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Review article

A concern on phthalate pollution of herbal extracts/medicines and detection methods

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Abstract
Esters of phthalates, mainly applied as plasticizer, cause several human health and environment hazards. Phthalates are widely used in pharmaceutical products, cosmetics, as well as other plastic commercial products, and can penetrate in foods, water dusts, and air leading to ingestion and inhalation exposure followed by skin absorption for human. These compounds cause serious adverse effects on human health like destroying the endocrine system, and consequently developmental alterations and reproductive changes through induction of inflammation and oxidative stress. Some phthalates are able to bio-accumulate in water and have been isolated from aquatic organisms. Mammals and birds may be influenced by these compounds through food chain. Therefore, simple and rapid method for identification and quantification of these compounds is a debate especially for developing countries. Gas Chromatography-Mass Spectroscopy has been successfully employed to determine and measure these compounds in volatile fractions of the plant or the algal materials without more essential chemical reactions. In this article, a rapid review on phthalate toxicity and related analysis methods to detect them in herbal extracts is presented.

Keywords: alternative substituents, detection methods, environment hazards, phthalates, plasticizer

Introduction
Phthalates, referred to the esters of phthalic acid, are mainly used as plasticizers. They are manufactured by reacting phthalic anhydride with alcohols [ranged from methanol (C_1) to tridecyl alcohol (C_{13})] in both straight and branching chains. These compounds make polyvinyl chloride (PVC), a brittle plastic, more flexible and durable. Due to the toxicity concerns related to lower molecular weight phthalates (3-6 C), they are now being slowly replaced in the USA, Canada, and European Union by high molecular weight phthalates (> 6 C). The reason might be behind their higher permanency and durability [1,2]. One of the main groups of plasticizers are phthalate esters like di-(2-ethylhexyl) phthalate (DEHP) and di-isononyl phthalate (DINP), the mostly used phthalates, that probably cause side effects on human health [2]. It is assumed that six million tons of plasticizers are consumed every year, of which phthalates used in a large number of products including enteric coated pharmaceutical pills and supplements (as viscosity control agents), gelling agents, film formers, stabilizers, dispersants, lubricants,

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binders, emulsifyers, and suspending agents [3]. Moreover, they are mainly applied in several commercial compounds like cosmetics, paper coating, paints, plastic food packages, infant toys, and adhesives [2]. Phthalates are employed as solvent to hold fragrance, reduce nail polish cracking, and decrease hair spray stiffness, as well as increasing products penetration and moisturizing of skin in cosmetics [1]. As a matter of fact, when phathalates are released into the water and soil, a consequent exposure and bio-accumulation of these compounds may happen in either plants or animals and even human [4]. Regarding the recent published articles on probable pollution of medicinal plants and other natural medicines like marine algae to phthalate [2,4,5], finding a detection and even quantification method, which can be accurate, fast and cost effective, seems an important and considerable challenge particularly in standardization of herbal extracts and phytopharmaceuticals.

Routs of exposure

Since these compounds are not in chemical bound with plastics, they can penetrate into foods, water dusts, and air leading to ingestion and inhalation exposure to human. They can even be absorbed dermally trough application of cosmetics on the skin [6]. Children normally consume more food than adults related to their body weights, and therefore they are supposed to expose higher concentration of phthalates from food. Additionally, children are in contact with phthalate containing products through mouthing toys, and children stuffs. Consequently, phthalates in these products dissolve in saliva during mouthing and are absorbed into their body [1]. Newborns or children may receive phthalates, mostly DHEP, trough medical devices like intestinal feeding and dialysis equipment, catheters, and gloves [1].

Accumulation of phthalates may occur in a variety of herbal medicines especially those that are used to grow up in water and rivers due to the exposure of plants' roots to the wastewater. The results of a study has revealed that *Benincasa hispida* uptake and accumulated DHEP in its inner parts of leaves, stems, and fruits from contaminated air [7]. Moreover, vegetables like lettuce (*Lactuca sativa*), hot pepper (*Capsicum frutescens*), Chinese cabbage (*Brassica parachinensis*), carrot root (*Daucus carota*), cucumber (*Cucumis sativus*), water spinach (*Ipomoea aquatica*), tomato (*Lycopersicon esculentum*) and pumpkin (*Cucurbita moschata*) are reported to accumulate DHEP from plastic mulch film. Some of these plants have been introduced as phytoremediation for absorbing phthalates from polluted soil and air [8]. Consequent exposure of animals and humans to phthalate by using polluted herbs, crops and vegetables is possible [4]. Vegetables growing in urban districts have shown high levels of contamination with phthalates and this contamination has decreased by increasing the distance from the city center [9].

Mechanisms of toxicity

Epidemiological studies have revealed that the human health may be impacted by phthalates plasticizers [1]. Phthalates disturb endocrine systems such as thyroid and sex hormones in humans [10], followed by induction of inflammation, oxidative stress, early puberty in girls, asthma, and allergic symptoms [1,6,11]. Additionally, literature review has demonstrated that these compounds could exhibit toxicity in liver, kidney, lung and testis in both animal and clinical studies [1,2]. Modulation of peroximal beta-oxidation, the peroxisome proliferator-activated receptor alpha-receptor and gap junction may be involved in hepatocarcinogenicity of phthalates [2].

Serious health effects of these compounds on reproductive system have been reported including decrease of sperm count and quality followed by histopathological alteration in testis in men, as well as endometriosis and uterine leiomyomata in women [4,12]. Furthermore, exposure to phthalates during pregnancy has produced serious adverse effects like miscarriage, low birth weight, and preterm birth trough induction of inflammation and oxidative stress [6]. Moreover, fetal exposure to phthalate is associated with behavioral and mental ability; for instance in the third trimester of pregnancy they have caused neurogical problems in children even until 4-9 years of age [13]. The results of a study has suggested that di-*n*-butyl phthalate (DBP) changed epididymal structure and function through alteration of epididymal antioxidant enzymes and enhancement of lipid peroxidation in a dose dependent manner in adult rats [14]. Testicular atrophy in rats has occurred by DEHP through decreasing zinc in testis and alteration of zinc transporter, ZnT-1. This compound has caused reduction of testosterone biosynthesis in Leydig cells as well as inhibition of follicle stimulating hormone (FSH) in Sertoli cells [2]. Additionally, a significant relation has been reported between metabolites of phthalate in the urine and C-reactive protein (CRP), ferritin, fibrinogen, alkaline phosphatase (ALP), and absolute neutrophil count (ANC), markers of inflammation and gamma glutamyltransferase (GGT), and eventually bilirubin and markers of oxidative stress [15,16].

Ecotoxicity and food chain

These compounds were also identified in seawater and rivers indicating that they polluted seawater in uncontrolled ways and produced toxic effects on phyto- and zooplanktons, fish, and arthropods. Diallyl phthalates have hormonal activity, and therefore they can change gonadal development and reproductive system in aquatic creatures. These compounds decreased phoshpoproteins in both males and females [2]. Some phthalates like benzyl butyl phthalate (BBP) are bio-accumulated in water, and have been isolated from aquatic organisms indicating endocrine disruption in fish. Mammals and birds may be influenced by these compounds from food chain [1].

Alternatives of phthalates

A number of alternatives have been identified for plasticizers including citrates, sebacates, adipates, and phosphates for producing toys, children articles, and medical devices. However, the effects of these alternative substances on human health and environment have not been studied well so far [1]. These compounds do not chemically bind to polymers like phthalates, and can leach out from the products. Although these alternative compounds have demonstrated promising application, the evidence-based studies have revealed their effects on liver, kidney, spleen, testis, and uterus, as well as toxic effects on aquatic organisms [1]. Other substitution approaches are petroleum-based plastics, which have been derived from non-renewable fossil fuel resources but these plastics also possess several health and environment concerns. During the manufacturing process of these compounds, greenhouse gases and pollutants including hydrogen sulfide, sulfuric acid, heavy metals, chlorofluorocarbons, polycyclic aromatic compounds, volatile organic compounds, and nitrogen and sulfur dioxides are generated. Bio-based plastics are the substituents for the two latter types of plastics including plastics with phthalates and petroleum-based plastics. These bio-based plastics may completely be manufactured using plant materials or a mixture of plant materials and petroleum-based. Production of these kinds of plastics causes their special hazards like large quantities of pesticides usied in agriculture, and hazardous chemicals like sodium hydroxide, carbon disulfide, and chlorine. They are also of concern for health and environment. Moreover, using genetically modified organisms (GMOs) and their influence on the environment for production of bio-based plastics are not fully recognized [1,17].

Methods of measurement

Actually, the Joint Research Centre (JRC) of the European Commission published a review of methods to measure phthalates in food in 2009 [18]. All methods used are able to detect even trace amounts of phthalates in tested materials. A bibliography has revealed that beside the different methods [HPLC-UV (High Performance Liguid Chromatography-Ultra Violet spectroscopy), GC-FID (Gas Chromatography-Flame Ionization Detector), HRGC-MS (High Resolution Gas-Chromatography- Mass Spectroscopy) and GC-ECD (Gas Chromatography-Electron Capture Detection)] for the determination of DEHP (Di (2-Ethylhexyl) Phthalate) in various biological fluids and tissues, wastewater and so on [19,20], Gas Chromatography-Mass Spectroscopy has been employed successfully to determine and measure both the presence and quantity of these compounds in volatile fractions of the plant or the algal materials without using more chemical reactions like esterifying the extracts [21].

But the first challenge in this way would be the preparation of a volatile fraction from natural materials. Interestingly, Clevenger type apparatus, which have commonly been used for essential oil extraction, may be applicable for extraction of volatile fractions even from non-flavor or non-essential oil-bearing plants. Combination of solvent extraction using high volatile solvents like hexane or petroleum ether with maceration of natural materials in water before performing the oil extraction is one of the successful techniques to extract the main parts of phthalate content from the tested materials. The results of a previous study have shown that DEHP was present in both essential oils of *L. salicaria* obtained using hydro-distillation and microwave assisted hydro-distillation methods. The amount of DEHP was higher in the oil obtained by microwave assisted hydro-distillation method compared to hydro-distillation method [5]. Moreover, oil of the brown algae *Nizamuddinia zanardinii* that had been extracted by hydro-distillation method contained 5.9% phthalate contamination [21]. It seems that this technique can be used by slight modification especially in material preparation just before the extraction process. Further investigation is highly recommended to support and develop this idea.

Conclusion

Plasticizers are chemically synthesized compounds, which improved physical properties of PVC and cosmetics [1,2]. Since these compounds are not in chemical binding to polymers, they can easily leach out to the environment [1]. Human are in contact with these chemicals through ingestion of contaminated food like plants and animals and polluted water, as well as inhalation of phthalates in the air [1,6,19,20]. These compounds also exhibited toxicity in liver, kidney, lung and testis followed by causing disturbance in endocrine systems of human and aquatic creatures [1]. Although there are several alternatives presented for plasticizers, these alternative substances are not studied well whether they can cause any adverse effects on human health and environment or not [1,17]. There are some analytical methods to detect and measure phthalates in polluted items including HPLC-UV, GC-FID, HRGC-MS, GC-MS, and GC-ECD suggested in previous papers [19,20,21], in which GC-MS has been suggested as a simple and accurate method for quantitative determination of these compounds in the contaminated plant oils.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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