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Multi Residual Pesticide Monitoring in Commercial Herbal Crude Drug Materials in South Korea

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Abstract In order to reduce health risks from residual pesticides in herbal drug products, the residue information of pesticides should be acquired. Therefore, 38 commodities (229 herbal crude drug materials) were analyzed for 44 different pesticides. Among the 229 samples (36 types of herbal crude drugs), seven imported and two domestic drug materials were contaminated with eight pesticides (0.034–0.579 mg/kg) such as BHC, procymidone, endosulfan, etc. Among the eight detected pesticides, four were fungicides, which were found in underground crude drug materials such as rhizoma or radix.

Keywords Herbal crude drug · Pesticides · Monitoring · Gas chromatography

Five hundred and eighteen herbal drug materials are registered in the Korean Pharmacopeia and Korean Herbal Pharmacopeia. Direct human exposure to herbal medicinal drugs that contain environmental contaminants, including residual pesticides, may induce health risks from crude, unpurified materials. There may be an increase in health risk due to the illegal use of some cultivation pesticides. Specifically, unlike domestic pesticides, imported herbal crude drugs may contain unpredictable chemicals. Therefore, in order to reduce health risks from residual pesticides in herbal drug products, crude drug materials must be subject to control. To determine the residue information of pesticides in herbal crude drug materials, 38 commodities were analyzed for 44 different pesticides. Analysis was performed using the modified pesticide multi-residual analysis method (MRM) No. 83 from the Korean Food Code, which is used by official spots of Korea food and drug administration (KFDA 2002). Recovery tests from three representative samples and monitoring results for 229 herbal crude drug materials are presented.

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Materials and Methods

Total of 229 samples consisted with 36 kinds of herbal crude drug materials are listed on Table 1. Most of the imported herbal crude drugs were purchased in Kyoung-dong market that is the largest herbal drug market in South Korea. Domestically produced, crude herbal drug materials were purchased at large herbal drug wholesale markets located in Daegu, Geumsan and Yeongcheon. To reduce the possibility of sample duplication, more than four samples were gathered at regular intervals between March and October 2001. Immediately after sampling, the crude herbal drug materials were identified by an herbal medicine expert. Forty-eight pesticide standards above the purity 95% were purchased from ChemService, INC (West Chester, PA, USA), Dr. Ehrenstofer (Augsburg, Germany) and Waco pure chemical Industries (Osaka, Japan).

Each pesticide standard was dissolved in acetone as a stock solution (1,000 mg/L) and preserved under the –4°C within 3 months. The working solution mixtures of pesticides were prepared in acetone as a concentration range of 0.5, 1.0, 5.0 and 10 mg/L. The pesticide analysis grade acetonitrile, acetone and hexane were ordered from Burdick & Jackson (Muskegon, MI, USA). And all other chemicals were purchased from Junsei Chemical Corp. (Tokyo, Japan).

Table 1 Tested herbal crude drugs confirmed to genuine with origin

Korean name	Latin name	Academic name	Origin (number of the samples)	
			Imported	Domestic
Galgun	Puerariae radix	*Pueraria lobata* Ohwi	China (3)	Kimcheon, Gyeongju, Jeongseon
Gamcho	Glycyrrhizae radix	*Glycyrrhiza uralensis* Fischer	China (6)	–
Geji	Cinnamonmi ramulus	*Cinnamomum cassia* Blume	China (3)	–
Gepi	Cinnamon bark	*Cinnamomum cassia* Blume	Vietnam (6)	–
Gugija	Lycii fructus	*Lycium chinense* Miller	China (4)	Jindo, Cheongyang, Cheongyang(2)
Gilgyeong	Playcodi radix	*Platycodon grandiflorum* A. De Candolle	China(4)	Muju, Andong, Unknown
Dansam	Salvia miltiorrhizae radix	*Salvia miltiorrhiza* Bunge	China (6)	–
Dangui	Angelica Gigantis radix	*Angelica gigas Nakai* (Domestic) *Angelica sinensis* (China)	China (4)	Jecheon, Pyeongchang, Samcheok
Dokhwal	Udo (Manchurian spikenard)	*Aralia cordata var. continentalis* Kitagawa	–	Yeongcheon, Imsil, Cheongyang, Imsil (2), Andong
Duchung	Eucommiae cortex	*Eucommia ulmoides* Oliver	China (4)	Seongju, Yeongcheon, Unknown
Maekmundong	Liriopus tuber	*Liriope platyphylla* Wang et Tang *Ophiopogon japonicus* Ker-Gawler	China (4)	Milynag, Milyang (2), Cheongyang
Baekchool	Atractylodis rhizoma alba	*Atractylodes japonica* Koidzumi	China (4)	Yeongyang, Jinbu, Yeongcheon
Bokryeong	Hoelen	*Poria cocos* Wolf	China (4)	Bonghwa, Yeongwol, Yeongwol (2), unknown
Jandae (Sasam)	Adenophorae radix	*Adenophora triphylla var. japonica* Hara	China (4)	Jinan, Hoengseong, Ulleungdo
Sain	Amomi fructus	*Amomum villosum* Loureiro	China (6)	–
Sansuyoo	Corni fructus	*Cornus officinalisSieboldii* et Zuccarini	–	Bonghwa, Uiseong, Yeongcheon, Gurye, Uiseong (2)
Sanyak	Dioscoreae rhizoma	*Dioscorea batatas* Decaisne	China (4)	Yeongju (1), Yeongju (2), Andong
Sanjoin	Zizyphi semen	*Zizyphus jujuba* Miller	China (6)	–
Gungang	Zingiberis rhizoma	*Zingiber officinale* Rosco	China (4)	Bongdong, Cheongyang, Bongdong (2)
Ogapi	Acanthopanax root bark	*Acanthopanax sessiliflorum* Seeman	China (4)	Andong, Jecheon,Andong (2)
Omija	Schisandrae fructus	*Schizandra chinensis* Baillon	China (4)	Inje, Sanchok, Muju
Yonganyook	Longanae arillus	*Dimocarpus longan* Lour.	Thailand (6)	–
Damo Usul (Tulsue Murup)	Acyranthis radix	*Achyranthes fauriei* H. Lev. & Vaniot	China (4)	Andong, Sungju, Yeongcheon
Wonji	Polygalae radix	*Polygala tenuifolia* Willdenow	China (6)	–
Yukdugu	Myristicae semen	*Myristica fragrans* Houttuyn	China (6)	–
Jakyak	Paeoniae radix	*Paeonia lactiflora* Pallas	China (4)	Bosung, yeongcheon, Uiseong
Gun Jihwang	Rehmanniae radix	*Rehmannia glutinosa Libschitz var. purpurea* Makino	China (4)	Geumsan, Andong, Gunwi
Suk Jihwang	Rehmanniae radix preparata	*Rehmannia glutinosa Libschitz var. purpurea* Makino	China (3)	Unknown, unknown (2), unknown (3)
Changchul	Atractylodis rhizoma	*Atractylodes lancea* D.C.	China (4)	Yeongcheon, Cheongsong, unknown
Chungung	Cnidii rhizoma	*Cnidium officinale* Makino	China (4)	Yeongyang, Yeongyang (2), Cheongsong
Chunma	Gastrodiae rhizoma	*Gastrodia elata* Blume	China (4)	Muju, unknown, unknown (2)
Chija	Gardeniae fructus	*Gardenia jasminoides* Ellis	–	Gurye, Wando, Goeje, Haenam, Jindo
Tosaja	Cuscuta semen	*Cuscuta chinensis* Lamark	China (6)	–
Baek Hasuo	Cynanchi wilfordii radix	*Cynanchum wilfordii* Hemsley	China (4)	Yeongju, Yeongju (2), unknown

Table 1 continued

Korean name	Latin name	Academic name	Origin (number of the samples)	
			Imported	Domestic
Juk Hasuo	Polygoni Mjltiflori Radix	*Polygonum multiflorum* Thunburg	China (3)	–
Mongo Hwangi	Astragali mongolici Radix	*Astragalus membranaceus Bunge var.* mongoricus Hsiao	China (4)	Jeongseon, Jecheon, Jecheon (2)

The sample was homogenized by Omni mixer homogenizer (Model 17105, Marietta, GA, USA). And the excess solvent was evaporated by N-EVAP111 of Organomation Associates, Inc. (Berlin, MA, USA). The residual pesticides were extracted with solid-phase extractor consisted with SPE vacuum manifold of Supelco (Bellefonte, PA, USA) and Florisil cartridge (1 g, 5 mL) of Waters (Milford, MA, USA)

The HP-5 (30 m × 0.32 mm ID) and HP-1701 capillary columns (30 m × 0.25 mm ID) with a film thickness of 0.25 μm (Agilent J&W Scientific, Folsom, CA, USA) were utilized for dual GC-μ electron capture detector (μECD) analysis. For the GC-nitrogen phosphorus detector (NPD) analysis, only HP-5 column (same dimension with above) was adapted. For GC-μECD and NPD analysis, Agilent 6890 GC (Agilent, Palo Alto, CA, USA) was used. The carrier gas nitrogen (1–1.2 mL/min) was adapted with constant flow mode. For the GC-MSD analysis, Agilent 5973N mass selective detector (MSD) equipped with DB-5MS (30 m × 0.25 mm ID, 0.25 μm; Agilent J&W Scientific, Folsom, CA, USA) was used with mobile phase helium 1 mL/min constant flow mode. The oven temperature condition for all GC and GC-MSD analyses was same as held constant at 80°C for 2 min, increasing to 280°C with 10°C/min and kept for 15 min. Each 1 μl of sample was injected with split mode (split ratio 50:1) at 260°C except GC-NPD in which splitless mode was adapted due to the low sensitivity comparing μECD. The detector temperature was 280°C for μECD and NPD detectors. The make up gas for μECD and NPD was 60 and 1 mL/min (N_2), respectively. The hydrogen gas flow for NPD was 3 mL/min. All samples were injected by auto-sampler. The mass range for GC-MSD was set from 50 to 550 amu with electron impact mode (70 eV).

The modified pesticides MRM No. 83 were applied to 36 types of herbal crude drug material. Forty-eight target pesticide standards were divided into the three groups and chlordane in which all peaks belong to each group were non-overlapping on a HP-5 GC capillary column. Each pesticides group mixture was prepared in acetone (20 μg/mL for each pesticide). For statistically validating the efficiencies of the method, the recovery tests were performed with Dioscoreae rhizoma, Lycii fructus and Playcodi radix after spiking the above pesticide group mixtures at two concentration levels (0.05 and 0.1 mg/kg). The 1 mL of each pesticide mixture was added to the sample (20 g) and the sample was pre-soaked in the 40 mL of distilled water for 4 h followed by homogenization for 3 min after adding 100 mL of acetonitrile. The rest is the same as Oh's sample preparation method (Oh 2006). The final sample was dissolved in 2 mL acetone in hexane (20/80, v/v) before GC and GC-MSD analysis. All injection was repeated three times. To determine the limit of detection (LOD), each group of pesticides from 0.001 to 1 μg/mL were prepared. For the quantitative analysis, standard mixtures of 0.01, 0.1, 1, 5 and 10 μg/mL were analyzed to make a calibration curve. Any positively identified peaks were confirmed by GC-MSD and/or quantified by GC-μECD or NPD.

Results and Discussion

Forty-four pesticides (consisting of 56 pesticide standards) were analyzed for LOD and recovery from herbal crude drug samples. LOD values for target pesticides with pesticide standard solutions ranged from 0.001 to 0.04 μg/mL. Cypermethrin and dicofol indicated an LOD of 0.04 μg/mL. Due to two-fold dilution during sample preparation (Oh 2006), the LOD value could be 0.02 μg/mL, which may be low enough to enforce the maximum residue limit (MRL) value 0.5 mg/kg of Anemarrhenae rhizoma.

To test recovery of the pesticide, Dioscoreae rhizoma, Lycii fructus and Playcodi radix were selected from 36 types of herbal crude drugs. Three preparations of each sample were performed and the results were averaged. Fifty-six pesticides indicated recoveries from 53% to 113%. Among the three herbal crude drug materials, iprodione had the lowest recovery, below 55%. According to the MRM No. 83 validation report, 40%, 70%, and 81% iprodione was recovered from Chinese cabbage, apple, and perilla leaf, respectively (Labfrontier Co. 2004). Additionally, bifenthrin (average 61%) and prothiofos (average 64%) had relatively low recoveries. The

Table 2 Monitoring results of the herbal crude drugs and detected pesticides with the diverse pesticide MRLs (unit = mg/kg)

Sample name	Origin	Pesticide	Limit of detection	Detected concentration	Korean MRLs in crude drug	MRLs of European pharmacy
Platycodi radix	Domestic	Chinomethionate	0.002	0.268	0.3	
Paeoniae radix	China	Carbofuran	0.006	0.034		
		BHC	0.002	0.215	0.2	
Cuscuta semen	China	BHC	0.002	0.160	0.2	
Atractylodes rhizoma alba	China	BHC	0.002	0.053	0.2	
		Procymidone	0.003	0.572	0.1	
		Captan	0.002	0.154	2.0	
Zingiberis rhizoma	China	Phosalon	0.020	0.191		0.1
Atractylodes rhizoma	China	Tolyfluanid	0.006	0.308	1.0	
Polygalae radix	China	Endosulfan	0.004	0.349	0.2	3.0
Myristicae semen	China	BHC	0.002	0.057	0.2	
Atractylodes rhizoma	Domestic	Procymidone	0.003	0.579	0.1	

remaining pesticides indicated more than 70% recovery from the three herbal crude drug samples.

Among the 229 samples (36 types of herbal crude drugs), seven imported and two domestic drug materials were contaminated with eight pesticides (Table 2). A very common worldwide fungicide, procymidone, was detected at 0.57 and 0.58 mg/kg in the imported Atractylodis rhizoma alba and domestic Atractylodis Rhizoma, respectively. Atractylol is the common active compound in both herbal crude drug materials. The medicinal usages of Atractylodis rhizoma and Atractylodis rhizoma alba are for dyspepsia and chronic gastroenteritis, respectively (Parziale 2000). Procymidone is a preventive and curative fungicide that is moderately systemic. Procymidone was a very frequently detected pesticide found in 135 of the 55,908 samples from Korean markets (Consumers Korea 2004). Carbofuran was the only carbamate pesticide not a target pesticide in this study; however, it was detected with a GC-MSD screening of an imported Paeoniae radix sample. It is a broad-spectrum carbamate pesticide that kills insects, mites, and nematodes on contact or after ingestion. The most commonly detected pesticide was BHC, which has a Korean MRL value of 0.2 mg/kg (sum of α, β, δ and γ-BHC isomers) for herbal crude drug materials. The gamma isomer (Lindane) is the only isomer that has insecticidal property. Lindane production, once thriving in the United States and Europe, is now limited to China, India, Romania and possibly Russia (Gerald 2005). BHC was detected in four products imported from China. Endosulfan was found in Polygalae radix as 0.35 mg/kg that was 1.75 times of MRL 0.2 mg/kg of 24 herbal crude drug materials including Polygalae radix in South Korea (KFDA 2005). The US EPA classifies endosulfan as Catagory Ib—Highly Hazardous. However because of the number of important advantages in that it is inexpensive, is soft on beneficial insects, and provides a different chemistry useful in resistance management, it is still widely used in many countries (APVMA 2004). Phosalone, which was found in Zingiberis rhizoma, is a non-systemic acaricidal and insecticidal organophosphate (BCPC 2002). Phosalone is not registered in the USA, however, it is registered in South Korea as a mixture with cypermethrine against for greenfly and *Phyllocnistis citrella* Stainton. There are 17 Korean MRL values of phosalone for sixteen agricultural products and one group MRL (for other citrus fruits). There is no phosalone MRL for Zingiberis rhizoma, a dried fresh ginger. Fresh ginger, among Oriental physicians, is reputed to be a good remedy for vomiting, coughing, abdominal distension, and fever, whereas the processed (steamed and dried) ginger is used for abdominal pain, lumbago, and diarrhea (Carper 1991). Tolylfluanid (Tolyfluanid) detected in imported Atractylodis rhizoma is a fungicidal and insecticidal phenylsulfamide registered for tomato, watermelon, ginseng, red pepper, mandarin and cucumber in South Korea since 1996. The MRL values of tolylfluanid were established for 12 fruit and vegetables in South Korea (1.0–5.0 mg/kg). US EPA established an import tolerance for residues of tolylfluanid in or on imported apple, grape, tomato and hop on 25 September 2002. In 2001, tolylfluanid ranked ninth out of the ten most frequently found pesticides in fruits and vegetables in the Netherlands (Schee 2002). A detected amount of Atractylodes rhizoma, 0.31 mg/kg, was a moderate residue value in trial results for head-lettuce from southern France, Italy, Portugal and Spain at a rate of 0.08 or 0.1 kg ai/hl (JMPR 2003). In addition to BHC and procymidone, the pesticide captan appeared in imported Atractylodis rhizoma alba at 0.15 mg/kg. Captan is a non-systemic phthalimide fungicide used to control diseases of many fruit, ornamental, and vegetable crops. It improves fruit finish by giving it a healthy, bright colored appearance (EXTOXNET 1996). Chinomethionat, detected in domestic Platycodi radix, is not a commonly detected residual pesticide that was just detected as the reporting level in two Strawberries out of 170 samples and one cucumber out of 115 samples in the pesticide monitoring in Finland, 2000 (NFA 2001). Chinomethionat, also known as oxythioquinox and quinomethionate, may be used for Platycodi radix for the purpose of the post-harvest due to the low residual behavior. According to the low residual character of chinomethionat, it may be used for Platycodi radix as post-harvest purpose. Platycodi radix has been used traditionally as an expectorant and a remedy for bronchitis, tonsillitis, laryngitis and suppurative dermatitis in South Korea, Japan and China (Han 2000). Among the eight detected pesticides, four were fungicides, which were found in underground crude drug materials such as rhizoma or radix. The captan (phthalimide) and tolylfluanid (sulphamide) pesticides are low risk groups with no signs of developing resistance to the majority of fungicides and no cross-resistance between group members (CSL 2006). To this end, confidential risk assessments of the aforementioned fungicides should be performed due to the high possibility of occurrence in underground crude drug materials and potential incorporation in herbal medicine and ordinary foods.

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