

# The effect of complex herbal remedy on the angiogenic activity of L-1 sarcoma cells, L-1 sarcoma tumour growth, and on the bacterial infection in mice

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## Abstract

The effect of herbal remedy (further named HR), a complex preparation consisting of three substances: *Echinacea purpurea* extract, *Allium sativum* extract, and cocoa, on the growth of L-1 sarcoma, tumour angiogenesis, VEGF tumour concentration and *Pseudomonas aeruginosa* infection in mice were studied.

A significant inhibitory effect of the remedy on tumour angiogenic activity using cutaneous angiogenesis test, and an inhibitory effect on L-1 sarcoma growth were observed. However, the concentration of VEGF was higher in tumours of mice treated with the remedy as compared to the control group.

It was found that *P. aeruginosa* infection intensity was significantly lower (after treatment with the remedy) than in the control group. The possible mechanisms of action of the preparation and perspectives of its use in human clinic are discussed.

**Key words:** L-1 sarcoma, tumour angiogenesis, VEGF, *Pseudomonas aeruginosa*, mice, herbal remedy

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## Introduction

In recent years an increase has been observed in the interest in drugs of natural origin having immunotropic activity. They may be a valuable complementation to treatment of infection, increasing cellular and humoral immunity of the organism in various clinical situations, among them in cancer patients after chemotherapy.

However, these natural substances should not stimulate tumour growth or vasculature development, since angiogenesis is one of the most important factors connected with the development and spreading of malignant tumours [1, 2].

Vascular endothelial growth factor (VEGF), potent mitogen for endothelial cells is one of the most important cytokines determining tumour development [3]. So far, we have not been

able to find any literature reports on the influence of *Echinacea* and cocoa, and only a few papers reporting the effect of garlic on angiogenesis and endothelial cells proliferation [4, 5].

Cocoa and *Echinacea* extracts contain polyphenols. Accumulating evidence demonstrates that diet-derived polyphenols (among them tea catechins) may inhibit tumour invasion and angiogenesis [6-8]. Some of these catechins are also present in cocoa.

In people immunocompromised due to severe burns, or immunosuppressive and cancer therapy, opportunistic infections e.g. with *Pseudomonas aeruginosa* are frequently observed [9-12].

The immunomodulatory and antiseptic action of extracts of some plants such as *Echinacea purpurea* and garlic (*Allium*

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*sativum*) is well known [13-15]. The immunomodulatory function and antibacterial properties of cocoa have been confirmed [16]. In recent years attention has been paid to *Echinacea purpurea*, that was found to augment the activity of NK cells in leukaemic mice [17, 18]. It is also known, that garlic has a broad range of beneficial effects, among them, antimicrobial activity [19]. The active ingredients in *A. sativum* are also responsible for the observed antitumour activity and immune stimulation [20].

We believe, that it could be of great importance to find new, non-toxic anti-angiogenic composition of agents among substances of plant origin, which simultaneously will exert antibacterial activity.

In the present study the combination was used consisting of three substances (*Echinacea purpurea*, *Allium sativum*, cocoa). The preparation partly suppressed tumour development, diminished tumour angiogenesis, and reduced the development of bacterial infection.

## Material and methods

### Drug

Syrup Alchinal (Gemi, Poland), designated by us herbal remedy (HR), containing in 1 ml 7 mg of *Echinacea purpurea* extract, 30 mg of *Allium sativum* extract and 50 mg of cocoa, was administered to mice orally (2x30 µl daily), directly to mouth by Eppendorff pipette, for 10 days or longer, depending on the type of experiment, with the exception of some angiogenesis assays, where mice obtained 3x30 µl daily, for 3 days. For eliminating eventual influence of mice handling, all control mice received water 2x30 ul or 3x30 ul daily, directly to mouth by Eppendorff pipette.

These daily doses, after calculation, taking into account differences in body area to body mass ratios between man and mouse, corresponded to 30 and 45 ml of Alchinal administered to a man weighting about 70 kg.

HPLC analysis of cocoa revealed the presence of catechin (1 mg per 1 g) and epigallocatechin (1.6 mg per 1 g).

### Animals

Estimation of the tumour growth and angiogenesis assays were performed on F1 hybrids of inbred strains BALB/c and C3H mice, of both sexes, at the age of 10-14 weeks. The tests with *P. aeruginosa* were performed on inbred C57BL/6 mice, at the age of 10-12 weeks. All experiments were accepted by the local Ethical Committee.

### Sarcoma growth

L-1 Sarcoma cells were delivered from Warsaw's Oncology Center Bank and then passaged through 4 generations of locally bred BALB/c mice. Briefly, tumour cells were grafted (1x10<sup>6</sup>/0,1 ml) into mice subcutaneously, into sub-scapular region. Mice were fed HR or water for

4 days before and 13 days after L-1 sarcoma cells grafting. Starting day 6-th after cell transplantation tumours were measured with electronic calipers. After 14 days tumors were removed, weighted and prepared from them cell suspensions were intradermally grafted to recipient mice (angiogenesis assay), or suspended in PBS (10<sup>7</sup> cells per ml), homogenized (Virsonic, USA) and frozen at -75°C for later VEGF examination..

### Cutaneous angiogenesis test

Tumors were cut for smaller pieces, rubbed through stainless sieve and suspended in 5 ml of phosphate buffered saline (PBS). The suspension was left for 10 min in room temperature. After sedimentation the supernatant was collected and centrifuged for 10 min, 1400 rpm. Obtained tumor cells were washed once in PBS for 10 min, 1500 rpm. Cells were resuspended in Parker medium in concentration of 4x10<sup>6</sup>/ml.

Cutaneous angiogenesis assay was performed according to Sidky and Auerbach [21] method with own modifications Skopińska-Różewska et al. [22]. Multiple samples of two hundred thousands sarcoma cells have been implanted intradermally (while suspended in 0.05 ml of Parker medium) into regionally shaved, anaesthetized (3.6% chloral hydrate) BALB/c mice. In order to facilitate the localization of cells injection sites later on, the suspension was coloured with 0.1% of trypan blue. On the day of cells grafting and on the following two days mice were fed HR, or water (as control, or in experiments where tumor cells were isolated from animals fed HR for 17 days before testing). 72 hours after cells grafting mice were killed with lethal dose of Morbital. All newly formed blood vessels on the inner skin surface were identified and counted in dissection microscope using criteria suggested by the authors of the method (small size, tortuosity and divarications).

### Measurement of VEGF concentration

Cytokine levels were determined in homogenates of L-1 sarcoma cell suspensions using ELISA kit (R&D) according to producers instruction. Optical density was measured at 450 nm. VEGF concentrations were expressed as pg/ml.

### Bacterial infection

Mice were fed HR (2x30 µl daily) or water (control) for 10 days and on the day 11-th were infected intraperitoneally (i.p.) with *Pseudomonas aeruginosa* strain ATCC (27853). Four hours after administration of 0.1 ml of bacteria suspension (3x10<sup>7</sup> CFU) the mice were anaesthetised with barbiturates and killed by spinal dislocation after which the livers were isolated. The livers were homogenised and the number of viable bacteria were estimated by plating.

### Statistical methods

Mann-Whitney and Student's tests were used for statistical analysis and the data with P values less than 0.05 were considered significant.

**Table 1.** The effect of HR administration (2x30 ul daily, since – 4 to +13 day) to Balb/c x C3H F1 mice on the growth of subcutaneously grafted L-1 sarcoma

Days after cell grafting	Control group (n=10)	Mice fed HR (n=12)	Significance of difference
	Mean value of tumour (x±SE/mm <sup>3</sup> )		
6	79±34	40±17	NS
7	133±29	56±21	p<0.05
8	348±70	195±48	p<0.05
10	915±109	511±96	p<0.05
13	1438±214	856±120	p<0.05

## Results

### Tumour growth

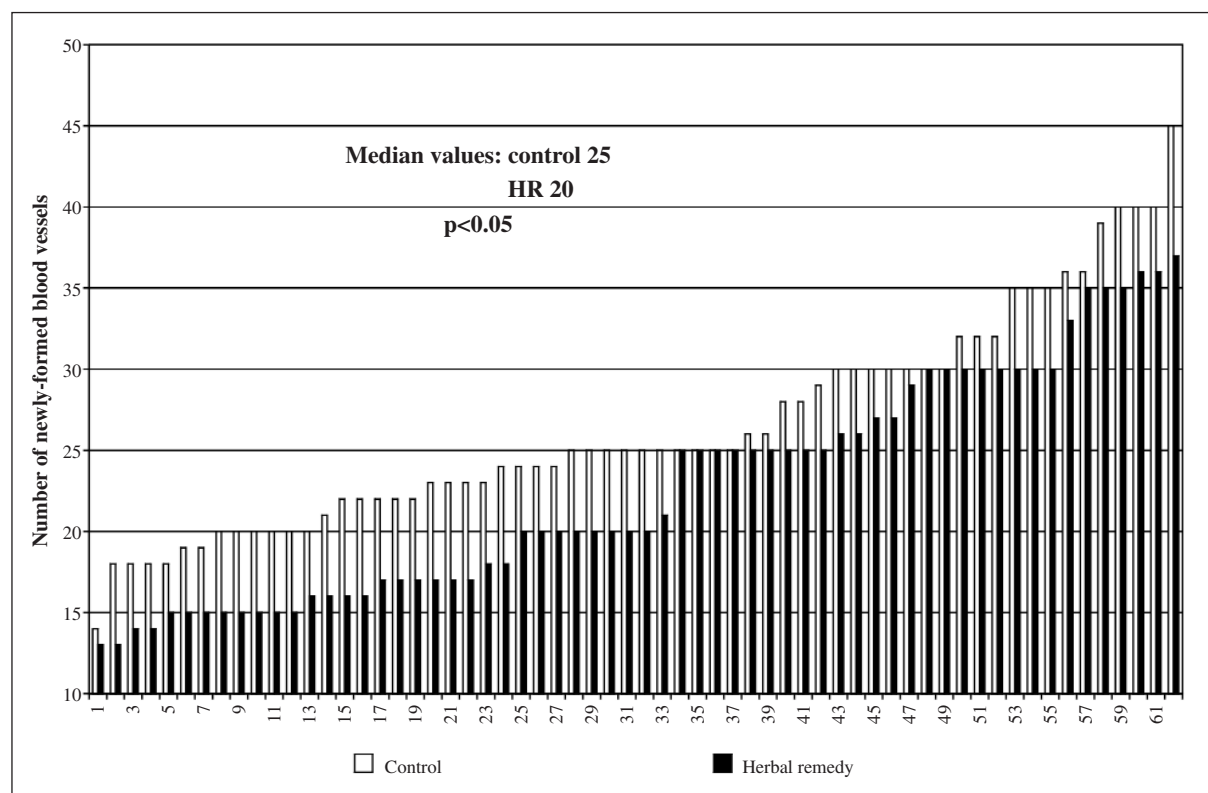
The effect of herbal remedy on L-1 sarcoma growth was presented in Table 1.

HR was administered to mice from 4<sup>th</sup> day before, until 13<sup>th</sup> day after grafting of tumour cells. Estimation of tumour volumes was accomplished on the days: 6, 7, 8, 10 and 13 after tumour cells grafting. Starting from day 7, mean volumes of tumours from HR – fed group were significantly lower (p<0.05) than in the control, water-fed animals. Angiogenic activity of cells isolated from tumours of HR-fed group,

**Table 2.** The effect of HR administration on VEGF concentration in L-1 sarcoma tumours

Group of mice	VEGF concentration (pg/ml) (x±SE)	Significance of difference
HR fed mice n=18	819±14	p<0.01
Controls n=14	515±29	

measured by skin test, was significantly lower than that of the controls (Fig. 1). However, VEGF concentration was significantly higher in tumour cell homogenates derived from HR-fed mice than in tumours from untreated mice (Table 2).



**Fig. 1.** Angiogenic activity of cells isolated from tumours of control or HR fed mice

**Table 3.** The effect of HR administration (3x30 µl daily, during 3 days after intradermal grafting of sarcoma L-1 tumour cells) on the cutaneous angiogenesis induced by these cells in mice

Group of mice	Number of tests	Mean number of newly formed blood vessels (x±SE)	Statistical significance of difference from the control
Controls	18	52.7±1.8	p<0.01
HR-fed mice	18	37.9±3.1	

### Cutaneous angiogenesis assay

When HR was fed to mice for 3 days after sarcoma cells intradermal grafting, diminution of neovascular response was observed in comparison to the control group (Table 3).

### Bacterial infection

A significantly decreased number of bacteria in *P. aeruginosa* – infected mice after administration of HR as compared with the control group was demonstrated (Table 4).

### Discussion

The studied complex remedy caused inhibition of L1 sarcoma development and tumour angiogenesis in spite of increased VEGF concentration in the tumour cells. The mechanism of this phenomena is not clear. However, it might be connected with the ability of catechins to interfere with VEGF binding to its receptors, such phenomenon was described by Kondo et al [23]. As a result, at the presence of undisturbed VEGF production, accumulation of this cytokine might arise. We observed similar phenomenon in embryos of pregnant mice fed chocolate – lower angiogenic activity and higher VEGF concentration than in controls [24]. In fact, HPLC analysis of cocoa, one of the compounds of our herbal remedy, revealed the presence of substantial amounts of catechin and epigallocatechin.

Khanna et al [25], in different experimental system reported increased VEGF expression in cultures of keratinocytes in the presence of polyphenolic bioflavonoids, proanthocyanidins.

Polyphenols are known to inhibit a wide variety of enzymatic activities associated with cell activation, cell proliferation and tumour progression. Some of these effects are mediated by blocking of VEGF binding to its receptors, inhibition of tyrosine kinase activity and VEGF receptor phosphorylation [26, 27]. It was also reported that tea polyphenols, among them epigallocatechin (also present in cocoa) and flavonoids (present in Echinacea extract) inhibit activities of important for angiogenesis enzymes, metalloproteinases and some serine proteases, in particular, the urokinase-type plasminogen activator - plasmin system [28, 29].

As mentioned above, we have not found in the literature papers about inhibitory action of cocoa or chocolate on

**Table 4.** Mean number of bacteria in livers of *Pseudomonas aeruginosa* – infected mice after administration of HR

CFU/g liver±SE (x10 <sup>5</sup> )		Statistical significance of difference from the control
HR fed mice	Controls	
2.22±0.51 n = 6	3.68±0.32 n = 6	p<0.01

\*Mice received orally HR/2x30 µl daily for 10 days before infection  
\*\*The control group received orally distilled water

angiogenesis, however, there are reports about such effect exerted by catechins present in green tea. Some of these catechins are common for tea and cocoa – for example catechin and epigallocatechin. Demeule et al [8] reported that green tea catechins, in addition to their antioxidative properties, also affect the molecular mechanisms involved in angiogenesis, extracellular matrix degradation, regulation of cell death and multidrug resistance.

Drinking green tea and its polyphenols significantly prevented corneal neovascularization induced by VEGF (30) and also inhibited angiogenesis in other standard animal angiogenesis models [6, 7].

Carneseccchi et al [31] have found, that flavanols and procyanidins of cocoa and chocolate inhibit growth and polyamine biosynthesis of human colonic cancer cells. Kozikowski et al [32] also described inhibition of cancer cell growth by epicatechin-derived procyanidins, through cell cycle arrest.

Regarding the third component of our remedy, garlic (*Allium sativum*), anti-angiogenic and antitumour activities of its compounds has been described. Shukla et al [4] reported inhibitory effect of diallyl sulfide on angiogenesis in Ehrlich ascites tumour-bearing mice. Thioallyl compounds were described as potent inhibitors of endothelial and tumour cells proliferation [5]. In the study of Tang et al [33] garlic prevented oral precancer induced in rats by chemical carcinogen. The potential application of garlic for the treatment of bladder cancer was discussed by Lamm and Riggs [20]. Diallyl disulfide caused growth retardation of human breast cancer cell lines transplanted to nude mice [34].

The results of the present study also demonstrated that the administered preparation caused inhibition of *Pseudomonas aeruginosa* growth. Analysing individual components of Alchinal, it can be seen that *Echinacea* extract contains many compounds with immunomodulatory activity – polysaccharides, alkamides, polyphenols, glycoproteins. Some of these compounds have also anti-inflammatory activity [35, 36]. Other authors [37, 38] reported that polysaccharides from *Echinacea purpurea* stimulate the activity of phagocytic cells. These authors described stimulatory effect of *Echinacea purpurea* on non-specific immunity of mice *in vivo* and *in vitro*, resulting

in protection against systemic infections with *Listeria monocytogenes* and *Candida albicans*.

Another component of Alchinal – cocoa, exerted inhibitory effect on the growth of a variety of bacteria (e.g. *Shigella*, *Staphylococci*, etc) in various media [16]. Cocoa may cause inhibition of the biosynthesis of polysaccharides by both cell-free and cell – associated streptococcal glucosyltransferases [39]. Another study suggested that antioxidant cocoa liquor polyphenols (major component of chocolate) exert immunoregulatory effects [40]. It has been also shown that garlic compounds, diallyl sulphide and diallyl disulphide possess bactericidal effects [19, 41].

In summary it can be said that the preparation used in our study caused modifications of angiogenesis and tumour growth and also reduction of bacterial infection in mice. Therefore, it seems that the substances contained in Alchinal modulate certain parameters of antitumour and antibacterial immunity.

Further studies could help elucidate the mechanism of these phenomena and create an interesting perspective of possible use of Alchinal as tumour angiogenesis inhibitor in the treatment of malignancies (in combination with classic methods) in humans, particularly in patients with impaired immunity. The preparation would also be used for limiting the development of opportunistic infections.

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#### References

1. Folkman J (1990): What is the evidence that tumours are angiogenesis-dependent? *J Nat Cancer Inst* 83: 4-6.
2. Folkman J (1995): Clinical applications of research on angiogenesis. *N Engl J Med* 333: 1747-1763.
3. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N (1989): Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 246: 1306-1309.
4. Shukla Y, Arora A, Singh A (2002): Antitumorogenic potential of diallyl sulfide in Ehrlich ascites tumour bearing mice. *Biomed Environ Sci* 15 (1): 41-47.
5. Lee ES, Steiner M, Lin R (1994): Thioallyl compounds: potent inhibitors of cell proliferation. *Biochim Biophys Acta* 10; 1221 (1): 73-77.
6. Cao Y, Cao R, Brakenhielm E (2002): Antiangiogenic mechanisms of diet-derived polyphenols. *J Nutr Biochem* 13 (7): 380-390.
7. Jung X D, Ellis LM (2001): Inhibition of tumour invasion and angiogenesis by epigallocatechin gallate (EGCG) a major component of green tea. *Int J Exp Pathol* 82 (6): 309-312.
8. Demeule M, Michaud-Levesque J, Annabi B, et al. (2002): Green tea catechins as novel antitumour and antiangiogenic compounds. *Curr Med Chem Anti-Canc Agents* 2 (4): 441-463.
9. Spencer RC (1996): Predominant pathogens found in the European prevalence of infection in intensive care study. *E J Clin Microb Infect Dis* 15: 281-285.
10. Pruitt BA, McManus AT, Kim SH, et al. (1997): Burn wound infections: current status. *World J Surg* 22: 135-145.
11. Griffith SJ, Nathan RK, Selender RK, et al. (1989): The epidemiology of *Pseudomonas aeruginosa* in oncology patients in a general hospital. *J Inf Dis* 160: 1033-1036.
12. Korvick JA, Marsh JW, Starzl TE, et al. (1991): *Pseudomonas aeruginosa* bacteremia in patients undergoing liver transplantation: an emerging problem. *Surgery* 109: 62-68.
13. Bodinet C, Mentel R, Wegner U, et al. (2002): Effect of Oral Application of an Immunomodulating Plant Extract on Influenza Virus Type A Infection. *Planta Medica* 68: 896-900.
14. Colic M, Vucevic D, Kilibarda V, et al. (2002): Modulatory effects of garlic extracts on proliferation of T-lymphocytes in vitro stimulated with concanavalin. *A. Phytomedicine* 9: 117-124.
15. Melchart D, Linde K, Worku F, Bauer R, Wagner H (1994): Immunomodulation with Echinacea—a systematic review of controlled clinical trials. *Phytomedicine* 1: 245-254.
16. Park CE, Stankiewicz ZK, Rayman MK, et al. (1979): Inhibitory effect of cocoa powder on the growth of a variety of bacteria in different media. *Can J Microbiol* 25 (2): 233-5.
17. Currier NL, Miller SC (2000): Natural killer cells from aging mice treated with extracts from *Echinacea purpurea* are quantitatively and functionally rejuvenated. *Exp Gerontol* 35 (5): 627-639.
18. Currier NL, Miller SC (2001): *Echinacea purpurea* and melatonin augment natural-killer cells in leukemic mice and prolong life span. *J Altern Complement Med* 7 (3): 241-251.
19. Chen GW, Chung JG, Ho HC, Lin JG (1999): Effects of the garlic compounds diallyl disulphide and diallyl disulphide on arylamine N-acetyltransferase activity in *Klebsiella pneumoniae*. *J Appl Toxicol* 19: 75-81.
20. Lamm DL, Riggs DRY (2000): The potential application of *Allium sativum* (garlic) for the treatment of bladder cancer. *Urol Clin North Am* 27 (1): 157-162.
21. Sidky YA, Auerbach R (1975): Lymphocyte-induced angiogenesis a quantitative and sensitive assay of the graft-vs-host reaction. *J Exp Med* 141: 1084-1100.
22. Skopińska-Różewska E, Piazza GA, Sommer E, et al. (1998): Inhibition of angiogenesis by sulindac and its sulfone metabolite (FGN-1): A potential mechanism for their antineoplastic properties. *Int J Tissue React* XX. 3: 85-89.
23. Kondo T, Ohta T, Igura K, Hara Y, Kaji K (2002): Tea catechins inhibit angiogenesis in vitro measured by human endothelial cell growth, migration and tube formation, through inhibition of VEGF receptor binding. *Cancer Lett* 28 (180): 139-144.
24. Skopińska-Różewska E, Borowska A, Skopiński P, et al. (2003): The effect of theobromine or chocolate feeding on pregnant mice and their offspring. *Immun Letters* 87: 286, Greece 15<sup>th</sup> Europ Immun Cong, June 8-12.
25. Khanna S, Roy S, Bagchi D, Bagchi M, Sen CK (2001): Upregulation of oxidant-induced VEGF expression in cultured keratinocytes by a grape seed proanthocyanidin extract. *Free Radic Biol Med* 31 (1): 38-42.
26. Liang YC, Lin-shiau SY, Chen CF, Lin JK (1997): Suppression of extracellular signals and cell proliferation through EGF receptor binding by (-)-epigallocatechin gallate in human A431 epidermoid carcinoma cells. *J Cell Biochem* 67 (1): 55-65.
27. Lamy S, Gingras D, Beliveau R (2002): Green tea catechins inhibit vascular endothelial growth factor receptor phosphorylation. *Cancer Res* 15; 62 (2): 381-385.
28. Saito M, Saito K, Kunisaki N, et al. (2002): Green tea polyphenols inhibit metalloproteinase activities in the skin, muscle, and blood of rainbow trout. *J Agric Food Chem* 20; 50 (24): 7169-7174.

29. Kim MH (2003): Flavonoids inhibit VEGF/bFGF-induced angiogenesis in vitro by inhibiting the matrix-degrading proteases. *J Cell Biochem* 89 (3): 529-538.
30. Cao Y, Cao R (1999): Angiogenesis inhibited by drinking tea. *Nature* 398: 381.
31. Carnasechci S, Schneider Y, Lazarus S, Coehlo D, Gosse F, Raul F (2000): Flavanols and procyanidins of cocoa and chocolate inhibit growth and polyamine biosynthesis of human colonic cancer cells. *Cancer Lett* 175: 157-156.
32. Kozikowski A, Tuckmantel W, Bottcher G, et al. (2003): Studies in Polyphenol Chemistry and Bioactivity. 4. Synthesis of Trimeric, Tetrameric, Pentameric, and Higher Oligomeric Epicatechin-Derived Procyanidins Having All -4beta, 8-Interflavan Connectivity and Their Inhibition of Cancer Cell Growth through Cell Cycle Arrest. *J Org Chem* 68: 1641-1658.
33. Tang Z, Sheng Z, Liu S, et al. (1997): The preventing function of garlic on experimental oral precancer and its effect on natural killer cells, T-lymphocytes and interleukin-2. *Human Yi Ke Da Xue Bao* 22 (3): 246-248.
34. Nakagawa H, Tsuta K, Kiuchi K, et al. (2001): Growth inhibitory effects on diallyl disulfide on human breast cancer cell lines *Carcinogenesis* 22 (6): 891-897.
35. Facino RM, Carini M, Aldini G, Saibene L, et al. (1995): Echinacoside and caffeoyl conjugates protect collagen from free radical-induced degradation: A potential use of Echinacea extracts in the prevention of skin photodamage. *Planta Med* 61: 510-514.
36. Clifford LJ, Nair MG, Rana J, Dewit DL (2002): Bioactivity of alkamides isolated from *Echinacea purpurea* (L) Moench. *Phytomedicine* 9: 249-253.
37. Roesler J, Steinmuller C, Kiderlen A, Emmendorffer A, Wagner H, Lohmann Mathes ML (1991): Application of purified polysaccharides from cell cultures of the plant *Echinacea purpurea* to mice mediates protection against systemic infections with *Listeria monocytogenes* and *Candida albicans*. *Int J Immunopharmacol* 13 (1): 27-37.
38. Steinmuller C, Roesler J, Grottrup E, Franke G, Wagner H, Lohmann-Mates ML (1993): Polysaccharides isolated from plant cell cultures of *Echinacea purpurea* enhance the resistance of immunosuppressed mice against systemic infections with *Candida albicans* and *Listeria monocytogenes*. *Int J Immunopharmacol* 15 (5): 605-614.
39. Paolino VJ, Kashket S (1985): Inhibition by cocoa extracts of biosynthesis of extracellular polysaccharide by human oral bacteria. *Arch Oral Biol* 30 (4): 359-63.
40. Sanbongi C, Suzuki N, Sakane T (1997): Polyphenols in chocolate, which have antioxidant activity, modulate immune functions in human in vitro. *Cell Immunol* 177 (2): 129-36.
41. Chung JG, Chen GW, Wu LT, et al. (1998): Effects of garlic compounds diallyl sulfide and diallyl disulfide on arylamine N-acetyltransferase activity in strains of *Helicobacter pylori* from peptic ulcer patients. *Am J Clin Med* 26: 353-364.