

Antiproliferative Activity of Korean Wild Vegetables on Different Human Tumor Cell Lines

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Abstract This study was conducted to determine the antiproliferative activity of 24 Korean wild vegetables. The methanol extracts of these wild vegetables were used against lung, breast, colon and gastric cancer cells, and the results were assessed by MTT assay. It was found that at the extract concentration of 400 mgL⁻¹ 14 plants exercised antiproliferative activity over 80% against the lung cancer cells, one plant among six—against breast cancer cells, and two plants among six—against colon cancer cells, respectively. Eighteen wild vegetables had the hyperplasia inhibition activity against gastric cancer cells over 23.6% at all extract concentrations, however, only six plants had the antiproliferative activity over 80% in 600 mgL⁻¹. It was

found that the extracts from *Youngia sonchifolia*, *Synurus deltooides*, *Syneilesis palmata*, and *Cephalonoplos segetum*, in concentration of 400 mgL⁻¹ inhibited the hyperplasia of lung cancer cells over 95% and *Angelica gigas*—both lung and colon cancer cells over 95%. In conclusion, the studied wild vegetables' methanol extracts possess dose dependent antiproliferative properties, based on their bioactive compounds, mainly polyphenols, but some of them as *Hypericum ascyron* against lung cancer are not effective and even cause harm.

Keywords Korean wild vegetables · Methanol extracts · Antiproliferative activity

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Abbreviations

Calus-6	human pulmonary carcinoma—human cancer cell line
FBS	fetal bovine serum
HCT-116	human colon carcinoma—human cancer cell line
MTT (3-(4, 5-dimethylthiazol -2-yl)-2,5-diphenyltetrazolium bromide)	assay for determination of anti-cancer activity
NAG-1	drug-activated gene
NCF-7	human breast carcinoma—human cancer cell line
PAB	diterpenoid pseudolaric acid B
PARP	poly ADP-ribose polymerase
RPMI 1640	A medium developed by Poswell Park Memorial Institute and used for the culture of human normal and neoplastic leukocytes
SNU-601	human gastric carcinoma—human cancer cell line

Introduction

In spite of the new measures of prevention and treatment, cancers remain one of the most dangerous diseases [1–4]. Each year the American Cancer Society (ACS) estimates the number of new cancer cases and deaths expected in the United States in the current year. ACS compiles the most recent data on cancer incidence, mortality, and survival based on incidence data from the National Cancer Institute, Centers for Disease Control and Prevention, and the North American Association of Central Cancer Registries and mortality data from the National Center for Health Statistics [3]. A total of 1,444,920 new cancer cases and 559,650 deaths for cancers are projected to occur in the United States in 2007. Notable trends in cancer incidence and mortality rates include stabilization of the age-standardized, delay-adjusted incidence rates for all cancers combined in men from 1995 through 2003; a continuing increase in the incidence rate by 0.3% per year in women; and a 13.6% total decrease in age-standardized cancer death rates among men and women combined between 1991 and 2004 [3].

In most cases chemo and radio therapy of cancer lead to serious side effects. Therefore, the search for anticancer plants, which have minimal side effects is on increase [5, 6]. In the last years some authors found that additional non known different plants possess some antiproliferative properties [7–9]. So, Sun et al. [7] reported about antiproliferative activities of common fruits. Investigation *in vitro* and *in vivo* revealed that also *Astragalus membranaceus* possesses anti-tumor properties [8]. We also investigated some plants and discovered that Korean salad plants possess antiproliferative properties [10, 11]. This time we decided to investigate 24 Korean wild vegetables in order to determine their possible anticancer activities. The polyphenols of these plants were extracted with methanol, the extracts were used against lung, breast, colon and gastric cancer cells, and the results were assessed by MTT assay.

As far as we know there are not such investigations.

Materials and Methods

Chemicals All chemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA. All reagents were of analytical grade. Deionized and distilled water was used throughout.

The cell lines were purchased from Korean Cell Line Bank.

Plant Material

All samples were whole wild plants or their young sprouts, leaves and roots gathered in April to July 2008 in habitats

in Suncheon, Jeollanam-do. The studied plants were (in alphabetical order) *Ainsliaea acerifolia*, *Amaranthus mangostanus*, *Angelica gigas*, *Arctium lappa*, *Aster scaber*, *Capsella bursa-pastori*, *Cephalonoplos segetum*, *Chenopodium album*, *Codonopsis lanceolata*, *Hemerocallis minor*, *Hypericum ascyron*, *Ixeris dentata*, *Lilium hansonii*, *Osmunda japonica*, *Pimpinella brachycarpa*, *Pteridium aquilinum*, *Rumex acetosa*, *Saxifraga stolonifera*, *Solidago virgaurea*, *Symplocarpus renifolius*, *Syneilesis palmata*, *Synurus deltoides*, *Taraxacum mongolicum* and *Youngia sonchifolia*.

The gathered samples were dried at 50 °C for 5 days, pulverized, passed through a 1 mm strainer and extracted (200 g in 2 L of 95% methanol) for 24 h. The extracts were taken for decompression concentration process at 50 °C by an evaporator IKA-Werke GmbH & Co. KG), dried by a freeze dryer (Samwon Co., Korea) and pulverized.

The methanol extracts of the plants were tested for polyphenol content and their possible anticancer properties on the lung cancer, breast cancer, colon cancer, and gastric cancer cell lines.

Methods

Polyphenols were determined by Folin-Ciocalteu method and the measurement was performed at 765 nm with gallic acid as the standard [10, 11].

Tumor cell line of the experiment has its origins in human bodies, including Calus-6 (ATCC, HTB-56), lung cancer cell line, breast cancer cell line, NCF-7, colon cancer cell line, HCT-116 and gastric cancer cell line SNU-601 from Korean Cell Line Bank. The cell lines were adapted to and nourished in a humid medium at 37 °C and 5% CO₂ with RPMI 1640 times paper added in 10% FBS (fetal bovine serum), penicillin G (25 unit/mL) and streptomycin (25 mgL⁻¹).

Inhibitory effects on the hyperplasia of these cancer cells were assessed using MTT assay [12, 13]. The tumor cells were adjusted to be in the extract concentration of 3×10⁴ cells/mL, divided per 90 μL/well in 96 well micro plates, cultivated in a medium (Forma, Germany) at 37 °C, 5% CO₂ for 12 h to attach the cells and added 10 μL, respectively, to be in the extract concentration of 50, 100, 200, 400 and 800 mgL⁻¹. To the control groups distilled water was added as the same amount as the sample and cultivated for 72 h, put 10 μL of MTT (3-(4,5-dimethylthiazol -2-yl)-2,5-diphenyltetrazolium bromide) solution with 5 mg/mL density in each well, incubated in mediums for 4 h, removed badges in MTT solution, added DMSO 150 μL to stir them for 30 min, dissolved each cell and measured absorbance at 540 nm using micro plate reader (Bio-Rad, USA). The relative cell growth rate was

converted at 100% non-sample-added groups of each cell [14].

Statistical Analysis

The values are means of three measurements. Dispersion analysis (ANOVA) in SAS program for the statistics took a significance test between the samples in 5% significance level by Duncan's multiple range test.

Results and Discussion

It was of interest to know how the content of the total polyphenols influences the anti-proliferative activity of the studied plants. Therefore, the content of the total polyphenols was determined. The statistical evaluation shows that the significantly highest content of polyphenols was in *Aster scaber* following by *Hypericum ascyron*: 88.9 ± 2.2 and 76.9 ± 1.9 mg kg⁻¹, respectively (Fig. 1).

The results of the inhibitory effects on the hyperplasia of lung cancer cells are summarized in the Table 1. As can be seen, the highest inhibitory effect was achieved at the extracts concentration of 800 mgL⁻¹.

Concentration of the methanol extracts (200 mgL⁻¹) from *Youngia sonchifolia*, *Solidago virgaurea*, *Syneilesis palmata*, *Angelica gigas* and *Aster scaber* on the cancer cell survival rate was below 27.8%. These wild vegetables also showed superior inhibitory effects on the hyperplasia of lung cancer

at the rate below 9.3% in the extract concentration of 400 mg L⁻¹. At concentration of the methanol extracts of 400 mg L⁻¹, the cell survival rate was below 70.7%, except of *Capsella bursa-pastori*, *Chenopodium album*, *Hypericum ascyron*, *Taraxacum mongolicum*, *Saxifraga stolonifera* and *Lilium hansonii*.

At concentration of 800 mg L⁻¹, the survival rate of 22 vegetables was below 9.9%, except for *Hypericum ascyron* (117.1%) and *Saxifraga stolonifera* (17.7%).

Kim et al. [15] conducted a research of 557 samples of wild vegetables. These authors found that only 44 of these samples had anticancer effect, including 17 of mulberry tree, *Amaranthus mangostanus*, tea plant, *Capsella bursa-pastori*, Winged spindle, Japanese angelica tree, *Peucedanum japonicum*, Chinese matrimony vine, Japanese lady bell, *Artemisia princeps*, *Cirsium japonicum*, *Ixeris dentata*, *Aster yomena*, *Petasites japonicus*, *Taraxacum mongolicum*, *Allium tuberosum*, *Erythronium japonicum*, and *Dioscorea japonica*. The results of the above cited study for the first time disclosed the antiproliferative properties of some additional wild vegetables. The results of our investigation show that one of the studied wild plants had a procancer effect. The survival rate of the lung cancer cells treated with methanol extract of *Hypericum ascyron* in all used concentrations of 50, 100, 200, 400, 600 and 800 mgL⁻¹ was 150.78, 143.76, 127.33, 122.85, 119.88 and 117.1%, respectively.

Therefore, not all wild vegetables could be helpful for treatment of lung cancer. Some of them (*Hypericum*

Fig. 1 Total polyphenols in the studied plants (values \pm standart deviations)

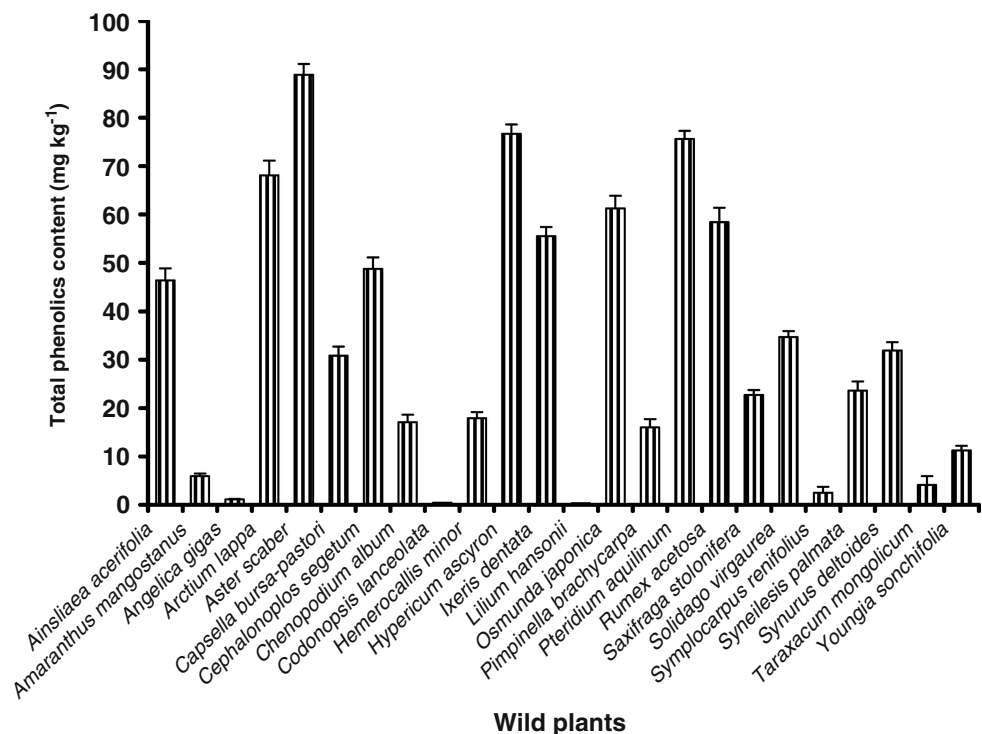


Table 1 Effects of methanol extracts from the 24 wild vegetables on the survival rate of lung cancer (%)

Wild vegetables	Extracting concentration (mg L ⁻¹)					
	50	100	200	400	600	800
<i>Youngia sonchifolia</i>	70.67	57.12	12.93	3.52	2.85	1.42
<i>Osmunda japonica</i>	66.18	62.80	60.27	12.77	10.27	8.54
<i>Pteridium aquilinum</i>	97.99	85.31	60.69	12.77	10.67	5.12
<i>Capsella bursa-pastori</i>	96.40	99.70	100.0	87.70	2.40	0.00
<i>Ainsliaea acerifolia</i>	60.10	53.72	47.58	25.44	2.80	2.15
<i>Codonopsis lanceolata</i>	90.90	78.13	65.85	45.20	4.95	0.49
<i>Chenopodium album</i>	101.12	94.45	88.24	80.47	23.83	4.31
<i>Hypericum ascyron</i>	150.78	143.76	127.33	122.85	119.88	117.10
<i>Solidago virgaurea</i>	91.15	72.45	10.40	7.12	2.65	1.79
<i>Taraxacum mongolicum</i>	99.70	95.80	82.60	77.50	22.90	2.80
<i>Saxifraga stolonifera</i>	121.79	121.17	119.19	70.66	18.62	17.66
<i>Amaranthus mangostanus</i>	80.56	77.14	66.71	17.90	1.05	0.78
<i>Lilium hansonii</i>	96.53	89.29	84.35	75.27	64.69	2.86
<i>Synurus deltooides</i>	97.95	94.37	50.73	4.52	3.44	1.53
<i>Rumex acetosa</i>	107.83	107.17	102.16	27.82	11.60	9.90
<i>Ixeris dentata</i>	88.20	86.20	73.40	32.40	9.50	3.90
<i>Symplocarpus renifolius</i>	89.78	85.14	57.85	9.94	6.85	4.73
<i>Hemerocallis minor</i>	83.69	77.45	72.71	10.20	1.67	1.10
<i>Syneilesis palmata</i>	94.15	85.11	27.79	3.76	2.57	1.55
<i>Arctium lappa</i>	80.53	78.04	68.59	16.24	9.34	8.14
<i>Cephalonoplos segetum</i>	79.10	74.72	66.90	3.31	0.76	0.56
<i>Pimpinella brachycarpa</i>	98.20	85.50	63.50	18.00	10.00	3.80
<i>Angelica gigas</i>	68.90	55.50	16.40	3.20	0.0	0.00
<i>Aster scaber</i>	85.60	73.30	25.70	9.30	5.40	1.60

The values are means of three measurements

ascyron) could even cause harm. The same is true for *Saxifraga stolonifera*: in concentrations of 50, 100 and 200 mgL⁻¹, the survival rate was 121.79, 121.17 and 119.19%, respectively (Fig. 2).

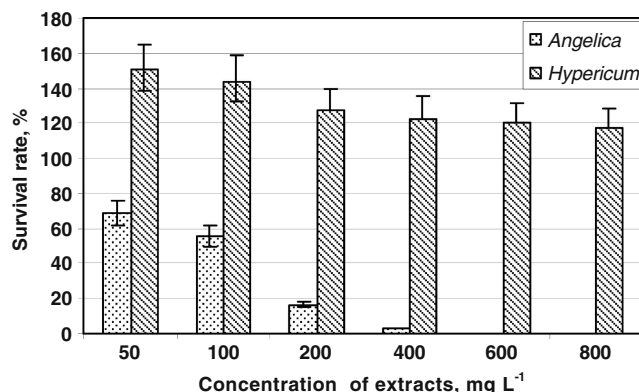


Fig. 2 Effects of *Angelica gigas* and *Hypericum ascyron* methanol extracts on lung cancer cells (%) survival

The results of the effect of methanol extracts from 6 wild vegetables on the survival rate of breast cancer are summarized in Table 2.

As can be seen, the survival rate of the breast cancer cells in methanol extract concentration of 200 mg L⁻¹ was over 74.4%, except of *Angelica gigas*—15.0%; in the extract of concentration of 400 mg L⁻¹—42.2%, except of *Angelica gigas*—8.4%, and in the extract with concentration of 800 mg L⁻¹—below 12.6%, except of *Ixeris dentata*—20.6%. According to the results of Table 2, *Ixeris dentata* methanol extract possesses the lowest level of inhibition effects on the hyperplasia of breast cancer cells and the *Angelica gigas*—the highest.

As reported by Kim et al. [16], methanol extracts of *Pimpinella brachycarpa* or *Taraxacum mongolicum* showed inhibition effects on lung cancer cells and on liver cancer cells (Hep3B) in acid fractions in concentration of 375 mg L⁻¹ of 70.6%, and 76% of inhibition effects on osteogenic sarcoma cancer cells. Therefore, it can be concluded that the anticancer activities of wild vegetables are different on various cancer types. Hence, it is required to confirm inhibitory effects on the

Table 2 Effects of methanol extracts from six wild vegetables on the survival rate of breast cancer (%)

Wild vegetables	Extracting concentration (mgL ⁻¹)					
	50	100	200	400	600	800
<i>Capsella bursa-pastori</i>	100.0	97.6	98.0	78.0	22.0	12.6
<i>Taraxacum mongolicum</i>	98.8	91.8	84.0	66.4	41.1	10.5
<i>Ixeris dentata</i>	100.0	100.0	94.1	83.6	63.2	20.6
<i>Pimpinella brachycarpa</i>	100.0	100.0	97.4	80.4	51.1	6.2
<i>Angelica gigas</i>	73.0	56.7	15.0	8.4	0.4	0.0
<i>Aster scaber</i>	96.0	90.8	74.4	42.2	15.4	3.1

The values are means of three measurements

hyperplasia of breast cancer cells by other vegetables, except of wild vegetables known for their anticancer effect.

The results of the effect of methanol extracts from 6 wild vegetables on the survival rate of colon cancer are summarized in Table 3.

In the methanol extract concentrations of 200 mg L⁻¹, the colon cancer cell survival rates were for *Angelica gigas* and *Aster scaber* of 26.9% and 33.5% (73.1% and 66.5% of the inhibition rate, respectively). However, the survival rates of four other used vegetables were over 65.8%. In the methanol extracts concentration of 400 mg L⁻¹ the survival rate for *Angelica gigas* and *Aster scaber* was 2.3% and 8.6%, respectively, a significant change vs. concentration of 200 mg L⁻¹.

However, the survival rates from four other used vegetables were over 48.5%. The survival rate of colon cancer cell at the extract concentration of 600 mg L⁻¹ vs. extract concentration of 400 mg L⁻¹, for *Capsella bursa-pastori* and *Pimpinella brachycarpa* was dramatic: 58.8 and 0.7 and 48.5 and 6.6%, respectively.

The best results were registered at the methanol extract concentrations of 800 mg L⁻¹: a clear sign of the dose dependent influence of the studied vegetables. The *Aster scaber*, *Pimpinella brachycarpa*, *Angelica gigas* and *Capsella bursa-pastori* had superior inhibitory effects on the hyperplasia of colon cancer cells and are distributed

especially through southern Korean local fifth day markets so that customers use them easily.

In this study the anticancer activity only of six wild vegetables against colon cancer, were tested. According to Kim et al. [17], colon cancer is found more frequently in western developed countries, however, owing to increasing westernized diets, Korea should take care of it. It is necessary further studies to test the anticancer activity of additional wild vegetables on colon cancer in order to use them as possible remedy in prevention and treatment of this dangerous disease.

The results of the influence of methanol extracts from 18 wild vegetables on the survival rate of gastric cancer are summarized in Table 4. The *Osmunda japonica* methanol extract concentration of 200 mg L⁻¹ was the most effective—the gastric cancer cell survival rate was 54.4%, and the range for other vegetables was from 61.0 to 101.1%.

As in the cases of lung, breast and colon cancers, we found a dose dependent anticancer influence.

So, the survival rate of gastric cancer cells decreased as the methanol extracts concentration increased: in 400 mg L⁻¹, the survival rates for 5 extracts were fewer than 50.5%, and in 800 mg L⁻¹ for 14 extracts—less than 15%, that is, over 85% of gastric cancer hyperplasia rate.

The exception was for: *Hypericum ascyron* (56.2%), *Lilium hansonii* (34.1%), *Ainsliaea acerifolia* (31.2%) and *Rumex acetosa* (21.9%).

Table 3 Effects of methanol extracts from six wild vegetables on the survival rate of colon cancer (%)

Wild vegetables	Extracting concentration (mgL ⁻¹)					
	50	100	200	400	600	800
<i>Capsella bursa-pastori</i>	100.0	100.0	94.1	58.8	0.7	0.0
<i>Taraxacum mongolicum</i>	96.7	95.7	84.4	76.6	48.9	1.9
<i>Ixeris dentata</i>	100.0	93.3	88.2	71.4	43.9	7.5
<i>Pimpinella brachycarpa</i>	90.0	79.4	65.8	48.5	6.6	2.1
<i>Angelica gigas</i>	56.0	40.7	26.9	2.3	0.6	0.6
<i>Aster scaber</i>	80.6	51.9	33.5	8.6	8.1	3.1

The values are means of three measurements

Table 4 Effects of methanol extracts from 18 wild vegetables on the survival rate of gastric cancer (%)

Wild vegetables	Extracting concentration (mg·L ⁻¹)					
	50	100	200	400	600	800
<i>Youngia sonchifolia</i>	75.78	67.86	60.95	29.65	6.18	0.78
<i>Osmunda japonica</i>	60.72	55.26	54.35	50.47	25.25	10.52
<i>Pteridium aquilinum</i>	84.24	82.93	75.29	58.00	20.09	14.95
<i>Ainsliaea acerifolia</i>	111.78	105.48	91.03	39.72	32.15	31.23
<i>Codonopsis lanceolata</i>	89.81	84.41	77.89	66.15	37.86	4.22
<i>Chenopodium album</i>	93.53	92.17	83.76	69.50	19.28	1.85
<i>Hypericum ascyron</i>	108.30	107.84	99.27	77.54	56.71	56.2
<i>Solidago virgaurea</i>	107.37	107.06	100.02	23.58	1.44	0.98
<i>Saxifraga stolonifera</i>	92.94	92.83	92.44	90.73	51.23	14.57
<i>Amaranthus mangostanus</i>	79.16	73.50	71.36	65.09	30.43	4.23
<i>Lilium hansonii</i>	97.5	97.24	95.80	91.05	81.38	34.08
<i>Synurus deltooides</i>	95.39	87.14	86.14	62.01	5.85	2.49
<i>Rumex acetosa</i>	104.33	102.76	100.50	100.20	44.91	21.92
<i>Symplocarpus renifolius</i>	91.53	90.17	83.76	69.50	19.28	1.85
<i>Hemerocallis minor</i>	84.45	83.91	82.97	80.05	24.42	2.37
<i>Syneilesis palmata</i>	105.95	105.4	94.77	24.73	3.16	2.56
<i>Arctium lappa</i>	87.91	86.72	85.35	83.64	51.03	6.65
<i>Cephalonoplos segetum</i>	100.45	99.03	90.28	76.82	42.95	5.30

The values are means of three measurements

Our results slightly correspond with others [18], showing that the aqueous crude extract of *Millingtonia hortensis* possess the apoptosis induction on RKO colon cancer cells. In this research were used only crude methanolic extracts, which possess a high bioactivity according to the results of polyphenols. Such methanolic extracts were used in other investigations, but not with the same plants [19], and the results were similar to ours. The methanolic extract of a Rubiaceae plant native to China *Uncaria rhynchophylla* (URE) showed highly cytotoxic effects via the MTT reduction assay on HT-29 cells. URE inhibited the growth of HT-29 cells in a dose-dependent manner. The methanolic URE of the 500 µg/ml showed 15.8% inhibition against growth of HT-29 cells. It induced characteristic apoptotic effects in HT-29 cells, including chromatin condensation and sharking occurring 24 h when the cells were treated at a concentration of 500 µg/ml. These results indicate that URE contains bioactive materials with strong activity, and is a potential chemotherapeutic agent candidate against HT-29 human colon carcinoma cells and correspond with our results. Organic solvent (methanol, ethanol, and acetone) extracts and water extracts of cherry (*Prunus serrulata* var. *spontanea*) blossoms plants [20] were prepared, and antioxidant activities of the extracts were evaluated. Methanolic extract (100 µg/ml) possess the highest total phenol content as in our results, and at 500 µg/ml showed 38.8% inhibition against growth of human colon cancer on

cell line HT-29. These results indicated that cherry blossoms could provide valuable bioactive materials, and our results indicated that some plants investigated in this research as well could provide high bioactivity.

Our results as well corresponded with another report [21], where the pulp of *Randia echinocarpa* fruit, which is a Rubiaceae plant native to Sinaloa, Mexico, was sequentially extracted with solvents of different polarity. A high extraction yield was obtained with methanol (72.17% d.w.), but the aqueous extract showed the highest content of phenolics (2.27 mg/g as ferulic acid equivalents). These results were higher than those in the plants shown in Fig. 1, but the conditions of extraction and the solvents were different in our experiment.

Eighty-eight extracts of different polarity obtained from 18 Lamiaceae medicinal and aromatic plants were screened for their antioxidant and antifungal properties [22]. In most cases the highest radical scavenger capacity was detected in methanolic and aqueous extracts, and polyphenols may be responsible. Our results correspond with this conclusion. Of course, not all the plants react in the same way: the most interesting antioxidant activity was observed in polar extracts obtained from *Lycopus europaeus*, *Melissa officinalis*, *Origanum vulgare* subsp. *virens* and *Lavandula latifolia*. On the contrary, the best results for the antifungal test against *Rhizopus stolonifer* were produced by non-polar herbal extracts [22].

Some vegetables, which showed inhibitory effects on the hyperplasia of gastric cancer cells and have fewer side effects, are expected to contribute to their wider use also as boiled vegetables, juice or other processed foods [15], therefore additional processing with the investigated plants are necessary in future studies.

More and more investigations were performed on plants in order to discover their possible anticancer properties. Recently also phytosterols were proposed as anticancer compounds [6]. It has been estimated that diets rich in phytochemicals can significantly reduce cancer risk by as much as 20%. Epidemiological data suggest that the phytosterol content of the diet is associated with a reduction in common cancers including colon, breast and prostate cancer. Molecular studies with tumorigenic research models showed how the dietary phytosterols achieving these effects. Phytosterols affect host systems potentially enabling more robust antitumor responses, including the boosting of immune recognition of cancer, influencing hormonal dependent growth of endocrine tumors, and altering sterol biosynthesis. In addition, phytosterols have effects that directly inhibit tumor growth, including the slowing of cell cycle progression, the induction of apoptosis, and the inhibition of tumor metastasis [6].

In conclusion, some of the studied wild vegetables' methanol extracts possess dose dependent antiproliferative properties and therefore could be recommended as a source of bioactive compounds. However, it must be taking into account that some of them as *Hypericum ascyron*, are not effective against lung cancer and even courses harm.

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