International Journal of
Translational Medicine

MDPI

Article

Antifungal Activity of *Angelica gigas* with Enhanced Water Solubility of Decursin and Decursinol Angelate by Hot-Melt Extrusion Technology against *Candida albicans*

Suji Ryu [1,†], Ha Yeon Lee [1,†], Seoul-Hee Nam [2,*] and Jong-Suep Baek [1,3,*]

[1] Department of Bio-Health Convergence, Kangwon National University, Chuncheon 24341, Korea
[2] Department of Dental Hygiene, Kangwon National University, Samcheok 25949, Korea
[3] Department of Herbal Medicine Resource, Kangwon National University, Samcheok 25949, Korea
* Correspondence: nshee@kangwon.ac.kr (S.-H.N.); jsbaek@kangwon.ac.kr (J.-S.B.); Tel.: +82-33-540-3324 (J.-S.B.)
† These authors contributed equally to this work.

Abstract: Hot-melt extrusion (HME) has been an alternative technique to improve the solubility and bioavailability of active molecules with low water solubility. In this study, HME-*Angelica gigas* Nakai (AGN) was prepared to increase the aqueous solubility of decursin (D) and decursinol angelate (DA), the active ingredients of AGN. Compared with unprocessed AGN, HME-AGN showed enhanced water solubility of D and DA. The HME-AGN exhibited improved antioxidant activity by the DPPH radical scavenging method. The antifungal activity was confirmed against *Candida albicans* (*C. albicans*). There was a decrease in CFU in the plate treated with the HME-AGN extract compared with the plate treated with the AGN extract, and F2 showed the highest antifungal activity.

Keywords: *Angelica gigas*; hot-melt extrusion; antifungal activity; *Candida albicans*

Citation: Ryu, S.; Lee, H.Y.; Nam, S.-H.; Baek, J.-S. Antifungal Activity of *Angelica gigas* with Enhanced Water Solubility of Decursin and Decursinol Angelate by Hot-Melt Extrusion Technology against *Candida albicans*. *Int. J. Transl. Med.* **2022**, *2*, 515–521. https://doi.org/10.3390/ijtm2040038

Academic Editor: Amol V. Janorkar

Received: 29 June 2022
Accepted: 27 September 2022
Published: 30 September 2022

1. Introduction

Angelica gigas Nakai (AGN), the traditional medicinal plant that grows in Korea, Japan, and China [1], has been widely used for the cure of amenorrhea, dysmenorrhea, infection, menopause, pain, injuries, articular rheumatism, and migraine headaches [2]. Type of coumarin compounds, such as decursin (D), decursinol angelate (DA), nodakenin, umbelliferon, and marmesin, are representative components of AGN [3]. In particular, D and DA are more abundant than other Angelica species, such as *Angelica sinesis* (Chinese) and *Angelica acutiloba* (Japanese). [4]. AGN has pharmacological activities such as anticancer [5], antioxidant [6], anti-inflammatory [7], and neuroprotective [8]. However, despite the beneficial activity of AGN, the low solubility of D and DA in water does not exert sufficient pharmacological efficacy and may limit the use of AGN.

To enhance the solubility and oral bioavailability of hydrophobic active molecules, various drug delivery systems have been investigated [9–12]. Among these systems, hot-melt extrusion (HME) is based on the solid material transfer through the heated barrel, equipped with single or twin screws that can be either corotating or counter-rotating [13]. HME is one of the technologies to form solid dispersions to improve the bioavailability and solubility of poorly soluble substances [14] and is a processing technology used in the pharmaceutical and food industries [15,16]. HME does not require organic solvents during the process, so there is no risk of toxicity to residual organic solvents, and it is an environmentally friendly processing system because of its short steps and continuous processes [17]. Although several studies have previously been conducted on hot-melt extrusion of AGN [18–21], the improved antibacterial activity was not investigated.

In this study, AGN solid dispersion (HME-AGN) was prepared using HME to increase the aqueous solubility of the active compounds (D and DA) of AGN. The prepared HME-

AGN was analyzed by HPLC to compare the contents of D and DA with AGN that was not processed with HME, and the antifungal activity was evaluated.

2. Materials and Methods

2.1. Material

Decursin (D) and decursinol angelate (DA) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and 2,2-diphenyl-1- picrylhydrazyl (DPPH) was purchased from Alfa Aesar (Ward Hill, MA, USA). All other chemicals used were of analytical grade.

2.2. Preparation of HME-AGN

Before HME processing, AGN was washed, cut into 3-5 mm pieces, and dried before use. HME-AGN was prepared through HME (STS-25HS tween screw extruder, Hankook E.M. Ltd., Pyoung-Taek, Korea) after mixing by adding AGN and additives in the combination, shown in Table 1. The processing temperature of HME was fixed at 100°C, and it was extruded under the conditions of a screw speed of 15 bar and 150 rpm. The extruded AGN was used for subsequent experiments after drying.

Table 1. The compositions of HME-AGN.

	Mixing Ratio (%)		
	AGN	**Ascorbyl Palmitate**	**Whey Protein Isolate**
Control (Nonextrudate)	100	-	-
F1 (Extrudate)	100	-	-
F2 (Extrudate)	80	10	10

2.3. Preparation of Extracts

The extract was prepared by weighing 1 g of dried HME-AGN and AGN powder in 50 mL of distilled water and sonicating in a 40 °C water bath for 1 h. Then, the extract was centrifuged at 1509 × *g*, and the supernatant was filtered by Whatman filter paper (No. 6) and dried through a freeze dryer. Finally, the powder was stored at 4 °C for further studies. Each sample was extracted in triplicate.

2.4. The HPLC Condition

Samples for analysis were filtered with a syringe filter (0.50 μm) before analysis. HPLC was performed using a Simadzu LC-20AT HPLC system with a UV–Vis detector. The analysis conditions are suggested in Table 2.

Table 2. The HPLC condition for analysis of D and DA.

Column	YMC—ODS AM C18 (250 * 4.6 mm, 5 μm, 12 nm)	
Detector	UV-vis detector (330 nm)	
Solvent A	Water	
Solvent B	Acetonitrile (ACN)	
Flow rate	0.8 mL/min	
Oven	28 °C	
Injection volume	10 μl	
	Gradient elution system	
Time (min)	% A	% B
Initial	45	55
10	30	70
15	20	80
25	10	90
35	5	95
40	45	55

2.5. Antioxidant Activity

The antioxidant activity of AGN and HME-AGN was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical with slight modifications to the method presented by Khan et al. [22]. The amount of 600 µL of DPPH solution was added to 20 µL of the extract, and the mixture was reacted for 30 min under dark conditions at room temperature. The absorbance measurement of the mixture was carried out using a UV–Vis spectrophotometer. As a control, a comparison was made using L-ascorbic acid. The percent inhibition activities for all samples were calculated using the following equation:

$$\text{Inhibition } (\%) = \frac{\textit{Control sample absorbance} - \textit{Extract sample absorbance}}{\textit{Control sample absorbance}} \times 100$$

2.6. Antifungal Activity

The antifungal activity of HME-AGN was tested using the colony-forming unit (CFU) method. The yeast strain of *C. albicans* (KCTC 7965/ATCC 10231) was used in this study. The strain of *C. albicans* was cultured in yeast mold broth and incubated in liquid media at 37 °C overnight. The cell was resuspended in phosphate-buffered saline (PBS) to a concentration of 5×10^5 CFU/mL, and 100 µL of cultured *C. albicans* (5×10^5 colony-forming units; CFUs/mL) was inoculated into a 24-microwell plate containing yeast mold broth in which AGN and HME-AGN extract were added at each concentration. The total volume of each mixture was 1.5 mL. The microplate was incubated anaerobically for 6 h at 37 °C, and then the mixture in each well was uniformly smeared in an agar medium and then cultured at 37 °C for 24 h to check the number of CFUs.

2.7. Statistical Analysis

The analyses were performed in triplicate, and the data were expressed as mean ± standard deviation. SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses. Significant differences between samples were assessed with a one-way analysis of variance (ANOVA) with Duncan's multiple range test (DMRT) at a 5% level ($p < 0.05$).

3. Results and Discussion

3.1. Preparation of HME-Processed AGN Samples

To enhance the water solubility of hydrophobic D and DA in AGN, HME was introduced with additives. As seen in Figure 1, the morphology of AGN was changed after the HME process. Before the HME process, unprocessed AGN exhibited irregular morphology. It was highlighted that the spherical shape was observed with a nanosized range after the HME process. Previous studies reported that HME could reduce particle size and increase the surface area [16,20]. The changes would enhance the solubility and the oral bioavailability of the hydrophobic active compounds [21].

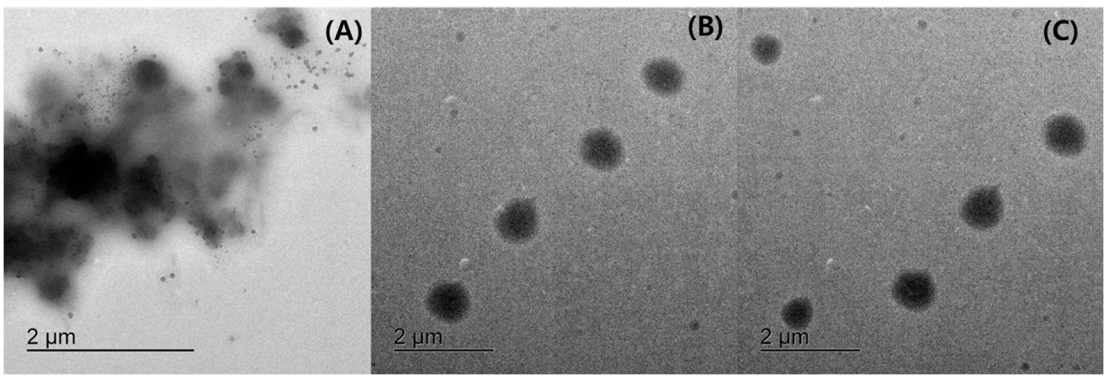

Figure 1. Transmission electron microscopy (TEM) images of (**A**) nonextruded AGN, (**B**) F1, and (**C**) F2. The scale bar represents 2 um.

3.2. The HPLC Analysis of D and DA in AGN and HME-AGN

The contents of D and DA from the AGN and HME-AGN are shown in Table 3. When AGN containing D and DA with low water solubility was extracted with water, it was confirmed through the content analysis of the control that there was a limitation in the extraction of the active compound. The D content in HME-AGN was confirmed to be about 5 times higher in F1 and 43 times higher in F2 than in control. The DA content in HME-AGN was confirmed to be about 3 times higher in F1 and 13 times higher in F2 than in control.

Table 3. D and DA contents of control and HME formulation of AGN.

	Decursin (D) (mg/g) *	**Decursinol Angelate (DA) (mg/g) ***
Control	0.07 ± 0.05	0.11 ± 0.02
F1	0.41 ± 0.20	0.29 ± 0.10
F2	3.01 ± 0.47	1.47 ± 0.23

* Data are expressed as means ± standard deviation (n = 3).

3.3. Antioxidant Activity of AGN and HME-AGN

The antioxidant activity of AGN and HME-AGN extracts was evaluated by DPPH assay. The IC_{50} value of AGN was 9478.26 ± 660.45 μg/mL, and that of HME-AGN was 4671.34 ± 392.72 μg/mL, and 4417.32 ± 330.82 μg/mL, respectively, and the IC_{50} value decreased about 2 times (Table 4). The IC_{50} value of ascorbic acid used as a positive control was 103.8 ± 6.29 μg/mL, and all samples had a higher IC_{50} value than ascorbic acid, but it was confirmed that the antioxidant activity of AGN was improved by HME.

Table 4. IC_{50} values of AGN and HME-AGN extracts against DPPH free radicals.

Sample	**IC_{50} (μg/mL) ***
Control	9478.26 ± 660.45 ^{a}
F1	4671.34 ± 392.72 ^{b}
F2	4417.32 ± 330.82 ^{c}
Ascorbic acid	103.8 ± 6.29 ^{d}

* Data are expressed as means ± standard deviation (n = 3). Different letters (^{a}–^{d}) indicate significantly different at $p < 0.05$ by one-way ANOVA.

The DPPH radical scavenging activity was investigated at various concentrations (1-5 mg/mL) (Figure 2). The maximum activity of AGN extract was 26.24%, and the maximum activity of HME-AGN extract was 52.84% and 54.72%, respectively, indicating that F2 showed the highest DPPH inhibitory activity.

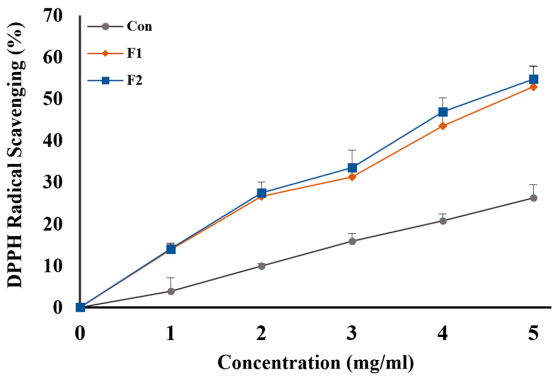

Figure 2. DPPH radical scavenging activity of AGN and HME-AGN extracts. Data are expressed as means ± standard deviation (n = 3).

Lee et al. demonstrated that D and DA isolated from AGN have antioxidant activity [23]. The content of D and DA detected in the extract was increased because of the improvement of the water solubility of D and DA, the active components of AGN, through HME processing, suggesting that it is related to the increase in antioxidant activity.

3.4. Antifungal Activity of AGN and HME-AGN

According to Figure 3, F1 and F2 at all concentrations showed greater growth inhibition than the control against *C. albicans* ($p < 0.05$). A decrease in CFU of the control extract was observed at a concentration of 10 mg/mL or more. HME-AGN extract also showed a decrease in CFU from 10 mg/mL and antifungal activity of more than 99% compared with the CFU of the plate untreated with the extract. The F2 showed higher antibacterial activity than F1 at all concentrations except 1 mg/mL. The activity was similar at 10 mg/mL, with no significant difference between F1 and F2 ($p > 0.05$).

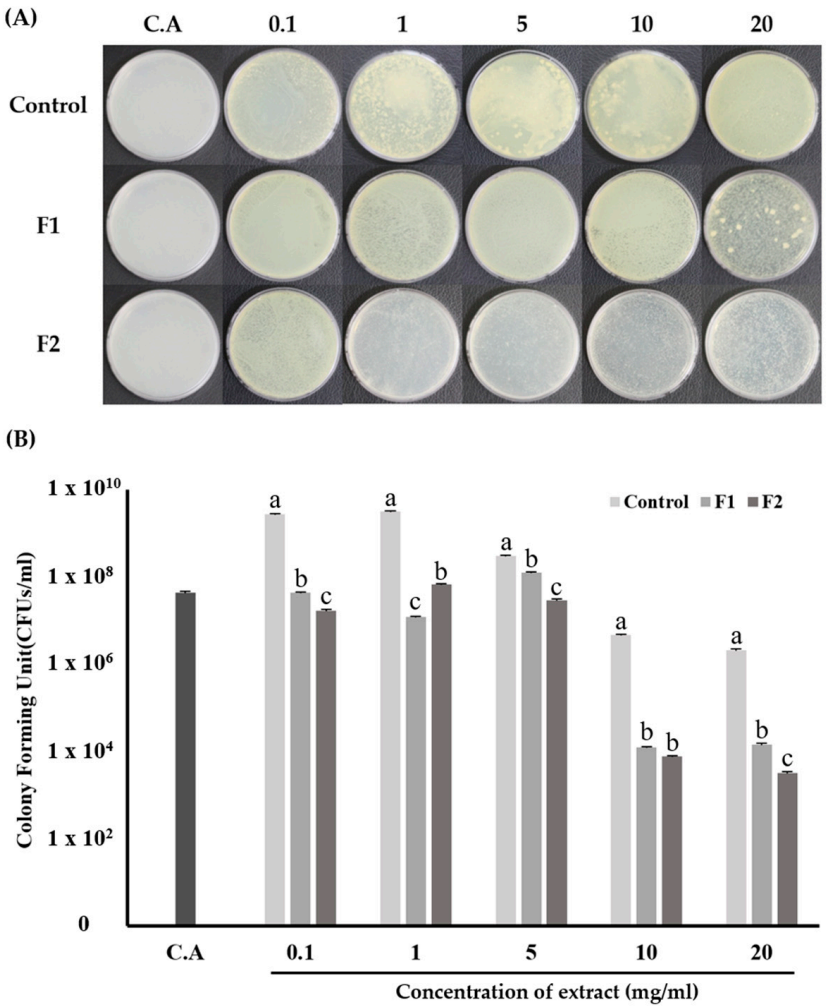

Figure 3. (**A**) Petri dish photos of antifungal activity assay of control and HME formulation of AGN against *C. albicans*; (**B**) colony forming unit of *C. albicans* after 24 h. Data are expressed as means ± standard deviation ($n = 3$). Different letters (a–c) indicate significant differences between control, F1, and F2 at the same concentration by one-way ANOVA, followed by Duncan's multiple range test at $p < 0.05$.

Antifungal activity was higher in F2 than in F1, and since high content of D and DA was shown in the HPLC analysis of F2 extract in the extrudate, the content increase is supposed to be related to the increase in antifungal activity.

4. Conclusions

HME decreased the particle size of AGN and increased the surface area, indicating an increase in the water solubility of the active ingredient. In the F2 formulation, the D content increased about 41 times, and the DA content increased about 13 times. The antioxidant activity of all extracts increased in a concentration-dependent manner, and it was confirmed that the F2 formulation exhibited the lowest IC_{50} value among the AGN extracts. In the antifungal activity investigation using the extract, it was found that HME-AGN exhibited higher activity than conventional AGN. Through these results, it was suggested that AGN with increased water solubility of the active ingredient using HME could be used for improving pharmacological activity.

Author Contributions: S.R. and H.Y.L. conceived, designed, and performed the experiments; S.-H.N. analyzed the data; J.-S.B. contributed reagents, materials, and analysis tools and wrote the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (No. 2022R1F1A1074747).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Shehzad, A.; Parveen, S.; Qureshi, M.; Subhan, F.; Lee, Y.S. Decursin and decursinol angelate: Molecular mechanism and therapeutic potential in inflammatory diseases. *Inflamm. Res.* **2018**, *67*, 209–218. [CrossRef] [PubMed]
2. Fontamillas, G.; Kim, S.W.; Kim, H.-U.; Kim, S.-J.; Park, T.S.; Park, B.-C. Effects of *Angelica gigas* Nakai on the production of decursin- and decursinol angelate-enriched eggs. *J. Sci. Food Agric.* **2019**, *99*, 3117–3123. [CrossRef] [PubMed]
3. Ko, M.-J.; Kwon, M.-R.; Chung, M.-S. Pilot-scale subcritical-water extraction of nodakenin and decursin form *Angelica gigas* Nakai. *Food Sci. Biotechnol.* **2020**, *29*, 631–639. [CrossRef] [PubMed]
4. Park, I.S.; Kim, B.; Han, Y.; Yang, H.; Cho, U.; Kim, S.I.; Kim, J.H.; Park, J.H.Y.; Lee, K.W.; Song, Y.S. Decursin and Decursinol Angelate Suppress Adipogenesis through Activation of β-catenin Signaling Pathway in Human Visceral Adipose-Derived Stem Cells. *Nutrients* **2019**, *12*, 13. [CrossRef] [PubMed]
5. Zhang, J.; Li, L.; Jiang, C.; Xing, C.; Kim, S.-H.; Lü, J. Anti-cancer and other bioactivities of Korean *Angelica gigas* Nakai (AGN) and its major pyranocoumarin compounds. *Anticancer Agents Med. Chem.* **2012**, *12*, 1239–1254. [CrossRef] [PubMed]
6. Lee, Y.R. Biological Activities of Extracts from Leaf of *Angelica gigas* Nakai. *Korean J. Food Preserv.* **2021**, *34*, 181–186.
7. Cho, J.H.; Kwon, J.E.; Cho, Y.; Kim, I.; Kang, S.C. Anti-Inflammatory Effect of *Angelica gigas* via Heme Oxygenase (HO)-1 Expression. *Nutrients* **2015**, *7*, 4862–4874. [CrossRef]
8. Sowndhararajan, K.; Kim, S. Neuroprotective and Cognitive Enhancement Potentials of *Angelica gigas* Nakai Root: A Review. *Sci. Pharm.* **2017**, *85*, 21. [CrossRef]
9. Ryu, S.; Jin, M.; Wang, M.-H.; Baek, J.-S.; Cho, C.-W. Effects of lipid nanoparticles on physicochemical properties, cellular uptake, and lymphatic uptake of 6-methoxflavone. *J. Pharm. Investig.* **2022**, *52*, 233–241. [CrossRef]
10. Lee, H.; Bang, J.-B.; Na, Y.-G.; Lee, J.-Y.; Cho, C.-W.; Baek, J.-S.; Lee, H.-K. Development and Evaluation of Tannic Acid-Coated Nanosuspension for Enhancing Oral Bioavailability of Curcumin. *Pharmaceutics* **2021**, *13*, 1460. [CrossRef]
11. Huh, H.W.; Na, Y.-G.; Kang, H.; Kim, M.; Han, M.; Pham, T.M.A.; Lee, H.; Baek, J.-S.; Lee, H.-K.; Cho, C.-W. Novel self-floating tablet for enhanced oral bioavailability of metformin based on cellulose. *Int. J. Pharm.* **2021**, *592*, 120113.
12. Na, Y.-G.; Pham, T.M.A.; Byeon, J.-J.; Kim, M.-K.; Han, M.-G.; Baek, J.-S.; Lee, H.-K.; Cho, C.-W. Development and evaluation of TPGS/PVA-based nanosuspension for enhancing dissolution and oral bioavailability of ticagrelor. *Int. J. Pharm.* **2020**, *581*, 119287. [CrossRef] [PubMed]
13. Genina, N.; Hadi, B.; Löbmann, K. Hot Melt Extrusion as Solvent-Free Technique for a Continuous Manufacturing of Drug-Loaded Mesoporous Silica. *J. Pharm. Sci.* **2018**, *107*, 149–155. [CrossRef] [PubMed]
14. Ashour, E.A.; Majumdar, S.; Alsheteli, A.; Alshehri, S.; Alsulays, B.; Feng, X.; Gryczke, A.; Kolter, K.; Langley, N.; Repka, M.A. Hot melt extrusion as an approach to improve solubility, permeability and oral absorption of a psychoactive natural product, piperine. *J. Pharm. Pharmacol.* **2016**, *68*, 989–998. [CrossRef] [PubMed]
15. Patil, H.; Tiwari, R.V.; Repka, M.A. Hot-Melt Extrusion: From Theory to Application in Pharmaceutical Formulation. *AAPS PharmSciTech* **2016**, *17*, 20–42. [CrossRef] [PubMed]

16. Go, E.-J.; Rut, B.-R.; Ryu, S.-J.; Kim, H.-B.; Lee, H.-T.; Kwon, J.-W.; Baek, J.-S.; Lim, J.-D. An Enhanced Water Solubility and Stability of Anthocyanins in Mulberry Processed with Hot Melt Extrusion. *Int. J. Mol. Sci.* **2021**, *22*, 12377. [CrossRef]
17. Hwang, I.; Kang, C.-Y.; Park, J.-B. Advances in hot-melt extrusion technology toward pharmaceutical objectives. *J. Pharm. Investig.* **2017**, *47*, 123–132. [CrossRef]
18. Jiang, Y.; Piao, J.; Liu, N.; Hou, J.; Liu, J.; Hu, W. Effect of Ultrafine Powderization and Solid Dispersion Formation via Hot-Melt Extrusion on Antioxidant, Anti-Inflammatory, and the Human Kv1.3 Channel Inhibitory Activities of *Angelica gigas* Nakai. *Bioinorg. Chem.* **2020**, *2020*, 7846176. [CrossRef]
19. Jiang, Y.; Piao, J.; Cho, H.-J.; Kang, W.-S.; Kim, H.-Y. Improvement in antiproliferative activity of *Angelica gigas* Nakai by solid dispersion formation via hot-melt extrusion and induction of cell cycle arrest and apoptosis in HeLa cells. *Biosci. Biotechnol. Biochem.* **2015**, *79*, 1635–1643. [CrossRef]
20. Azad, M.; Kang, W.S.; Lim, J.D.; Park, C.H. Bio- Fortification of *Angelica gigas* Nakai Nano-Powder Using Bio-Polymer by Hot Melt Extrusion to Enhance the Bioaccessibility and Functionality of Nutraceutical Compounds. *Pharmaceuticals* **2020**, *13*, 3. [CrossRef]
21. Piao, J.; Lee, J.-Y.; Weon, J.B.; Ma, C.J.; Ko, H.-J.; Kim, D.-D.; Kang, W.-S.; Cho, H.-J. *Angelica gigas* Nakai and Soluplus-Based Solid Formulations Prepared by Hot-Melting Extrusion: Oral Absorption Enhancing and Memory Ameliorating Effects. *PLoS ONE.* **2015**, *10*, e0124447. [CrossRef] [PubMed]
22. Khan, R.A.; Khan, M.R.; Sahreen, S.; Ahmed, M. Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (L.) Hill. *Chem. Cent. J.* **2012**, *6*, 12. [CrossRef] [PubMed]
23. Lee, S.; Lee, Y.-S.; Jung, S.-H.; Shin, K.H.; Kim, B.-K.; Kang, S. S Antioxidant Activities of Decursinol Angelate and Decursin from Angelica gigas Roots. *Nat. Prod. Sci.* **2003**, *9*, 170–173.