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Neuronal Protection Effect of Hwanggi (*Astragalus membranaceus*) Sprouts in MPP+ treated SH-SY5Y cells

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Abstract

Background : This study began with the aim of developing food materials using Hwanggi (*Astragalus membranaceus*) sprouts to find new ways to use Hwanggi, mostly using roots. It was confirmed that the shoots of medicinal crops had the functionality suitable for processed foods.

Methods and Results : Hwanggi Extracts were manufactured using Huanggi seeds and Hwanggi sprouts grown for 5, 10 and 15 days. Extracts were frozen and dried with Huanggi seeds and shoots, then crushed, and reflux extracted at 70 percent ethanol for 2 hours, and 3 times at 85 degrees Celsius. The extracted solution was decompressurized and freeze dried. HPLC analyzes the content of tryptophan in Huanggi seeds and Huanggi sprout extracts. During seed cultivation up to 15 days, tryptophan content increased significantly and dramatically. Hwanggi sprouts grown on the 15th contained 4.57 times more tryptophan than Huanggi seeds. Neurotoxic inhibitory effect of Huanggi sprout extract was confirmed in Parkinson's disease neuronal cells model induced by MPP+ (1-methyl-4-phenylpyridinium) treatment in neronal cells SH-SY5Y. SH-SY5Y was treated with 10 μg/mL of Huanggi sprout extract (1, 5, 10, 15 days) and treated with MPP+ 1 mM, incubated for 24 hours, and the cell survival rate was measured. The neuronal protection effect of Huanggi sprout extract was confirmed in the Parkinson's disease nerve cell model. MPP+ is a neurotoxic metabolite of primates that accumulates in nigrostric neurons through dopamine transporters and is transported to mitochondria by membrane potential. MPP+ shows very similar symptoms to Parkinson's disease and is widely used in cell and animal models.

Conclusion : Hwanggi sprouts grown on the 15th contained 4.57 times more tryptophan than Huanggi seeds. Furthermore, we confirmed that the Hwanggi sprout extract treatment group cultivated on the 10th and 15th showed a significant neuronal protection effect. This was the similar as the tryptophan content change pattern. These results suggest that Hwanggi sprouts are likely to grow neurons and protect neurons. Moreover, the results will be provided as basic data for the development of materials for processed foods using medicinal crops such as residues. In addition, it could be helpful to the industry involved in the processing of medicinal crops.

Materials and Mehtods

● Hwanggi (*Astragalus membranaceus*) Sprouts

- Hwanggi sprouts was grew for 15 days indoors, where it remained at 23 degrees Celsius.
- Hwanggi seeds and Hwanggi sprouts grown for 1, 5, 10 and 15 days were extracted.

● Hwanggi seed and sprouts extract

- freeze drying, 70% ethanol extraction, freeze drying,

● Analysis

- analyze with SPME-GC/MS
- content of tryptophan using HPLC

● Protection of neronal cells in Hwanggi sprouts in Parkinson's disease cell model

- The Hwanggi sprout extract by period (1, 5, 10, 15 days) was treated with 10 μg/mL in Neuronal cells SH-SY5Y. Thereafter, MPP+ was treated 1 mM each and cultured for 24 hours.
- The cell survival rate was measured to confirm the degree of cell death of the Hwanggi sprout extract in the Parkinson's disease cell model.

Fig. 1. Hwanggi seeds and sprouts

Tabel 1. HPLC Analysis Conditions

HPLC : Agilent 1260
Elution :
A : Water 0.1% Trifluoroacetic acid (HPLC grade)
B : ACN (HPLC grade)
Column : Kinetex 5 ㎛ XB-C18, 4.6 x 250 ㎜
B/N : 5705-034
S/N : 725782-5
Column Temperature : 35 ℃
UV-wavelength : 290.4 ㎚

Time	Flow (㎖)	A (%)	B (%)
0	1	95	5
5	1	95	5
8	1	90	10
14	1	75	25
20	1	73	27
40	1	0	100

Tabel 2. Yield of Hwaggi seeds and Hwaggi sprout extract

Sample	Yield (%)
Seeds (1days)	42.8
5-day sprout	40.6
10-day sprout	42.1
15-day sprout	39.2

Results

Table 3. Content of tryptophan in the Hwanggi extract.

Content	L-Tryptophan (mg/g)	RSD* (%)
1 days	1.475 ± 0.029	3.407
5 days	1.769 ± 0.040	3.901
10 days	5.527 ± 0.046	1.446
15 days	6.368 ± 0.136	3.706

Values are the mean ± SEM.
***RSD : relative standard deviation.**

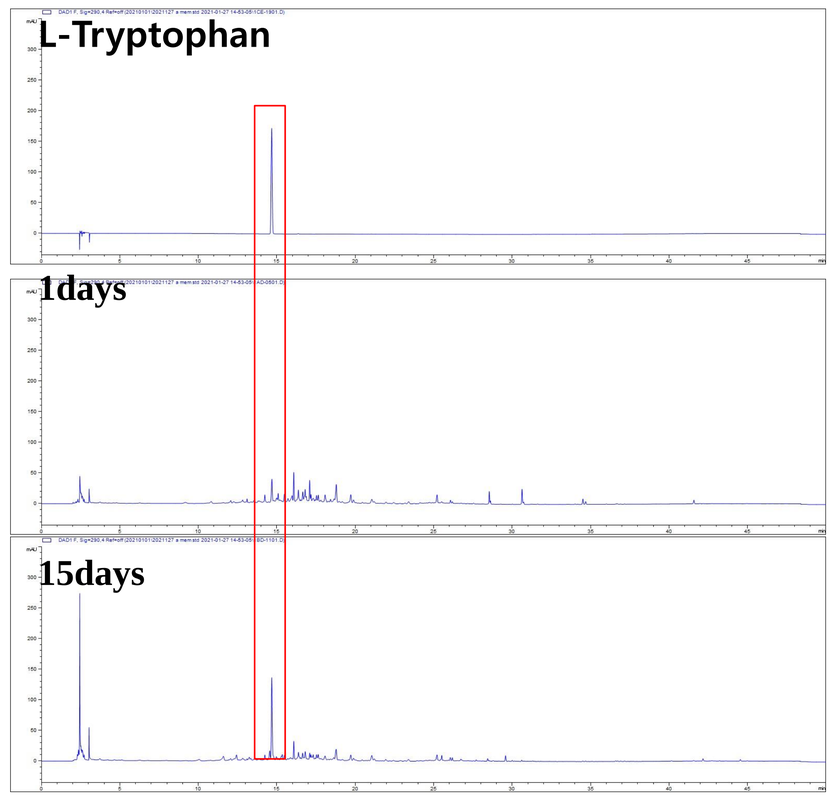

Fig. 2. Comparison of tryptophan content in standard and 1, 15-day sprouts

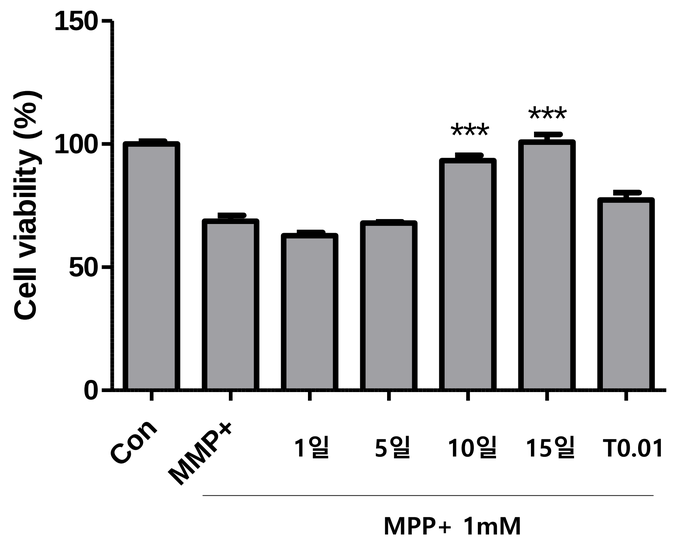

Fig. 3. Neural cell protection effect of Hwanggi sprout extract in Parkinson's disease cell model.

Conclusion

● **Hwanggi (*Astragalus membranaceus*) sprouts**

- Hwanggi sprouts grown on the 15 days contained 4.57 times more tryptophan than Hwanggi seeds.
- The Hwanggi sprout extract treatment group cultivated on the 10th and 15th confirmed a significant neuronal protection effect.
- Neuronal cells protection effect similar to tryptophan content change pattern.

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