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General Characteristics of Korean Propolis

Soon Ok Woo*, Sang Mi Han, Inpyo Hong, Yong Soo Choi, Kwang Gill Lee, Joo Hong Yeo, Hae Yong Kweon, Myoung Ryeol Lee, and Man Young Lee

Department of Agricultural Biology, National Academy of Agricultural Science(NAAS), Rural Development Administration(RDA), Suwon, 441-853, Korea

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The propolis was collected from the whole part of Korea Peninsula, such as central, southern, and Jeju island, to analyze the general composition including total flavonoid and phenolic contents, heavy metals, colors, amino acids, and crude lipid. The total flavonoid and phenolic contents of middle region in Korea Peninsula (4.26% and 13.06 g/100 g gallic acid) were higher than southern region (2.53%, 10.9 g/100 g gallic acid) and Jeju island (0.03%, 7.22g/100 g gallic acid), respectively. The heavy metals contents showed that Zn contents were found in 0.44±0.2 ppm and a harmful heavy metal such as Cr, Cd, Pb, Ni, Cu, As, and Hg were not detected at all in our experiment. The colors of extracted propolis showed a various spectrum from yellow to red purple. Various amino acids were also detected as proline, valine, methionine and the average crude lipid contents of propolis were 42.4%. All data collected in South Korea were very first executed in our institution for the purposes of utilizing and developing the industrial insects, honeybees as well as silkworm.

Key words: Propolis, Characteristics, South Korea, Flavonoids, Phenolics

Introduction

Propolis, or 'bee glue', is a complex resinous mixture of plant-derived products gathered, modified and used by bees as a general purpose sealer, draught excluder and antibiotic in their hives. Propolis typically consists of waxes, resins, water, inorganics, phenolics and essential oils, the exact composition of which is dependent upon the source plant(s). The presence of propolis within the hive may also provide an environment not suitable for the growth of bacteria and other microorganisms. Propolis composition is directly related to that of bud exudates collected by bees from various trees (Mochida *et al*., 1985). The geographical dependence of propolis constituents is exemplified in analyses of, e.g. European, South American, Chinese, Canadian and Spanish sourced samples. Very little work has been carried out on the quantification of individual propolis components. Propolis in general contains a variety of chemical compounds such as polyphenols (flavonoid aglycones, phenolic acids, and their esters, phenolic aldehydes, alcohols, and ketones), terpenoids, steroids, amino acids, and inorganic compounds (Dimov *et al*., 1991; Volpert and Elstner, 1993; Moreno *et al*., 2000). Many biological properties, including antibacterial, (Mochida *et al*., 1985; Velikova *et al.*, 2000), antifungal (Dimov *et al*., 1991; Murad *et al.*, 2002), antiviral (Amoros *et al.*, 1992; Amoros *et al*., 1994), anti-inflammatory (Strehl *et al.*, 1994; Miyataka *et al.*, 1997), antioxidant (Sun *et al*., 2000; Isla *et al.*, 2001), hepatoprotective (Gonzales *et al.*, 1995) and immunostimulating (Dimov *et al.*, 1991) activities have been ascribed to propolis. Antibacterial activity of propolis is reported to be due to flavonoids, aromatic acids, and its esters. The biological properties of propolis are attributed to a synergism between phenolic and other compounds in the resin (Burdock, 1998). Therefore, the present study investigated the general composition including total flavonoid and phenolic contents, heavy metals, colors, amino acids, and crude lipid on the south Korean propolis. The propolis collected from the whole part of South Korea, such as central, southern and Jeju island.

*To whom the correspondence addressed
Department of Agricultural Biology, National Academy of Agricultural Science(NAAS), Rural Development Administration(RDA), Suwon, 441-853, Korea
Tel: 031-290-8508, 290-8516, E-mail: wooso1@korea.kr
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Materials and Methods

Propolis samples

The propolis samples collected by *Apis mellifera* were obtained from the whole part of Korea peninsula, such as middle(Suwon, Yeoju, Yeongwol, Hongcheon, Dangjin, Daejeon, Chungju, Danyang), southern region(Daegu, Mungyeong, Jinju, Changnyeong, Jeonju, Namwon, Gurye, Hwasoon) and Jeju island.

The crude propolis samples were ground into a fine powder, and 5 g of the propolis powder was mixed with 50 ml of 80% Ethanol in a test tube and shaken at RT for 48 hours. After extraction, the mixture was centrifuged to obtain the supernatants, which were designated as an ethanol extract of propolis(EEP). The EEPs were lyophilized, which were used to analyze for total phenolic contents and amino acids, the total flavonoid contents, heavy metals and crude lipids.

Determination of total flavonoids concentration

Flavonoids concentration was determined as follows: EEP(0.1 mg) was extracted with 90% aqueous ethanol (20 ml), and centrifuged(3000 rpm, 10 min). Supernatant was collected, the residue was extracted with 80% ethanol (8 ml), three times, total supernatants volumes were 50 ml with 80% ethanol.

An aliquot of 0.5 ml was added to test tubes containing 0.1 ml of 10% aluminum nitrate, 0.1 ml of 1 M aqueous potassium acetate, 1.5 ml of ethanol and 2.8 ml of distilled water. After 40 min at room temperature, the absorbance was determined spectrophotometrically at 415 nm on a Perkin-Elmer Lambda 10 UV/VIS Spectrophotometer. Total flavonoid concentration was calculated using quercetin as standard (Moreno *et al*., 2000).

Determination of total phenolic contents

The amount of total phenolic in the extracts was determined according to a modification of the Folin-Ciocalteu method (Kuyala *et al*., 2000). A 0.5 ml aliquot of diluted extract (extract-80% ethanol, 1:5000 (w/v), three replicates) was introduced into a test tube and mixed with 0.5 ml of 1 N Folin-Ciocalteu`s reagent. The mixture was allowed to stand for a 2 to 5 min period which was followed by the addition of 0.5 ml of 10% Na_2CO_3. After 50 min incubation at room temperature, the mixture was centrifuged for 10 min (150 g) and the absorbance of the supernatant was measured at 760 nm on a Perkin-Elmer Lambda 10 UV/VIS Spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram dry material.

The heavy metals contents

The metal(Cadmium, Chrome, Copper, Nickel, Lead, Zinc, Arsenic, Mercury) concentrations of the propolis were determined using a ICP-MS(Agilent 7500a), the samples for analysis of heavy metal content were degraded with ternery solution.

Amino acids analysis

Approximately 5 mg of the EEP extract was dissolved in 0.5 ml of 80% ethanol, added in 0.5 ml of 12 N HCl and evacuated in a vacuum hydrolysis glass tube (Kontes, Vineland, NJ). Traces of oxygen were replaced by repeated flushing with nitrogen. The closed tube was heated at 110°C for 24 hours to achieve complete hydrolysis. The solvent was removed *in vacuo*. at 40°C, and applied to an amino acid analyzer (Pharmacia Biotech System Biochrom 20 Plus type amino acid analyzer) after filtering.

The crude lipid

The crude lipid was weighed extracted lipid of propolis. Triplicate samples from crude propolis were weighed to 3.0 g and crude lipids were extracted in ether (Poon *et al.,* 1956).

Table 1. Total flavonoids and phenolic contents of Korean propolis

Region		Total flavonoids contents		Total phenolic contents	
		(%)	Average	(g/100g gallic acid)	Average
Middle	Suwon	5.37	4.26	19.78	13.05
	Yeoju	4.33		13.79	
	Yeongwol	5.22		7.97	
	Hongcheon	2.60		11.91	
	Dangjin	6.67		13.69	
	Daejeon	4.72		12.77	
	Chungju	2.49		10.21	
	Danyang	2.67		14.25	
Southern	Daegu	2.95	2.53	9.65	10.91
	Mungyeong	1.44		9.18	
	Jinju	3.44		11.10	
	Changnyeong	2.08		9.51	
	Jeonju	2.08		12.27	
	Namwon	1.76		7.84	
	Gurye	3.34		14.57	
	Hwasoon	3.16		14.45	
Jeju	Jeju 1	0.08	0.03	4.42	7.22
	Jeju 2	0.00		12.05	
	Jeju 3	0.00		5.18	

Table 2. Heavy metal contents of Korean propolis (unit : ppm)

Region		Cd	Cr	Cu	Ni	Pb	Zn	As	Hg
Middle	Suwon	ND	ND	ND	ND	ND	0.301	ND	ND
	Yeoju	0.008	ND	ND	0.019	0.184	0.315	ND	ND
	Yeongwol	ND	ND	0.027	0.001	0.114	0.402	ND	ND
	Dangjin	ND	ND	ND	ND	ND	0.256	ND	ND
	Daejeon	ND	ND	ND	ND	0.013	0.265	ND	ND
	Chungju	ND	ND	ND	ND	ND	0.848	ND	ND
	Danyang	ND	ND	ND	ND	ND	0.354	ND	ND
Southern	Daegu	ND	ND	0.001	0.067	ND	0.672	ND	ND
	Mungyeong	ND	ND	ND	ND	0.010	0.445	ND	ND
	Jinju	ND	ND	0.016	0.002	0.070	0.449	ND	ND
	Changnyeong	ND	ND	ND	ND	0.403	0.280	ND	ND
	Jeonju	ND	ND	ND	0.001	ND	0.229	ND	ND
	Namwon	ND	ND	0.130	0.004	0.089	0.579	ND	ND
	Gurye	ND	ND	0.053	ND	0.017	0.515	ND	ND
	Hwasoon	ND	ND	ND	ND	ND	0.242	ND	ND
Jeju	Jeju 1	ND	ND	0.014	0.001	0.159	0.651	ND	ND
	Jeju 2	ND	ND	0.016	0.028	0.269	0.507	ND	ND
	Jeju 3	0.018	ND	0.021	0.013	0.538	0.600	ND	ND
Average							0.440		

* ND : Not Detected

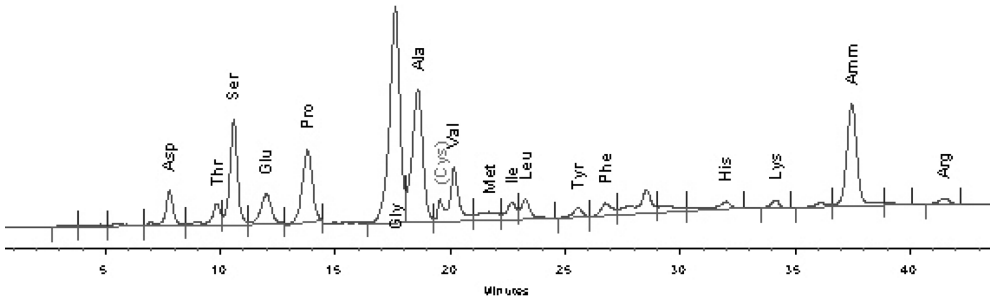

Fig. 1. Amino acids spectrum of Jeju island.

Results

To analyze the characteristics of Korean propolis according to the region of Korean peninsula, total flavonoids and phenolic contents were measured(Table 1). Total flavonoids and phenolic contents of middle region in Korean peninsula (4.26% and 13.05 g/100 g gallic acid) were higher than southern region (2.53%, 10.9 g/100 g gallic acid) and Jeju island(0.03%, 7.22 g/100 g gallic acid), respectively. Our results demonstrate that the qualities and quantities of flavonoids are different among the regional provinces of Korea.

A comparative study of total phenolic content of the propolis was undertaken. Total phenolic contents appeared similarly as those of flavonoids did. Moreno *et al.*(2000) reported that total flavonoids content of Argentine propolis was 1 to 4%. In our studies, the flavonoids and phenolic contents of Korean propolis which collected from middle region of Korean peninsula was higher than Argentine propolis. These meant quantity and quality of Korean propolis were similar or even superior than Argentine propolis.

Although several propolis were collected in Korea contained Pb, they were less than 1 ppm(Table 2), we concluded that Korean propolis would be suitable for the sources of health food because a harmful heavy metal including Cr, As and Hg were not detected at all in our experiments. On the other hand, Zn contents were found in 0.44 ± 0.2 ppm.

While various amino acids(Table 3) including aspartic

Table 3. Amino acids content of Korean propolis

Region		Asp	Thr	Ser	Glu	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	Pro
Middle	Suwon	○	-	○	○	○	○	○	○	○	-	○	○	-	-	-	○	○
	Yeoju	○	-	○	○	○	○	○	○	○	-	○	○	-	-	-	-	○
	Yeongwol	○	-	○	○	○	-	○	○	○	-	○	-	-	○	○	○	○
	Dangjin	○	○	○	○	○	-	○	○	○	-	○	-	-	○	○	○	○
	Daejeon	○	-	○	○	○	○	○	○	○	-	-	○	-	-	-	○	○
	Chungju	○	○	○	○	○	○	○	○	○	○	○	○	-	○	○	○	○
	Danyang	○	○	○	○	○	○	○	○	○	○	○	○	-	○	○	○	○
Southern	Daegu	○	○	○	○	○	○	○	○	○	○	○	○	-	-	○	○	○
	Mungyeong	○	○	○	○	○	○	○	○	○	○	○	○	-	-	○	○	○
	Jinju	○	-	○	○	○	○	○	○	○	-	-	-	-	-	-	-	○
	Chang-nyeong	○	-	○	○	○	○	○	○	○	○	○	○	-	○	○	○	○
	Jeonju	○	-	○	○	○	-	○	○	○	○	-	-	-	-	-	-	○
	Nam won	○	-	○	○	○	○	○	○	○	-	-	-	-	-	-	-	○
	Gurye	○	-	○	○	○	○	○	○	○	○	○	○	-	-	-	-	○
	Hwasoon	○	-	○	○	○	○	○	○	○	-	○	○	-	-	-	-	○
Jeju	Jeju 1	○	○	○	○	○	○	○	○	○	○	○	○	-	-	○	-	○
	Jeju 2	○	○	○	○	○	○	○	○	○	-	○	-	○	-	○	○	○
	Jeju 3	○	○	○	○	○	○	○	○	○	○	○	○	-	-	○	○	○

d : detected, - : not detected

Table 4. The crude lipid contents of Korean propolis

Region		Crude lipid (%)	Average
Middle	Suwon	54.0	45.1
	Yeoju	45.2	
	Yeongwol	53.8	
	Hongcheon	37.3	
	Dangjin	50.2	
	Daejeon	51.6	
	Chungju	34.5	
	Danyang	34.1	
South	Daegu	43.4	39.7
	Mungyeong	33.8	
	Jinju	57.3	
	Changnyeong	38.4	
	Jeonju	36.1	
	Namwon	27.7	
	Gurye	41.3	
	Hwasun	39.4	
Jeju	Jeju 1	20.7	27.0
	Jeju 2	31.0	
	Jeju 3	29.3	

acid(Asp), serine(Ser), glutamic acid(Glu), glycine(Gly), cystein(Cys), proline(Pro), valine(Val), and methionine(Met) were detected in propolis of all area, phenylalanine(Phe) was detected in only Jeju propolis. That means uniqueness of Jeju region which is separated by channel and which might have a unique race of source plants. Finally, the average crude lipid contents(table 4) of propolis were 45.1% and 39.7% in middle and southern region, respectively and that of Jeju region were 27%.

In conclusion, the central and southern region propolis are similar, but shows a lot of difference in Jeju Island. These results shows the general characteristics of propolis originates from the vegetation, the southern and central regions are similar vegetation, but Jeju Island due to different vegetation compared with inland areas.

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