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ORIGINAL PAPER

Total Polyphenols, Antioxidant and Antiproliferative Activities of Different Extracts in Mungbean Seeds and Sprouts

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Abstract The aim of this investigation was to evaluate the biological, alcohol dehydrogenase (ADH) and antiproliferative activities of different extracts of mungbean seeds and sprouts. All extracts from the sprouts showed higher contents of total phenolics (TP), total flavonoids (TF), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity than from seeds. The highest DPPH and tyrosinase inhibition activities were registered in ethyl acetate (EtOAc) extract. ADH activity of methanol (MeOH), n-hexane (n-hexane) and n-butanol (n-BuOH) extracts from sprouts was significantly higher ($P<0.05$) than from seeds. However, the highest ADH activity was found in water extract of seeds. According to 3-(4, 5-dimethylthiazol -2-yl)-2, 5-diphenyltetrazolium bromide) (MTT) assay, extracts from sprouts were more effective against Calu-6 (human pulmonary carcinoma) and SNU-601 (human gastric carcinoma) cells than from seeds. EtOAc extract showed the highest antiproliferative activity in both sprouts and seeds, followed by n-hexane extraction. During sprouting of mungbean, all the studied components significantly increased. In conclusion, the extracts of sprouts are more effective than from seeds and could be a potential source of antioxidants linked with health benefits.

Keywords Mungbean seeds · Sprouts · Total phenolics · Total flavonoids · Antioxidant and antiproliferative activities

Abbreviations

ADH	Alcohol dehydrogenase
Calu-6	Human pulmonary carcinoma cell line
DPPH	1, 1-diphenyl-2-picrylhydrazyl radical
MTT	3-(4, 5-dimethylthiazol -2-yl)-2, 5-diphenyltetrazolium bromide
SNU-601	Human gastric carcinoma cell line

Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] is one of the most important short-season, summer-growing legumes grown widely throughout the tropics and subtropics [1]. Methanol extracts of plants growing in Korea, such as sorghum, brown rice, mungbean and foxtail millet were analyzed [2], and the antioxidant activities of different seeds, including mungbean were compared [3]. The impact of germination on phenolic content and antioxidant activity in 13 edible seed species was analyzed [4]. Vitexin and isovitexin were found as the major antioxidant components in mungbean [5]. Mungbean cultivars [6] were a potential source of essential fatty acids, antioxidants, minerals and proteins. The seeds and sprouts are excellent examples of functional foods that lower the risk of various diseases [7]. The seeds and sprouts have health-promoting effects, in addition to their nutritive value [8]. Today there is an increasing interest

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in Western countries in the sprouting of seeds as consumers demand minimally processed, additive-free, more natural, nutritional and healthy foods. There has been a worldwide trend towards the use of wild plants, including Korean wild vegetables and salads, due to their bioactive phytochemicals, of which phenolics are the most important [9–12]. Antiproliferative activity of essential oils extracted from 17 Thai medicinal plants on human mouth epidermal carcinoma (KB) and murine leukemia (P338) cell lines using MTT assay was investigated [13]. The potential use of mungbean protein hydrolysate prepared from tryptic hydrolysis as an antioxidative hydrolysate and as a carrier for anticancer asiatic acid was applied on HepG2 (Human hepatocellular liver carcinoma) cells [14]. However, there are no studies on the difference in anticarcinogenic properties between seeds and mungbean sprouts. Therefore, the aim of our study was to compare the physiological value between seeds and sprouts of mungbean as a good source of antioxidants.

Material and Methods

Chemicals Folin-Ciocalteu reagent (FC), butylated hydroxytoluene (BHT), MTT, and DPPH were obtained from Sigma Chemical Co. (St. Louis, MO).

Plant Materials Seeds of mungbean (*Vigna radiata* L., cv "Owool") were harvested from a field at the Jeollanamdo Agricultural Research and Extension Services, Naju, Korea, in 2009. The seeds were planted in a container and grown for five days to produce young sprouts. Dry seeds and sprouts were freeze-dried at—60 °C for 120 h and finely ground. The ground grains (1 kg) were extracted by boiling under reflux with 80% methanol for 12 h. The extract was filtered through No. 5 paper (Whatman, Clifton, NJ) to remove the fiber debris.

Total Phenolics (TP) Content and Total Flavonoids (TF) Level TP content was determined by Folin-Ciocalteu assay [15] and measured at 640 nm using a UV-1650 spectrophotometer (Shimadzu, Japan). The assay contained 5 ml of Nanopure water, 0.5–1 ml of sample, 1 ml of Folin-Ciocalteu reagent and 10 ml of 7% sodium carbonate solution. Flavonoids were determined according to Bao et al. [16]. The mixture contained 0.5 ml of sample, 2 ml of water, 0.15 ml of 5% $NaNO_2$, 0.15 ml of 10% $AlCl_3$ and 2 ml of 4% NaOH and measured at 510 nm.

DPPH Radical-Scavenging Activity Each MeOH extract at various concentrations was added to a 1.5×10^{-4} M solution of DPPH in methanol. DPPH activity of the extracts was measured at 520 nm [17].

Tyrosinase Inhibition Activity The reaction media (3 ml) for monophenolase and diphenolase activities contained 2 mM Tyrosine (Tyr) or 0.5 mM L-DOPA in 50 mM Na_2HPO_4 – NaH_2PO_4 buffer (pH 6.8) and measured at 475 nm [18].

ADH Activity Liver cytosolic solution was incubated in a reaction system containing 0.01 M NADH, 0.05 M semicarbazide and 0.01 M ethanol in 2 ml of Tris buffer

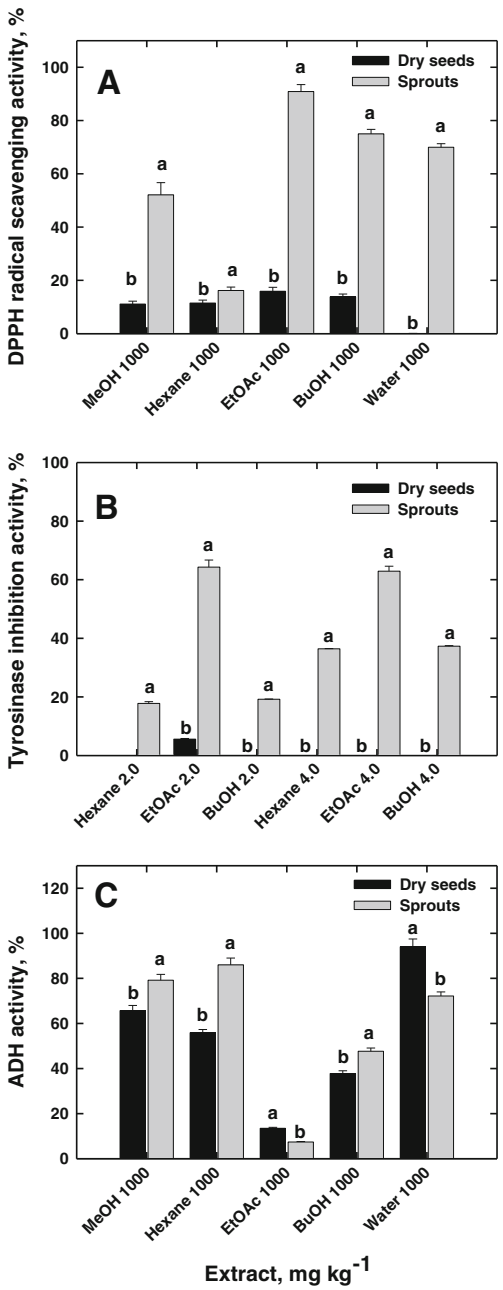

Fig. 1 DPPH radical scavenging activity (**A**), tyrosinase inhibition activity (**B**) and alcohol dehydrogenase activity (**C**) of methanol extract and its fractions from dry seeds and sprouts of mungbean. Means with same letters within bars are not significantly different ($P<0.05$)

(pH 8.5). The formation of NADH was monitored at 340 nm for 1 min [19].

Antiproliferative Activity The cancer cell lines were purchased from Korean Cell Line Bank (KCLB). Antiproliferative activity on human cancer cell lines Calu-6 and SNU-601 was measured using MTT assay as described in detail by us [9, 10]. The cell lines were nourished in a humid medium at 37 °C and 5% CO_2 with RPMI 1640 added in 10% FBS (fetal bovine serum), penicillin G (25 unit/ml) and streptomycin (25 mg/l) and incubated for 24 h. The optical density was recorded using a micro plate reader at 540 nm. Controls and samples were assayed in duplicate for each concentration and replicated three times for each cell line.

Statistics To verify the statistical significance, mean±SD of three independent measurements were calculated. Differences between groups were tested by two-way ANOVA. The data were analyzed by Student's *t*-test. The *P* values of <0.05 were considered significant [20].

Results and Discussion

Total Phenolics and Flavonoid Levels TP of methanol extracts [mg ferulic acid equivalents (FAE) kg^{-1} DW] for sprouts of mungbean were ranged from 166.5±4.5 to 191.7±2.2, and were higher than in dry seeds (ranged from 97.8±1.3 to 101.1±1.0). The highest content of TP was present in the extracts from sprouts of cultivar "Owool" and the lowest - "Soseon" ($P<0.05$). However, no significant difference in TP content among dry seeds of cultivars was observed (data not shown). Therefore, methanol extract from cultivar "Owool" was used for the next experiments. The highest amount of TF was registered in the sprouts of mungbean cultivar "Owool" [69.2±1.7 mg rutin equivalents (RE) kg^{-1}], following by "Samkang", "Sohyeon", and "Soseon" (the lowest). However, no significant difference in TF content among dry seeds of cultivars was observed (23.4±1.7 mg RE kg^{-1}). Sprouting of seeds is known to increase the nutritive value such as phenolics and flavonoids and the health qualities of foods in a natural way. Our results are in accordance with others [4]. Accumulated phenolics and total antioxidant capacity (TAC) showed the general trend distribution of seven days: sprouts>dormant seeds>imbibed seeds. Increases in phenolics from dormant seed to seven days sprout differ among seeds, ranging from 2010% (mungbean) to 11% (kale), while increases in TAC ranged from 1928% (mungbean) to 0% (lentil). Similar results [8] that the total phenolic compounds in the sesame sprouts were rapidly increased with the increase of DPPH were supported by others. Our study also showed that mungbean sprouts are an excellent source of dietary phenolic antioxidants.

DPPH Radical Scavenging Activity Methanol extracts of young sprouts in cultivar "Owool" (Fig. 1A) had higher DPPH activity than seeds. At 2,000 mg kg^{-1} methanol extracts, DPPH activities of seeds and young sprouts were 24.9% and 74.2%, respectively, showing higher activity in sprouts. DPPH activities of all fractions from young sprouts and seeds at 2,000 mg kg^{-1} were ranged from 18.5 to 90.9% and 13.5 to 24.9%, respectively. All EtOAc fractions showed the highest activity in both seeds and sprouts, 32.8 and 90.9%, respectively. BHT showed high DPPH activity, which ranged from 75.8 to 93.9%. These data are in accordance with our previous results that all samples of plant species proved that DPPH activity is dose-dependent [21]. The methanol extract of *Ulmus davidiana* [22] exhibited strong antioxidant activity in the tested model systems. Our results also showed high phenolics content and antioxidant activity in the methanol extracts from mungbean plants, and were highly consistent with the finding of DPPH activity [23]. Others have also reported [24] that total phenolic content of the tested vegetable extracts was correlated with the DPPH activity, suggesting that total phenolics can play a major role in the antioxidant activity of plant materials. Our results can be compared with the data of methanolic extract of *Cassia hirsuta* L. seed materials

Table 1 IC_{50} values of the different extracts from dry seeds and sprouts of mungbean on the human cancer cell lines by MTT assay

Extract	Cytotoxicity, IC_{50} (μg/ml)			
	Calu-6		SNU-601	
	Dry seeds	Sprouts	Dry seeds	Sprouts
MeOH	787.5±24.2[b]	2,962.9±145.2[a]	745.3±23.1[b]	1,951.2±102.[a]
Hexane	604.0±20.1[a]	448.1±19.1[b]	465.9±13.1[a]	320.8±12.2[b]
EtOAc	384.3±13.6[a]	169.4±9.9[b]	368.3±12.3[a]	148.8±3.1[b]
BuOH	1,261.8±43.6[a]	589.2±11.4[b]	20,400.6±610.[a]	377.9±11.4[b]
Water	9,948.3±234.7[a]	1,108.0±96.3[b]	6451.6±1345[a]	699.6±17.6[b]

The values are means±SD of three measurements. [a,b] Different superscript letters for each extract within a human cancer cell line means significant differences between dry seeds and sprouts ($P<0.05$).

[25]. The methanolic extract of raw seeds contained a total free phenolic content of 15.82±1.69 g CE/100 g extract DW and the scavenging activity against DPPH of 64.40%. Our results are consistent with Samotyja et al. [3], where the phenolics in the seed extracts from germinated wheat, lentil, sunflower, radish, and mungbean seeds varied between 4.1–31.7 mg caffeic acid equivalent/g extract DW. Our results are in accordance with other reports [6], where methanolic extracts of the seeds of the mungbean cultivars exhibited a good antioxidant activity as determined in TP contents (from 0.62 to 1.08 g/100 g DW), percent inhibition of peroxidation (from 49.8% to 89.2%), reducing power (from 1.19% to 1.45%), and bleaching of β-carotene assay. Our results are consistent with Choi et al. [2], where the polyphenolics in mungbean grown in Korea were about 45 mg GAE/100 g grains on wet weight (WW).

Tyrosinase Inhibition Activity MeOH extracts and the fractions from sprouts showed higher tyrosinase inhibition activity than from seeds. EtOAc fraction from sprouts showed the highest tyrosinase inhibition activity of 62.9%, followed by n-BuOH and n-hexane fractions (Fig. 1B).

ADH Activity Methanol extracts from sprouts showed higher ADH activity than from seeds. However, water extract from dry seed showed the highest activity of 94%, followed by hexane extract from sprouts of 86%, water extract from sprouts - 72.2% and hexane extract from seeds - 55.9% (Fig. 1C).

Antiproliferative Effect All extracts from sprouts, except MeOH extract, showed higher anticancer activity than from seeds (Table 1). Human gastric carcinoma cell line SNU-601 was more sensitive to the methanol and other extracts than human pulmonary carcinoma cell line Calu-6. EtOAc fraction of sprouts exhibited the highest influence on the two human cancer cell lines Calu-6 (IC_{50}=169.4 mg kg^{-1}) and SNU-601 (IC_{50}=148.8 mg kg^{-1}), followed by hexane and butanol fractions (Table 1). Our results correspond with others [13] that guava (*Psidium guajava* L.) leaf and Sweet Basil oils exhibited the highest antiproliferative activity in KB and P388 cell lines, respectively. These results, however, were not consistent with the findings of DPPH activity or TP content. During sprouting in mungbean, TP and TF levels significantly increased and improved free radical scavenging, tyrosinase inhibition, anticancer, and ADH activities. The potential application of mungbean biological activities is based on its nutritional and antioxidant properties: flavonoids and antioxidants [5, 6] and aroma compounds such as eugenol [26]. Some specific food-grade microbial polysaccharides as potential elicitors of mungbean phenolic content and gelling agents can be accounted for health-promoting effects [14, 27, 28].

Conclusions

This investigation shows that the content of TP and TF from mungbean sprouts was higher than from the seeds. The EtOAc extract of the mungbean sprouts was characterized by the highest DPPH radical scavenging and tyrosinase inhibition activities. The extracts dose-dependently increased the free radical scavenging activity. MeOH extracts from sprouts showed higher ADH activity than from seeds. Water extract from dry seeds showed the highest ADH activity, followed by hexane extract from sprouts, water fraction from sprouts, and hexane fraction from seeds. Antiproliferative effect of all extracts from the sprouts on two human cancer cell lines Calu-6 and SNU-601 showed significantly higher activity than seeds, and the highest was found in the EtOAc extract of mungbean sprouts. Seeds and sprouts of mungbean with high levels of TP and TF, DPPH, tyrosinase inhibition, antiproliferative and ADH activities could be recommended as preventative or/and therapeutic agents mainly for human diseases in addition to proper prescription drugs.

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