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A Comparative Survey of Leguminous Plants as Sources of the Isoflavones, Genistein and Daidzein: Implications for Human Nutrition and Health

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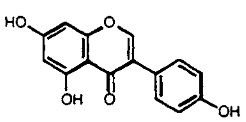
ABSTRACT

Over 80 taxa of mostly agriculturally important legumes were surveyed as sources of the metabolites, genistein and daidzein. Remarkably high concentrations (over 2 g · kg^{-1} dry weight) of the anticancer metabolite, genistein, were found in the leaves of Psoralea corylifolia (Indian bread root). All other legumes, with the exception of fermented soybean miso, had genistein levels <400 mg · kg^{-1} dry weight. Concentrations of over 1 g · kg^{-1} dry weight and 0.95 g · kg^{-1} dry weight of the anticancer metabolite, daidzein, were found in the stems of the fava bean (*Vicia faba*) and roots of kudzu vine (*Pueraria lobata*), respectively. From this survey, our results indicate that the legumes, lupine (*Lupinus* spp.), fava bean, (*Vicia faba*), soybeans (*Glycine max*), kudzu (*Pueraria lobata*), and psoralea (*Psoralea corylifolia*), are excellent food sources for both genistein and daidzein. Miso, a fermented soybean product, is also a rich source of both isoflavones.

INTRODUCTION

Many legumes (members of the bean family, Fabaceae) are important sources of the isoflavone secondary metabolites, genistein (1) and daidzein (2), in addition to their being excellent sources of dietary protein. In one of these legumes, namely seedlings of soybeans (*Glycine max*), the isoflavones, including genistein and daidzein, are the predominant metabolites present (Graham, 1995). They, or derivatives from them, are known to play a role in deterring attack of host plants by pathogenic fungi (Dixon et al., 1983; Graham, 1995; Lindsay et al., 1993; Oomen et al., 1994). Furthermore, daidzein, and perhaps genistein, act as signaling molecules in the establishment of symbiotic nitrogen-fixing bacteria in roots of leguminous plants such as soybeans (Zhang and Smith, 1995).



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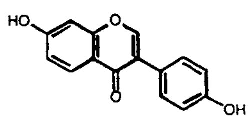

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Our interest in these compounds derives from the fact that genistein and daidzein are important medicinal compounds (reviewed by

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Boik, 1996, and Duke, 1995). Genistein is a promising anticancer agent that inhibits platelet aggregation, induces apoptosis, inhibits leukotriene production, inhibits DNA topoisomerase II, inhibits angiogenesis, reduces the bioavailability of sex hormones, and induces differentiation in cancer cells (reviewed by Boik, 1996; Fotsis et al., 1995; Kennedy, 1995; Matsukawa et al., 1993; and Peterson, 1995). Its ability to act in these ways may be related to its action involving the inhibition of protein tyrosine kinases (Uckun et al., 1995). It is of interest, in this connection, that the epidemiological evidence has linked high soybean, adzuki bean, and mung bean diets in Asian populations with reduced cancer risks (Duke, 1987; Kennedy, 1995). Daidzein acts as an anticancer agent by inducing differentiation in B16 melanoma and HL-60 human leukemia cells (Boik, 1996). Its glucoside, daidzin, obtained from the kudzu vine (*Pueraria lobata*), has been shown to inhibit the enzyme ALDH-I, an NAD-dependent aldehyde dehydrogenase that catalyzes the oxidation of acetaldehyde, the primary product of alcohol metabolism (Duke, 1995). As such, it has been investigated as a treatment for alcoholism.

It should be mentioned here that both genistein and daidzein have phytoestrogen activity. In the human intestine, soy foods produce equol, an estrogen-like compound with an activity of approximately 0.2% of estradiol (Boik, 1996). Although this is a low activity level, the amount produced from soy food products can result in levels of equol that result in significant competition for estrogen receptors. Because of the estrogenic activity of these two isoflavones, this effect could be quite serious if they were taken by women of child bearing age. Further, there is also a lot of concern about the long-term effects of cumulative small doses of such compounds on male fertility. Finally, a word of caution needs to be mentioned about the phytotoxicity of psoralens in some plants, for example, *Psoralea* species. Photoactivated psoralens have been used to treat psoriasis since the 1950s. This therapy can be carcinogenic and is only performed in carefully qualified and monitored regimes. High levels of photoactivated psoralens can also cause immunosuppression (Boik, 1995).

The primary objective of the present study was to survey a wide variety of legumes, most of which are agriculturally important crop plants, in order to identify those with the highest concentrations of genistein and daidzein. Such plants, used as food sources by humans, may potentially also serve as excellent sources of these medicinally important isoflavones. To date, the lack of data on optimal plants sources, as pointed out by Stafford (1990), has hindered the development of genistein and daidzein-containing formulations for use in cancer prevention, cancer treatment, and for treatment of individuals who are alcoholics. In this paper, we present new findings on which of the nutritionally important legumes and legume food products surveyed are the best sources of these isoflavones.

MATERIALS AND METHODS

Legume seeds were obtained from commercial sources by Dr. James A. Duke, National Germplasm Resources Laboratory, United States Department of Agriculture, Beltsville, Maryland. Seeds were stored at 4°C until use for genistein and daidzein analysis or for germination. Voucher specimens of plants were made for every accession used for the analysis of genistein and daidzein in vegetative plant parts and are on deposit in our laboratory in the Department of Biology at the University of Michigan. Voucher seed specimens were also kept for every accession tested.

Plant growth

Seeds of legumes germinated in the greenhouse were planted at a depth of twice the diameter of the seeds. Plants were grown under fluorescent lamps operating 24 hr per day with a light intensity of 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ at the level of the plants. Plants were watered daily. After 30 days of growth, the plants were harvested. The seedlings were washed with distilled water and partitioned into the following fractions: roots, stems, seed leaves (cotyledons), and true leaves. They were immediately stored at −80°C prior to extraction and HPLC analysis.

Extraction

Methods for genistein and daidzein extraction were as follows: Tissues were ground in 4:1 MeCN/H_2O (MeCN, acetonitrile), and the mixture was filtered. Distilled water was then added to reduce the concentration of MeCN to 25%. Organic materials were concentrated on a disposable C18 column, and were then eluted from the column with 100% MeCN. This final solution was evaporated to dryness and dissolved in a small volume of MeCN. These methods for extraction and partial purification of genistein and daidzein are the same as cited by O'Neill and Saunders (1994) for extraction of the phytoalexin, medicarpin, from alfalfa (*Medicago sativa*).

Assay

Fifteen microliters of each sample were assayed by reverse phase (ODS) HPLC (4.6 · 250-mm column size) at 280 nm and 3000 psi using a linear gradient from MeCN:H_2O:TFA (20:79.9:0.1) to MeCN:H_2O:TFA (86:13.9:0.1) (TFA, trifluoroacetic acid). Genistein and daid-zein concentrations were determined using standard samples of each of these metabolites, with confirmation by mass spectrometry.

RESULTS AND DISCUSSION

Determination of levels of genistein and daidzein in vegetative parts of legume plants

80 different taxa of legumes were surveyed for their genistein and daidzein contents (Table I). Analyses were conducted on vegetative parts of plants grown from the seeds of these plants or on commercial edible food products made from the plant material. Of these samples, eighteen had significant levels (>50 mg · kg^{-1} dry wt) of either genistein or daidzein, with four samples containing over 700 mg of these isoflavones per kilogram of dry weight (Table 1). Of special interest are *Psoralea corylifolia* (Indian breadroot, entry 1a) leaves, which contained over 2 g of genistein per kilogram of dry weight. It should be mentioned in this connection that the fruit and seed of *P. corylifolia* are commonly used in traditional Chinese medicine for skin diseases and as a tonic (Bensky and Gamble, 1990; Chang and But, 1986; Tierra, 1988). Duke (1987) mentions that as an ancient Hindu remedy in India, the seeds of this plant have long been used effectively in treatments for leukoderma and vitiligo. All other legumes had genistein levels <400 mg · kg^{-1} of dry weight except for soy miso (entry 3g), which contained ca 430 mg · kg^{-1} of dry weight. Daidzein levels, on the other hand, were highest in fava bean stems (*Vicia faba;* ca 1,025 mg · kg^{-1} of dry weight, entry 3b) and kudzu vine roots (*Pueraria lobata;* ca. 950 mg · kg^{-1} of dry weight, entry 2d). Lesser amounts were found in all other samples except soy miso, which contained 327 mg · kg^{-1} of dry weight. It is notable that both daidzein and genistein levels were relatively high in this commercial fermented soybean product.

Determination of levels of genistein and daidzein in seeds of selected legumes

Levels of daidzein and genistein in seeds for a general selection of legumes are presented in Table 2. Of all the types of legume seeds analyzed, *Psoralea* seeds were confirmed to have the highest levels of both genistein and daidzein. Seeds having lesser but significant amounts of daidzein included pinto beans and soybeans. Lower but still significant amounts of genistein occurred in yellow split peas, baby lima beans, and black turtle beans. The fact that the level of genistein in soybean seeds is relatively low (as compared with soybean seedlings) is probably due to the fact that most of the genistein found in the seeds occurs as glucosyl or malonyl-glucosyl conjugates (Graham and Graham, 1996; Graham et al., 1990; Matsuura et al., 1989) and, therefore, were not detected in this analysis.

Results from the present study indicate that legumes such as *Pueraria lobata* (kudzu vine), *Lupinus albus* (lupine), *Vicia faba* (fava beans), *Glycine max* (soybeans), and *Psoralea corylifolia* (Indian bread root) are excellent sources of the isoflavones, genistein, and daidzein. The levels of these compounds, in general, are greater in seedlings than in the seeds (see Tables I and II). This could be explained by either enhanced isoflavone synthesis and/or release of the

TABLE 1. AMOUNTS OF GENISTEIN AND DAIDZEIN IN VEGETATIVE PARTS OF SELECTED LEGUMES

Sample/source[a]	Species (common name)	Daidzein mg • kg dry wt	Genistein mg • kg dry wt	Total	Sample/source[a]	Species (common name)	Daidzein mg • kg dry wt	Genistein mg • kg dry wt	Total
1a	*Psoralea corylifolia*	99.2	2151.0	2250.2	40b	*Vigna unguiculata* (wild cowpea)	9.8	8.1	17.9
2d	*Pueraria lobata* (kudzu)	949.8	316.9	1266.7	41a	*Phaseolus vulgaris* (great north bean)	7.1	9.2	16.3
3b	*Vicia faba* (fava bean; broadbean)	1032.8	92.1	1124.9	42b	*Phaseolus vulgaris* (Hutterite bush bean)	8.1	7.7	15.8
3g	*Glycine max* (fermented soybean miso)	327.6	427.6	755.2	43b	*Phaseolus vulgaris* (common bean)	8.2	7.4	15.4
4a	*Baptisia australis* (baptisia)	34.0	350.7	394.7	44a	*Phaseolus vulgaris* (Anasazi bean)	4.1	11.0	15.1
5f	*Trifolium sp.* (clover)	73.6	110.0	183.6	45f	*Medicago sativa* (alfalfa)	7.2	7.3	14.5
6d	*Sophora japonica* (chinese scholar tree)	0	181.6	181.6	46b	*Phaseolas vulgaris* (red chile bean)	7.4	7.0	14.4
7a	*Lupinus luteus* (lupine, Poland)	38.9	86.7	124.6	47a	*Phaseolus vulgaris* (haelan bean)	12.1	2.3	14.4
8a	*Amphicarpa americana* (hog peanut)	18.9	96.7	115.6	48b	*Phaseolus acutifolius* (black tepary bean)	5.9	7.7	13.6
9c	*Lupinus albus* var. *Kiev* (edible lupine bean)	46.6	59.2	105.8	49a	*Phaseolus lunatus* (sieva bean)	5.9	7.9	13.8
10c	*Glycine max* cv. edamame (vegetable soybean)	62.7	42.9	105.6	50b	*Pueraria lobata* var. montana (Japan)	6.4	5.6	12.0
11a	*Lupinus albus* L-2043-N (lupine)	0	101.0	101.0	51a	*Phaseolus vulgaris* (navy beans)	4.2	7.0	11.2
12c	*Lupinus albus* L-044-P (lupine)	19.0	65.2	84.2	52b	*Phaseolus lunatus* (baby lima bean)	0	11.1	11.1
13c	*Glycine max* cv. nakesennari	3.7	71.7	75.4	53a	*Trifolium resupinatum*	6.1	4.7	10.8
14b	*Phaseolus acutifolius* var. *tenuifolius*	32.5	40.2	72.7	54a	*Vigna radiata* var. radiata	3.6	7.0	10.6
15b	*Pueraria phasioloides* (Kudzu)	30.9	39.3	70.2	55a	*Vigna unguiculata* spp. (black-eyed pea)	5.9	4.5	10.4
16a	*Apios americana* (Indian potato)	34.7	33.8	68.5	56b	*Vicia faba* (fava bean)	4.6	5.4	10.0
17b	*Cajanus cajan* (pigeon pea)	16.8	50.1	66.9	57f	*Astragalus* sp. (locoweed)	0	9.0	9.0
18b	*Trigonnella balansae*	0	66.3	66.3	58b	*Lupinus mutabilus*	0	8.7	8.7
19c	*Lupinus luteus* (yellow lupine)	0	61.5	61.5	59b	*Phaseolus vulgaris* cv. wadex	4.1	4.5	8.6
20a	*Phaseolus leptostachys*	25.9	31.2	57.1	60a	*Lablab purpureus* (hyacinth bean)	3.3	4.5	7.8
21a	*Pueraria lobata* var, *montana* (USA)	44.4	9.0	53.4	61a	*Vigna umbellata*	7.7	0	7.7
22a	*Glycyrrhiza* sp.	0	51.9	51.9	62b	*Phaseolus vulgaris* (pinto bean)	3.1	4.5	7.6
23a	*Lens esculenta* (green lentils)	16.5	33.4	49.9	63b	*Phaseolus vulgaris* (giant snap pole bean)	7.1	0	7.1
24b	*Lupinus albus* 1-2085 var, *Nibs* (white lupine)	0	48.0	48.0	64b	*Phaseolus coccineus* (blackcoat runner bean)	0	6.9	6.9
25a	*Cicer arietinum* (garbanzo bean)	0	46.1	46.1	65b	*Mucuna deeringiana*	0	6.7	6.7
26b	*Phaseolus vulgaris* (light pod kidney)	15.7	27.7	43.4	66b	*Phaseolus coccineus* (core pole bean)	0	6.3	6.3
27a	*Trigonella foenicum graecum* (USA; fenugreek)	23.1	9.1	32.2	67a	*Trifolium incarnatum* (crimson clover)	6.1	0	6.1
28b	*Tifolium pratense* (red clover)	5.0	26.1	31.1	68b	*Phaseolus vulgaris* (kidney bean)	0	5.0	5.0
29b	*Glycine max* cv. suzuyutaka	15.7	12.2	27.9	69	*Astragalus* sp.	0	4.9	4.9
30a	*Glycine max* cv. HP-201	11.1	16.6	27.7	70b	*Phaseolus vulgaris* (black bean)	0	4.7	4.7
31b	*Pueraria lobata* var. montana	11.5	16.0	27.5	71b	*Vicia faba* (aporacho largo bean)	4.7	0	4.7
32b	*Phaseolus vulgaris* cv. topmost	8.2	19.2	27.4	72b	*Lathyrus sativa* (grass pea)	4.3	0	4.3
33a	*Vigna aconitifolia*	14.0	11.4	25.4	73a	*Pisum sativum* (garden pea)	3.4	0	3.4
34b	*Glycine max* (Agate soybean)	13.0	10.6	23.5	74a	*Vicia faba* (yellow faba bean)	2.8	0	2.8
35a	*Vigna radiata* (mung bean)	14.2	8.3	22.5	75b	*Glycyrrhiza echinata*	0	2.7	2.7
36b	*Vigna angularis* (Adzuki bean)	9.1	13.0	22.1	76b	*Vigna unguiculata* var. unguiculata	0	0	0
37b	*Vicia faba* (Guatemalan purple fava bean)	10.5	11.5	22.0	77f	*Miletta* sp.	0	0	0
38a	Sophora subprostrata	5.9	14.6	20.5	78a	*Apios americana* (Indian potato, healthy)	1.4	0.9	2.3
39b	*Trigonella foenicum graecum* (Ethiopia)	18.1	0	18.1	79a	*Apios americana* (Indian potato, fungus-infected)	34.7	33.8	78.5

[a]Section of plant chosen corresponds to highest daidzein + genistein concentration. a = leaf, b = stem, c = cotyledon, d = root, e = bark f = flower, g = commercial product.

TABLE 2. AMOUNTS OF DAIDZEIN AND GEINSTEIN IN SEEDS OF SELECTED LEGUMES

Species (common name)	*Daidzein mg•kg dry wt*	*Genistein mg•kg dry wt*	*Total*
1. *Psoralea corylifolia*	539.7	1528.0	2067.7
2. *Glycine max* (soybean)	37.6	24.1	61.7
3. *Pisum sativum* (yellow pea)	0.4	45.8	46.2
4. *Phaseolus vulgaris* (pinto bean)	23.2	22.3	45.5
5. *Phaseolus vulgaris* (black turtle bean)	0.4	45.1	45.5
6. *Phaseolus vulgaris* (baby lima bean)	0.4	40.1	40.5
7. *Phaseolus vulgaris* (Anasazi bean)	6.5	29.8	36.3
8. *Phaseolus lunatus* (large lima bean)	0.3	34.4	34.7
9. *Phaseolus vulgaris* (red kidney bean)	2.7	29.3	32.0
10. *Lens esculenta* (red lentil)	5.2	25.0	30.2
11. *Vigna angularis* (Adzuki bean)	4.6	21.2	25.8
12. *Phaseolus vulgaris* (great northern bean)	7.2	17.7	24.9
13. *Vicia faba* (fava bean)	5.0	19.9	24.9
14. *Vigna unguiculata* (black-eyed pea)	0.3	23.3	23.6
15. *Vigna radiata* (mung bean)	0.3	21.8	22.1
16. *Phaseolus vulgaris* (red Mexican bean)	1.4	16.3	17.7

isoflavones from stored glucosyl or malonyl–glucosyl conjugates through the action of glucosidases during seedling development (Graham et al., 1990, Graham and Graham, 1996). Furthermore, we found that roots of mature kudzu vine (*Pueraria lobata*) had levels of genistein and daidzein that were almost an order of magnitude higher than in leaves and stems of the seedlings (Table I). Similar findings have been reported by Graham (1991) for soybean seedling root-tips as compared with other seedling organs of soybean.

Our findings on amounts of genistein and daidzein isoflavones in legumes have several important implications for human health and nutrition in addition to the concerns on their use cited in the introduction. They are as follows: (1) Sprouts of the edible species of legumes (e.g., fava beans, Adzuki beans, and soybeans) may be a preferred source of these isoflavones over the seeds for reasons of their higher content of genistein and daidzein, and perhaps, greater availability than with the stored conjugates of these isoflavones that occur in the seeds. Such sprouts, routinely used in Asian diets mainly because of their palatability, high vitamin C content, high levels of soluble protein, and anticancer properties, should be more widely used in our diets for these very reasons. (2) Roots of the edible legume sprouts might be consumed as well as the shoots. A combination of the roots and the cotyledons (seed leaves) of the shoots (the sites of greatest synthesis of genistein and daidzein) provide the highest levels of these isoflavones. (3) Kudzu vine roots, not palatable and edible as such, may provide us with new and rich sources of these isoflavones that could be extracted commercially. Starch from the roots is already available in Oriental stores here in the United States. Kudzu vine is a pernicious and highly competitive vine-type introduced weed in southeastern forests of the United States, and this would certainly be a good use for this otherwise undesirable plant. (4) It would be worthwhile to look at other species of *Psoralea*, such as *P. esculenta*, the edible Indian potato of the United States. It is a staple starch crop of native Amerinds. Roots of its relative, *P. corylifolia*, possess the highest levels of both isoflavones of any species we have examined to date (Kaufman et al., 1996).

Finally, the yield data for isoflavones in any legumes must *not* be looked at in terms of absolute amounts for any given species. The levels of those isoflavones may vary according to environmental conditions, pathogen attack, or chemical races within the germplasm (genotype) of a given legume species.

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