in vivo *15:* 437-442 (2001)

Biological Activity of a Fruit Vegetable, "Anastasia Green", a Species of Sweet Pepper

NOBORU MOTOHASHI[1], TERUO KURIHARA[2], HIDETSUGU WAKABAYASHI[2], MIKAKO YAJI[2], ILONA MUCSI[3], JOSEPH MOLNÁR[3], SHICHIRO MARUYAMA[4], HIROSHI SAKAGAMI[4], HIDEKI NAKASHIMA[5], SATORU TANI[6], YOSHIAKI SHIRATAKI[6] and MASAMI KAWASE[6]

[1]*Meiji Pharmaceutical University, Kiyose, Tokyo, Japan;*[2]*Faculty of Science, Josai University, Sakado, Saitama, Japan;*
[3]*Faculty of Medicine, Institute of Microbiology, Albert Szent-Györgyi Medical University, Szeged, Hungary;*
[4]*Department of Dental Pharmacology, Meikai University School of Dentistry, Saitama, Japan;*
[5]*Department of Microbiology, St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan;*
[6]*Faculty of Pharmaceutical Sciences, Josai University, Sakado, Saitama, Japan*

Abstract. *Russian green sweet pepper (Anastasia Green) was successively extracted with hexane, acetone, methanol and 70% methanol and the extracts were further separated into a total of twenty fractions by silica gel or ODS column chromatographies. The biological activities of these extracts and fractions were compared. The extracts and fractions showed higher cytotoxic activity against two human oral tumor cell lines than against normal human gingival fibroblasts, suggesting their tumor-specific action. Several fractions [**H3, H4, A4**] reversed the multidrug resistant gene (MDR1) against L5178 mouse T-cell lymphoma more effectively than (±) verapamil (positive control). All extracts and fractions showed no anti-human immunodeficiency virus (HIV) nor anti-Helicobacter pylori activity. These data suggest the medicinal importance of an Anastasia Green extract.*

Sweet peppers, like tomatoes, cucumbers and melons, are popular "fruit vegetables". The Russian green sweet pepper(Anastasia Green) (Figure 1) of the *Solanaceae* is also an excellent fruit vegetable for raw consumption and cooking. Daily diets should keep and enhance our healthly condition, by containing many phytochemical nutrients such as amino acids, fats, dietary fibers, vitamins, minerals, flavonoids, carotenoids, polyphenols and other micronutrients, which can reduce the incidence of age-related diseases such as diabetes, hypertension and cancer (1).

It has been suggested that a low cancer incidence is associated with the comsumption of fiber rich-foods, fresh fruits, vegetables, vitamins and minerals in the diet (2). Fruits and vegetables are good for health, since they are rich in ß-carotene, vitamins (A, C, E), polyphenols, minerals, dietary fibers and chlorophyll, which might reduce mutagenicity, carcinogenicity and the incidence of other diseases (3, 4).

Carotenoids include carotenes and xanthophylls of carotenoid's oxygenated derivatives. ß-Carotene, a vitamin A precursor, has the most important physiological function in animal organisms (5). Some cancer cells are sensitive to antioxidants such as α-carotene, ß-carotene, xanthophylls and retinoids (5). Sweet pepper provides the carotenoids, ß-carotene (major component) and also α-carotene and ß-cryptoxanthin (6).

Sweet pepper, *Capsicum annuum*, is also an important source of ß-carotene and vitamin A with antimutagenic and anticarcinogenic activities. Surprisingly, Bell pepper (Pimiento) extract of one type of *Capsicum annuum* showed higher antimutagenic activity than ß-carotene or xanthophylls, and the effects of those nutrients were apparently synergistic (7).

The purpose of this paper was to investigate the biological activitly of sweet pepper named as "Anastasia Green", one species of *Capsicum annuum* L. var. angulosum Mill. (Solanaceae).

Materials and Methods

Materials. The following chemicals and reagents were obtained from the indicated companies: RPMI1640 medium, Dulbecco's modified Eagle medium (DMEM) (Gibco BRL, Grand Island, NY, USA), McCoy's 5A medium (Gibco BRL, Grand Island, NY, USA); fetal bovine serum (FBS) for RPMI medium (JRH Bioscience, Lenexa, KS, USA); horse serum for McCoy's 5A medium (Gibco BRL, Grand Island, NY, USA); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Wako Pure Chem. Ind., Ltd., Osaka, Japan); dextran sulfate (8 kD) (Kowa Chem. Co., Tokyo, Japan); curdlan sulfate (Ajinomoto Co., Tokyo, Japan); 3'-azido-2',3'-dideoxythymidine (AZT) (Sigma Chem. Co., St. Louis, MO, USA); metronidazole (Wako Pure Chem. Ind., Ltd., Osaka, Japan); erythromycin (Wako Pure Chem. Ind., Ltd., Osaka, Japan); rhodamine 123 (Sigma Chem. Co., St. Louis, MO, USA); (±)-

Correspondence to: Dr. Noboru Motohashi, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose-shi, Tokyo 204-8588, Japan. Tel and Fax: (+)-81-424-95-8953, e-mail: <motohasi@my-pharm.ac.jp>

Key Words: Russian green sweet pepper (Anastasia Green) extracts, cytotoxic activity, anti-HIV activity, anti-*H. pylori* activity, MDR reversal.

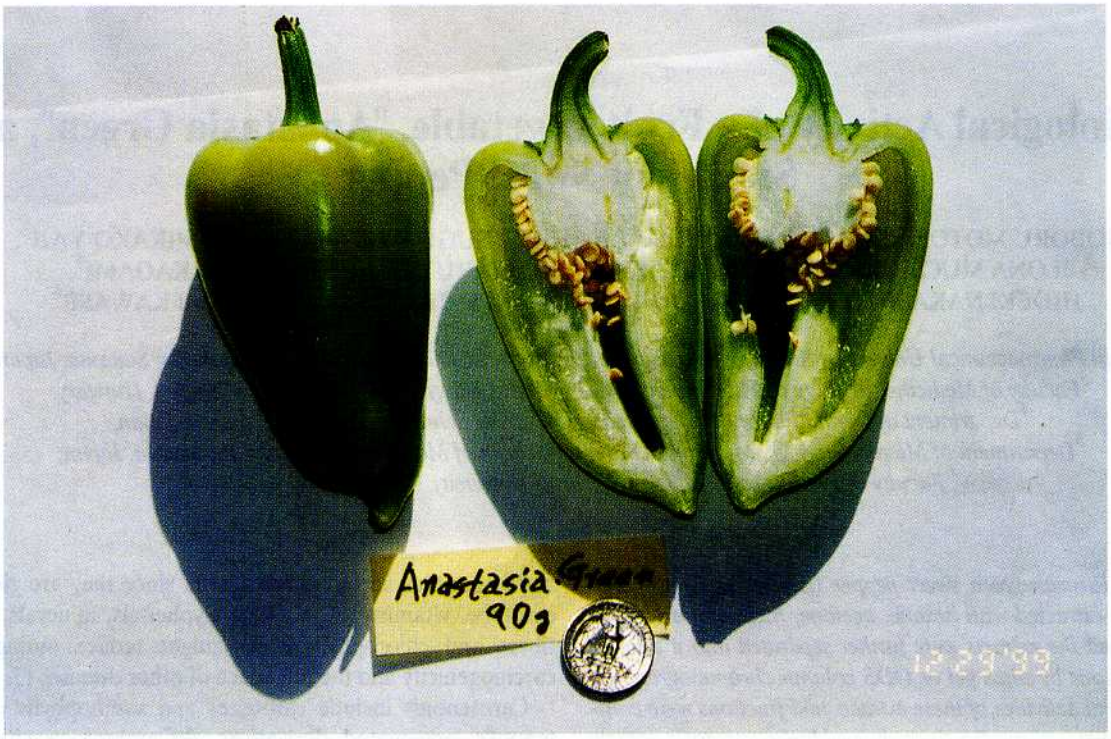

Figure 1. *(Color photo). Russian green sweet pepper (Anastasia Green).*

verapamil (Sigma Chem. Co., St. Louis, MO, USA). Clarithromycin was gifted from Taisho Pharmaceutical Co. (Tokyo, Japan). A strain of *Helicobacter pylori* (ATCC43504) was purchased from American Type Culture Collection (Rockville, MD, USA).

Dried powder of Anastasia Green, a variety of sweet pepper which is an unripe fruit of *Capsicum annuum* L. var. angulosum Mill. (Solanaceae) cultivated in heated greenhouses in the suburbs of Moscow and/or St. Peterburg in Russia, was kindly donated by Field Co. Ltd. (Shin-Beppu machi, Miyazaki, Japan). This is also cultivated in greenhouse in the laboratory farm attched to Field Co. Ltd. (Address: 1401-221 Maehama, Shin-Beppu machi, Miyazaki 880-0834, Japan; Tel: +81-985-24-1960; Fax: +81-985-24-1891) and a voucher specimen is deposited in the Herbarium of Josai University, Japan.

Preparation and fractionation of Anastasia Green extracts. Dried powders of Anastasia Green (1 kg) were successively extracted with hexane, acetone, MeOH and 70% MeOH at room temperature. After evaporation of the solvent *in vacuo,* the hexane extract **[H0]** (5.8 g), acetone extract **[A0]** (8.8 g), MeOH extract **[M0]** (237.0 g) and 70% MeOH extract **[70M0]** (284 g) were obtained, respectively (Figure 1). First, the aliquot of hexane extract **[H0]** (5.5 g) was applied to silica gel column chromatography, which was then eluted with a hexane-acetone gradient. The hexane-acetone (24:1) fraction **[H1]** (2.60 g), hexane-acetone (9:1) fraction **[H2]** (0.55 g), hexane-acetone (4:1) fraction **[H3]** (1.90 g) and acetone fraction **[H4]** (0.36 g) were step-wisely eluted. Secondly the acetone extract **[A0]** (7.3 g) was applied to silica gel column chromatography, which was then eluted with a benzene-EtOAc gradient. The benzene-EtOAc (24:1) fraction **[A1]** (0.55 g), benzene-EtOAc (9:1) fraction **[A2]** (0.37 g), benzene-EtOAc (4:1) fraction **[A3]** (0.99 g), benzene-EtOAc (1:1) fraction **[A4]** (0.32 g) and EtOAc fraction **[A5]** (1.60 g), were step-wisely eluted. Thirdly, the MeOH extract **[M0]** (34 g) was applied to silica gel column chromatography, which was then eluted with a CH_2Cl_2-MeOH gradient. The CH_2Cl_2 fraction **[M1]** (0.63 g), CH_2Cl_2-MeOH (49:1) fraction **[M2]** (0.85 g), CH_2Cl_2-MeOH (24:1) fraction **[M3]** (1.40 g), CH_2Cl_2-MeOH (9:1) fraction **[M4]** (1.30 g), **[M5]** (6.00 g), CH_2Cl_2-MeOH (4:1) fraction **[M6]** (18.0 g) and MeOH fraction **[M7]** (12.00 g) were step-wisely eluted. Finally, the 70% MeOH extract **[70M0]** (32 g) was applied to ODS column chromatography, which was then eluted with a H_2O-MeOH gradient. The H_2O-MeOH (2:1) fraction **[70M1]** (27.0 g), **[70M2]** (0.65 g), H_2O-MeOH (1:1) fraction **[70M3]** (0.12 g) and MeOH fraction **[70M4]** (1.20 g) were step-wisely eluted (Figure 2).

Assay for cytotoxic activity. Human oral squamous cell carcinoma (HSC-2) and human salivary gland tumor (HSG) cell lines and human gingival fibroblasts (HGF) (5-7 population doubling levels) were cultured in DMEM medium supplemented with 10% heat-inactivated FBS. These cells were incubated for 24 hours with the indicated concentrations of the test samples. The relative viable cell number (absorbance at 540 nm (A_{540})) was then determined by MTT assay. The 50% cytotoxic concentration (CC_{50}) was determined from the dose-response curve (Table I) (8).

Assay for anti-human immunodeficiency virus (HIV) activity. Human T-cell leukemia virus 1 (HTLV1)-bearing CD4-positive human T-cell lines, MT-4 cells, were infected with HIV-1_{IIIB} at a multiplicity of infection (m.o.i.) of 0.01. HIV- or mock-infected MT-4 cells (1.5×10^5/mL, 200 μL) were placed into 96-well microtiter plates and incubated in the presence of varying concentrations of each extract or fraction in RPMI1640 medium supplemented with 10% FBS. After incubation for 5 days at 37°C in a 5% CO_2 incubator, the cell viability was quantified by a colorimetric assay (at 540 nm and 690 nm), monitoring the ability of viable cells to reduce MTT to a blue formazan product. All the data represent the mean values of triplicate determinations. CC_{50} was determined with mock-infected cells, whereas the 50% effective concentration (EC_{50}) was determined with HIV-infected cells.

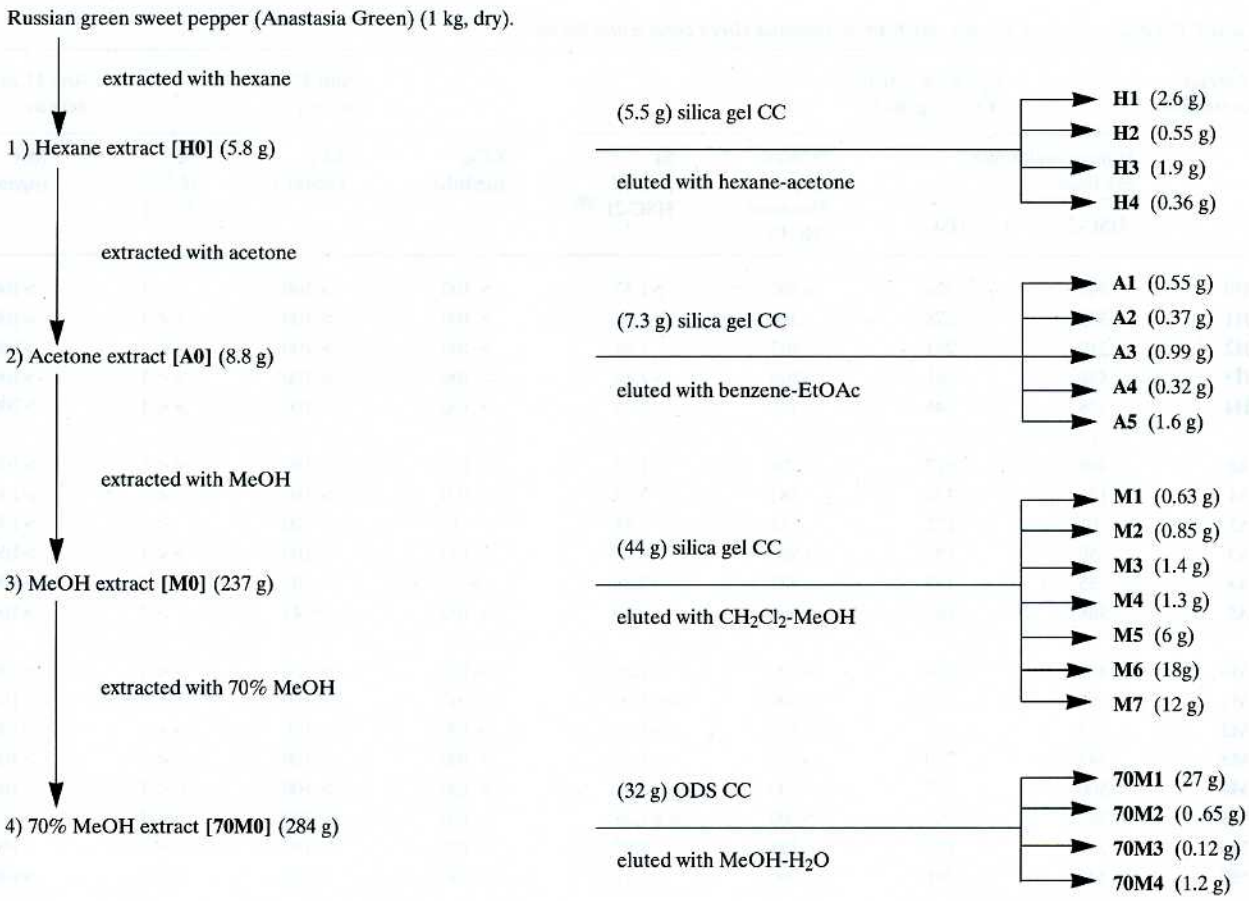

Figure 2. *Fractional separation of Anastasia Green extracts. CC: column chromatography.*

Selectivity index (SI) was defined as follows: $SI=CC_{50}/EC_{50}$ (Table I) (9).

Anti-Helicobacter pylori activity. Mueller-Hilton broth containing 5% FBS was used as the medium and was cultured in a jar conditioned with Campylo Pack (Dia Iatron) for 48 hours. Briefly, *H. pylori* strains were inoculated on a Brucella agar plate containing 10% horse serum and cultured at 37°C for 48 hours. The bacterial colonies collected were diluted to 10^7 colony forming unit (CFU)/mL with 0.9% saline. The Anastasia Green extracts were dissolved in DMSO and then diluted with Mueller-Hilton broth. To the solution of the extracts and fractions, each bacterial suspension was added to a density of 10^6 colony forming units (CFU)/100 mL/well. The mixture was incubated at 37°C for 48 hours. The minimum inhibitory concentration (MIC) of each extracts and fraction was calculated from the dose-response curve (10, 11) (Table I).

Reversal of multidrug resistance of tumor cells. (Assay for MDR reversal). The L5178 mouse T-cell lymphoma cell line was transfected with a multidrug resistant gene *MDR1/A*-containing retrovirus, as previously described (12). *MDR1*- expressing cell lines were selected by culturing the infected cells with 60 ng/mL colchicine to maintain the expression of *MDR1* phenotype. The L5178 *MDR1* T cell line and the L5178Y parent cell line were grown in McCoy's 5A medium with 10% heat-inactivated horse serum, L-glutamine and (±)-Verapamil. The cells were adjusted to a density of 2 x 10^6/mL and resuspended in serum-free McCoy's 5A medium; 0.5 mL aliquots of the cells were distributed into the Eppendorf centrifuge tubes. Then, 2.0 μL of 10 mg/mL of the tested fractions were added and incubated for 10 minutes at room temperature. Ten μL rhodamine 123 (R123) indicator (5.2 μM final concentration) was then added and incubated for further 20 minutes at 37°C. After washing twice and re-suspending in 0.5 mL phosphate-buffered saline (PBS), the fluorescence of the cell population was measured by flow cytometry, using Beckton Dickinson FACScan instrument (cell sorter). (±)-Verapamil was used as a positive control in the R123 accumulation experiments (13). The R123 accumulation was calculated from the fluorescence intensity of the samples. The percentage of control of untreated mean fluorescence intensity was calculated for parental and *MDR1* cell lines and compared to the fluorescence intensity of treated cells. An *MDR1* reversal activity was calculated by the following equation (13, 14) (Table II):

$$MDR1 \text{ reversal activity} = \frac{(MDR1 \text{ treated}/MDR1 \text{ control})}{(\text{parental treated}/\text{parental control})}$$

Results

We first investigated the relative cytotoxic activity of four extracts of Anastasia Green (Table I). The cytotoxic activity

Table I. *Cytotoxic activity and tumor specificity of Anastasia Green extracts and fractions.*

Extract or fraction	Cytotoxic activity[1] (CC_{50}: μg/mL)				Anti-HIV activity			Anti-H. *pyrori* activity[2]
	Human oral tumor cell lines		Human gingival fibroblast (HGF)	SI (HGF/ HSC-2)	CC_{50} (μg/mL)	EC_{50} (μg/mL)	SI (CC_{50}/ EC_{50})	MIC (μg/mL)
	HSC-2	HSG						
H0	365	324	>500	>1.37	> 100	> 100	>< 1	>100
H1	332	278	358	1.08	> 100	> 100	>< 1	>100
H2	210	264	407	1.94	> 100	> 100	>< 1	>100
H3	438	481	>500	>1.14	> 100	> 100	>< 1	>100
H4	178	246	401	2.25	> 100	> 100	>< 1	>100
A0	400	427	>500	>1.25	> 100	> 100	>< 1	>100
A1	126	330	383	3.04	= 100	> 100	< 1	>100
A2	61	121	212	3.48	= 49	> 100	< 1	>100
A3	59	186	>500	>8.47	> 100	> 100	>< 1	>100
A4	35	150	422	12.06	= 62	> 100	< 1	>100
A5	188	164	>500	>2.66	> 100	= 45	> 2	>100
M0	461	>500	>500	>1.08	> 100	> 100	>< 1	>100
M1	>500	229	>500	><1.00	> 100	> 100	>< 1	>100
M2	478	225	>500	>1.05	> 100	> 100	>< 1	>100
M3	345	250	>500	>1.45	> 100	> 100	>< 1	>100
M4	>500	317	>500	><1.00	> 100	> 100	>< 1	>100
M5	382	>500	>500	><1.31	> 100	> 100	>< 1	>100
M6	202	188	398	1.97	> 100	> 100	>< 1	>100
M7	448	241	>500	>1.12	> 100	> 100	>< 1	>100
70M0	146	>500	>500	>3.42	> 100	> 100	>< 1	>100
70M1	250	>500	>500	>2.00	> 100	> 100	>< 1	>100
70M2	143	475	>500	>3.50	> 100	> 100	>< 1	>100
70M3	333	>500	>500	>1.50	> 100	> 100	>< 1	>100
70M4	172	>500	>500	>2.91	> 100	> 100	>< 1	>100
Dextran sulfate(DS)					> 1000	= 1.1673	> 857	
Curdlan sulfate(CRDS)					> 1000	= 0.6132	>1631	
AZT(μM)					= 50	= 0.0060	= 8370	
Metronidazole								7.4
Clarithromycin								1.9
Erythromycin								1.8

1) Near confluent HSC-2, HSG and HGF cells were incubated for 24 hours with various concentrations of Anastasia Green extracts and fractions and the relative viable cell number (A_{540}) was determined by MTT method. 50% cytotoxic concentration (CC_{50}) was determined from the dose-response curve. Each value represents the mean from duplicate determinations. Control A_{540} values of HSC-2, HSG and HGF cells were 1.444, 1.600 and 0.337, respectively. 2) -: inactive (> 100 μg/m L) .

against two human oral tumor cells roughly decreased with increase in-water solubility: hexane extract [**H0**] (CC_{50}=365, 324 μg/mL) > acetone extract [**A0**] (CC_{50}=400, 427 μg/mL) > methanol extract [**M0**] (CC_{50}=461, >500 μg/mL) > 70% methanol extract [**70M0**] (CC_{50}=146, >500 μg/mL). By appling **A0** extract to silica gel column chromatography, the cytotoxic activity was concentrated to fractions **A2** (CC_{50}=61 μg/mL), **A3** (CC_{50}=59 μg/mL) and **A4** (CC_{50}=35 μg/mL). It is apparent that normal cells (human gingival fibroblast: HGF) were relatively resistent to all fractions.

Anti-human immunodeficiency virus (HIV) activity. All extracts and column fractions showed no anti-HIV activity (selectivity index (SI)<1), in contrast to popular anti-HIV agents, such as dextran sulfate (SI>857), curdlan sulfate (SI>1631) and AZT (SI=8370) (Table I).

Table II. *MDR reversing activity of Anastasia Green extracts and fractions in lymphoma-5178 cells.*

Extract or fraction	Concentration (μg/mL in DMSO)	Forward scatter height (cell size ratio)	Side scatter height	Fluorescence one height (FL-1)[a)]	Fluorescence activity ratio[b)]
Par(control) [c)]		457	371	2816	
Par control DMSO[b)]		486	481	2961	
MDR control		562	737	**110**	**1**
MDR control DMSO		493	533	59	
(±) -verapamil(positive control)	5	438	519	420	4.96
H0	100	458	553	915	10.80
H1	100	446	517	429	5.10
H2	100	443	510	284	3.30
H3	100	452	549	1321	15.60
H4	100	464	515	2049	24.20
A0	100	457	551	105	1.23
A1	100	427	483	58	0.68
A2	100	376	573	145	1.71
A3	100	439	516	105	1.23
A4	100	435	519	3040	35.90
A5	100	350	558	1138	13.40
M0	100	435	502	70	1.20
M1	100	421	547	71	0.83
M2	100	421	504	95	1.10
M3	100	417	517	129	1.50
M4	100	413	518	98	1.50
M5	100	430	501	223	2.60
M6	100	450	511	246	2.90
M7	100	439	499	52	0.60
70MO	100	436	494	302	3.60
70M1	100	428	498	69	0.80
70M2	100	421	492	1094	12.90
70M3	100	435	509	276	3.20
70M4	100	421	487	55	0.60
Par(control)		480.19	124.74	926.27	
MDR+ R123(mean)	5	455.62	121.39	829.73	
MDR control		476.30	143.25	**43.80**	**1**
MDR+ DMSO	5	459.21	138.74	35.47	0.81
(±) -verapamil	5	452.16	150.08	269.29	6.15
A4	20	448.42	136.06	542.35	12.38
A5	20	447.29	139.83	180.12	4.11
H3	20	435.20	135.68	498.34	11.38
H4	20	429.41	132.64	732.10	16.71
70M2	20	412.77	127.22	31.79	0.73

a) References 13,14.
b) The R123 accumulation was calculated from fluorescence of one height value using:

$$\log(y)=\log_{10}\frac{x}{256}$$

then the fluorescence activity ratios were calculated according to the formula given below:

$$MDR1 \text{ reversal activity} = \frac{(MDR1 \text{ treated}/MDR1 \text{ control})}{(\text{parental treated}/\text{parental control})}$$

c) Par: a parental cell without *MDR1* gene. d) *MDR1*: a parental cell with *MDR1* gene.

Anti-Helicobacter pyrori activity. All extracts and fractions showed no anti-*H. pylori* activity (MIC>100 μg/mL), in contrast to metronidazole (MIC=7.4 μg/mL), clarithromycin (MIC=1.9 μg/mL) and erythromycin (MIC=1.8 μg/mL) (Table I).

Reversal of multidrug resistance (MDR). Enforcement of the expression of *MDR1/A* in the L5178 mouse T-cell lymphoma cell line resulted in *MDR,* as reflected by the reduced intracellular accumulation of R123, while addition of (±) -verapamil reversed the *MDR1,* as reflected by the increase in R123 accumulation (4.96-fold increase) (Table II). Surprisingly, some column fractions of Anastasia Green (100 μg/mL) showed higher *MDR1,*reversal activity when compared with *MDR1,* control (Fluorescence activity ratio=1) or (±)-verapamil (Fluorescence activity ratio=4.96). Acetone fraction [**A4**] (Fluorescence activity ratio=35.90) had the highest *MDR1,* reversal activity, and followed by [**H4**] (Fluorescence activity ratio=24.20) and [**H3**] (Fluorescence activity ratio=15.60), respectively (Table II). These fractions also could slightly, but significantly, reverse the *MDR1,*, even at lower concentration (20 μg/mL), demonstrating their dose-dependent effects.

Discussion

The present study demonstrated that Anastasia Green extract and fractions showed tumor-specific cytotoxic action and reversed the *MDR1.* P-glycoprotein (Pgp), reduces the cytotoxic activity of anticancer agents such as etoposide, vinblastine, mitomycin C and actinomycin D by pumping the agents out of the cells (15-17). Pgp-mediated drug resistance can be circumvented by the treatment regimens which either exclude Pgp substrate agents, or include Pgp inhibitory agents such as (±) -verapamil (18). It is suggested that the extracts and fractions of Anastasia Green might also contribute to the *MDR1,* reversal and enhance the incorporation of anticancer drug into the cells. Further purification of the active ingredients in Anastasia Green is underway in our laboratory.

Acknowledgements

We are very grateful to Mr. Kazutoshi Kobayashi, President of Field Co. LTD. (Shin-Beppu, Miyazaki, Japan), for his kind supply of Anastasia Green. This study was also supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan (No. 11671853) and the Szeged Foundation for Cancer Research, Hungary.

References

1 Schneeman BO: Dietary influences on health. Prev Med *25:* 38-40, 1996.

2 Odin AP: Vitamins as antimutagens: advantages and some possible mechanisms of antimutagenic action. Mutation Res *386:* 39-67, 1997.

3 Ho C: Phenolic compounds in food. *In:* Ho C, Lee CY, Huang M (eds.), Phenolic Compounds in Food and Their Effects on Health, I. Analysis, Occurrence and Chemistry. American Chemical Society, Washington, DC, pp1-7, 1992.

4 Kuo M, Lee K and Lin J: Genotoxicities of nitropyrenes and their modulation by apigenin, tannic acid, ellagic acid and indole-carbinol in the *Salmonella* and CHO system. Mutation Res *270:* 87-95, 1992.

5 Gross J: Carotenoids. *In:* Gross J (ed.), Pigments in Vegetables, Chlorophylls and Carotenoids, New York, 1991, pp75-79.

6 Mejía LA, Hudson E, de Mejía EG and Vázquez F: Carotenoid content and vitamin A activity of some common cultivars of Mexican peppers (*Capsicum annuum*) as determined by HPLC. J Food Sci *53:* 1448-1451, 1988.

7 Gonzaez de Mejía E, Quintanar-Hernández A and Loarca-Piña G: Antimutagenic activity of carotenoids in green peppers against some nitroarenes. Mutation Res *416:* 11-19, 1998.

8 Sakagami H, Jiang Y, Kusama K, Atsumi T, Ueha T, Toguchi M, Iwakura I, Satoh K, Ito H, Hatano T and Yoshida T: Cytotoxic activity of hydrolyzable tannins against human oral tumor cell lines. - A possible mechanism. Phytomedicine *7:* 39-47, 2000.

9 Nakashima H, Murakami T, Yamamoto N, Sakagami H, Tanuma S, Hatano T, Yoshida T and Okuda T: Inhibition of human immunodeficiency viral replication by tannins and related compounds. Antiviral Res *18:* 91-103, 1992.

10 Numao N, Iwahori A, Hirota Y, Sasatsu M, Kondo I, Onimura K, Sampe R, Yamane S, Itoh S, Katoh T and Kobayashi S: Antibacterial activity of two alkylamines integrated an indane scaffold: mimicry of a complementary unit on magainin 2. Biol Pharm Bull *20:* 800-804, 1997.

11 Kawase M, Harada H, Saito S, Cui J and Tani S: *In vitro* susceptibility of *Helicobacter pylori* to trifluoromethyl ketones. Bioorg Med Chem Lett *9:* 193-194, 1999.

12 Pastan I, Gottesman MM, Ueda K, Lovelace E, Rutherford AV and Willingham MC: A retrovirus carrying an *MDR1* cDNA confers multidrug resistance and polarized expression of P-glycoprotein in MDCK cells. Proc Natl Acad Sci USA *85:* 4486-4490, 1988.

13 Weaver JL, Szabo G, Pine PS, Gottesman MM, Goldenberg S and Aszalos A: The effect of ion channel blockers, immunosuppressive agents, and other drugs on the activity of the multidrug transporter. Int J Cancer *54:* 456-461, 1993.

14 Kessel D: Exploring multidrug resistance using Rhodamine 123. Cancer Commun *1:* 145-149, 1989.

15 Endicott JA and Ling V: The biochemistry of P-glycoprotein-mediated multidrug resistance. Annu Rev Biochem *58:* 137-171, 1989.

16 Gerlach JH, Endicott JA, Juranka PF, Henderson G, Sarangi F, Deuchars KL and Ling V: Homology between P-glycoprotein and a bacterial haemolysin transport protein suggests a model for multidrug resistance. Nature *324:* 485-489, 1986.

17 Juliano RL and Ling V: A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta *455:* 152-162, 1976.

18 Sharom FJ: The P-glycoprotein efflux pump: how does it transport drugs? J Membr Biol *160:* 161-175, 1997.

Received March 5, 2001
Accepted June 8, 2001