Isolation of di(2-ethylhexyl) phthalate from a commercial South African cognate herbal mixture

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Abstract

Traditional medicinal trade in South Africa is a lucrative enterprise, estimated to be worth in excess of R3 billion, representing roughly 6% of the National Health budget. With approximately 27 million consumers, a rich local floral biodiversity platform and a substantial base of traditional medicinal knowledge, its impact and growth potential has not gone unnoticed by the South African government. In this regard, several draft policy bills have passed through parliament to facilitate the integration of traditional medicine into the mainstream South African healthcare system. Despite these measures, trade in traditional medicine remains fragmented and largely unregulated. The present findings highlight this aspect of the industry via identification of the common plasticizer di(2-ethylhexyl) phthalate (DEHP) in a local cognate herbal remedy. Given the highly toxic nature of this substance and the strict international controls in place for regulation of its consumption, the amount of DEHP isolated (43.3 mg/L) from the mixture was seen to be unacceptably high. These observations raise further questions pertaining to quality, safety and efficacy structures within the traditional medicine sector.

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Keywords: Di(2-ethylhexyl) phthalate; Herbal remedy; Traditional medicine

1. Introduction

The southern African region is home to an abundant floral diversity comprising some 30 000 species, representing at least 10% of the world’s flowering plant population (Goldblatt, 1978). The use of many of these plants in the traditional medicinal practice systems of the indigenous peoples of the area has been known for several centuries (Watt and Breyer-Brandwijk, 1962). Conservative estimates have put a figure of around 1000 to the number of ethnomedicinal plant taxa currently exploited in the South African traditional healthcare sector (Arnold et al., 2000). As a consequence, many related scientific investigations have been guided by the rich oral tradition of ethnic medicine, in conjunction with the substantial base of traditional medicinal knowledge which has appeared in the literature over the past two decades (Light et al., 2005). Such endeavors have propelled the potential of our local biodiversity onto the international stage, catalysed by the commercialization of several endemic species such as Aloe ferox, Agathosma betulina, Harpagophytum procumbens, Sutherlandia frutescens and Hoodia gordonii as global herbal remedies for diverse medical conditions (Van Wyk, 2002).

Trade in traditional medicines (TM)s in South Africa is a vibrant, widespread and lucrative enterprise, estimated to be worth in excess of R3 billion, representing roughly 6% of the National Health budget (Mander, 1998). With approximately

Abbreviations: COSY, correlation spectroscopy; EI, electron impact; HMBC, heteronuclear multiple bond correlation; HSQC, heteronuclear spin quantum correlation; LRMS, low resolution mass spectroscopy; NMR, nuclear magnetic resonance; TM, traditional medicine.

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27 million, mainly Black South African, consumers and at least 133,000, largely rural women employees, the TM plant trade is a key industrial and business incubator (Williams et al., 2000). The reliance of such a large proportion of the population on TM for primary health care needs has been attributed to a number of factors, including availability and accessibility to plant material, affordability, and extensive traditional knowledge and expertise within local communities (Fennell et al., 2004). Contrary to popular belief, use of TMs occurs across all sectors of the consumer population and is not confined to the poor, rural or uneducated end of the market (Mander et al., 2007). Furthermore, a recent study has shown that 97% of patients consulting traditional healers do so out of choice, rather than issues associated with access and cost of western medicine (Mander et al., 2007).

Given the expanse and potential of TM trade, the South African government, in line with World Health Organization (WHO), African Union (AU) and Southern African Development Community (SADC) initiatives, has formulated a program for integration of African Traditional Medicine (ATM) into the mainstream South African healthcare system (Government Gazette No. 31271, 2008). Towards this end, the Directorate of Traditional Medicine was established in 2006 to co-ordinate and manage initiatives within the Department of Health, as well as to facilitate the Traditional Practitioners Act (No. 22 of 2007) (Government Gazette No. 30660, 2008). Further to this, greater responsibility towards TMs was encouraged within statutory bodies such as the Council for Scientific and Industrial Research (CSIR), Medical Research Council (MRC) and Medicines Control Council (MCC). Despite these far-reaching measures, trade in TM in South Africa remains largely unregulated and as such raises serious questions about quality, safety and efficacy structures within the sector (Street et al., 2008).

Against this backdrop, we became interested in elucidating the pharmacological basis to the usage of popular herbal preparations available commercially on the local TM market in Pietermaritzburg (Ndhlala et al., 2010a,b, 2011a,b). Not surprisingly, this work uncovered the potential of several herbal preparations as antioxidant and antimicrobial agents, as well as inhibitors of the enzymes HIV-1 reverse transcriptase and acetylcholinesterase (Ndhlala et al., 2010b, 2011a). Despite these promising pharmacological profiles, several of these preparations were seen to possess in vitro cytotoxic and mutagenic properties, thus cautioning against their extended use (Ndhlala et al., 2010a, 2011a). As a consequence of its broad observed pharmacological profile (Ndhlala et al., 2010b), ‘Sejeso’ herbal mixture (Ingwe® brand) (Fig. 1) was here selected for phytochemical investigation to elucidate the underlying active constituents. In the process, the known commercial plasticizer di(2-ethylhexyl) phthalate (DEHP) (Fig. 2) was isolated at a concentration of 43.3 mg/L from the preparation and identified by spectroscopic means, including 2D NMR techniques. The presence of this well-known toxic substance in the cognate mixture, and at such high levels, is alarming and raises questions pertaining to quality control measures within the TM sector.

2. Materials and methods

2.1. General

IR spectra were measured on a Bio-Rad FTS-40 series spectrometer in dry film. EIMS were run on a Micromass Quattro Ultima spectrometer fitted with a direct injection probe (DIP) with ionization energy set at 70 eV and HRMS (EI) were performed with a Micromass Q-Tof Ultima spectrometer. $^1$H and $^{13}$CNMR, DEPT, COSY, HSQC and HMBC spectra were recorded on a Bruker AV400 spectrometer in CDCl$_3$, chemical shifts are reported in units of δ (ppm) and coupling constants (J) are expressed in Hz. Silica gel Merck KGaA (70–230 mesh) was used for CC and TLC silica gel 60 F$_{254}$ for analytical and preparative TLC (both Merck KGaA). Spots on chromatograms were detected under UV light (254 and 365 nm) and by anisaldehyde reagent stain.

Fig. 1. Commercially available ‘Sejeso’ herbal mixture (Ingwe® brand).

Fig. 2. Structure of phthalate diester isolated from ‘Sejeso’ herbal mixture (Ingwe® brand).
2.2. ‘Sejeso’ herbal mixture (Ingwe® brand)

The herbal preparation, bottled in a deep yellow plastic container (Fig. 1), was purchased from a known ‘muthi’ shop in the Pietermaritzburg area of KwaZulu-Natal, with dates of manufacture and expiry indicated as 20/03/2005 and 03/2012 respectively. According to the label material, the product was formulated by T/Dr. Seth Seroka and traded under ‘Guideline Trading’ of Alberton North. The product is indicated for symptomatic relief of heartburn, constipation, stomach ache, loss of appetite, vomiting and indigestion. Side effects may include a mild fever during use, while the product is contra-indicated for children and pregnant women. The recommended dosage is one-quarter of a cup taken three times daily after meals. Ingredients (each 125 mg) as listed per 125 mL are: Lesoko (Alepidia amatymbica), Momnanela, Poo-Ishheila, Setimamollo (Pentanisia prunelloides), Mositsane (Elephantorrhiza elephantina), deionized water (92.5% m/v), and 1.2% m/v potassium sorbate as preservative. A warning is also issued on the bottle label that the product should be kept out of reach of children and stored below 25 °C away from direct sunlight.

2.3. Extraction and isolation of di(2-ethylhexyl) phthalate

The herbal mixture (2 × 500 mL) was filtered through Whatman No.1 filter paper and subsequently freeze dried. The resultant extract (4.1 g) was subjected to gravity column chromatography over silica gel by gradient elution with hexane/ethyl acetate (80:20), further purification was achieved via preparative TLC using hexane/ethyl acetate (80:20) as developing solvent (80:20). Further purification was achieved via preparative TLC using hexane/ethyl acetate (80:20) as developing solvent to give DEHP (43.3 mg) as a colorless oil. The spectroscopic data as shown below closely matched those that have been reported for DEHP (Cohen et al., 1991).

2.4. Physical and spectroscopic data for di(2-ethylhexyl) phthalate

IR νMAX/cm−1 (dry film): 2945, 1726, 1600, 1460, 1277, 1128, 1065, 750. HRMS (EI): calcd. 390.2770 for C24H38O4, found 390.2778. LRMS (EI) 70 eV, 168.1 (s, CO). Identification of the structure of di(2-ethylhexyl) phthalate (DEHP) (Fig. 2) was based on spectroscopic data analysis. Infrared absorption bands at 1726 and 1600 cm−1 were indicative of ester carbonyl and phenyl ring moieties, respectively. Dissociation of the molecule via electron impact (EI) showed the molecular ion [M]+ peak as the base peak at m/z 390, correct for the molecular mass of DEHP, with diagnostic fragment ions detected at m/z 277 [M-C8H17]+ and 113 [C8H17]+. This was further substantiated by HRMS analysis which gave a mass of 390.2778 g/mol for the compound, correct for the formula C24H38O4 with calculated mass 390.2770 g/mol. The low field region of the 1H NMR spectrum was populated by two resonance signals, characteristic of an A2B2 spin system, in this case derived from the aryl protons H-3/H-6 (δ 7.70, 2H, dd, J = 5.68, 3.32 Hz) and H-4/H-5 (δ 7.52, 2H, dd, J = 5.68, 3.32 Hz). 2D proton-carbon HSQC analysis showed these protons to be correlated to respective carbon signals at δ 129.1 (d, C-3/C-6) and 131.2 (d, C-4/C-5). The C-2′ oxygen-related methylene proton signals were found further upfield in the 1H NMR spectrum at δ 4.20 (2H, dd, J = 10.88, 6.12 Hz, H-1a′) and 4.24 (2H, dd, J = 10.92, 5.76 Hz, H-1b′), correspondent with a carbon resonance at δ 68.5 (t, C-1′). The C-2′′ methine proton was resonant at δ 1.67 (2H, m, H-2′), and had HSQC connectivity to the carbon signal at δ 39.1 (d, C-2′-). Both H-1′ and H-2′ were seen to share pronounced COSY contours with each other, in accordance with their vicinal relationship, confirming the branched nature of the alkyl chain of the ester as opposed to the straight chain present in the isomeric diocetyl phthalate (DOP). In addition, three-bond HMBC correlation clearly linked H-1′ to the ester carbonyl (δ 168.1, s, CO), thus precluding the possibility of the 1-methylheptyl ester isomer. Further signals at δ 0.89 (6H, t, J = 6.84 Hz, H-6′), 0.92 (6H, t, J = 7.48 Hz, H-8′), 1.46 (4H, m, H-7′), 1.39 (4H, m, H-5′), 1.35 (4H, m, H-3′), 1.32 (4H, m, H-4′), 1.67 (2H, m, H-2′), 4.20 (2H, dd, J = 10.88, 6.12 Hz, H-1a′), 4.24 (2H, dd, J = 10.92, 5.76 Hz, H-1b′), 7.52 (2H, dd, J = 5.68, 3.32 Hz, H-4/H-5), 7.70 (2H, dd, J = 5.68, 3.32 Hz, H-3/H-6). 13CNMR (100 MHz, CDCl3); δ 11.3 (q, C-8′), 14.4 (q, C-6′), 23.3 (t, C-5′), 24.1 (t, C-7′), 29.3 (t, C-4′), 30.7 (t, C-3′), 39.1 (d, C-2′), 68.5 (t, C-1′), 129.1 (d, C-3/C-6), 131.2 (d, C-4/C-5), 132.8 (s, C-1/C-2), 168.1 (s, CO).

3. Results and discussion

Identification of the structure of di(2-ethylhexyl) phthalate (DEHP) (Fig. 2) was based on spectroscopic data analysis. Infrared absorption bands at 1726 and 1600 cm−1 were indicative of ester carbonyl and phenyl ring moieties, respectively. Dissociation of the molecule via electron impact (EI) showed the molecular ion [M]+ peak as the base peak at m/z 390, correct for the molecular mass of DEHP, with diagnostic fragment ions detected at m/z 277 [M-C8H17]+ and 113 [C8H17]+. This was further substantiated by HRMS analysis which gave a mass of 390.2778 g/mol for the compound, correct for the formula C24H38O4 with calculated mass 390.2770 g/mol. The low field region of the 1H NMR spectrum was populated by two resonance signals, characteristic of an A2B2 spin system, in this case derived from the aryl protons H-3/H-6 (δ 7.70, 2H, dd, J = 5.68, 3.32 Hz) and H-4/H-5 (δ 7.52, 2H, dd, J = 5.68, 3.32 Hz). 2D proton-carbon HSQC analysis showed these protons to be correlated to respective carbon signals at δ 129.1 (d, C-3/C-6) and 131.2 (d, C-4/C-5). The C-2′ oxygen-related methylene proton signals were found further upfield in the 1H NMR spectrum at δ 4.20 (2H, dd, J = 10.88, 6.12 Hz, H-1a′) and 4.24 (2H, dd, J = 10.92, 5.76 Hz, H-1b′), correspondent with a carbon resonance at δ 68.5 (t, C-1′). The C-2′′ methine proton was resonant at δ 1.67 (2H, m, H-2′), and had HSQC connectivity to the carbon signal at δ 39.1 (d, C-2′-). Both H-1′ and H-2′ were seen to share pronounced COSY contours with each other, in accordance with their vicinal relationship, confirming the branched nature of the alkyl chain of the ester as opposed to the straight chain present in the isomeric diocetyl phthalate (DOP). In addition, three-bond HMBC correlation clearly linked H-1′ to the ester carbonyl (δ 168.1, s, CO), thus precluding the possibility of the 1-methylheptyl ester isomer. Further signals at δ 0.89 (6H, t, J = 6.84 Hz, H-6′), 0.92 (6H, t, J = 7.48 Hz, H-8′), 1.46 (4H, m, H-7′), 1.39 (4H, m, H-5′), 1.35 (4H, m, H-3′), and 1.32 (4H, m, H-4′), 1.67 (2H, m, H-2′) accounted for the remaining methyl and methylene group protons of DEHP. The 13CNMR spectrum also indicated the presence of aryl ester carbonyl groups (δ 168.1, s, CO) and the C-1/C-2 quaternary carbons (δ 132.8, s), thus accounting for the 24-carbon skeleton of DEHP.

Phthalates are a well-known group of plasticizers used to make polyvinyl chloride (PVC) plastic materials viable (Chen et al., 2004). Most PVC products contain DEHP plasticizers in the range 30% to 80% by weight (Chen et al., 2004). DEHP is found in products as diverse as construction materials, consumer goods, medical products and packaging (Aignasse et al., 1995). Since DEHP is not chemically bound to the plastic, it poses a threat to the environment by readily leaching to the surrounding medium or fluids (Chen et al., 2004). This is exacerbated by the fact that DEHP exhibits reproductive and developmental toxicity, is carcinogenic and teratogenic, and produces neurodegenerative effects in animal model studies (Koch et al., 2006; Wittassek and Angerer, 2008). The most striking effect of DEHP in short term toxicity studies is the proliferation of hepatic peroxisomes, indicated by increased peroxisomal enzyme activity and histopathological changes (Koch et al., 2006; Wittassek and Angerer, 2008).
al., 2006; Wittassek and Angerer, 2008). Furthermore, it has been shown that DEHP is hydrolyzed enzymatically to mono(2-ethylhexyl) phthalate (MEHP), which is known to be even more toxic than the parent compound (Albro and Lavenhar, 1989). Given the widespread usage of DEHP, its inherent toxic properties and the serious health risks it poses on a global scale, organizations such as WHO have implemented stringent guidelines pertaining to tolerable daily intake (TDI) levels (WHO, 1996, 2003). In the case of DEHP, this has been set at 8 μg/L for drinking water and 1.5 mg/kg of food consumed. Based on the WHO drinking water guideline ratio for DEHP, patients consuming ‘Sejeso’ herbal mixture at the recommended dose (see experimental) would have an intake of around 7.8 mg DEHP per day, in large excess of the levels suggested for either water or food consumption. This level of DEHP contamination in the mixture is alarming and highlights the lack of effective control mechanisms within the informal sector of TM trade in South Africa. Presumably, DEHP contamination of the herbal remedy would have originated from the plastic bottling material.

In summary, based on the promising pharmacological profile of the commercially available ‘Sejeso’ preparation and its cognate herbal constituents, a phytochemical investigation was carried out to identify and quantify the active ingredients. During the process, the common plasticizer material DEHP was isolated at a concentration of 43.3 mg/L, which is seen to be unacceptably high given its toxic nature as well as its restricted via international regulatory guidelines. The prominence of TM within the South African cultural landscape, its economic potential as well as its anticipated integration into the mainstream South African healthcare system, has benefits for all players within the sector. However, for it to be a sustainable industry, a concerted effort is required to implement relevant structures to ensure that the quality, safety and efficacy of TMs are not compromised.

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