed by a serial dilution technique using 96-well microtitre plates. The investigated compounds were dissolved in 5% DMSO (1 mg/ml) and added in broth medium with inoculum. The microplates were incubated for 48 h at 37°C for bacteria and or 72 h at 28°C, for fungi. The lowest concentrations without visible growth were defined as MICs. The minimum bactericidal concentrations (MBCs) and fungicidal concentrations (MFCs) were determined by serial subcultivation of a 2 ml into microtitre plates containing 100 ml of broth per well and further incubation 48 h at 37°C or 72 h at 28°C, respectively. The lowest concentration with no visible growth was defined as MBC/MFC respectively, indicating 99.5% killing of the original inoculum. All experiments were performed in duplicate and replicated three times. Streptomycin, amplicillin and commercial fungicide, bifonazole and ketoconazole, were used as positive controls (0.1 to 3 mg/ml).

RESULTS AND DISCUSSION

Liquid chromatography-mass spectrometry (LC-MS)

The citotoxicity and antimicrobial activity of allicin (73% purity), obtained by synthesis and the transformation products of allicin obtained by microwave-assisted syntesis were tested using *in vitro* methods. The identification of compounds in the mixture after the transformation of allicin was done using LC-MS and it was based on the comparison of their retention times and mass spectra with authentic samples and literature data. The identified compounds in the reaction mixture were, (E) - and (Z)-ajoene, 2-vynil-4H-1,3-dithiine, 3-vynil-4H-1,2-dithiine and diallyl disulfide. Mass spectra data and retention times from LC-MS analysis were: allicin R_t = 2.993 min, MS data: 162 (M^+, 10), 144 (24), 73 (28), 72 (40), 45 (48), 41 (100); (E)-ajoene: R_t = 3.2 min, 234 (M^+, 10), 145 (24), 111 (11), 103 (91), 73 (39), 68 (32), 45 (100), 41 (91)); (Z)-ajoene: R_t = 3.4 min 234 (M^+, 10), 145 (22), 111 (13), 103 (93), 73 (39), 68 (37), 45 (100), 41 (91); 3-vynil-4H-1,2-dithiine: Rt = 4.3 min, 144 (M^+, 49), 111 (75), 103 (31), 97 (48), 72 (25), 71 (60), 45 (100), 39 (20); 2-vynil-4H-1,3-dithiine: R_t= 4.7 min, 144 (M^+, 35), 111(34), 103 (31), 72 (100), 71 (25), 45 (60), 39 (43) and diallyl disulfide: R_t = 6.7 min, 146 (M^+, 41), 113 (46), 105 (40), 81 (55), 79 (33), 45 (78), 41 (100), 39 (50).

Table 1. Cytotoxic effects of allicin and the transformation products of allicin on FemX and MRC-5 cells.

Cell line	IC_{50} (μg/ml ± S.D.)*	
	Allicin	**Transformation products of allicin**
FemX	0.71±0.20	0.64±0.52
MRC-5	66.48±3.75	64.5±1.50

*Concentrations of examined compounds that induced a 50% decrease in FemX and MRC-5 cell survival (expressed as IC50, μg/ml ± S.D.). Allicin and the transformation products of allicin were incubated with cells for 72 h. At the end of this incubation period, antiproliferative activity *in vitro* was determined by the MTT assay. Results are presented as the mean value ± SD of three independent experiments.

Cytotoxic effects of allicin and its transformation products

Allicin and the transformation products of allicin, (E)- and (Z)-ajoene, 2-vynil-4H-1,3-dithiine, 3-vynil-4H-1,2-dithiine and diallyl disulfide, were tested for their ability to inhibit the proliferation of human cancer cell lines such as melanoma cell line (FemX) and human embryonic lung fibroblast (MRC-5). The IC50 values of allicin and transformation products of allicin are presented in Table 1. The *in vitro* assays showed that both tested compounds significantly decreased cell survival in Fem X cells. In contrast, allicin had less effect on the normal cells (MRC-5). Fifty percent inhibition of cell survival occurred with 0.71 μg/ml of allicin and 0.64 μg/ml of transformation products of allicin in FemX cells, and with 66.48 μg/ml of allicin and 64.5 μg/ml of transformation products in MRC-5 cells. Allicin is 93 times more active against malignant FemX cell lines than to normal fibroblast MRC-5 cells. Similarly, the transformation products of allicin were 100 times more active towards FemX cells than to normal fibroblast MRC-5 cells.

Allicin and its transformation products exhibited the highest selectivity against FemX. The results showed that both tested samples significantly inhibited the proliferation of FemX cells. According to the IC50 values of transformation products of allicin against FemX cells, we can conclude that the mixture of sulphur compounds, formed by transformation of allicin ((E)- and (Z)-ajoene; 2-vynil-4H-1,3-dithiine, 3-vynil-4H-1,2-dithiine and diallyl disulfide) has slightly higher activity than pure allicin. Cytotoxicity against non-tumor MRC-5 cells was similar for both samples.

Several pieces of evidences suggested that *Allium* genus possess anticancer properties as shown by their ability to suppress tumor proliferation *in vivo* and *in vitro* (Ahmed et al., 2001; Miron et al., 2002; Zhang et al., 2007). Sulphur containing compounds, especially garlic compounds are the most chemopreventing promising compounds because their potent chemopreventing activity was confirmed in many *in vivo* and *in vitro* studies. Garlic extract and its sulphur compounds have shown chemopreventive activity on malignant breast, colon, skin, uterus, oesophagus and lung cell lines (Wu et al., 2005).

This antineoplastic effect was greater for lipidsoluble than for water-soluble allyl sulfides (Rabinkov et al., 1998; Tattelman, 2005). Diallyl disulfide suppressed the growth of human colon tumor cell xenografts in athymic nude mice (Miron et al., 2003; Zhang et al., 2007), while only diallyl trisulphide was tested against MRC-5 cells (Sakamoto et al., 1997). The exact pharmacologic mechanism for anticarcinogenic and antitumorigenic activity of garlic has not yet been determined. Both water-soluble and lipid-soluble diallyl sulphides can influence a number of molecular events involved with cancer. These include inhibiting mutagenesis, blocking carcinogen DNA adduct formation, scavenging free radicals, as well as blocking cell proliferation, differentiation, and angiogenesis (Omar et al., 2007).

Active cellular proliferation appears to be a factor in enhancing the growth inhibitory effects described for diallyl sulfides. Scharfenberg et al. (1990) found that A549 lung and BJA-B Burkitt lymphoma cells were more than twice as sensitive to the antiproliferative effects of diallyl trisulfide (DATS) and ajoene than were nonneoplastic MRC-5 lung and FS4/BHK fibroblasts cells.

However, the mechanism of their antitumor action is not revealed yet, so further studies are needed, especially in the field of their cytotoxic effects on non-tumour cells. We tested the activity of allicin and its transformation sulphur products against a malignant cell line (FemX) and healthy lung fibroblasts (MRC-5). The activity of allicin and some of the transformation products against this two cell lines have not been studied yet. Sakamoto et al. (1997) showed that diallyl trisulphide inhibited proliferation in human lung A549 cells, but not in MRC-5 cells. Ajoenes induced apoptosis in human acute myeloid leukaemia cell line HL-60, as well in peripheral blood mononuclear cells (PBMC) isolated from a chronic leukaemia patients but not against PBMC isolated from

Table 2. Minimum inhibitory (MIC mg x 10^{-1}/ml)* and bactericidal concentration (MBC mg x 10^{-1}/ml)** of allicin and the transformation products of allicin.

Bacteria	Allicin	Transformation products of allicin	Streptomycin	Ampicillin
	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC
Bacillus cereus	0.03/0.06	0.12/0.25	0.12/0.25	1.0/1.5
Micrococcus flavus	0.06/0.06	0.5/0.5	0.25/0.5	1.0/1.5
Staphylococcus aureus	0.06/0.12	0.5/0.5	0.5/1.0	1.0/1.5
Listeria monocytogenes	0.12/0.25	0.5/0.5	1.5/3.0	1.5/3.0
Escherichia coli	0.12/0.25	0.25/0.5	0.5/1.0	1.5/2.0
Enterococcus faecalis	0.12/0.25	0.12/0.25	0.5/1.0	1.5/2.0
Pseudomonas aeruginosa	0.06/0.06	0.25/0.5	0.5/1.0	3.0/5.0
Salmonella typhimurium	0.06/0.06	0.25/0.25	0.5/1.0	1.0/2.0

*The lowest concentrations without visible growth were defined as MICs. **The lowest concentration with no visible growth was defined as MBC respectively, indicating 99.5% killing of the original inoculum. All experiments were performed in duplicate and replicated three times.

Table 3. Minimal inhibitory (MIC mg × 10^{-1}/ml)* and fungicidal concentration (MFC mg × 10^{-1}/ml)** of allicin and the transformation products of allicin.

Fungi	Allicin	Transformation products of allicin	Bifonazole	Ketoconazole
	MIC/MFC	MIC/MFC	MIC/MFC	MIC/MFC
Penicillium funiculosum	0.004/0.03	0.008/0.03	2.0/2.5	2.0/5.0
Penicillium ochrochloron	0.002/0.015	0.015/0.03	1.5/2.0	10.0/10.0
Trichoderma viride	0.008/0.015	0.008/0.03	2.0/2.5	25.0/30.0
Aspergillus fumigatus	0.008/0.03	0.008/0.03	1.5/2.0	2.0/5.0
Aspergillus niger	0.004/0.015	0.015/0.06	1.5/2.0	2.0/5.0
Aspergillus flavus	0.008/0.015	0.008/0.03	2.0/2.5	15.0/20.0
Candida albicans	0.004/0.004	0.015/0.015	1.0/1.5	2.0/5.0
Candida kruzei	0.001/0.002	0.008/0.015	0.5/1.5	1.0/2.0

*The lowest concentrations without visible growth were defined as MICs. *The lowest concentration with no visible growth was defined as MFC respectively, indicating 99.5% killing of the original inoculum. All experiments were performed in duplicate and replicated three times.

healthy donors (Dirsch et al., 1998).

According to our results, allicin exhibited strong antiproliferative action on FemX cells, but this effect was lower in human foetal lung fibroblasts. Also, the mixture of the transformation products (ajoenes, vinyldithiins and diallyl disulfide) shows a similar effect against human melanoma cells and human foetal lung fibroblasts.

Antimicrobial activity

The results of antimicrobial activity of allicin (73% purity) and the transformation products of allicin against a panel of selected Gram positive, Gram negative and fungi are presented in Tables 2 and 3 in the comparison with those of the reference drugs streptomycin, ampicillin, bifonazole and ketoconazole, respectively.

The antibacterial activities are reported in Table 2. The both samples, allicin and the transformation products of allicin showed strong antibacterial activity against all bacteria species, but on different level. Minimal inhibitory concentration for allicin ranged between 0.03 to 0.12 mg x× 10^{-1}/ml and 0.12 to 0.25 mg × 10^{-1}/ml for the transformation products of allicin. Minimal bactericidal concentration was 0.06 to 0.25 mg x 10^{-1}/ml for allicin and 0.25 to 0.5 mg × 10^{-1}/ml for transformation products of allicin. The most sensitive bacterial species was *B. cereus* with MIC at 0.03 to 0.12 mg × 10^{-1}/ml and MBC at

0.06 to 0.25 mg × 10^{-1}/ml. *E. faecalis* was the most resistant species with inhibitory activity at 0.12 mg × 10^{-1}/ml and bactericidal activity at 0.25 mg × 10^{-1}/ml for both tested samples. Standard drugs used as a positive control, streptomycin and ampicillin, were also active against all bacteria. Range of MIC for streptomycin was 0.12 to 1.5 mg × 10^{-1}/ml and, of MBC 0.25 to 3.0 mg × 10^{-1}/ml, while ampicillin showed slightly lower antibacterial potential with MIC 1.0 to 3.0 mg × 10^{-1}/ml and MBC 1.5 to 5.0 mg × 10^{-1}/ml.

Allicin showed better antibacterial activity than streptomycin and ampicillin, while the transformation products of allicin exhibited same or slightly better activity than streptomycin and better than ampicillin (Table 2).

The results of antifungal activity are presented in Table 3. Both tested compounds showed great antifungal activity in different range. Allicin possessed stronger antifungal activity with MIC at 0.001 to 0.008 mg × 10^{-1}/ml and MFC at 0.004 to 0.03 mg × 10^{-1}/ml. The transformation products of allicin showed inhibitory activity at 0.008 to 0.015 mg × 10^{-1} /ml and fungicidal at 0.015 to 0.06 mg × 10^{-1}/ml. *C. cruzei* was the most sensitive tested fungi with MIC at 0.001 to 0.008 mg x 10^{-1}/ml and MFC at 0.02 to 0.015 mg × 10^{-1}/ml, while *A. niger* was the most resistant species with MIC at 0.004 to 0.015 mg × 10^{-1}/ml and MFC at 0.015 to 0.06 mg × 10^{-1}/ml.

Antimycotics (bifonazole and ketoconazole) used as a positive control showed inhibitory activity at 0.5 to 2.0 mg × 10^{-1}/ml and 1.0 to 25.0 mg × 10^{-1}/ml while fungicidal activity was at 1.5 to 2.5 mg × 10^{-1}/ml and 2.0 to 30.0 mg × 10^{-1}/ml, respectively. Both tested samples exhibited better antifungal activity than antimycotics used, with better antifungal potential of allicin compared to transformation products of allicin (Table 3).

Our results support a specific antifungal more than an antibacterial activity of the both tested samples. Allicin showed better antibacterial and antifungal activity than the transformation products of allicin (ajoenes, vinyldithiins and diallyl disulfide). Inhibition of certain thiol-containing enzymes in the microorganisms by the rapid reaction of thiosulfinates with thiol groups was assumed to be the main mechanism involved in the antimicrobial effect of allicin (Cavallito et al., 1944). The mode of action of allicin on the fungal cell has not yet been elucidated but it is assumed to function on thiol enzymes as in other microorganisms. Other requirements such as molecular accessibility and lipophilicity seem to play an important role for their antifungal activity (Yamada and Azuma, 1997).

Very low concentration obtained for antibacterial and antifungal activity suggested high activity of allicin and the transformation products of allicin which could be comparable to commercially available antibiotics and become a prime candidate for therapeutic use.

Also, IC50 values for inhibition of proliferation of human cancer cell lines such as melanoma cell line (FemX) and human embryonic lung fibroblast (MRC-5) suggested significant cytotoxicity of tested compounds. According our results, allicin and its transformation products exhibited strong antiproliferative action on FemX cells, but this effect was lower in human foetal lung fibroblasts.

ACKNOWLEDGEMENT

REFERENCES

Ahmed N, Laverick L, Sammons J, Zhang H, Maslin DJ (2001). Ajoene, a garlic-derived natural compound, enhances chemotherapy-induced apoptosis in human myeloid leukaemia CD34-positive resistant cells. Anticancer Res., 21: 3519-3523.

Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y (2001). Intake of Garlic and Its Bioactive Components. J. Nutr., 131: 955S-962S.

Ankri S, Mirelman D (1999). Antimicrobial properties of allicin from garlic. Microbes. Infect. 2: 125-129.

Block E, Ahmad S, Cataltamo JL, Jain MK, Apitz-Castro R (1986). Antithrombotic Organosulfur Compounds from Garlic, Structural, Mechanistic and Synthetic Studies. J. Am. Chem. Soc., 108: 7045-7055.

Booth C (1971). Fungal Culture Media. In J. R. Norris, and D. W. Ribbons, Methods in Microbiology, London and New York: Academic Press, pp. 49-94.

Cavallito C, Bailey JH (1944). Allicin, the antibacterial principle of *Allium sativum*. Isolation, physical properties and antibacterial action. J. Am. Chem. Soc., 66: 1944 - 1952.

Canizares P, Gracia I, Gomez LA, Martin de Argila C, De Rafael L, Garcia A (2002). Optimization of *Allium sativum* solvent extraction for the inhibition of *in vitro* growth of *Helicobacter pylori*. Biotechnol. Prog., 18: 1227 - 1232.

Cavagnaro PF, Camargo A, Piccolo RJ, Lampasona SG, Burba JL, Masuelli RW (2005). Resistance to *Penicillium hirsutum* Dierckx in garlic accessions. Eur. J. Plant Pathol., 112: 195-199.

Chen YY, Chiu HC, Wang YB (2009). Effects of Garlic Extract on Acid Production and Growth of *Streptococcus mutans*. J. Food Sci., 17: 59-63.

Cruz-Villalon G (2001). Synthesis of Allicin and Purification by Solid-Phase Extraction. Anal. Biochem., 290: 376-378.

Daouk RK, Dagher SM, Sattout JE (1995). Antifungal activity of the Essential oil of *Origanum syriacum* L. J. Food Prot., 58: 1147-1149.

Davis LE, Shen J, Royer R (1994). *In vivo* activity of ajoene against rodent malaria. Planta Med,. 60: 546 - 549.

Dirsch VM, Antlsperger DSM, Hentze H, Vollmar AM (2002). Ajoene, an experimental anti-leukemic drug: mechanism of cell death. Leukemia 16: 74-83.

Dirsch VM, Gerbes AL, Vollmar AM (1998). Ajoene, a compound of garlic, induces apoptosis in human promyeloleukemic cells, accompanied by generation of reactive oxygen species and activation of nuclear factor kappaB. Mol. Pharmacol., 53: 402 - 407.

Gao Y, Liu YQ, Cao WK, Chen XF, Wan YY, Heng C, Xu LJ (2009).

Effects of allicin on invasion and metastasis of colon cancer LoVo cell line *in vitro*. Zhonghua Yi Xue Za Zhi., 89: 1382-1386.

Ghannoum MA (1988). Studies on the anticandidal mode of action of *Allium sativum* (garlic). J. Gen. Appl. Microbiol., 134: 2917-2924.

Han J, Lawson L, Han G, Han P (1995). A Spectrophotometric Method for Quantitative Determination of Allicin and Total Garlic Thiosulfinates. Anal. Biochem., 225: 157-160.

Hanel H, Raether W (1998). A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example. Mycoses, 31: 148-154.

Hasan N, Siddiqui M, Toossi Z, Khan S, Iqbal J, Islam N (2007). Allicin-induced suppression of *Mycobacterium tuberculosis* 85B mRNA in human monocytes. Biochem. Biophys. Res. Commun., 335: 471-476.

Hassan HT (2004). Ajoene (natural garlic compound): a new anti-leukaemia agent for AML therapy. Leuk. Res., 28: 667-671.

Ilić D, Nikolić V, Nikolić Lj, Stanković M, Stanojević Lj (2010). Thermal degradation, antioxidant and antimicrobial activity of the synthesized allicin and allicin incorporated in gel. Chem. Ind., 6: 85-93.

Jung EM, Jung F, Mrowwietz C, Kiesewetter H, Pindur G, Wenzel E (1991). Influence of garlic powder on cutaneous microcirculation. A randomized placebo-controlled double-blind cross-over study in apparently healthy subjects. Drug. Res,. 41: 626-630.

Kang NS, Moon EY, Cho CG, Pyo S (2001). Immunomodulating effect of garlic component, allicin, on murine peritoneal macrophages. Nutr. Res., 21: 61 -626.

Khodavandi A, Alizadeh F, Harmal NS, Sidik SM, Othman F, Sekawi Z, Jahromi MAF, Ng KP, Chong PP (2011). Comparison between efficacy of allicin and fluconazole against *Candida albicans in vitro* and in a systemic candidiasis mouse model. FEMS Microbiol. Lett., 315: 87 - 93.

Kritchevsky D (1991) The effects of Dietary Garlic on the Development of Cardiovascular Disease. Trends Food Sci. Technol., 4: 141 -144.

Ledezma E, Apitz-Castro R, Cardier J (2004). Apoptotic and anti-adhesion effect of ajoene, a garlic derived compound, on the murine melanoma B16 F19 cells: Possible role of caspase-3 and alpha (4) beta (1) integrin. Cancer Lett., 206: 35-41.

Li M, Ciu JR, Ye Y, Min JM, Zhang LH, Wang K, Gares M, Cros J, Wright M, Leung-Tack J (2002). Antitumor activity of Z-ajoene, a natural compound purified from garlic: antimitotic and microtubule-interaction properties. J. Carcinogen., 23: 573 - 579.

Miron T, Mironchik M, Mirlman D, Wilchek M, Rabinkov A (2003). Inhibition of tumor growth by a novel approach: in situ allicin generation using targeted alliinase delivery. Mol. Cancer Ther., 2: 1295 -1301.

Miron T, Shin I, Feigenblat G, Weiner L, Mirelman D (2002). A spectrophotometric assay for allicin, alliin, and alliinase (alliin lyase) with a chromogenic thiol: reaction of 4-mercaptopyridine with thiosulfinates. Anal. Biochem., 307: 76- 83.

Miron T, Wilchek M, Sharp A, Nakagawa Y, Naoi M, Nozawa Y, Akao Y (2008). Allicin inhibits cell growth and induces apoptosis through the mitochondrial pathway in HL60 and U937 cells. J. Nutr. Biochem., 19: 524 - 535.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65: 55 - 63.

Nikolić V, Stanković M, Nikolić Lj, Cvetković D (2004). Mechanism and kinetics of synthesis of allicin. Pharm., 59: 10-14.

Nikolić V, Stanković M, Nikolić Lj, Cvetković D, Kapor A, Cakić M (2005). The canal inclusion complex of allicin with carbamide: preparation, characterization and microbiological investigation. Chem. Ind., 2: 69-73.

Ohno M, Abe T (1991). Rapid colorimetric assays for the quantification of leukemia inhibitory factor (LIF) and interleukin-6 (IL-6). J. Immunol. Methods, 145: 199-203.

Omar SH, Abshar UH, Nehal M (2007). Anticarcinogenic and antitumorigenic effect of garlic and factors affecting its activity: a review. Pharmacog. Rev., 1: 215-221.

Prasad K, Laxdal V, Yu M, Raney B (1995). Antioxidant activity of allicin, an active principle in garlic. Mol. Cel. Biochem., 148: 183-189.

Rabinkov A, Miron T, Konstantinovski L, Wilchek M, Mirelman D, Weiner L (1998). The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. Biochim. Biophys. Acta., 1379: 233-244.

Ross ZM, O'Gara EA, Hill DJ, Sleightholme HV, Maslin DJ (2001). Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Appl. Environ. Microbiol., 67: 475 - 480.

Sakamoto K, Lawson LD, Milner JA (1997). Allyl sulphides from garlic suppress the *in vitro* proliferation of human A549 lung tumor cells. Nutr. Cancer, 29: 152 - 156.

Scharfenberg K, Wagner R, Wagner KG (1990). The cytotoxic effect of ajoene, a natural product from garlic, investigated with different cell lines. Cancer Lett., 53: 103-108.

Sigounas G, Hooker JL, Li W, Anagnostou A, Steiner M (1997). S-Allylmercaptocysteine, a stable thioallyl compound, induces apoptosis in erythroleukemia cell lines. Nutr. Cancer, 28: 153 -159.

Tattelman E (2005). Health effects of garlic. Am. Fam. Physician, 72: 103 -106.

Terrasson J, Xu B, Li M, Allart S, Davignon JL, Zhang LH, Wang K, Davrinche C (2007). Activities of Z-ajoene against tumour and viral spreading *in vitro*. Fund. Clin. Pharm., 21: 281-289.

Turos E, Revell KD, Ramaraju P, Gergeres DA, Greenhalgh K, Young A, Sathyanarayan N, Dickey S, Lim D, Alhamadsheh MM, Reynolds K (2008). Unsymmetric aryl–alkyl disulfide growth inhibitors of methicillin-resistant *Staphylococcus aureus* and *Bacillus anthracis*. Bioorg. Med. Chem., 16: 6501 - 6508.

Weisberger AS, Pensky J (1958). Tumour inhibition by a sulfhydryl-blocking agent related to an active principle of garlic (*Allium sativum*). Cancer Res., 18: 1301 - 1308.

Wu X, Kassie F, Mersch-Sundermann V (2005). Induction of apoptosis in tumor cells by naturally occurring sulfur-containing compounds. Mut. Res/Rev. Mut. Res., 589: 81 -102.

Yamada Y, Azuma K (1997). Evaluation of the *in vitro* antifungal activity of allicin. Antimicrob. Agents Chemother., 1: 743 - 749.

Zhang ZM, Yang XY, Shu-ha D, Wei X, Hai-qing G (2007). Anti-tumor effects of polybutylcyanoacrylate nanoparticles of diallyl trisulfide on orthotopic transplantation tumour model of hepatocellular carcinoma in BALB/c nude mice. Chin. Med. J., 120: 1336 -1342.