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Efficient Utilization of Plant Resources by Alkaline Extraction

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Abstract

As compared to the studies with hot water extracts of plants, those with alkaline extracts were limited. Both alkaline and hot water extracts from green tea leaf, oolong tea leaf and orange flower were compared for their biological activities. Plant materials were successively extracted first with hot-water and then alkaline solution, or extracted directly with alkaline solution. Viable cell number of HIV-infected and UV-irradiated cells was determined by MTT method. Antibacterial activity against *Porphyromonas gingivalis* 381 was determined by turbidity assay. Cytochrome P-450 (CYP)3A4 activity was measured by β-hydroxylation of testosterone using human recombinant CYP3A4 (Figure 5). Radical intensity of superoxide and hydroxyl radical was determined by ESR spectroscopy. Alkaline extraction recovered twice as much as dried materials as compared with water extraction. Water extracts showed higher anti-bacterial, CYP3A4 inhibitory and superoxide scavenging activities, whereas alkaline extract showed higher anti-HIV and hydroxyl radical scavenging activity. Both water and alkaline extracts showed comparable anti-UV activity. The present study suggests the usefulness of alkaline extraction for the efficient utilization of the natural resources.

Keywords: Alkaline extraction; Hot-water extraction; Anti-HIV activity; Anti-UV activity; Anti-bacterial activity; CYP3A4 inhibition; Radical scavenging activity

Introduction

We have previously reported that lignin-carbohydrate complex (LCC) fractions prepared by acid precipitation of the alkaline extracts of pine cone, pine seed shell, catuaba bark, cacao husk, cacao mass, *Lentinus edodes* mycelia potently protected the cells from HIV-infection [selectivity index (SI)=7~311] [1], and from UV irradiation (SI=7.6~ >38.1) [2]. Similarly, crude alkaline extract of the leaves of *Sasa senanensis* Rehder (SE) showed comparable anti-HIV (SI=36-45) and anti-UV activity (SI=20~39) with LCC fractions [3]. On the other hand, hot-water extracts of a total of 35 Kampo medicines and their constituent plants had much lower anti-HIV (SI=1~8) and anti-UV activity (SI=1~4.4) [4]. This raised a possibility that the use of alkaline extraction is more advantageous than hot-water extraction to obtain higher amounts of anti-HIV and anti-UV substances. However, this possibility has not yet been tested with water and alkaline extracts prepared from the same plant species. To clarify this point, we prepared hot-water extract (Fr. I), alkaline extract of its residue (Fr. II), and total alkaline extract (Fr. III) from green tea leaf (GT), oolong tea leaf (OT) and orange flower (OF) (Figure 1), and compared their anti-HIV, anti-UV, anti-bacterial, cytochrome P-450 (CYP)3A4 inhibitory and radical scavenging activities, together with their compositional analysis with HPLC.

Materials and Methods

Materials

The following chemicals and reagents were obtained from the indicated companies: Dulbecco's modified Eagle's medium (DMEM): Gibco BRL, Grand Island, NY, USA; fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), hypoxanthine (HX), xanthine oxidase (XOD), diethylenetriaminepenta-acetic acid (DETAPAC), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) (Dojin, Kumamoto, Japan), RPMI–1640 medium, azidothymidine (AZT), 2',3'-dideoxycytidine (ddC), gallic acid (Sigma-Aldrich Co. St. Louis, MO, USA), dimethyl sulfoxide (DMSO), caffeine, hesperidin, dextran sulfate (5 kDa) (Wako Pure Chemical Ind., Ltd., Osaka, Japan), sodium ascorbate (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCg), (-)-epicatechin gallate (ECg) (Nagara Science Co., Ltd., Gifu, Japan), curdlan sulphate: 79 kDa (Ajinomoto Co. Inc., Tokyo, Japan).

Preparation of water and alkaline extracts

Five g green tea leaf (GT) (Kimpo, Satoen Co. Ltd., Shizuoka, Japan), oolong tea leaf (OT) (Mitsui Norin Co. Ltd., Tokyo, Japan) or orange flower (OF) (Tochimoto Tenkaido Co.,Ltd., Osaka, Japan) were extracted at 80°C for 30 min with 100 ml of water, and filtered through filter paper (No. 5A, Kiriyama glass Co., Tokyo, Japan) (Figure 1). The filtrate was concentrated and lyophilized to give the water extract (Fr. I: GT-I, OT-I, OF-I) at the yield of 19.3, 15.6 and 45.2%, respectively. The residue was extracted at 80°C for 30 min with 100 ml of 0.15 M NaOH and filtered. The filtrate was neutralized with HCl, concentrated and lyophilized to give the alkaline extract of the residue (Fr. II: GT-II, OT-II, OF-II) at the yield of 34.0, 30.0 and 19.0%, respectively (18.1, 18.1, 9.7%, respectively, after correction for NaCl present in the extracts). Alternatively, GT, OT or OF (3 g) were directly extracted with 100 ml

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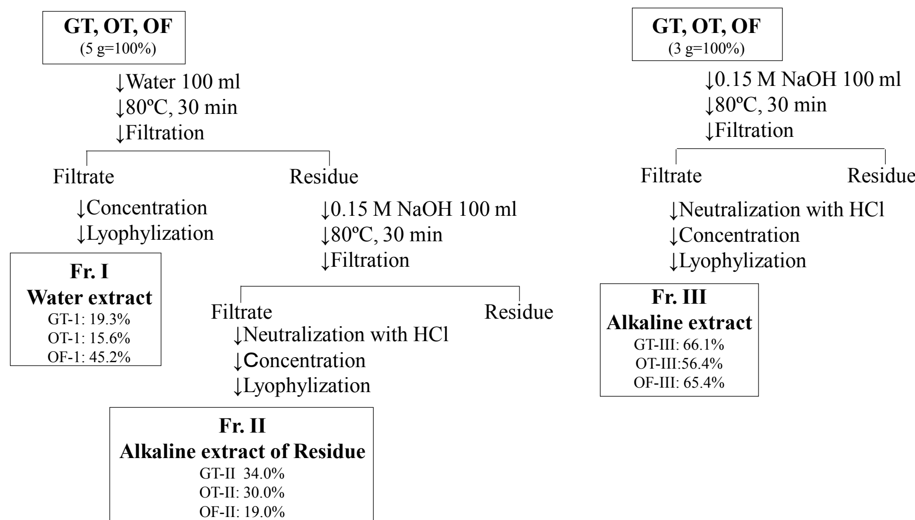

Figure 1: Fractional preparation of hot-water and alkaline extract from green tea leaf (GT), oolong tea leaf (OT) and orange flower (F).

of 0.15 M NaOH, without hot-water extraction to give the alkaline extract (Fr. III: GT-III, OT-III, OF-III) at the yield of 66.1, 56.4 and 65.4%, respectively (47.3, 35.8 and 42.6%, respectively after correction for NaCl present in the extracts). NaCl, present in GT-II, GT-III, OT-II, OT-III, OF-II and OF-III, determined by atomic absorption spectrometry (HITACHI Z-2010 Polarized Zeeman Atomic Absorption Spectrophotometer, Hitachi High-Technologies Corporation, Tokyo, Japan) was 46.7, 28.4, 39.6, 36.6, 49.0 and 34.8% of dried weight, respectively.

HPLC separation of water and alkaline extracts

HPLC was performed on a Shimadzu HPLC apparatus with a Lab Solutions Ver. 5.54 SP3 System and a model SPD-M20A Diode Array Detector [column: Develosil XG-C30M-3, 3.0 mm ID×15 cm, Nomura Chemical Co., Ltd.; mobile phase: 0 to 5 min, 5% CH_3CN in 0.1% trifluoroacetic acid (TFA), 5 to 20 min, linear gradient of 5-35% CH_3CN in 0.1% TFA, 20 to 25 min, 35% CH_3CN in 0.1% TFA; flow rate: 0.75 ml/min; detection: UV 210-400 nm, max; column temperature: 40°C], using gallic acid, EGC, EGCg, ECg, caffeine and hesperidin as standards. Five µl of 4 mg/ml each sample was injected to HPLC.

Assay for anti-HIV activity

Human T-cell leukemia virus I (HTLV-I)-bearing CD4-positive human T-cell line, MT-4, was cultured in RPMI-1640 medium supplemented with 10% FBS and infected with HIV-1IIIB at a multiplicity of infection of 0.01. HIV- and mock-infected MT-4 cells ($3×10^4$ cells/96-microwell) were incubated for 5 days with different concentrations of samples and the relative viable cell number was determined by MTT assay. The concentration that reduced the viable cell number of the uninfected cells by 50% (CC_{50}) and the concentration that increased the viable cell number of the HIV-infected cells to the 50% that of control (mock-infected, untreated) cells (EC_{50}) were determined from the dose–response curve with mock-infected and HIV-infected cells, respectively. The anti-HIV activity was evaluated by the selectivity index (SI), which was calculated using the following equation: $SI=CC_{50}/EC_{50}$ [5].

Assay of anti-UV activity

Human oral squamous cell carcinoma HSC-2 cells (Riken Cell Bank, Tukuba, Japan) were inoculated into 96-microwell plates ($3×10^3$ cells/well, 0.1 ml/well) and incubated for 48 hours to allow cell attachment. The culture supernatant was replaced with 100 µl phosphate-buffered saline without calcium and magnesium [PBS(−)] that contained different concentrations of samples in triplicate, placed at 21 cm distance from a UV lamp (wavelength: 253.7 nm) and exposed to UV irradiation (6 J/m^2/min) for 1 minute. The cells were then incubated for a further 48 hours in DMEM containing 10% FBS to determine the relative viable cell number by the MTT assay. From the dose–response curve, the CC_{50} and the concentration that increased the viability of UV-irradiated cells up to 50% that of control cells (EC_{50}) was determined. The SI was determined using the following equation: $SI=CC_{50}/EC_{50}$ [6,7].

Assay for antibacterial activity

Porphyromonas gingivalis 381 (ATCC33277) ($1×10^6$ cfu/ml) was incubated for 24 hours at 37°C in Gifu Anaerobic Medium (GAM) containing containing serially diluted samples, 5 µg/ml hemin and 1 µg/ml menadione under anaerobic conditions with mixed gas of nitrogen (83%), hydrogen (7%) and CO_2 (10%), and then the absorbance at 595 nm of the bacterial suspension was measured [8]. From the dose–response curve, the concentration that reduced the bacterial growth by 50% (IC_{50}) was determined.

Measurement of CYP3A4 activity

CYP3A4 activity was measured by β-hydroxylation of testosterone using human recombinant CYP3A4 [9,10]. The reaction mixture, containing 200 mM potassium phosphate buffer (pH 7.4), NADPH regenerating system (1.3 mM NADPH, 1.3 mM glucose-6-phosphate, 0.2 U/ml glucose-6-phosphate dehydrogenase, and 3.3 mM $MgCl_2$) along with 0, 10, 30, 100, 300, 600 and 1000 µg/mL of the test samples or vehicle in triplicate and the human recombinant CYP3A4 (16.5 pmol/ml), was preincubated at 37°C for 5 min. The reaction was started by the addition of 300 µM testosterone substrates. The final volume of the reaction mixture was 250 µl with a final DMSO concentration of 0.5%. The reaction was stopped by the addition of 500 µl ethyl acetate after 15 min. After centrifugation (15,000 g, 5 min), 400 µl of supernatant was collected, dried, and resuspended in 100 µl of methanol. Analyses of the metabolites were performed by HPLC (JASCO PU2089, AS2057,

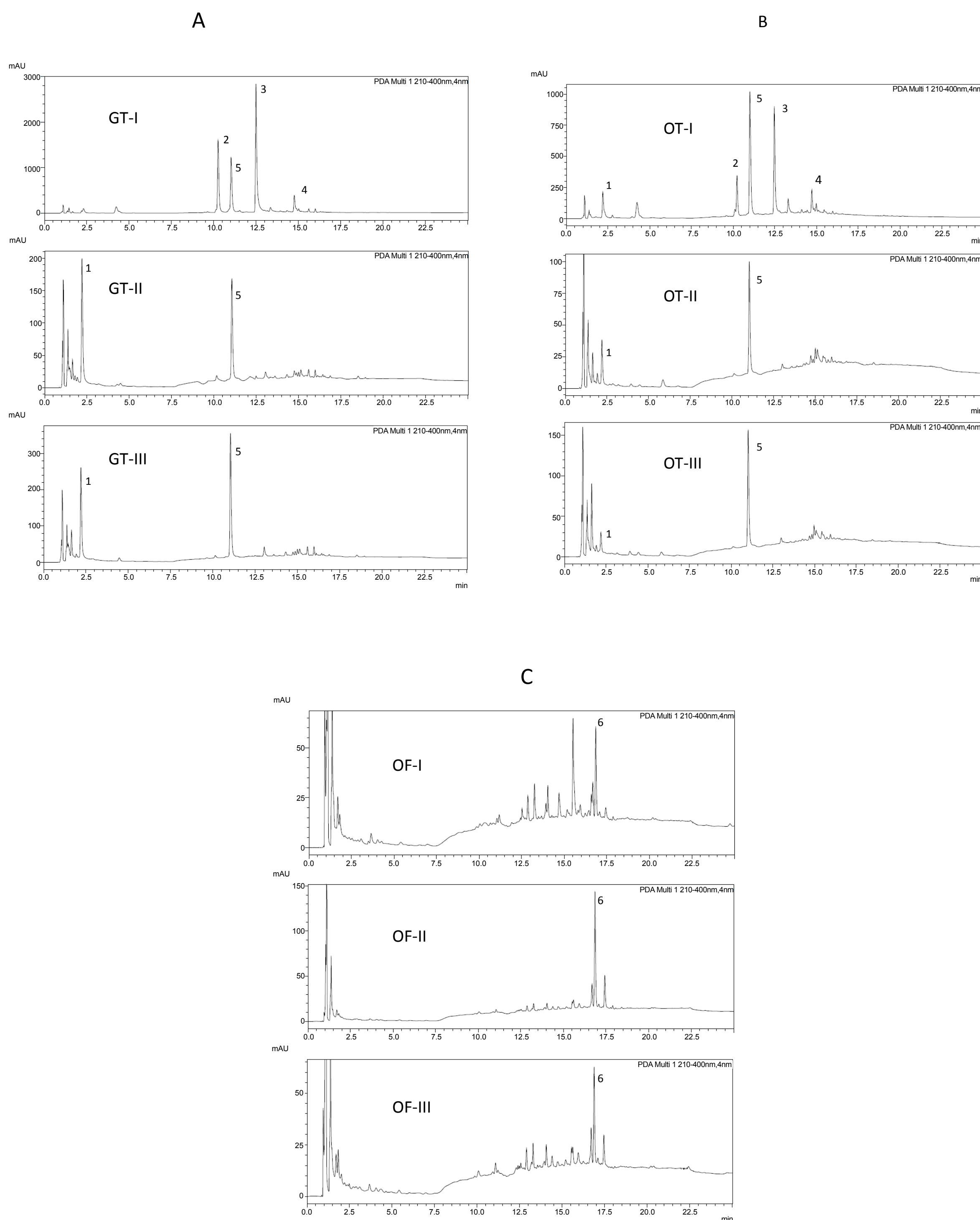

Figure 2: HPLC separation of hot-water and alkaline extracts from green tea leaf (GT), oolong tea leaf (OT) and orange flower (OF). S Standard: 1, gallic acid; 2, EGC; 3, EGCg; 4, ECg; 5, caffeine; 6, hesperidin.

UV2075 ChromNAV) equipped with TSKgel ODS-120A, 4.6 mm ID×25 cm, 5 μm column (TOSOH, Tokyo, Japan). The mobile phase consisted of 70% methanol and 30% water. The metabolites were separated using an isocratic method at a flow rate of 1.0 ml/min. Quantification of the metabolites was performed by comparing the HPLC peak area at 254 nm to that of 11α-progesterone, the internal standard. The retention times for 6 β-hydroxytestosterone and 11α-progesterone were about 4.3 and 6.0 min, respectively. From the dose-response curve, the concentration

	Exp. 1			*Exp. 2*			*Exp. 3*	*Exp. 4*	*Exp. 5*	*Exp. 6*
	Anti-HIV activity			Anti-UV activity			Anti-bacterial activity	CYP3A4 inhibitory activity	O_2- radical scavenging activity	・OH radical scavenging intensity
	CC_{50} (μg/mL)	EC_{50} (μg/mL)	SI (CC50/EC50)	CC_{50} (μg/mL)	EC_{50} (μg/mL)	SI (CC_{50} /EC_{50})	IC_{50} (mg/ml)	IC_{50} (mg/ml)	IC_{50} (mg/ml)	IC_{50} (mg/ml)
Green tea extract										
GT-1	21.97	>1000	<0.022	>4000	372 ± 42	>10.8	0.275	0.0534	0.00167	0.154
GT-II	59.61	>533	<0.11	>2132	204 ± 13	>10.4	1.405	0.272	0.00815	0.091
GT-III	79.28	23.8	3	>2864	299 ± 21	>9.6	0.952	0.3872	0.00916	0.101
Oolonng tea extract										
OT-I	32.9	>1000	<0.033	>4000	428 ± 55	>9.3	0.234	0.0267	0.00187	0.131
OT-II	66.02	6.99	9	>2416	326 ± 63	>7.4	1.082	0.0883	0.00991	0.092
OT-III	71.67	5.52	13	>2536	258 ± 13	>9.8	0.872	0.1405	0.00888	0.099
Orang flower extract										
OF-I	498.7	>1000	<0.50	>4000	1152 ± 21	>3.5	3,828	0.5608	0.154	0.888
OF-II	>510	39.7	>13	>2040	508 ± 47	>4.0	1.671	>1.0000	0.2	0.685
OF-III	>652	43.5	>15	>2608	725 ± 147	>3.6	1.731	>1.0000	0.19	0.698
Dextran sulfate (μg/ml)	>1000	0.706	>1417							
Curdlan sulfate (μg/ml)	>1000	0.174	>5753							
AZT (μM)	258.9	0.03	8558							
ddC (μM)	2547.7	1.01	2535							
Sodium ascorbate (mM)				>16	0.275 ± 0.029	>58.2				
Grapefruit juice EtOAc Fr. (mg/ml)							0.0645			

All values in alkaline extracts were corrected for NaCl present in the extracts.

Table 1: Biological activities of hot water and alkaline extracts of three plant materials.

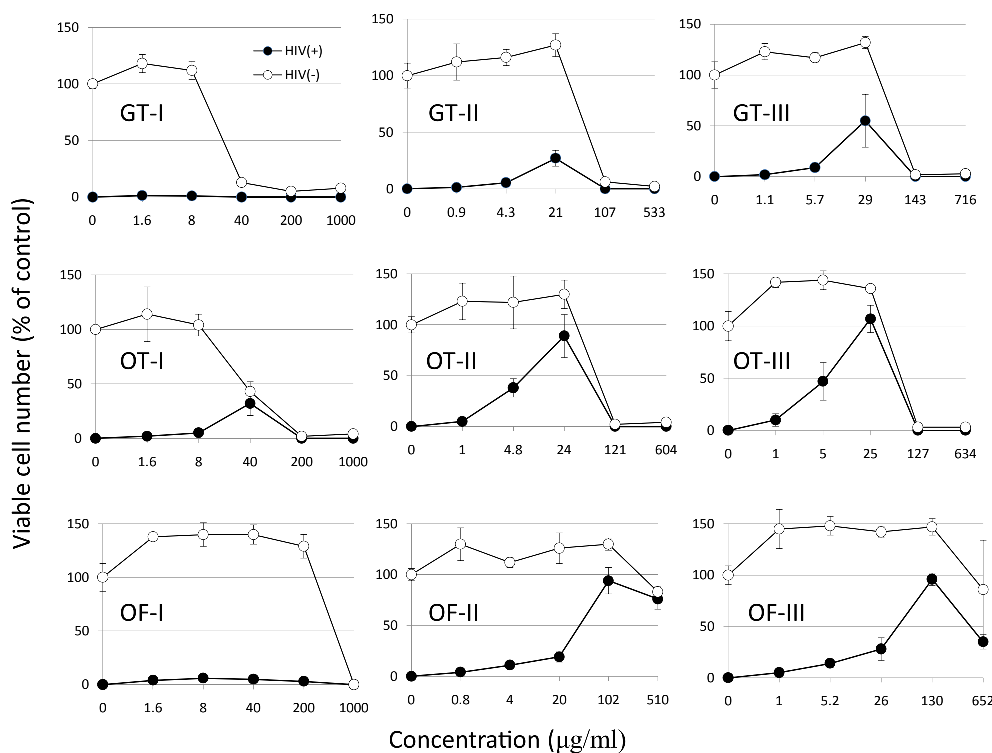

Figure 3: Anti-HIV activity of water and alkaline extracts. HIV-1_{IIIB}-infected (HIV+) (●) and mock-infected (HIV–) (○) MT-4 cells were incubated for 5 days with the indicated concentrations of each sample, and the viable cell number was determined by the MTT assay and expressed as a percentage that of the control. Data represent the mean ± S.D. from triplicate assays. NaCl present in the extracts was corrected.

that inhibited the CYP3A4 activity by 50% (IC_{50}) was determined.

Radical-scavenging activity

The free radical intensity was determined at 25°C, using electron-spin resonance (ESR) spectroscopy (JEOL JES REIX, X-band, 100 kHz modulation frequency; JEOL Ltd., Tokyo, Japan) [11]. The instrument settings were: centre field, 335.5 ± 5.0 mT; microwave power, 16 mW; modulation amplitude, 0.1 mT: gain, 630; time constant, 0.03 s and scanning time, 2 minutes. For the determination of the superoxide anion (in the form of DMPO-OOH), produced by the HX-XOD reaction (total volume: 200 µl) [2 mM HX in 0.1 M phosphate buffer (PB) (pH 7.4) 50 µl, 1 mM DETAPAC 10 µl, 10% DMPO 30 µl, test sample (in PB) 40 µl, PB 40 µl, XOD (0.5 U/ml in PB) 30 µl], the time constant was changed to 0.03 s [11]. For the determination of the hydroxyl radical (in the form of DMPO-OH), produced by the Fenton reaction (200 µl) [1 mM $FeSO_4$ (containing 0.2 mM DETAPAC) 50 µl, 0.1 M PB (pH 7.4) 50 µl, 92 mM DMPO 20 µl, test sample (in H2O) 50 µl, 1 mM H_2O_2, 30 µl], the gain was changed to 160 [11]. The concentration that reduced the radical intensity of DMPO-OOH and DMPO-OH by 50% (IC_{50}) was determined by the dose–response curve of triplicate samples.

Statistical treatment

Experimental values are expressed as the mean±standard deviation (SD). Statistical analysis was performed by using Student's *t*-test. A *p*-value <0.01 or <0.05 was considered to be significant.

Results

HPLC separation of the hot-water and alkaline extracts

Major components of GT-I were identified as EGC, EGCg, ECg and caffeine (Figure 2A). On the other hand, major components of GT-II and GT-III were identified as gallic acid and caffeine, while EGC, EGCg and ECg disappeared. Elevated background peaks (retention time: 7.5~25 min) suggests the accumulation of numerous degradation products.

Major products of OT-I were gallic acid, EGC, EGCg, ECg and caffeine (Figure 2B), whereas major components of OT-II and OT-III were gallic acid and caffeine, and many peaks of degradation products. Major peaks of OF-I, OF-II and OF-III were hesperidin and many degradation products (Figure 2C).

Anti-HIV activity

All three water extracts prepared from green tea leaf, oolong tea leaf and orange flower (GT-I, OT-I, OF-I) showed no anti-HIV activity (SI=<0.022, <0.033 and <0.50, respectively) (Exp. I, Table 1) (Figure 3). Alkaline extract prepared from the residue of water extraction (GT-II, OT-II, OF-II) showed slightly higher anti-HIV activity (SI=<0.11, 9 and >13, respectively). Total alkaline extract (Fr. III) (GT-III, OT-III, OF-III) showed the highest anti-HIV activity (SI=3, 13 and >15, respectively), although their SIs were 100- to 600-fold lower than that of popular anti-HIV agents (dextran sulfate, curdlan sulfate, AZT, ddT (SI=>1417, >5753, 8558 and 2535, respectively) (Table 1). Alkaline extracts of orange flower (OF-II, OF-III) (SI=>13, >15) showed the highest anti-HIV activity, followed by those of oolong tea leaf (OT-II, OT-III) (SI=9, 13), and green tea leaf (GT-III) (SI=3). The lower activity of green tea and oolong tea leaves may be due to interfering action of cytotoxic substances.

Anti-UV activity

We recently reported that UV irradiation induced non-apoptotic cell death without induction of internucleosomal DNA fragmentation in HSC-2 cells [6]. UV irradiation (6 J/m^2/min, 1 minute) significantly reduced the viable cell number after 48 hours' incubation. Addition of tea extracts during the UV irradiation protected the cells from UV-induced cell injury. Green tea leaf extracts showed the highest anti-UV activity, regardless of water extraction (GT-I) (SI=>10.8) or alkaline extraction (GT-II, GT-III) (SI=>10.4, >9.6), although its anti-UV activity was approximately 5-times lower than sodium ascorbate (SI=>58.2) (Exp. 2 in Table 1). Oolong tea leaf extracts showed slightly lower anti-UV activity, regardless of water extraction (OT-I) (SI=>9.3) or alkaline extraction (OT-II, OT-III) (SI=>7.4, >9.4). Orange flower

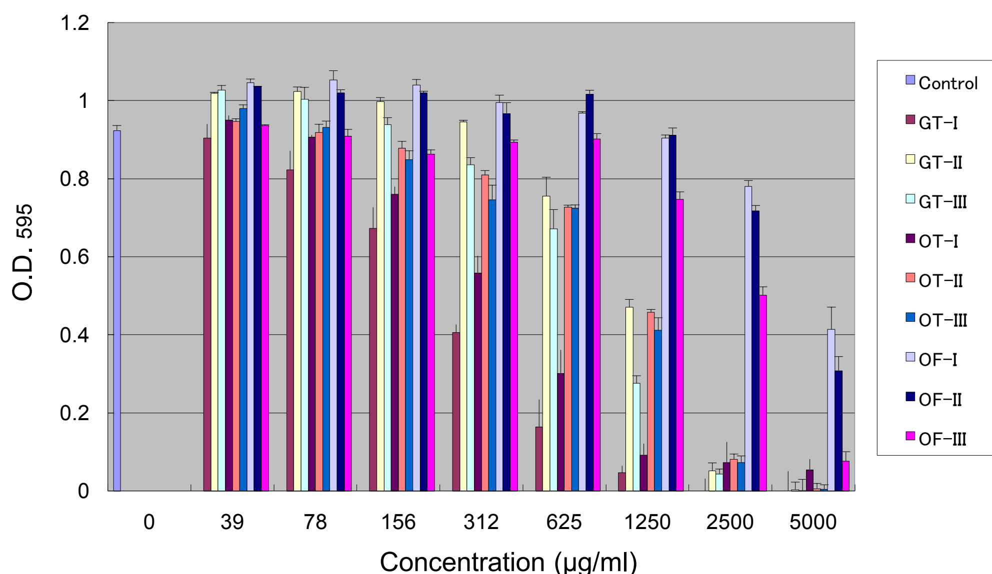

Figure 4: Antibacterial activity of GT, OT and OF extracts. Each value represents the mean ± S.D. of triplicate assays. *p<0.01 compared with control. NaCl present in the extracts was not corrected

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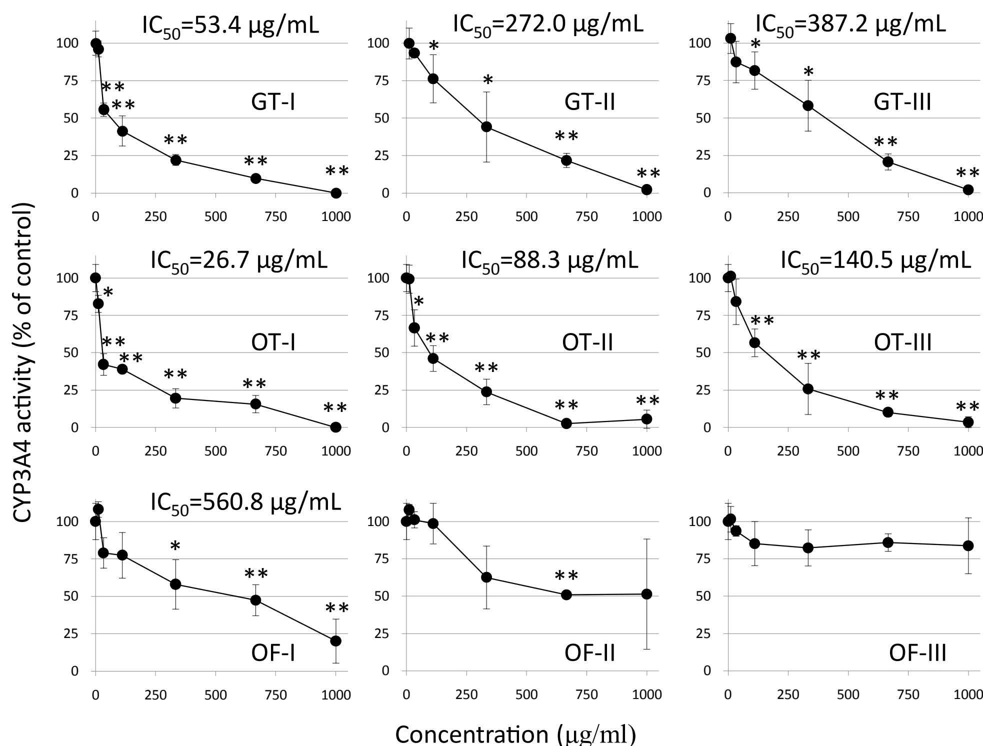

Figure 5: Cytochrome P450 enzyme (CYP)3A4 inhibitory activity of GT, OT and OF extracts. Each value represents the mean ± S.D. of triplicate assays. ***p*<0.01, **p*<0.05 relative to the control (0%). NaCl present in the extracts was corrected

extracts showed the least anti-UV activity, regardless of water extraction (OF-I) (SI=>3.5) or alkaline extraction (SI=>4.0, >3.6).

Antibacterial activity

OT-I showed the highest anti-bacterial activity against P. gingivalis 381 (IC_{50}=0.234 mg/ml, determined after correction of NaCl present in the extracts), followed by GT-I (0.275 mg/ml), OT-III (0.872 mg/ml), GT-III (0.952 mg/ml), OT-II (1.082 mg/ml), GT-II (1.405 mg/ml), OF-II (1.671 mg/ml), OF-III (1.731 mg/ml) and OF-I (3.828 mg/ml) (Figure 4, Exp. 3 in Table 1). Water extracts of GT and OT were 4 to 5- times more potent than alkaline extracts. OF extracts were much less potent. All extracts did not show any hormetic stimulation (known as growth stimulation at lower concentration ranges [12]), in contrast to alkaline extract of the leaves of Sasa senanensis Rehder (SE) [8].

CYP3A4 inhibitory activity

Water extracts (GT-I, OT-I, OF-I) inhibited CYP3A4 (IC_{50}=53.4, 26.7 and 560.8 μg/ml, respectively) more potently than alkaline extracts (GT-II, GT-III=272.0, 387.2 μg/ml; OT-II, OT-III=88.3, 140.5 μg/ml; OF-II. OF-III=>1000, >1000 μg/ml) (Figure 3; Exp. 4, Table 1). Grapefruit juice is known to inhibit the CYP3A4 activity [13]. EtOAc extractable fraction of grapefruit juice inhibited CYP3A4 activity to comparable extent with GT-I (IC_{50}=64.5 μg/ml). On the other hand, the CYP3A4 inhibitory activity of alkaline extracts were one order lower than that of the EtOAc extractable fraction of grapefruit juice (Exp. 4, Table 1).

Radical-scavenging activity

Water extract of green tea leaf (GT-I) most potently scavenged the superoxide anion (detected as DMPO-OOH), generated by HX and XOD reaction (IC_{50}=0.00167 mg/ml) (Exp. 5, Table 1). Alkaline extract of green tea leaf (GT-II, GT-III) showed 5-time lower superoxide scavenging activity (IC_{50}=0.00815, 0.00916 mg/ml). Water extract of oolong tea leaf (OT-I) showed comparable superoxide scavenging activity with GT-I, and alkaline extract of it was also 5-times less active. Orange flower extracts showed the weakest superoxide scavenging activity, regardless of water or alkaline extraction.

Water extract of green tea leaf (GT-I) scavenged the hydroxyl radical (detected as DMPO-OH), generated by the Fenton reaction (IC_{50}=0.154 mg/ml) (Exp. 6, Table 1). Alkaline extracts of green tea leaf (GT-II, GT-III) were slightly more active. Oolong tea leaf extracts showed comparable activity with green tea leaf extracts, whereas orange flower extracts were 7-times less active. However, in all of these cases, alkaline extracts were slightly more activity than water extracts.

Discussion

The present study demonstrated for the first time that alkaline extracts of green tea, oolong tea leaves and orange flower with 0.15 M NaOH consistently gave much higher anti-HIV activity, as compared with water extracts. The low anti-HIV activity of water extracts (GT-I and OT-I) may be due to the presence of gallic acid, EGC, EGCg and ECg, that had essentially no anti-HIV activity (SI<1) [5]. On the other hand, alkaline extracts (GT-II, GT-III, OT-II, OT-III) contained no detectable amount of EGC, EGCg and ECg, but higher amounts of gallic acid and numerous degradation products. Degradation products rather than gallic acid may be involved in the anti-HIV activity induction. We have recently purified the anti-UV substances (SEE-1) from the alkaline extract of *Sasa senanensis* Rehder, and identified it as *p*-coumaric acid derivative(s), a lignin precursor, by recycled HPLC and structural analysis with ^{1}H- NMR, ^{13}C-NMR, and UV absorption

[14]. Further studies are required to identify the active principle(s) of alkaline extracts of GT, OT and OF. In contrast to higher anti-HIV activity, alkaline extracts of GT and OT showed 4 to 5-times lower anti-bacterial activity, suggesting that lower molecular weight polyphenols may be involved in the anti-bacterial activity induction.

The present study demonstrated that alkaline extracts (GT-II, GT-III, OT-II, OT-III, OF-II, OF-III) inhibited the CYP3A4 activity to much lesser extent than water extracts (GT-I, OT-I, OF-I) (Figure 4). Alkaline extracts thus seem likely to be safer as compared with water extracts, since the latter are expected to enhance the side-effects of CYP3A4-metabolizable drugs that are administered together.

We also found that alkaline extracts scavenged hydroxyl radical more efficiently than water extracts, whereas water extracts were more active in scavenging superoxide. Hydroxyl radical is known to be highly cytotoxic and mutagenic [15,16], and therefore alkaline extract may prevent or reduce the incidence of hydroxyl radical-induced adverse effects.

We have recently found that alkaline extracts of GT, OT and OF more effectively inhibited the COX-2 activity, as compared with corresponding water extract (Fukuda, manuscript in preparation), suggesting their possible anti-inflammatory activity. Recent clinical research demonststrated that alkaline extracts of of *Sasa senanensis* Rehder leaf and pine cone of *Pinus parviflora* Sieb et. Zucc significantly improved the conditions of patients infected with lichenoid dysplasia [17] and herpes-simplex virus [18], respectively.

There was a possibility that some chemical entities in the plant might have been transformed or conversed during alkaline extraction. To test this possibility, gallic acid, EGCg, caffeine or hesperidin (insoluble material removed by filtration through 0.45 μm filter) (1 mg/ml) in 50% MeOH was mixed with 10 volume of 0.15 M NaOH, and stood at 80°C for 30 min, or at room temperature for overnight, and then subjected to HPLC analysis. We observed that (i) both gallic acid and EGCg were completely degraded and disappeared, (ii) caffeine was significantly, but not completely degraded, and (iii) hesperidin was stable, with its peak height rather increased, possibly due to increasing solubility under either alkaline conditions (data not shown). This observation was apparently inconsistent from the present results that these compounds were relatively stable in the alkaline extract. Alkaline solution increases the extractability of phenolic compounds, which may reduce the acidity of the extract and then enhance the stability. Gallic acid in the alkaline extracts of green tea may be produced from the degradation of catechin gallates such as EGCg or lignin-related compounds.

Conclusion

The present study demonstrates that water extracts showed higher anti-bacterial, CYP3A4 inhibitory and superoxide scavenging activity, whereas alkaline extracts showed higher anti-HIV and hydroxyl radical scavenging activity. Both water and alkaline extracts showed comparable anti-UV activity. Considering that alkaline extraction gave twice as much as dried materials, as compared with water extraction (Figure 1), it is very useful method to effectively utilize the natural resources. Application of the present alkaline extraction to other plant species may hopefully manufacture products that enrich our daily life.

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