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Diethyl phthalate and dioctyl phthalate in *Plantago major* L.

Ahmed A. Romeh

Plant Production Department, Faculty of Technology and Development, Zagazig University, Zagazig, Egypt.

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Phthalic acid esters commonly referred to as phthalates and are group of industrial chemicals that have become ubiquitous environmental contaminants because of their widespread usage and high persistence in the environment. Public health concerns of phthalates include carcinogenic, teratogenic, hepatotoxic and endocrine effects. The aim of this study was to analyze dioctyl phthalate and diethyl phthalate from *Plantago major* leaves and roots by using gas chromatography/mass spectrometry (GC/MS) analysis. Dioctyl phthalate (DOP) obtained from *P. major* leaves and roots were about 3.26 to 80.55% and 2.52 to 19.69%, respectively. Diethyl phthalate (DEP) obtained from *P. major* leaves and roots were about 13.57 to 36.50% and 0.08 to 2.13%, respectively. The percent of Dioctyl phthalate and diethyl phthalate in leaf samples higher than that of root sample. *P. major* leaves and roots may possess a stronger ability to accumulate dioctyl phthalate and diethyl phthalate as a cleanup technology from environmental pollution.

Key words: *Plantago major*, gas chromatography/mass spectrometry (GC/MS), dioctyl phthalate, diethyl phthalate.

INTRODUCTION

Diethyl phthalate and phthalic acid derivatives such as Di-n-octyl phthalate (DOP) are commercially important chemicals used predominantly as plasticizers in high molecular- weight polymers, which are toxic to humans, animals, microorganisms, algae, aquatic invertebrates, and fish (Chen and Sung, 2005; Hu et al., 2005), as well as plants (Saarma et al., 2003). These chemicals have been thought to be environmental pollutants and detected in soils, sediments, terrestrial and marine waters, and also living organisms (Namikoshi et al., 2006) but that their levels are low because they are subject to relatively rapid photochemical and biological degradation (Hurford et al., 1989). Phthalates are reported to affect human beings. DOP was reported to affect the functioning of the liver, kidney and thyroid (Hinton et al., 1986). It was recently classified as an endocrine disrupter and its use is prohibited (Sekiguchi et al., 2006).

There is a growing trend in the use of medicinal plant because of their medical effectiveness, low toxicity and many natural anticancer agents derived from these plants (Ozaslan et al., 2007). The common broad leaved plantain *Plantago major* belongs to the family Plantaginaceae and the highly diverse genus *Plantago* comprising approximately 256 species (Mabberley, 1997). *P. major* L. is a very familiar perennial weed and may be found anywhere by roadsides, in meadow-land, cultivated fields, waste areas, and canal water. The seed and husks contain high level of fiber; they expand and become highly gelatinous when soaked in water. It is an old medicinal plant that has been known for centuries, but it is regarded as weed by many people (Samuelsen, 2000). It is renowned as a traditional herbal medicine throughout the world (McCutcheon et al., 1995). The remarkable medicinal properties of *P. major* are due to

E-mail: ahmedromeh2006@yahoo.com. Tel: 020553712971. Fax: 020552364612.

the high content of phenols, flavonoids and tannin especially in its leaves (Mohamed et al., 2011). Jamilah et al. (2012) found that chromatographic analysis by gas chromatography/mass spectrometry (GC/MS) of five different extracts that enabled the identification of 51 compounds. The main constituents ethyl acetate extract was glycerine (30.70%), benzene (21.81%) and dibutyl phthalate (16.22%); n-butanol were phthalic acid (24.62%), benzene propanoic acid (16.83%) and group of phenol (10.20%).

Even after evaluating the processes associated with the use of phytoremediation by P. major L., using a cleanup technology of water and soils contaminated with chlorpyrifos (Romeh and Hendawi, 2013) and diazinon pesticides, it is clear that diethyl phthalate and dioctyl phthalate showed on GC-MS chromatograms. This paper presented the analysis of dioctyl phthalate (DOP) and diethyl phthalate (DEP) from *P. major* leaves and roots using GC-MS analysis.

MATERIALS AND METHODS

The whole plant of broadleaf plantain (*P. major* L.) was collected from meadow-land in Zagazig University, Zagazig, Sharkia governorate, Egypt. Plant roots were rinsed in running tap water for 2 min and were blotted dry. The plants dissected into individual leaves and roots then 8 g of leaves and 4 g of roots were analyzed for the phthalates. Leaf and root samples were homogenized with dichloromethane and extracted with 10% acetone in dichloromethane for three times. The extract was filtered and the organic phase was separated using liquid-liquid extraction method in a separatory funnel, dried over Na_2SO_4 and then concentrated to 1 ml for GC/MS analysis (Wang et al., 2002). Di ethyl and di octyl phthalates in the organic phase were analyzed by GC/DSQ II MS with TR5 column (30 m × 0.25 mm × 0.25 um) coated with 5% phenyl. Helium was used as the carrier gas with the flow rate of 1.75 ml min. The GC temperature program was set as follows: initial temperature 82°C, held 5 min; rate of 8°C min^{-1} up to 280°C, held 1 min, then rate of 25°C min^{-1} up to 300°C, held 2 min. The mass spectrometer was operated in electron impact ionization mode at 70 eV and the temperatures of transfer lines, ion source and quadrupole were set at 200, 250 and 250°C, respectively. Mass spectra in full-scan mode were collected at the rate of 2400 scan min^{-1} over the mass range (m/z) of 75-400.

RESULTS AND DISCUSSION

Di ethyl and di octyl phthalates were identified by GC-MS. The spectra of the compounds were matched with NIST and Willey library. Their structures were identified by the percentage similarity values (Figures 1 and 2). Dioctyl phthalate (DOP) obtained from *P. major* leaves and roots were about 3.26 to 80.55% and 2.52 to 19.69%, respectively (Figures 1 and 2). There are certain reports of the occurrence of the phthalates from plants. DOP has been reported from *Limonium bicolor* Kuntze (Wei and Wang, 2006), *Dracaena cochinensis* (Lour) SC Chen (Wei et al., 1998), *Caesalpinia sappan* (Sarumathy et al., 2011), stem bark of *Schleichera oleosa* (51.15%), *Dipteracanthus patulus* (9.89) (Gopalakrishnan et al., 2011), marine isolates of *Penicillium lividum* and *Trichoderma lignorum* (Ushadevi, 2008), *Sansevieria roxburghiana* (17.30%) (Philip et al., 2011), *Kappaphycus alvarezii* (3.20%) (Venkatesh et al., 2011), *Leea indica* (96.93%) (Srinivasan et al., 2009), and DOP isolated from the dried seeds of *Nigella glandulifera* (0.62 g/kg) (Nguyen et al., 2007). DOP has already been found in many vegetables, such as Chinese tomato fruit and cabbage, yet the cumulative content is less than 0.01 g/kg fresh weight (Du et al., 2006). DOP is reported to have antimicrobial activity (Ushadevi, 2008; Philip et al., 2011; Senthilkumar et al., 2011; Shafaghat et al., 2012). DOP isolated from *Nigella glandulifera* Fryen was identified as inhibiting melanogenesis (Nguyen et al., 2007).

Diethyl phthalate (DEP) obtained from *P. major* leaves and roots were about 13.57 to 36.50% and 0.08 to 2.13%, respectively (Figures 1 and 2). The results of phytochemical screening of the stem of *Mallotus philippensis* var. *philippensis* revealed the presence of organic esters, diethyl phthalate (DEP) as major constituents with highest peak area of 94.47% and retention time 11.29 (Jayaraman et al., 2011). DEP and derivatives of phthalic acids were isolated from the root exudates of barnyard grass *Echinochloa crusgalli* (Xuan et al., 2006), *Ipomoea carnea* (Adsul et al., 2009), *Kappaphycus alvarezii* (Venkatesh et al., 2011).

Data in Figures 1 and 2 show the percent of Dioctyl phthalate and diethyl phthalate in leaf samples higher than that of root sample. Data also show that DOP accumulated in leaf and root samples higher than that of DEP (Figures 1 and 2). The higher DOP and DEP contents in *P. major* leaves may indicate that this plant possesses a stronger ability to accumulate DOP and DEP. The percent of DOP and DEP different from sample to another may be due to the difference of age sample. Usually, the presence of DOP and DEP in plants is from environmental pollution, such as the release of DOP from plastic films.

Also, the presence of DOP and DEP might be caused by contaminants from either the sample preparation or analytical instruments. DOP and DEP have been thought to be environmental pollutants and detected in soils, sediments, terrestrial and marine waters, and also living organisms (Namikoshi et al., 2006) but that their levels are low because they are subject to relatively rapid photochemical and biological degradation (Hurford et al., 1989). Therefore, the existence of these compounds in the leaves and roots of *P. major* might not be derived from contamination because of plasticizers used during extraction and sample preparation or due to solvent impurities. Keire et al. (2001) reported that *Helicobacter pylori* secreted diethyl phthalate as a chemotactic factor. It was the first example of a phthalate ester that is produced by a bacterium. Elzaawely et al. (2005) reported that *Rumex janonicus* Houtt., a perennial herb

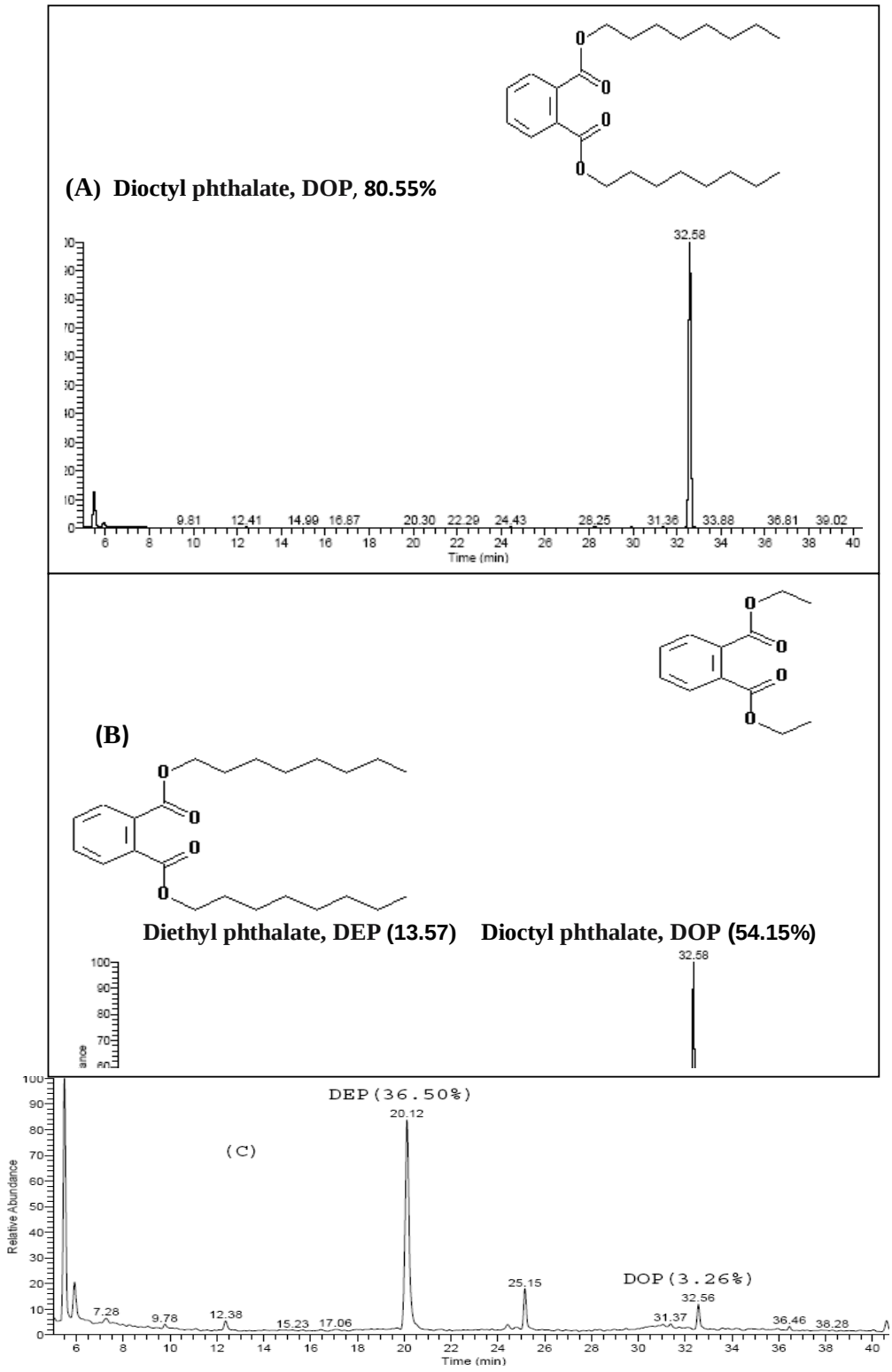

Figure 1. Mass spectra of diethyl phthalate (DEP) and 1, 2-Benzenedicarboxylic acid, diisooctyl ester (Di-n-octyl phthalate, DOP) in samples of *P. major* leaves

widely distributed in the subtropics produces diethyl phthalate.

The author suggests that diethyl phthalate and dioctyl phthalate may be derived from plants and act as a new

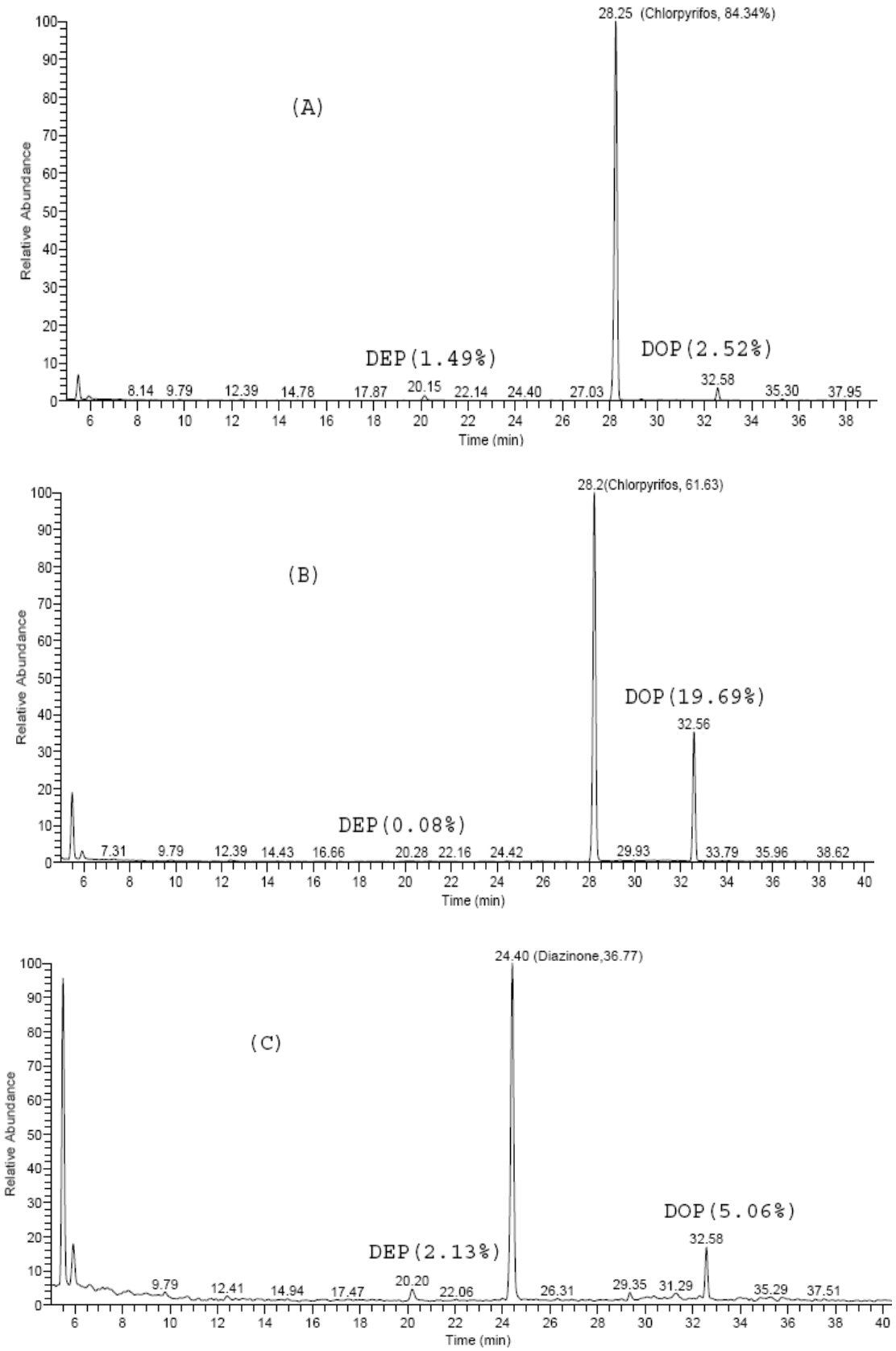

Figure 2. Mass spectra of diethyl phthalate (DEP) and 1, 2-Benzenedicarboxylic acid, diisooctyl ester (Di-n-octyl phthalate, DOP) in samples of *P. major* roots.

class of plant phytotoxins. However, this needs confirming in other plant species, and the mechanism of producing these compounds by plants should be examined before concluding that these substances are natural products. Also, *P. major* leaves and roots may have possessed a stronger ability to accumulate DOP and DEP as a cleanup technology from environmental pollution. However, *P. major* become seriously polluted by Phthalate esters PEs when used as a traditional herbal medicine.

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