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Antiproliferative Effects of Native Plants on Prostate Cancer Cells

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Abstract – As part of the research for the natural products about prostate-related disease, this study screened 159 plant species from 46 families, which included a total of 213 different kinds of local native plants and these plants were tested for the ability to inhibit LNCaP proliferation, an androgen-sensitive prostate cancer cell line, and DU145 proliferation, which is a more aggressive androgen-insensitive prostate cancer cell line. The results indicated that nineteen of 213 types of plants exhibited antiproliferative activity (cell viability < 30%, 500 μg/mL) on the growth of androgen-sensitive LNCaP cell lines, and five of them exhibited DU145 cell antiproliferative activity (cell viability < 30%, 500 μg/mL). The methanol extracts of *Eurya emarginata* (stems), *Gleditsia japonica* var. *koraiensis* (leaves), *Photinia glabra* (leaves) and *Elaeagnus macrophylla* (leaves) showed antiproliferative activity on both the androgen-sensitive LNCaP cells (cell viability < 30%) and androgen-insensitive DU145 cells (cell viability > 100%). The study also found that the methanol extracts of *Styrax japonica* (fruits), *Aralia continentalis* (leaves), *Fagus crenata* var. *multinervis* (stems), *Thuja orientalis* (stems) and *Poncirus trifoliate* (branches) presented the strongest activity and demonstrated potent antiproliferative activity on both cell lines (LNCaP and DU145 cell viability < 30%).

Keywords – Local native plants, Prostate cancer, Antiproliferative activity, LNCaP cells, DU145 cells.

Introduction

Prostate cancer (PCa) is a very common male-specific malignancy, being the second most common cancer found among men worldwide (Jemal *et al*., 2007), and it is generally believed that androgens play a critical role in this disease (Coffey and Walsh, 1990). In normal prostate tissue, androgens regulate the growth and differentiation of epithelial cells. During the early stages of PCa, androgens increase cell proliferation but can be kept in check by various therapies aimed at either decreasing the circulating androgens or blocking the androgen receptors using antagonists such as flutamide (Agarwal *et al*., 2002). However, in advanced stages of PCa, growth and development typically become refractory to androgen effects, and cells continue to grow in an unregulated manner. Interestingly, the majority of PCa patients show a refractory phase to antiandrogenic therapy within 1.5 - 2 years after beginning treatment (Long *et al*., 2000). The current therapies have significant limitations because the tumor eventually becomes resistant to the therapy (Chen *et al*., 2006). Recently, many anticancer drugs have been developed that are used for clinically controlling PCa, although additional specific and efficacious anticancer and chemopreventive agents are needed for PCa treatment.

Several plant compounds and mixtures have been shown to be effective against PCa cell growth including grape seed polyphenol extracts, lycopene and tomato preparations, soy isoflavones, and green tea extracts (Agarwal *et al*., 2002; Singh *et al*., 2004; Kotake-Nara *et al*., 2001; Vij and Kumar, 2004; Lee *et al*., 2004). One of the herbal mixtures frequently used by individuals with PCa is the Chinese herbal mixture represented PC-SPES (Darzykiewicz *et al*., 2001; Moyad *et al*., 1999). Previous studies showed that PC-SPES mediated an antiproliferative effect on PCa cells *in vivo* (Taille *et al*., 1999; Moyad *et al*., 1999; Hsieh *et al*., 1997). Saw Palmetto (Serenoa repens) is one of the components of PC-SPES. It has been studied exclusively in China and has been shown to possess anti-tumor activity. However, the mechanism by which Saw Palmetto inhibits growth of PCa cells remains to be fully elucidated.

This paper reports the antiproliferative activity of 159 plant species from 46 families of local native plants on two prostate cancer cell lines, LNCaP and DU145, which

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are androgen-sensitive and androgen-insensitive, respectively. The potential of these plants to treat early and advanced stages of PCa was the main focus of this research.

Experimental

Plant materials and sample preparation – The methanol extracts of 159 plant species from 46 families were purchased from Plant Extract Bank of Korea (KRIBB, Daejeon) in September 2010. Voucher specimens (Table 1, specimens 1-213) were deposited at Pharmacognosy Lab, College of Pharmacy, Chung-Ang University. For the antiproliferative activity tests, the extracts were dissolved in DMSO and distilled water to a final concentration of 500 μg/mL.

Cell culture – Androgen-sensitive prostate cancer cell line (LNCaP) and androgen-insensitive prostate cancer cell line (DU145) cells were purchased from Korean Cell Line Bank (KCLB, Seoul). These cell lines were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma, St. Louis, MO, USA) with 0.25% glucose, 0.238% HEPES, 0.011% sodium pyruvate, 0.15% $NaHCO_3$, 10% fetal bovine serum (FBS), 100 IU/mL penicillin G and 100 mg/mL streptomycin (Gibco BRL, Grand Island, NY, USA). Both cell lines were kept in a humidified incubator at 37 °C with 5% CO_2 and were subcultured after 75 mm flasks were determined to be 90% confluent. Cell culture experiments were conducted during the 6 - 12 passage numbers of cells.

Antiproliferation assays – Antiproliferation assay was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT), which is based on the reduction of MTT to formazan by mitochondrial dehydrogenase. LNCaP cells were plated at a minimum density of 2×4 10^4 cells/mL (180 μL/well) in a 96-well plate, with a treatment incubation period of 72 h to adjust for the slow doubling time. The methanol extracts were dissolved in dimethylsulfoxide (DMSO) and administered in RPMI1640 medium supplemented with 10% fetal bovine serum, and the final DMSO concentrations were less than 1.0%. The plates were incubated for 24 h at 37 °C. After the incubation, the MTT reagent (0.5 mg/mL) (Sigma, St. Louis, MO, USA) was added to medium and incubated for additional 4 h. The medium was then removed, and the MTT-formazan was dissolved in 200 μL DMSO. The extent of MTT reduction to formazan was quantified by measuring the absorbance at 540 nm using the microplate reader. DU145 cells were handled in a similar manner except that the cell density was adjusted to 1×10^4 cells/mL (180 μL/well) in a 96-well plate, and the cells were incubated for 48 h, after which the bioassays were performed.

Statistical analysis – All of the experiments were performed in duplicate, and the results are expressed as the average of three replications.

Results and Discussion

This study examined a total of 159 species from 46 plant families of known local native plants that were evaluated for antiproliferative activity. The bioactive results of these plants are shown in Table 1. Antiproliferative activity (cell viability < 30%) on the growth of androgen-sensitive LNCaP cells was demonstrated by the methanol extracts of *Aralia continentalis* (leaves), *Poncirus trifoliate* (branchs), *Styrax japonica* (fruits), *Thuja orientalis* (stems), *Fagus crenata* var. *multinervis* (stems), *Juglans mandshurica* (fruits), *Betula chinensis* (stems), *Eurya emarginata* (stems), *Gleditsia japonica* var. *koraiensis* (leaves), *Cornus macrophylla* (leaves), *Photinia glabra* (leaves), *Juniperus rigida* (leaves), *Elaeagnus macrophylla* (leaves), *Cudrania tricuspidata* (stems), *Evodia daniellii* (leaves), *Zanthoxylum schinifolium* (stems), *Tilia insularis* (stems), *Thuja orientalis* (leaves) and *Alisma canaliculatum* (whole plant). Additionally, the methanol extracts of *Eurya emarginata* (stems), *Gleditsia japonica* var. *koraiensis* (leaves), *Photinia glabra* (leaves) and *Elaeagnus macrophylla* (leaves) showed antiproliferative activity on both the androgen-sensitive LNCaP cells (cell viability < 30%) and androgen-insensitive DU145 cells (cell viability > 100%).

Previous studies have indicated that eutigosides B and C can be isolated from the stems of *Eurya emarginata* (Park *et al*., 2005a), and studies have also indicated that the leaves of *Gleditsia japonica* var. *koraiensis* contain vitexin, isovitexin, orientin, isoorientin, quinic acid derivative, caffeic acid, quercetin, isoquercitrin, and luteolin 7-*O*-glucopyranoside, all of which were isolated and reported (Hwang *et al*., 1994).

The results from these studies suggest that the four extracts mentioned above could potentially be useful for treating androgen-sensitive disorders. Androgens have also been implicated in the etiology of the two most common disorders of the prostate, benign prostatic hyperplasia (BPH) and PCa (Huggins and Hodges, 1941). In addition, it has been well established that androgens, such as testosterone and 5á-dihydrotestosterone (DHT), play an essential role in stimulating hyperplasia and carcinoma of hormone-sensitive tissue such as the prostate (Kokontis

Table 1. Antiproliferative effects of local native plants on LNCaP and DU145 prostate cancer cell lines

No.	Family	Scientific name	Part	Cell viability (%)	
				LNCaP	DU 145
1	Aceraceae	*Acer palmatum var. nakaii*	leaves	30.74	71.05
2	Aceraceae	*Acerpictum* var. *mono*	leaves	35.36	50.51
3	Aceraceae	*Acer barbinerve*	leaves	47.12	54.77
4	Aceraceae	*Acerpictum* var. *mono*	stems	48.32	–
5	Aceraceae	*Acer truncatum*	stems	57.94	79.92
6	Aceraceae	*Acer pseudo-sibolianum*	leaves	80.27	84.90
7	Aceraceae	*Acer palmatum*	leaves	–	–
8	Actinidiaceae	*Actinidia rufa*	leaves	33.07	81.73
9	Actinidiaceae	*Actinidia kolomikta*	stems	68.50	–
10	Actinidiaceae	*Actinidia arguta* var. *platyphylla*	leaves	87.50	–
11	Alismataceae	*Alisma canaliculatum*	whole	29.33	44.32
12	Anacardiaceae	*Rhus chinensis*	leaves	40.03	41.60
13	Anacardiaceae	*Rhus trichocarpa*	leaves	42.22	65.98
14	Anacardiaceae	*Rhus sylvestris*	leaves	43.71	69.52
15	Anacardiaceae	*Rhus succedanea*	leaves	46.20	52.23
16	Anacardiaceae	*Rhus succedanea*	stems	48.38	53.29
17	Aquifoliaceae	*Ilex crenata* var. *microphylla*	stems	51.06	94.49
18	Aquifoliaceae	*Ilex crenata*	stems	55.85	–
19	Aquifoliaceae	*Ilex cornuta*	leaves	60.60	–
20	Aquifoliaceae	*Ilex integra*	fruits	73.79	85.85
21	Aquifoliaceae	*Ilex integra*	leaves	74.22	–
22	Aquifoliaceae	*Ilex crenata*	leaves	76.63	–
23	Aquifoliaceae	*Ilex rotunda*	leaves	78.49	–
24	Aquifoliaceae	*Ilex crenata* var. *microphylla*	leaves	78.72	–
25	Aquifoliaceae	*Ilex serrata* var. *sieboldii*	fruits	86.48	–
26	Aquifoliaceae	*Ilex x wandoensis*	leaves	94.41	–
27	Aquifoliaceae	*Ilex macropoda*	leaves	98.36	–
28	Araceae	*Arisaema ringens*	fruits	–	98.83
29	Araliaceae	*Aralia continentalis*	leaves	17.07	26.79
30	Araliaceae	*Aralia continentalis*	stems	30.37	59.84
31	Araliaceae	*Aralia elata*	stems	55.72	71.35
32	Araliaceae	*Fatsia japonica*	fruits	81.47	–
33	Araliaceae	*Kalopanax pictus*	stems	84.06	–
34	Araliaceae	*Aralia elata*	leaves	88.96	–
35	Araliaceae	*Fatsia japonica*	leaves	–	–
36	Betulaceae	*Betula chinensis*	stems	23.88	61.80
37	Betulaceae	*Carpinus tschonoskii*	stems	32.69	34.67
38	Betulaceae	*Alnus pendula*	stems	40.88	40.69
39	Betulaceae	*Alnus pendula*	leaves	49.66	57.50
40	Betulaceae	*Alnus hirsuta*	bark	51.77	72.50
41	Betulaceae	*Corylus heterophylla* var. *thunbergii*	leaves	57.03	84.01
42	Betulaceae	*Corylus sieboldiana* var. *mandshurica*	stems	59.65	86.54
43	Betulaceae	*Carpinus tschonoskii*	leaves	62.37	–
44	Betulaceae	*Corylus heterophylla* var. *thunbergii*	stems	–	–
45	Caprifoliaceae	*Viburnum sargentii*	fruits	46.62	–

Table 1. continued

No.	Family	Scientific name	Part	Cell viability (%)	
				LNCaP	DU 145
46	Caprifoliaceae	*Lonicera maackii*	fruits	56.64	88.63
47	Celastraceae	*Celastrus orbiculatus*	fruits	36.80	85.17
48	Celastraceae	*Euonymus sieboldiana*	fruits	37.49	65.23
49	Compositae	*Xanthium strumarium*	fruits	57.68	–
50	Cornaceae	*Cornus macrophylla*	leaves	25.08	89.05
51	Cornaceae	*Aucuba japonica*	stems	30.56	81.33
52	Cornaceae	*Cornus officinalis*	leaves	37.87	92.49
53	Cornaceae	*Cornus controversa*	leaves	40.20	–
54	Cornaceae	*Cornus macrophylla*	stems	49.21	71.56
55	Cornaceae	*Cornus walteri*	stems	70.20	87.72
56	Crassulaceae	*Rhodiola sacchalinensis*	roots	61.57	89.16
57	Cupressaceae	*Thuja orientalis*	stems	20.90	28.05
58	Cupressaceae	*Juniperus rigida*	leaves	25.46	49.76
59	Cupressaceae	*Thuja orientalis*	leaves	28.22	64.03
60	Cupressaceae	*Juniperus chinensis*	leaves	31.46	79.78
61	Cupressaceae	*Chamaecyparis obtusa*	stems	61.85	88.45
62	Ebenaceae	*Diospyros lotus*	fruits	46.90	82.36
63	Elaeagnaceae	*Elaeagnus macrophylla*	leaves	25.97	–
64	Elaeagnaceae	*Elaeagnus umbellata*	leaves	83.96	93.04
65	Elaeagnaceae	*Elaeagnus glabra*	fruits	–	–
66	Elaeagnaceae	*Elaeagnus glabra*	leaves	–	–
67	Ericaceae	*Rhododendron weyrichii*	stems	30.49	82.78
68	Ericaceae	*Vaccinium oldhami*	stems	31.37	–
69	Ericaceae	*Vaccinium koreanum*	stems	60.36	–
70	Ericaceae	*Vaccinium oldhami*	fruits	98.31	–
71	Ericaceae	*Vaccinium koreanum*	leaves	–	–
72	Ericaceae	*Rhododendron weyrichii*	leaves	–	–
73	Ericaceae	*Vaccinium oldhami*	leaves	–	–
74	Euphorbiaceae	*Phyllanthus ussuriensis*	whole	63.51	93.93
75	Fagaceae	*Fagus crenata* var. *multinervis*	stems	21.35	27.79
76	Fagaceae	*Castanopsis cuspidata* var. *sieboldii*	stems	32.55	83.74
77	Fagaceae	*Quercus gilva*	leaves	32.85	–
78	Fagaceae	*Quercus serrata*	stems	35.94	56.53
79	Fagaceae	*Quercus glauca*	stems	39.16	–
80	Fagaceae	*Castanea crenata*	leaves	41.07	–
81	Fagaceae	*Castanea crenata*	stems	54.19	68.99
82	Fagaceae	*Quercus acuta*	leaves	55.69	–
83	Fagaceae	*Quercus salicina*	leaves	60.64	75.49
84	Fagaceae	*Quercus serrata*	leaves	67.03	–
85	Fagaceae	*Quercus dentata*	stems	67.62	73.10
86	Fagaceae	*Quercus glauca*	leaves	69.06	–
87	Fagaceae	*Quercus mongolica*	leaves	84.50	86.32
88	Fagaceae	*Castanopsis cuspidata* var. *sieboldii*	stems	85.61	82.40
89	Fagaceae	*Quercus dentata*	leaves	91.40	89.80
90	Fagaceae	*Castanopsis cuspidata* var. *sieboldii*	leaves	–	–

Table 1. continued

No.	Family	Scientific name	Part	Cell viability (%)	
				LNCaP	DU 145
91	Flacourtiaceae	*Idesia polycarpa*	fruits	91.00	–
92	Hamamelidaceae	*Corylus sieboldiana*	stems	67.22	94.71
93	Hamamelidaceae	*Distylium racemosum*	leaves	69.25	–
94	Illiciaceae	*Illicium religiosum*	stems	38.72	–
95	Illiciaceae	*Illicium religiosum*	leaves	73.31	–
96	Juglandaceae	*Juglans mandshurica*	fruits	22.32	45.72
97	Juglandaceae	*Platycarya strobilacea*	fruits	34.45	67.02
98	Juglandaceae	*Juglans sinensis*	fruits	50.32	80.44
99	Lauraceae	*Neolitsea sericea*	fruits	41.06	79.98
100	Leguminosae	*Gleditsia japonica* var. *koraiensis*	leaves	24.26	–
101	Leguminosae	*Gleditsia japonica* var. *koraiensis*	stems	32.03	65.23
102	Leguminosae	*Sophora japonica*	stems	33.16	89.45
103	Leguminosae	*Caesalpinia japonica*	leaves	72.81	95.35
104	Leguminosae	*Maackia amurensis*	fruits	81.93	–
105	Leguminosae	*Maackia amurensis*	leaves	84.31	–
106	Leguminosae	*Cercis chinensis*	leaves	84.96	–
107	Leguminosae	*Robinia pseudo-accacia*	leaves	92.37	–
108	Liliaceae	*Smilax china*	fruits	40.46	93.17
109	Liliaceae	*Liriope platyphylla*	fruits	87.57	–
110	Magnoliaceae	*Photinia glabra*	leaves	25.17	–
111	Magnoliaceae	*Magnolia kobus*	stems	41.38	78.27
112	Moraceae	*Cudrania tricuspidata*	stems	26.11	63.68
113	Moraceae	*Morus alba*	stems	31.46	80.93
114	Moraceae	*Ficus stipulata*	leaves	31.76	–
115	Moraceae	*Cudrania tricuspidata*	leaves	37.03	45.02
116	Moraceae	*Broussonetia kazinoki*	stems	56.17	89.42
117	Moraceae	*Morus alba*	leaves	78.03	82.24
118	Moraceae	*Broussonetia kazinoki*	leaves	87.22	–
119	Moraceae	*Morus tiliaefolia*	leaves	87.67	–
120	Moraceae	*Ficus erecta*	fruits	88.14	–
121	Moraceae	*Morus bombycis*	stems	88.63	89.07
122	Moraceae	*Cudrania tricuspidata*	fruits	–	–
123	Myricaceae	*Myrica rubra*	leaves	45.19	97.76
124	Oleaceae	*Syringa velutina*	stems	48.45	–
125	Oleaceae	*Osmanthus heterophylla*	leaves	49.35	96.01
126	Oleaceae	*Ligustrum obtusifolium*	fruits	61.11	–
127	Oleaceae	*Fraxinus mandshurica*	stems	62.12	–
128	Oleaceae	*Ligustrum lucidum*	fruits	67.66	–
129	Oleaceae	*Osmanthus fragrans* var. *aurantiacus*	leaves	72.77	–
130	Oleaceae	*Ligustrum obtusifolium*	stems	73.18	–
131	Oleaceae	*Osmanthus fragrans*	stems	75.30	88.75
132	Oleaceae	*Fraxinus rhynchophylla*	stems	76.43	–
133	Oleaceae	*Ligustrum japonicum*	leaves	80.43	94.54
134	Oleaceae	*Osmanthus heterophylla*	fruits	90.23	–
135	Oleaceae	*Osmanthus fragrans*	leaves	–	–

Table 1. continued

No.	Family	Scientific name	Part	Cell viability (%)	
				LNCaP	DU 145
136	Oleaceae	*Syringa reticulata* var. *mandshuriea*	leaves	–	–
137	Oleaceae	*Ligustrum obtusifolium*	leaves	–	–
138	Oleaceae	*Fraxinus rhynchophylla*	fruits	–	–
139	Punicaceae	*Punica granatum*	stems	43.75	77.04
140	Rhamnaceae	*Hovenia dulcis*	leaves	48.66	51.50
141	Rhamnaceae	*Rhamnus davurica*	stems	49.46	–
142	Rhamnaceae	*Sageretia theezans*	fruits	54.21	–
143	Rhamnaceae	*Paliurus ramosissimus*	leaves	54.39	55.76
144	Rhamnaceae	*Zizyphusjujuba* var. *inermis*	fruits	79.08	96.32
145	Rhamnaceae	*Hovenia dulcis*	stems	–	–
146	Rhamnaceae	*Hovenia dulcis*	fruits	–	–
147	Rosaceae	*Prunus pendula for. ascendens*	stems	30.35	47.02
148	Rosaceae	*Pyracantha angustifolia*	fruits	41.64	90.96
149	Rosaceae	*Rosa multiflora*	roots	45.44	–
150	Rosaceae	*Crataegus maximowiczii*	fruits	48.20	85.02
151	Rosaceae	*Pyrus pyrifolia*	leaves	52.36	–
152	Rosaceae	*Prunus yedoensis*	stems	52.90	–
153	Rosaceae	*Prunus salicina*	stems	53.55	–
154	Rosaceae	*Sorbus alnifolia*	leaves	55.93	77.75
155	Rosaceae	*Pyrus calleryana* var. *fauriei*	leaves	59.12	85.41
156	Rosaceae	*Raphiolepis umbellata*	fruits	66.24	74.02
157	Rosaceae	*Prunus padus*	stems	73.87	–
158	Rosaceae	*Sorbus alnifolia*	fruits	88.46	–
159	Rosaceae	*Sorbus commixta*	fruits	93.11	–
160	Rubiaceae	*Rubia akane*	fruits	51.21	64.54
161	Rubiaceae	*Gardenia jasminoides for. grandiflora*	stems	57.63	–
162	Rubiaceae	*Gardenia jasminoides for. grandiflora*	leaves	83.77	–
163	Rutaceae	*Poncirus trifoliata*	branches	19.13	29.92
164	Rutaceae	*Evodia daniellii*	leaves	26.86	49.91
165	Rutaceae	*Zanthoxylum schinifolium*	stems	27.20	46.30
166	Rutaceae	*Evodia daniellii*	stems	31.40	76.57
167	Rutaceae	*Zanthoxylum ailanthoides*	leaves	45.04	70.52
168	Rutaceae	*Poncirus trifoliata*	stems	45.22	67.30
169	Rutaceae	*Poncirus trifoliata*	fruits	47.18	69.65
170	Rutaceae	*Zanthoxylum ailanthoides*	fruits	62.05	–
171	Rutaceae	*Zanthoxylum ailanthoides*	stems	80.66	–
172	Rutaceae	*Citrus junos*	leaves	93.47	–
173	Rutaceae	*Citrus junos*	stems	94.21	–
174	Rutaceae	*Phellodendron amurense*	leaves	–	–
175	Rutaceae	*Zanthoxylum piperitum*	leaves	–	–
176	Salicaceae	*Populus deltoides*	stems	30.94	86.74
177	Salicaceae	*Salix hultenii*	leaves	48.36	–
178	Salicaceae	*Salix koreensis*	leaves	–	–
179	Saxifragaceae	*Ribes fasciculatum* var. *chinense*	fruits	87.59	–
180	Scrophulariaceae	*Paulownia coreana*	fruits	49.16	76.91

Table 1. continued

No.	Family	Scientific name	Part	Cell viability (%)	
				LNCaP	DU 145
181	Solanaceae	*Tubocapsicum anomalum*	fruits	52.21	98.28
182	Staphyleaceae	*Euscaphis japonica*	fruits	44.93	53.13
183	Styracaceae	*Styrax japonica*	fruits	20.49	23.05
184	Taxaceae	*Torreya nucifera*	stems	55.65	71.71
185	Taxaceae	*Torreya nucifera*	leaves	56.05	69.25
186	Taxaceae	*Taxus cuspidata*	stems	58.54	–
187	Taxaceae	*Taxus cuspidata*	leaves	61.51	87.86
188	Taxaceae	*Taxus cuspidata* var. *latifolia*	leaves	61.55	97.35
189	Theaceae	*Eurya emarginata*	stems	24.20	–
190	Theaceae	*Cleyera japonica*	branches	34.04	94.12
191	Theaceae	*Camellia japonica*	stems	41.96	72.85
192	Theaceae	*Cleyera japonica*	leaves	47.20	52.15
193	Theaceae	*Eurya emarginata*	leaves	54.11	91.59
194	Theaceae	*Ternstroemia japonica*	leaves	55.09	89.28
195	Theaceae	*Camellia japonica*	leaves	69.50	–
196	Theaceae	*Thea sinensis*	stems	83.73	83.07
197	Theaceae	*Eurya japonica*	leaves	86.89	–
198	Thymelaeaceae	*Edgeworthia papyrifera*	stems	33.76	78.31
199	Thymelaeaceae	*Daphne genkwa*	stems	44.51	–
200	Thymelaeaceae	*Daphne kiusiana*	leaves	58.94	98.30
201	Tiliaceae	*Tilia insularis*	stems	27.77	71.72
202	Tiliaceae	*Tilia amurensis*	stems	35.86	92.45
203	Tiliaceae	*Grewia biloba* var. *parviflora*	leaves	50.32	90.73
204	Tiliaceae	*Grewia biloba* var. *parviflora*	fruits	74.38	–
205	Ulmaceae	*Ulmus davidiana for. suberosa*	stems	30.10	–
206	Ulmaceae	*Ulmus parvifolia*	stems	31.00	–
207	Ulmaceae	*Hemiptelea davidii*	stems	31.10	–
208	Ulmaceae	*Celtis choseniana*	leaves	31.43	90.67
209	Ulmaceae	*Ulmus pumila*	stems	54.91	–
210	Ulmaceae	*Celtis choseniana*	fruits	–	–
211	Verbenaceae	*Callicarpa japonica* var. *luxurians*	fruits	45.07	–
212	Verbenaceae	*Callicarpa dichotoma*	fruits	62.20	84.15
213	Verbenaceae	*Callicarpa japonica*	fruits	94.18	–

–: no activity at tested concentrations

and Liao, 1999), and soy isoflavones have been found to inhibit the production of DHT, an active androgen, in the prostate (Vij and Kumar, 2004). Another study found that the abundant and widely distributed quercetin decreased the function of the androgen receptor protein in the LNCaP androgen-sensitive human prostate cancer cell line (Xing *et al*., 2001). Zand et al. (2002) found that a range of flavonoids significantly inhibited the androgen-related protein prostate-specific antigen (PSA) in the PC-3$(AR)_2$ prostate cancer cell lines. This study evaluated a total of 213 kinds of plants for their antiproliferative activity on the growth of androgen-insensitive DU145 cells. The results indicated that *Styrax japonica* (fruits), *Aralia continentalis* (leaves), *Fagus crenata* var. *multinervis* (stems), *Thuja orientalis* (stems) and *Poncirus trifoliate* (branches) showed significant antiproliferative activity (cell viability < 30%). Furthermore, these results indicated that more than five extracts demonstrated potent antiproliferative activity against both cell lines (LNCaP and DU145 cell viability < 30%). The leaves of *Styrax*

species were found to be a rich source of benzofurans, benzofuran esters, benzofuran glycosides and sapogenins (Akgul and Anil, 2003; Anil, 1980; Kitagawa *et al*., 1975). The *Aralia continentalis* roots were found to contain diterpenic acid, which were isolated for analysis in previous studies (Lim *et al*., 2009). Diterpenoids, essential oils, lignans and flavonoids were isolated from *Thuja orientalis* (Lee *et al*., 2008; Guleria *et al*., 2008; Yoon *et al*., 2008; Xu *et al*., 2009), and the dried immature fruits of *Poncirus trifoliata* have been found to contain compounds of interest, including flavonoids and coumarins (Park *et al*., 2005b; Guiotto *et al*., 1976). In addition, standardized grape seed extracts that contained 95% oligomeric proanthocyanidins have been reported to reduce PSA production and induce apoptotic death in androgen-insensitive DU145 cells (Agarwal *et al*., 2002). The results of this study indicated that the methanol extracts of five plants such as *Styrax japonica* (fruits), *Aralia continentalis* (leaves), *Fagus crenata* var. *multinervis* (stems), *Thuja orientalis* (stems) and *Poncirus trifoliate* (branches) could potentially be useful and applied in treatment of androgen-insensitive disorders such as advanced stages of PCa.

Although the results discussed above were limited to *in vitro* antiproliferative activities, the results of this study suggest that many natural plants could be applied as excellent sources for the treatment of prostate related diseases.

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