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Mutagenic and antimicrobial evaluation of methanol extract from *Allium kharputense* Freyn Et. Sint

Allium kharputense Freyn Et. Sint metanol ekstraktının mutajenik and antimikrobiyal yönüyle değerlendirmesi

Elif Ayse Erdogan[1], Erdal Yabalak[2], Ayse Everest[1], Ahmet Murat Gizir[2]

[1]Department of Biology, Faculty of Arts and Science, Mersin University, TR-33343, Turkey
[2]Department of Chemistry,Faculty of Arts and Science, Mersin University, TR-33343, Turkey

ABSTRACT
Aim: *Allium kharputense* Freyn Et. Sint (Liliaceae) is a wild species and grows naturally in eastern Turkey, Iraq, and Iran. This plant is widely used in traditional medicine in the world and to the best of our knowledge its antimicrobial and mutagenic activities had never been investigated before. For this, we firstly reported antimicrobial and mutagenic properties of the methanol extract (MtE) from *Allium kharputense*.
Methods: In the present study, antimicrobial and mutagenic activity of methanol extract (MtE) from *Allium kharputense* wasinvestigated with microdilution method and *Umu*-test *Salmonella typhimurium* TA1535/pSK1002, respectively.
Results: We reported that MtE of *A. kharputense* is quite active on *Bacillus subtilis* and *Candida neoformans* with the MIC (Minimum Inhibition Concentration) concentrations of 62.5 mg/ml and 7.8 mg/ml, respectively. Furthermore, we determined that the concentration of 31.2 mg/ml of methanol extract (MtE) of the plant has the mutagenic potencial with metabolic activation S9 mix.
Keywords:*Allium kharputense*, *Umu*-test, Microbroth, Mutagenic, Antimicrobial.

ÖZET
Amaç:*Allium kharputense* Freyn Et. Sint (Liliaceae) Doğu Türkiye, Irak ve İran'da doğal olarak büyüyen yabani bir türdür. Bu bitki, dünyada geleneksel tıpta yaygın olarak kullanılmakta ve bildiğimiz kadarıyla bitkinin antimikrobiyal ve mutajenik aktivitesi daha önce araştırılmamıştır. Bunun için, *Allium kharputense* metanolik ekstraktının antimikrobiyal ve mutajenik özelliklerini ilk kez araştırdık.
Yöntemler: Bu çalışmada, *Allium kharputense* metanolik ekstraktınınantimikrobiyal ve mutajenik aktivitesi sırasıyla, mikrodilüsyon ve *Umu*-test *Salmonella typhimurium* TA1535/pSK1002 testleriyle araştırıldı.
Bulgular: *A. kharputense* metanolik ekstraktının, *Bacillus subtilis* ve *Candida neoformans* üzerinde, sırasıyla MIC (Minimal İnhibisyon Konsantrasyon); 62.5 mg/ml ve 7.8 mg/ml konsantrasyonlarında oldukça etkili olduğunu ortaya çıkardık. Ayrıca, bitkinin 31.2 mg/ml konsantrasyonundaki metanolik ekstraktının (MtE), S9 metabolik aktivasyon varlığında, mutajenik potansiyele sahip olduğunu belirledik.
Anahtar kelimeler:*Allium kharputense*; *Umu*-test; Mikrobroth; Mutajenik; Antimikrobiyal.

CorrespondingAuthor:
Elif Ayse erdogan,
Mersin University Biology Department
eliferdogan81@gmail.com

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INTRODUCTION

Allium species, (Liliaceae), have been used worldwide for centuries [1-3]. Containing powerful cysteine sulphoxides and other numerous phenolic compounds biological activity of *Allium* species have been investigated by various researchers and it is shown that this genus has a variety of pharmacological effects such as antitumor [4,5], antifungal [6], antioxidant [7], antimicrobial, antibacterial [8,9], tumor cell growth inhibitory effect and chemopreventive activity [10].

Several compounds such as cysteine sulfoxides [11], fatty acid [12], phenolic constituents [7], and essential oil [13] have been investigated and identified from the genus *Allium*. Especially, *A. vineale*, *A. cepa*, *A. sativum*, *A. tripedale*, *A. siculum* are very important species in traditional medicine and as a daily nutrient [2,8,11].

The biological activity of plant extracts has formed the basis of pharmaceuticals, ethnopharmacology and natural therapies. The species of either wild or cultivated *Allium* have long been known for their antimicrobial activity against Gram-positive, Gram-negative and fungi for years. Thispowerful antimicrobial effect is believed to be due to their thiosulfinates enzymatically generated from *S*-alk(en)yl-Lcysteine sulfoxides [14].

Allium kharputense is a wild species and grow naturally in Eastern Turkey [15], and Iran [16]. To the best of our knowledge, its antimicrobial and mutagenic activity have never been researched before.

In the present study, *Umu*-test and Broth microdilution technique were chosen for a detailed investigation of MtE of *A. kharputense*for mutagenicity and antimicrobial effect, respectively.

Umu-test system is a method to detect gene mutations, damages in DNA or genes caused by a external agent, using the expression of one of the SOS genes. SOS is a global response in bacteria against to genotoxic agent and an error-prone repair system in which the cell cycle is arrested and mutagenesis induces. *Salmonella typhimurium* TA1535/pSK1002 carries the plasmid pSK1002 in which the umuC' gene is fused inframe to the lacZ'gene which is the structural gene for β-galactosidase. Therefore, when SOS response is activated by the genotoxic chemical, the activity of β-galactosidase is induced and easily measured with a chromogenic substrate by hydrolyzing this colorless compound to a colored one [17]. It has been determined mutagenic and genotoxic agent, using the expression of the SOS genes has some advantages because of its simplicity, precision and rapidity. Furthermore, *Umu*-test has a good correlation to those of the Ames test in which the revertant colonies are counted. [18, 19].

MATERIALS AND METHODS

Plant material

Allium kharputense were collected in April from Sırnak, Turkey, and the botanical identification of the plant has been done according to Davis's book [20].

Methanol Extraction (MtE)

Samples (10g) were extracted with 200 ml of methanol in a Soxhlet apparatus. Then, they were filtered and reduced to approximately 10 ml at 40 ºC by using rotary evaporator.

Antimicrobial activity

Minimal Inhibitory Concentration (MIC) of MtE of *A. kharputense* was determined by broth microdilutiontechnique and interpreted according to the guidelines set by Clinical and Laboratory Standards Institute (CLSI) [21]. The antimicrobial effects of extract were assessed on several pathogens, namely *Escherichia coli* (ATCC 25293), *Enterococcus feacalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633), *Salmonella thyphimurium*, *Staphylococcus aureus* (ATCC 25925), *Klebsiella pneumoniae* (10031), *Candida albicans* (clinic strain), *Candida neoformans*, *Candida parapsilosis* (ATCC 22019). The bacteria inoculum was prepared in 4 ml-Triptic Soy Broth medium and incubated at 37 ^{o}C, overnight. The yeast culture was grown in Sabouraud Dextrose Broth medium at 28 ^{o}C for 24 h before being used. Then, the culture suspensions were adjusted to 0.5 McFarland Standard Turbidity (~ 10^4 for bacteria and ~ 10^3) for yeast colony forming unit (CFU) per milliliter [22].

Ampisiline and fluconazole antibiotics were used as positive reference standards for bacteria and fungal strains, respectively. Two-fold serial dilutions of *A. kharputense* MtE were added to the wells of a 96-well microtiter plate. Then, the cell suspensions (100 µl) were added to the wells. For the negative control, the suspensions without microorganisms were used. The plates were incubated at 37 ºC for 24 hours and the growth (turbidity) was measured at 600 nm.

Umu-test

For mutagenicity evaluation, using the*umu* test kit, *Salmonella typhimurium* TA1535/pSK1002, was bought from (DSMZ, Germany). Lyophilized *Salmonella typhimurium* TA1535/pSK1002 was thawed with 2 ml of TGA medium (1 % trypton, 0.5 % NaCl, 0.2 % glucose) and incubated at 37 °C for 3 h. Then, the bacteria suspension was adjusted the absorbance of 0.25–0.3 at a wave length of 600 nm.

A 10 ml portion of the *A. kharputense* MtE was poured into each well of a 96-well microplate. At least six concentrations were tested for *A. kharputense* MtE. Three replicate wells were prepared for each concentration of the extract. The mutagenicity test of *A. kharputense* MtE was performed with and without metabolic activation (S9) which is a mixture of the microsome fraction of a rat liver with coenzymes such as reduced nicotinamide adenine dinucleotide phosphate and reduced nicotinamide adenine dinucleotide. Two positive control reagents were used: 2-aminoanthracene (2AA) for the test with metabolic activation and furylfuramide (AF-2) for the test without metabolic activation. The concentrations of 2AA tested were 0.05 $mg.ml^{-1}$, 0.1 $mg.ml^{-1}$, 0.5 $mg.ml^{-1}$, 1 $mg.ml^{-1}$, 5 $mg.ml^{-1}$, and 10 $mg.ml^{-1}$. Those of AF-2 were 0.5 $ng.ml^{-1}$, 1 $ng.ml^{-1}$, 5 $ng.ml^{-1}$, 10 $ng.ml^{-1}$, 15, and 100 $ng.ml^{-1}$. The 10 ml portion of 2AA or AF-2 solutions was added into the positive control well. Negative control wells were also prepared on each microplate by pouring the 10 ml portion of %10 methanol. For the mutagenic evaluation without S9, A 100 µL portion of the bacteria suspension was added into each well of a 96-well microplate into which a 10 µL portion of *A. kharputense* MtE solution had already been poured. For the mutagenic evaluation with S9, a 0.5 ml portion of S-9 mix solution was mixed to the remainder (5 ml) of the bacteria suspension and a 100-ml portion of this suspension was added to each well into which the 10 µL portion of *A. kharputense* MtE solution had already been poured. Then, All of the plates were incubated at 37°C for 2 h. After incubation, a 100-ml portion of O-Nitrophenil-β-D-galactopyranoside solution was added to each well.

The bacteria suspension with substrat was incubated for 1 h at 37°C. After last incubation, a 100 ml portion of DMSO was added to each well to stop the enzymatic reaction. Then, the absorbance at 620 nm was measured by a spectrophotometer for microplates. The relative activity of the β-galactosidase, which indicates the mutagenicity of *A. kharputense* MtE at that concentration, was calculated by subtracting the absorbance of the negative control well from that of the sample well ([18].

SPSS 22 software and one-way ANOVA followed by Tukey's multiple comparison test were used. The only value $p \leq 0.05$ was considered at Table 1 and 2.

Table 1. Antimicrobial activity of *Allium kharputense* against Gram positive, Gram negative and yeast. Minimal inhibitory concentrations (MIC) of methanol extraction was determined as the essential concentration that gave no visible growth after over night incubation. Amp: ampicillin, Flu: Fluconazole. Values were expressed as Mean ± SD, n = 9*; compared between groups. $P \leq 0.05$.

Microorganisms	mg/ml	Amp/Flu
Escherichia coli g(-)	>125 ± 0.002	0,48
*Klebsiella pneumoniae*g (-)	>125 ± 0.001	0,48
*Salmonella thyphimurium*g (-)	>125 ± 0.001	0,48
*Enterococcus feacalis*g (+)	>125 ± 0.012	0,48
*Staphylococcus aureus*g (+)	>125 ± 0.005	0,48
Bacillus subtilis g (+)	62,5 ± 0.003	1,9
Candida albicans	>125 ± 0.013	0,48
Candida neoformans	7,8 ± 0.001	0,48
Candida parapsilosis	>125 ± 0.002	0,48

RESULTS

The antimicrobal activity and mutagenic activity of methanol extract from *A. kharputense* have been shown in Table 1, Table 2 and Figure 1. The plant showed maximum antibacterial activity with 62.5 mg/ml against *Bacillus subtilis* G (+) strain among the test bacteria used. Slightly, no antibacterial effect was observed against *Escherichia coli*, *Enterococcus feacalis*, *Salmonella thyphimurium*, *Staphylococcus aureus* and *Klebsiella pneumonia* (Table 1) in the range of 125 mg/ml and 3.9 mg/ml. The plant inhibited *Candida neoformans* at 7.8mg/ml MIC. It did not inhibit the growth of *Candida albicans* (clinic strain),*Candida parapsilosis* (Table 1) in the range of 125 mg/ml and 3.9 mg/ml.

Results of the mutagenic property of different concentrations of MtE from *A. kharputense* are presented in Table 2. We reported that any mutagenicity was not determined in the SOS response of S. *typhimurium* TA1535/pSK1002 induced with MtE of *A. kharputense* in the experiment without S9. However, MtE of *A. kharputense* has mutagenic effect in the presence S9 at 31,25 mg/ml. When incubated with S9, MtE of *A. kharputense* was more effective than the one without S9 cultures. Because, absorbance value of relative β-galactosidase activity of MtE of *A. Kharputense* (31,25 mg/ml) was 1.019 and this value was two times greater than the absorbance of negative control (0.461). Our study demonstrated that MtE of *A. kharputense* induced moderate mutagenic activity on S. *typhimurium* TA1535/pSK1002.

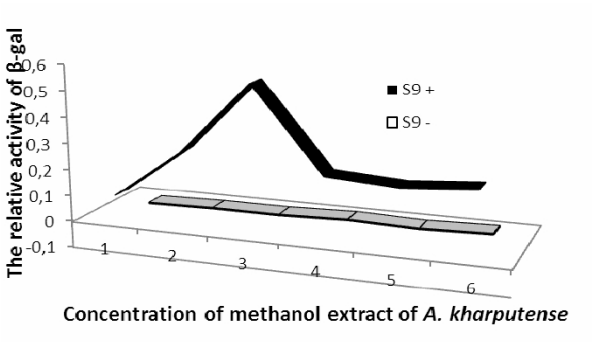

Figure 1. The relative activity of the β-galactosidase. The activity was calculated by substrating the absorbance of negative control well from that of sample well. Data are representative of three experiments performed in triplicate.

DISCUSSION

In the present study, the MtE of *A. kharputense* was screneed its antimicrobial activity and genotoxic properties in different concentrations. It was shown that *A. kharputense* have specific activities to some microorganisms. *Bacillus subtilis* (MIC:62.5 mg/ml) and *Candida neoformans* (MIC:7.8 mg/ml) were found to be more susceptible to *A. Kharputense.* Dziri and coworkers reported that MtE of *A. roseum* var. *odoratissimum* leaves, flowers, stalks and bullbs have strong antimicrobial effect on *Bacillus subtilis* according to disk difusion method [7]. Santas and coworkers reported that methanol extract of *Allium cepa* had antimicrobial activity on *Bacillus subtilis* because of the onion's quercetin and kaempferolcompounds [23]. They also indicated that methanolic leaf extract, an interspesific hybrid of *A. cepa* and *A. sativum* had high antimicrobial activity to fungi [24].

Table 2. The average absorbance (Avg. Abs) value of β-galactosidase at 600 nm. Conc: Concentration. Values were expressed as Mean±SD (standard deviation), n=6*; compared between groups. P ≤ 0.05. S9(**+**)**:** fraction with metabolic activation S9(**+**)**:** fraction without metabolic activation.

Conc. (mg/ml)	**Avg. Abs.**		**Avg. Abs.**	
	S9 +	**Sd**	**S9 -**	**Sd**
125	0.556	± 0.089	0.076	± 0.005
62.5	0.747	± 0.040	0.077	± 0.002
31.2	1.019	± 0.071	0.077	± 0.001
15.6	0.704	± 0.144	0.086	± 0.005
7.8	0.687	± 0.198	0.078	± 0.004
3.9	0.559	± 0.259	0.086	± 0.004
Negative Control	**0.461**	± **0.136**	**0.079**	± **0.005**

According to Table 1, the MtE of *A. kharputense* has lower antimicrobial avtivity than some *Allium* species in the literature. The differences of antimicrobial activities may be due to different antimicrobial procedure and ecological factor of plants. It is common knowledge that Allium species has different kinds and levels of sulfoxides distinctive to each species [14].

Recently, the *Umu*-test has been performed to evaluate the mutagenic and antimutagenic effects of several plant extracts by measuring the induction of the bacterial SOS response in *Salmonella typhimurium* TA1535/pSK1002 [25-27]. As shown in Table 2, mutagenicity of MtE of *A. kharputense* was performed at six concentrations (125 mg/ml, 62.5 mg/ml, 31.2 mg/ml, 15.6 mg/ml, 7.8 mg/ml, 3.9 mg/ml). Relative β-galactosidase activity was increased with the increase of extract concentration. After the point of maximum relative β-galactosidase activity level (31.2 mg/ml), this activity decreased with the increase of *A. kharputense* extract concentration. This concentration point is mutagen for *S.typhimurium* TA1535/pSK1002. Mutagenic strength at 31.2 mg/ml is presented in Figure 1. When the concentration of extract is at the strength of 31.2 mg/ml, MtE of *A. kharputense* is estimated to be mutagenic in the presence of S9 for human body. The starting range of β-galactosidase curve was approximately zero, and the enzyme activity increased with the increase of concentration of plant extract. After maximum level of β-galactosidase enzyme, β-galactosidase activity decreased because the proliferation of bacterial cells was inhibited by the mutagen agent. When the cells are exposed with a lot of DNA lesions, the cells tend to die, whereas those with fewer lesions can survive, exhibiting revise cellular functions or nature. Sometimes, such cells may be a reason for developmental abnormalities in human body. Therefore, mutagenicity has been known to be correlated with to carcinogenicity positively for years. It is determined that some herbs can be efficient in preventing mutagenic effect, so for this activity, they need to be metabolised by S9 to produce antimutagenic activity [19]. Some *Allium* species like *Allium sativum* [28] and *Allium cepa* [29]have antimutagenic activity and have demonstrated their chemopreventive activity because of organosulfur compounds derived from *Allium* [30].

In our study, antimicrobial and mutagenic activity of methanol extract of plant have been investigated at the same concentration. We demonstrated that low antimicrobial activity of an extract have a highly mutagenic potential with the S9 fraction in human body.

CONCLUSION

Data which obtained in antimicrobial and mutagenic activity (with S9 fraction) in this study exhibit the importance of MtE of Allium kharputence as a possible medical plant in the development of specific effected products.

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CONFLICT OF INTEREST

The authors of this study declare there are not any conflicts of interest.

REFERENCE

1. Block E. The chemistry of garlic and onions. SciAm. 1985;252:114–119.
2. Najjaa H, Ammar E, Neffati M. Antimicrobial activities of protenic extracts of Allium roseum L., a wild edible species in North Africa. J Food Agric Environ. 2009; 7(3&4):150–154.
3. Demirtas I, Erenler R, Elmastas M, Goktasoglu A. Studies on the antioxidant potential of flavones of Allium vineale isolated from its water-soluble fraction. Food Chem. 2013; 136(1): 34–40.
4. Koch HP, Lawson LD. Garlic. The Science and Therapeutic Application of Allium sativum L. and Related Species. Baltimore, MD: Williams and Wilkins. pp. 1996; 213–220.
5. Gao CM, Takezaki T, Ding JH, Li MS, Taijima K. Protective effect of Allium vegeTables against both esophageal and stomach cancer: a simultaneous case-referent study of high-epidemic area in Jiangsu province, China. Cancer Science. 1999;90(6): 614–621.
6. Corea G, Fattorusso E, Lanzotti V. Saponins and flavonoids of Allium triquetrum. J Nad Prod. 2003;66(11): 1405–1411.
7. Dziri S, Hassen I, Fatnassi S, Mrabet Y, Casabianca H, Hanchi B, Hosni K.. Phenolic constituents, antioxidant and antimicrobial activities of rosy garlic (Allium roseum var. odoratissimum). Journal of Functional Foods. 2012;4(2): 423-432.
8. Fritsch RM, Keusgen M. Occurrence and toxonomic significance of cysteine sulphoxides in the genus Allium L. (Alliaceae). Phytochemistry. 2006:67(11):1127-1135.
9. Panomket P, Wanram S, Srivorasmas T, Pongprom N. Bioactivity of plant extract against Burkholderia pseudomallei. Asian Biomedicine. 2012;6(4): 619-623.
10. Reuther HD. Allium sativum and Allium ursinum, Part 2, Pharmacology and medicinal application. Phytomedicine, 1995;2(1): 73–91.
11. Kusterer J, Keusgen M. Cysteine sulfoxides and volatile sulfur compounds from Allium tripedale. J Agric Food Chem. 2010;58(2): 1129-1137.
12. Hu G, Lu Y, Wei D. Fatty acid composition of the seed oil of Allium tuberosum. Bioresour Technol, 2005;96(14): 1630-1632.
13. Najjaa H, Neffati M, Zourai S, Ammar E. Essential oil composition and antimicrobial activity of different extracts of Allium roseum L., a North African endemic species. Comptes Rendus Chimie. 2007;10(9): 820-826.
14. Kyung K.H. Antimicrobial properties of Allium species. Curr Opin Biotech. 2012;23(2): 142-147.
15. Tuncturk M, Sekeroglu N, Tuncturk Y, Ozgokce F. Vitami C contents of wild herbs used in the production of Van Herby Cheese. Proceedings of the Fifth Conference on Medicinal and Aromatic Plants of Southeast European Countries. 5. Proceedings of the Fifth Conference on Medicinal and Aromatic Plants of Southeast European Countris. Cabdirect. [on line]. 2008. Available at: Accessed on 2-5 september.
16. Eftekharinasab N, Zarei D, Paidar S, Moghadam MJ, Kahrizi D, Khanahmadi M. Chenari. P. Identification of wild medicinal plant in Dalahoo mountain and their used indigenous knowledge (Khermanshah, Iran). Ann Biol Res. 2012;3(7): 3234-3239.
17. Nakamura S, Oda Y, Shimada T, Oki I, Sugimoto K. SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: examination with 151 chemicals. Mutat Res Lett. 1987;192(4): 239-246.
18. Yamomoto A, Kohyama Y, Hanaws T. Mutagenicity evaluation of fortyone metal salts by the Umu-test. J Biomed Mater Res. 2002;59(1): 176-183.
19. Caillet S, Lessard S, Lamoureux G, Lacroix M. Umu-test applied for screening natural antimutagenic agents. Food Chem. 2011;124(4): 1699-1707.
20. Davis PH, Tan K, Mill RR. Flora of Turkey and The East Aegean Islands (Suppl.) Edinburg University Press, United Kingdom. 1988.
21. Dabur R, Gupta A, Mandal TK, Deepak Singh D, Bajpai Gurav AM, Lavekar GS. Antimicrobial activity of some Indian medicinal plants. Afr J Tradit Complement Altern Med. 2007;4(3): 313-318.
22. McFarland J. Standardizasyon bacteria culture for the disc diffusion assay. J Am Med Assoc. 1987;49: 1176-1178.
23. Santas J, Almajano MP, Carbo R. Antimicrobial and antioxidant activity of crude onion (Allium cepa, L.) extracts. Int J Food Sci Technol. 2010;45(2): 403-409.
24. Satya VK, Radhajeyalakshmi R, Kavitha K, Paranidharan V, Bhaskaran R, Velazhahan R. In vitro antimicrobial activity of zimmu (Allium sativum L. Allium cepa L.) leaf extract. Arch Phytopathology Plant Protect. 2005;38(3): 185-192.
25. Uenobe F, Nakamura S, Miyazawa M. Antimutagenic effect of resveratrol against Trp-P-1. Mutat Res. 1997;373(2): 197-200.
26. Miyazawa M, Hisama M. Suppression of chemical mutagen-induced SOS response by alkylphenols from clove (Syzygium aromaticum) in the Salmonella typhimurium TA1535/pSK1002 umu test. J Agric Food Chem. 2001;49(8): 4019-4025.
27. Verschaeve L, Van Staden J. Mutagenic and antimutagenic properties of extracts from South African traditional medicinal plants. J Ethnopharm. 2008;119(3): 575-587.
28. Kim S, Kim J, Lee S. Antimutagenic compounds identified from the chloroform fraction of garlic (Allium sativum). J Korean Soc Food Sci Nutr. 1991;20(3): 253–259.
29. Singh BN, Singh BR, Singh RL, Prakash D, Singh DP, Sarma BK, Upadhyay G, Singh HB. Polyphenolics from various extracts/fractions of red onion (Allium cepa) peel with potent antioxidant and antimutagenic activities. Food Chem Toxicol. 2009;47(6): 1161-1167.
30. Thomsan M, Ali M. Garlic (Allium sativum): A rewiev of its Potential Use an an Anti-cancer Agent. Curr Cancer Drug Targets. 2003;3(1): 67-81.