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Artículo / Article

Phthalate contamination of some plants and herbal products

[Contaminación de algunas plantas y productos herbales con ftalatos]

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Abstract: Phthalate derivatives cause a number of risks to human health and the environment. Essential oil and volatile fractions of some vegetables and herbal products were extracted by hydrodistillation and percolation methods to analyze using gas chromatography and mass spectrometry (GC-MS) for evaluation of phthalate contaminations. The results revealed that four vegetables and all aromatic waters were contaminated by phthalate derivatives including di-n-butyl phthalate (DBP), diisobutyl phthalate and di-(2-ethylhexyl) phthalate (DEHP) (0.1-7.95%). Butylated hydroxytoluene (BHT), a widely used synthetic antioxidant, was also found in the most of the aromatic waters in the range of 3.15-61.3%. In addition, three vegetable samples contained diazinon (0.36-4.61%), an organophosphorus insecticide. Plants and herbal preparations may be contaminated by the absorption of phthalates from contaminated water or soil or by the migration of phthalates from inexpensive recycled plastic. Regarding the widespread use and associated health risks of phthalates, effective quality and safety regulations for herbal products should be implemented with respect to their phthalate content.

Keywords: Aromatic water, BHT, DBP, DEHP, diazinone, diisobutyl phthalate

Resumen: los derivados de ftalato causan una serie de riesgos para la salud humana y el medio ambiente. El aceite esencial y las fracciones volátiles de algunos vegetales y productos a base de hierbas fueron extraídos mediante hidrodestilación y métodos de percolación y luego fueron analizados mediante cromatografía de gases y espectrometría de masas (GC-MS) con el propósito de identificar contaminación con ftalatos. Los resultados revelaron que cuatro productos herbales y todas las aguas aromáticas analizadas estaban contaminadas con derivados de ftalato, incluyendo el ftalato de dibutulo (DBP), ftalato de diisobutilo y ftalato de bis(2-etilhexilo) (DEHP) (0.1-7.95%). El butilhidroxitolueno (BHT), un antioxidante sintético ampliamente utilizado, también se encontró en aguas aromáticas en el rango de 3.15-61.3%. Además, tres muestras vegetales contenían diazinón (0.36-4.61%), un insecticida organofosforado. Las plantas y las preparaciones herbales pueden ser contaminadas a partir de absorción de ftalatos del agua o el suelo contaminados o por la migración de ftalatos desde plástico reciclado de bajo costo. Con respecto al uso generalizado y los riesgos asociados a la salud de los ftalatos, deben implementarse normas efectivas de calidad y seguridad para los productos a base de hierbas con respecto a su contenido de ftalato.

Palabras clave: agua aromática, BHT, DBP, DEHP, diazinón, ftalato de diisobutilo

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INTRODUCTION

Phthalic acid esters have been used as plasticizer to improve flexibility, adhesion, and solubility of polymers. As plasticizer, phthalates are present in cosmetics, pharmaceutical coatings, medical devices, food containers, paints, floor and wall coverings (Saeidnia & Abdollahi, 2013; Saeidnia, 2014; Zorníková *et al.,* 2014). Phthalates ubiquitously distributed into the environmental sources like air, soil, sediments and water since these compounds physically dissolved in plastics with no covalent bounding (Hongjun *et al.,* 2013; Martine *et al.,* 2013). Two mostly applied phthalates are di-n-butyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) (Zorníková *et al.,* 2014). These toxic substances also accumulate in plants through their root systems or aerial parts (Yin *et al.,* 2003). Therefore, there are several ways of human daily exposure to phthalates. Phthalates possess various toxic effects depending on their chemical structures in kidney, liver, testes, and thyroid. For example, DEHP has been classified as probable carcinogen and identified as productive and developmental toxicant (Herr *et al.,* 2009). Increasing incidence of developmental abnormalities in neonates, neurological problems in children who exposed to phthalates in pregnancy and male infertilities due to antiandrogen effect of phthalates cause a serious concern regarding phthalate toxicity in human (Crinnion, 2010; Saeidnia, 2014). Additionally, essential processes in ovary including folliculogenesis and steroidogenesis are adversely targeted by phthalates. Proper regulation of ovarian steroidogenesis is prominently affect reproductive and non-reproductive health of women (Craig *et al.,* 2012; Hannon & Flaws, 2015). These compounds are rapidly metabolized in the gut, liver, and blood by esterases and lipases to their respective monoesters in which one alkyl chain remains on phthalic acid back bone. The monoester metabolites mostly produce toxicity in the body (Hannon & Flaws, 2015).

There are several reports of phthalates contamination in plants such as agricultural crops, medicinal plants, and marine algae (Gu *et al.,* 1990; Chen, 2004; Saeidnia & Abdollahi, 2013; Manayi *et al.,* 2014a; Manayi *et al.,* 2014b; Chandrasekar *et al.,* 2015;). Accumulation of phthalates in the natural sources has risen to the serious challenge of consumption of these compounds through contaminated food, vegetables or commercial herbal preparations (Heudorf et al., 2007; Saeidnia and Abdollahi, 2013). Although, there is a general public perception that herbal products are safe, effective and non-toxic, quality control of herbal products remains the biggest challenge despite their prevalent usage. Thus, the present study evaluated presence of phthalates derivatives in some vegetables and herbal products using gas chromatography-mass spectrometry (GC-MS) method to highlight the necessity of determination of plasticizers contamination as an effective quality control in herbs and traditional medicines.

METHODS AND MATERIALS

Plant materials and extraction

Aerial parts of the vegetables including *Ocimum basilicum*, *Mentha spicata* (3 samples), *Mentha piperita*, *Satureja hortensis* (4 samples), *Coriandrum sativum* (2 samples), *Artemisia dracunculus*, *Petroselinum crispum* (6 samples), and *Apium graveolens* (3 samples) were purchased from markets Tehran, Iran in summer of 2015.

The samples of the plants were cleaned of sand or dust and chopped into small pieces to extract at room temperature (20-22° C). Plant materials (500 g for each) were separately hydro-distilled to extract their essential oils using Clevenger apparatus for 4 h. All the oils were dried by anhydrous sodium sulfate (Merck, Germany). Some samples including *O. basilicum*, *M. spicata*, *M. piperita*, *S. hortensis* (2 samples), were also subjected to obtain their volatile compounds using n-hexan (Merck, Germany) by percolator apparatus three times each 48 h (100 g for each). The n-hexan was removed from extracts at room temperature.

Aromatic waters of *Achillea* spp., *Tribulus terrestris*, *Tanacetum balsamita*, *Salix* spp., *Apium graveolens*, and *Teucrium polium* were also purchased from a shop in Tehran, Iran in autumn of 2015. The volatile compounds of aromatic waters (200 mL) were extracted using liquid-liquid extraction method by equal volume of diethyl ether (Merck, Germany) three times (Shahani *et al.,* 2011). The organic layer was separated and evaporated at room temperature. All the extracts stored in dark glasses at refrigerator (4° C) prior to analysis.

Analysis of essential oils and volatile fractions

The oils and extracts were analyzed by GC-MS method on an Agilent 6890 (Agilent, US) and HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) with carrier gas of helium, linear

velocity of 36.4 cm/sec, flow of 1 mL/min and split ratio equal to 1/20. The oven temperature was held for 5 min at 50° C and transfer line temperature was 290° C for 10 min. Injection volume was 1.0 µL with runtime of 60 min. The quadrupole mass spectrometer was scanned over ionizing voltage of 70 eV and an ionization current of 150 µA. The compounds were identified by comparison of retention indices relative to C8-C28 n-alkanes with those reported in the literature together with comparison of their mass spectra with the Wiley, Adams and NIST libraries (Adams, 2007; NIST, 2016).

RESULTS AND DISCUSSION

The essential oils and volatile fractions of vegetables and herbal preparations were analyzed by GC-MS to evaluate contamination of phthalate derivatives. The main constituents (> 5%) and phthalate contaminations of the samples along with weight of extracted fractions were summarized in Table 1. Essential oils of a sample of *C. sativum*, *M. spicata*, and *P. crispum* have been contaminated by a phthalate derivative of dibutyl phthalate (0.51-0.86%). Additionally, diisobutyl phthalate (0.51%) was also found in the same *P. crispum* sample. DEHP was detected in volatile fraction of a *S. hortensis* sample (1.79%), while phthalates were not detected in the essential oil of the plant. All the tested aromatic waters have been also contaminated with phthalate derivatives and DEHP found in all of them in range of 0.1-7.95%. In addition to DEHP, aromatic waters of *Salix* spp. and *T. polium* have been polluted by diisobutyl phthalate and DBP. These phthalate contaminations in aromatic waters could either be emanating from their plastic containers and/or during the manufacturing process (Ndhlala *et al.,* 2012). Contaminated plants could probably be another origin of phthalates in herbal preparations like examined aromatic waters in the present study. A highly toxic phthalate, DEHP, has been isolated from oils of *Lythrum salicaria* obtained by hydro-distillation and microwave methods (29.2 and 43.2%, respectively) (Manayi *et al.,* 2014b). Essential oil of root of another medicinal plant, *Achillea tenuifolia*, was found to be contaminated by phthalate derivatives that revealed absorption of these compounds from water and soil (Manayi *et al.,* 2014a). Presence of DEHP has been indicated in a popular herbal product in South Africa (Nair *et al.,* 2012). Other phthalate derivatives including diisooctyl phthalate (9.71%) and phthalic acid, isobutyl octadecyl ester (6.55%) have been identified by GC-MS analysis of Nimbapatradi Choornam, an Ayurvedic medicine (Chandrasekar *et al.,* 2015). Phthalates are used in a wide variety of products and applications with no chemical bound resulting in their migration to the environment (Clewell *et al.,* 2008). A strict guideline has been implemented by World Health Organisation (WHO) for DEHP due to its associated health risks and extensive use in a variety of consumer products. Tolerable daily intakes of 8 µg/L and 1.5 mg/kg for drinking water and food consumed, respectively has been set by WHO for DEHP (World Health Organization, 2003). According to the toxicity of phthalates, health of current and future generations of population could affected by these chemical substances (Ndhlala *et al.,* 2012). Although literature regarding phthalate in herbal products is scarce in Iran, it could be hypothesized that these contaminations are not only limited to the tested plants and products but could be common characteristic of the other similar products.

Butylated hydroxytoluene (BHT), a most useful synthetic antioxidant, were found in all the aromatic waters in range of 3.15-61.3% of volatile fractions except for *A. graveolens* aromatic water in the present study. BHT is extensively used in the food and pharmaceutical industries to prevent damages done by free radicals. Since 1999, BHT was used between 0.0002-0.5% in industries, however according to historical data it was added to formulations up to 1% (Yehye *et al.,* 2015). Acceptable daily intake (ADI) of 0.25 mg/kg bw/d for BHT has been suggested according to the effect of the compound on reproduction and hepatic enzyme induction in two independent 2-generation studies in rats by European Food Safety Authority (EFSA) (EFSA-Panel on Food Additives and Nutrient Sources added to Food (ANS), 2012). These findings represent exposure of consumers to phthalates through consumption of the contaminated herbal products as drug, food or vegetables. Considering a potential health hazard by accumulation of these toxic substances in human body not only by consumption of herbal preparations but from other sources, mandatory quality and safety regulations should therefore be implemented by relevant authorities for herbal products, their plants source as well as their manufacturing and packaging in plastic containers. Further investigation to evaluate the amount of phthalates and BHT in herbal preparations

is required to estimate potential additive effects of them in other products.

Table 1
The main constituents (>5%) and phthalate contaminations of the oils and volatile fractions

Samples (wt.)	*Compounds*	*%*	*RT*	*KI*	*Known (%)*
O. basilicum[a] oil (83 mg)	Estragole	49.07	16.95	1210	91.48
	cis-Citral	6.67	18.00	1248	
	Caryophyllene oxide	6.67	26.67	1595	
O. basilicum[a] ext. (1127 mg)	Tetradecane, 2,6,10-trimethyl	8.4	31.34	1813	81.55
	Octacosane	71.3	48.90	2712	
M. spicata oil (230 mg)	1,6-Dihydrocarveol	38.18	16.78	1205	100
	Dihydrocarveol acetate	15.09	20.26	1332	
	Caryophyllene	5.69	22.72	1429	
	Caryophyllene oxide	10.92	26.66	1595	
	Dibutyl phthalate*	0.57	34.38	1968	
M. spicata oil (235 mg)	cis-Dihydrocarvone	32.16	17.02	1213	99.42
	(+)-Carvone	25.45	18.40	1302	
	β-Bourbonene	6.80	21.84	1394	
	trans-Caryophyllene	15.75	23.84	1475	
	Germacrene-D	5.09	24.08	1485	
M. spicata[b] oil (342 mg)	D-Limonene	7.00	11.89	1037	100
	1,6-Dihydrocarveol	28.97	16.96	1211	
	(+)-Carvone	32.62	18.40	1302	
M. spicata[b] ext. (1254 mg)	Eucalyptol	5.78	11.93	1038	89.51
	trans-Dihydrocarvone	18.86	17.03	1213	
	Carvotanaceton	21.24	18.56	1269	
	Caryophyllene	5.01	22.74	1430	
M. piperita[c] oil (212 mg)	Decanal	8.37	16.95	1210	76.88
	n-Nonyl cyclopropane	15.17	18.79	1277	
	Dodecanal	9.20	22.28	1411	
	trans-2-Dodecen-1-ol	16.75	23.86	1476	
	Ttridecanal	7.87	24.73	1512	
M. piperita[c] ext. (1301 mg)	Thymoquinone	15.84	18.29	1229	97.17
	Carvacrol	48.88	19.89	1318	
	Pentacosane	8.21	43.23	2500	
	Octacosane	18.6	48.88	2799	
S. hortensis[d] oil (172 mg)	α-Terpinen	43.38	11.49	1024	94.13
	Carvacrol	45.10	19.89	1318	
S. hortensis[d] ext. (1011 mg)	Eicosane	6.92	34.92	1996	93.04
	Tetracosane	9.92	41.70	2390	
	DEHP*	1.79	44.10	2543	
S. hortensis[e] oil (131 mg)	α-Terpinen	31.33	11.49	1024	100
	o-Cymol	6.13	11.73	1032	
	Carvacrol	58.00	19.78	1317	
S. hortensis[e] ext. (1355 mg)	Dihydrocarveol	29.7	16.80	1205	97.42
	Carvone	34.44	18.14	1254	
	Octacosane	14.26	48.88	2799	

S. hortensis oil (122 mg)	γ-Terpinene	19.14	12.74	1066	99.73
	Carvacrol	72.92	19.89	1318	
S. hortensis oil (145 mg)	γ-Terpinene	20.64	12.74	1066	100
	Carvacrol	71.74	19.89	1318	
C. sativum oil (48 mg)	Myristicin	74.57	25.32	1537	91.75
	Dibutyl phthalate*	0.86	34.38	1968	
C. sativum oil (56 mg)	Dodecanal	7.19	22.28	1411	58.09
	β-Ionone	10.86	24.37	1497	
	Apiol	5.14	27.55	1634	
	1,2,3,5,6,7-Hexahydro-inden-4-one	31.92	30.10	1753	
A. dracunculus oil (36 mg)	Estragole	80.30	16.95	1210	80.30
P. crispum oil (71 mg)	Methyl 7,10,13-hexadecatrienoate	8.12	33.06	1899	69.52
	Hexadecanoic acid, methyl ester	5.15	33.53	1924	
	Methyl linolenate	5.7	36.78	2099	
	Octacosane	10.48	48.86	2799	
P. crispum oil (64 mg)	β-Myrcene	9.00	10.71	997	94.27
	o-Cymene	5.19	11.86	1033	
	D-Limonene	13.64	11.90	1037	
	p-Cymenene	8.06	13.69	1098	
	Cryptone	7.24	16.55	1196	
	Myristicin	30.61	25.32	1537	
P. crispum oil (68 mg)	Myristicin	19.27	25.32	1537	67.27
	Phenyl ethyl phenyl acetate <2>	7.46	33.09	1901	
P. crispum oil (74 mg)	Myristicin	77.22	25.32	1537	96.23
P. crispum oil (59 mg)	α-Phellandrene	18.61	12.24	1049	78.60
	1,3,8-p-Menthatriene	23.81	14.93	1141	
	Myristicin	12.40	25.32	1537	
	(Z,E)-Farnesyl acetate	18.38	31.90	1841	
P. crispum oil (78 mg)	Myristicin	88.74	25.32	1537	96.37
	Diisobutyl phthalate*	0.51	32.56	1878	
	Dibutyl phthalate*	0.51	34.38	1968	
A. graveolens oil (72 mg)	α-Phellandrene	9.85	11.14	1012	99.64
	Carvacrol	8.85	19.89	1318	
	Germacrene D	8.47	24.22	1491	
	Apiol	47.28	27.54	1634	
	Farnesyl acetate	12.80	31.79	1836	
A. graveolens oil (57 mg)	α-Phellandrene	13.23	11.15	1012	100
	Apiol	5.17	27.55	1634	
	(2Z, 6E)-Farnesyl acetate	12.08	31.89	1841	
	10-Nonadecanone	67.11	51.44	2045	
A. graveolens oil (64 mg)	3,9-Epoxy-1-p-menthene	5.36	16.58	1197	95.11
	Germacrene D	11.37	24.23	1491	
	Myristicin	5.28	25.32	1537	
	Apiol	60.25	27.51	1633	
	Phytol	6.46	37.02	2101	
Achillea spp. AW (11 mg)	L-Camphor	17.19	15.43	1158	80.47
	Borneol	7.5	16.01	1178	
	cis-Carveol	8.91	17.46	1229	

	Thymol	5.17	19.37	1298	
	BHT	7.31	24.99	1523	
	DEHP*	0.91	44.13	2545	
T. terrestris AW (17 mg)	Pulegone	21.02	18.07	1251	74.72
	Piperitenone	24.24	20.79	1353	
	BHT	14.23	24.98	1523	
	DEHP*	2.28	44.13	2545	
T. balsamita AW (13 mg)	Dihydrocarveol	5.25	16.80	1205	75.86
	D-Carvone	33.1	18.28	1258	
	Thymol	8.06	19.38	1298	
	BHT	3.15	24.99	1523	
	DEHP	0.24	44.14	2545	
Salix spp. AW (21 mg)	BHT	61.3	24.99	1523	94.59
	Diisobutyl phthalate*	1.55	32.56	1878	
	Dibutyl phthalate*	1.57	34.38	1968	
	DEHP*	7.95	44.13	2545	
A. graveolens AW (34 mg)	Phenol, 4-heptyl-	66.50	30.04	1750	70.81
	DEHP*	0.10	44.13	2545	
T. polium AW (27 mg)	BHT	46.44	24.99	1523	65.09
	Dibutyl phthalate*	2.41	34.38	1968	
	DEHP*	3.55	44.13	2545	

wt.: Weight, RT: Retention time on HP-5MS column relative to C8-C28 n-alkanes, KI: Kovats index, ext.: Extract, DEHP: Di-(2-ethylhexyl) phthalate, *: Phthalates contaminations, BHT: Butylated hydroxytoluene, Plants with similar alphabets are same.

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