



Evaluation of Antioxidant Activity of the Extract and Subfractions of *Saussurea grandifolia*

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Dear Editor-in-Chief

Plants are natural reservoirs of various phytochemicals that are nutritionally and medicinally valuable for humans (1). Natural products contain a wide variety of antioxidants including phenolic acids, flavonoids, tocopherols, and ascorbic acid (2). Phenolic compounds reduce the risk for many chronic diseases by acting as antioxidants and scavenging free radicals (3, 4).

Saussurea grandifolia (commonly referred to as chwinamul in Korean) is widely distributed in Eastern Asia including the Republic of Korea and is commonly consumed by Koreans. Chwinamul is used as a flavoring agent and to prevent or treat hepatitis, cholesterolemic conditions, viral infections, diabetes, and atherosclerosis (5). A few studies have evaluated the caffeoylquinic acid derivatives and peroxynitrite-scavenging capacities of *S. grandifolia* (6, 7). However, detailed information on these phenolic compounds and their antioxidant properties are lacking.

Thus, we quantified the phenolic acid and flavonoid contents of *S. grandifolia* using spectrophotometry according to the Korean National Institute of Food and Drug Safety Evaluation (NIFDS) Guidelines (8). In addition, we measured 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity, 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical-scavenging activity, and the ferric reducing antioxidant power (FRAP) of different fractions

to assess the antioxidant capacities of *S. grandifolia*.

Phenol content of methanol extract of *S. grandifolia* was expressed in mg of gallic acid equivalent (GAE) per gram (mg GAE/g). The results also showed that the ethyl acetate fraction had higher phenol content (431.1 ± 2.5 mg GAE/g) than the other solvent fractions. Butanol, methanol, and water fractions had phenol contents of 331.3 ± 0.3 mg GAE/g, 193.8 ± 2.8 mg GAE/g, and 188.6 ± 2.3 mg GAE/mg, respectively. The flavonoid content of the crude extracts was determined using quercetin as a standard and expressed as its equivalent (mg QE/g). The ethyl acetate extract had the highest flavonoid content of 125.3 ± 0.4 mg QE/g, while the hexane extract had the lowest amount (6.1 ± 0.4 mg QE/g). The butanolic and methanolic extracts resulted in 40.1 ± 0.1 mg QE/g and 36.3 ± 0.5 mg QE/g of flavonoid, respectively.

The DPPH radical-scavenging activities of various solvent extracts were compared to those of the antioxidant butylated hydroxytoluene (BHT) at 0.5 mg/mL. Almost all solvent fractions exhibited inhibitory activities, ranging from 94.5% (the ethyl acetate fraction) to 2.6% (the hexane extract) (Fig. 1a). For comparison, the DPPH radical scavenging activities of pure quercetin and rutin standards at same concentration were 96.0 ± 0.21 and 90.9 ± 0.4 , respectively. Similarly, all

extracts exhibited ABTS radical-scavenging activities at concentrations of 1.0 mg/mL (Table 1). The ethyl acetate, methanol, and butanol extract values were >80%. The order of activity was ethyl acetate > methanol > butanol > water > chloroform > hexane (Fig. 1b). In the FRAP assay, the reducing powers of solvent extracts

ranged from 4.46 mM FeSO₄ equivalent/mg in ethyl acetate fractions to 0.31 mM FeSO₄ equivalent/mg in hexane fractions at concentrations of 1.0 mg/mL (Fig. 1c). In all assays, ethyl acetate fractions exhibited the highest antioxidant capacity, and hexane fractions had the lowest.

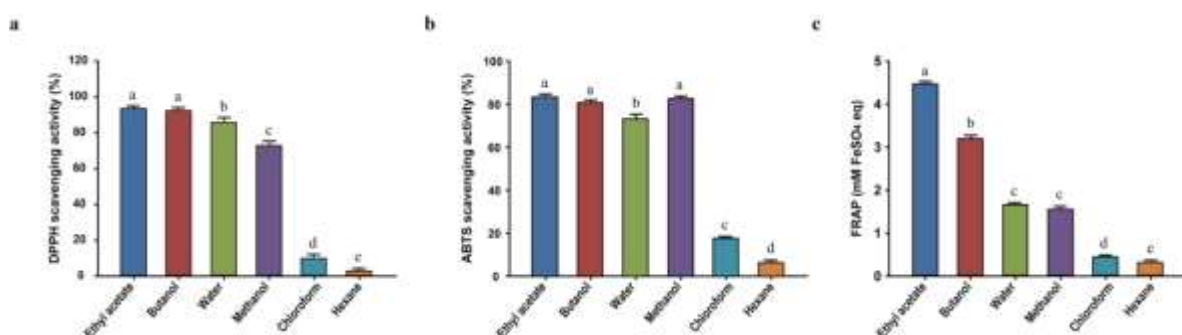


Fig. 1: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (a); 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging activity (b); and ferric reducing activity (c) of the different solvent fractions of *S. grandifolia*. Values are means ± standard deviation of three replications. The different letters means statistically significant at 0.01 by Duncan test

Antioxidants such as phenolics and flavonoids reduce the incidence of heart disease, several cancers, and degenerative disease (9, 10). Here, ethyl acetate fractions of *S. grandifolia* was shown to be a rich source of antioxidant compounds. Therefore, our results suggest that *S. grandifolia*, particularly ethyl acetate fractions, may serve as a

useful functional food and health-promoting herbal medicine. To the best of our knowledge, this is the first study to report the phenolic acid and flavonoid contents and antioxidant activities of *S. grandifolia* extracts. However, the associations between specific components and defined biological or therapeutic activities remain unclear.

Table 1: Total phenolics and flavonoids of different fractions extract from *Saussurea grandifolia*

<i>Extract solvent *</i>	<i>Total phenolics (mg GAE/g DW)</i>	<i>Total flavonoids (mg QE/g DW)</i>
Methanol	193.8±2.8	40.1±0.1
Hexane	33.6±0.1	6.1±0.4
Chloroform	61.2±0.3	9.9±0.3
Ethyl acetate	431.1±2.5	125.3±0.4
Butanol	331.3±0.3	87.7±0.2
Water	188.6±2.3	36.3±0.5

*Each sample concentration was 1.0 mg/mL. Values are mean±SD (n=3)

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Chen H, Liu RH (2018). Potential mechanisms of action of dietary phytochemicals for cancer prevention by targeting cellular signaling transduction pathways. *J Agric Food Chem*, 66(13): 3260-3276.
2. Kalt W (2005). Effects of production and processing factors on major fruit and vegetable antioxidants. *J Food Sci*, 70(1): R11-R19.
3. Ben Jannet S, Hymery N, Bourgou S, et al (2017). Antioxidant and selective anticancer activities of two Euphorbia species in human acute myeloid leukemia. *Biomed Pharmacother*, 90: 375-385.
4. Makhafola TJ, Elgorashi EE, McGaw LJ, et al (2016). The correlation between antimutagenic activity and total phenolic content of extracts of 31 plant species with high antioxidant activity. *BMC Complement Altern Med*, 16(1): 490.
5. Park HJ (2010). Chemistry and pharmacological action of caffeoylquinic acid derivatives and pharmaceutical utilization of chwinamul (Korean mountainous vegetable). *Arch Pharm Res*, 33(11): 1703-20.
6. Nugroho A, Lim SC, Lee CM, et al (2012). Simultaneous quantitative determination and validation of quercetin glycosides with peroxynitrite-scavenging effects from *Saussurea grandifolia*. *J Pharm Biomed Anal*, 61: 247-51.
7. Nugroho A, Kim KH, Lee KR, et al (2009). Qualitative and quantitative determination of the caffeoylquinic acids on the Korean mountainous vegetables used for chwinamul and their peroxynitrite-scavenging effect. *Arch Pharm Res*, 32(10): 1361-7.
8. NIFDS (2016). Health functional food testing method. NIFDS, Korea. Available from: http://www.nifds.go.kr/nifds/03_info/sub_02.jsp?mode=view&article_no=10055&pager.offset=0&board_no=167
9. Karakaya S (2004). Bioavailability of phenolic compounds. *Crit Rev Food Sci Nutr*, 44(6): 453-64.
10. Cartea ME, Francisco M, Soengas P, Velasco P (2010). Phenolic compounds in Brassica vegetables. *Molecules*, 16(1): 251-280.