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Determination of Organochlorine Pesticide Residue Levels in Chewable Parts of the Khat (*Catha edulis*) Plant

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Abstract In this study, the levels of DDT and its metabolite residues were determined in chewable parts of *Catha edulis* plants grown in the southern part of Ethiopia. The levels of *p*,*p*′-DDT and *p*,*p*′-DDE were found to be in the range of 10.8–19.7 and 3.5–18.6 μg/kg, respectively. These data revealed that the residue being detected is from recent applications. The estimated daily intake of total DDT from *C. edulis* consumption was calculated to be in the range between 0.0278 and 0.0747 μg/kg, which is significantly lower than the FAO/WHO guideline. However, this may not guarantee safety, as the application of DDT extends to vegetables as well. Even though the use of DDT was banned in Ethiopia for agriculture purposes, detectable levels are still being observed. The results of the study necessitate the need for awareness creation among the people in the community.

Keywords *Catha edulis* · Estimated daily intake · Maximum residual limit · DDT

Organochlorine pesticides (OCPs) are very persistent, bioaccumulative and toxic; hence, they have created adverse effects upon humans and the natural environment (Imo et al. 2007; Biego et al. 2010). Due to these reasons an international treaty known as the Stockholm Convention was ratified by many nations. The convention banned twelve 'persistent organic pollutants' (POPs), DDT being one (Linderholm et al. 2010; Zhu et al. 2005; Hussen et al. 2006). The occurrence of organochlorine compounds in water, soil, sediment, fish, meat, honey, vegetables and fruits has been reported (Blasco et al. 2004; Rodrigues et al. 2007; Hussen et al. 2006, 2007; Guo et al. 2007; Darko and Acquaah 2007; Odhiambo et al. 2009; Fouial-Djebbar et al. 2010; Ssebugere et al. 2010; Crentsil et al. 2012). However, studies are very limited on OCP residues in khat, *Catha edulis*, a plant that is grown in Ethiopia and commonly used by its population. The only published study that was found reported concentrations of *p*,*p*′-DDT ranging from 141.2 to 999.0 μg/kg in khat samples collected from eastern and western parts of Ethiopia (Daba et al. 2011).

Khat is a psychoactive shrub chewed by the people for its stimulating effects. It develops addiction on prolonged intake. The number of people chewing is increasing daily, particularly among the youth. The practice is also extending to western countries by immigrants from the traditional use regions of the Horn of Africa and the Middle East (Gebissa 2010). Regional and countrywide surveys revealed that an average of 30 % of Ethiopians have adopted the practice of chewing (Belew et al. 2000; Arrafaine 2004). The Sidama zone is one of the *C. edulis* growing areas in the southern part of Ethiopia. A survey conducted on 226 farmers in the Sidama zone revealed that 174 farmers (77 %) use DDT for agricultural pest control, especially for this plant (Daneil 2009). Since *C. edulis* is directly consumed by the chewers, it is very important to determine the residual status of DDT and its metabolites in the chewable parts of the plant. This study was carried out to determine the residues of DDT and its metabolites in chewable parts (leaves and tender stems) of the khat plant, and the magnitude of its hazard.

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Materials and Methods

Sampling sites were chosen in seven different districts of Sidama zone in Southern Ethiopia. They are located within 5°45′–6°45′N latitude and 38°–39°E longitude, covering a total area of 7,672 km^2. The districts were Tula, Shebedino, Dale, Lokabaya, Boricha, Wonsho and Chuko. Khat plants are regularly sprayed with DDT in these districts. The leaves with tender stems were randomly collected from farms of the selected districts. In each district, 110–120 plants were selected for collection of leaves with tender stems. The plant materials were wrapped with filter papers separately for individual districts. They were then placed into sealed paper envelopes, to which a small sachet of silica gel was added, and transported to the laboratory.

A standard solution of OCPs (2,000 μg/mL) was obtained from the Quality Monitoring and Testing Laboratory of the Ministry of Agriculture, Addis Ababa, Ethiopia. Anhydrous sodium sulfate (Samir Tech-chem Pvt. Ltd, Gujarat, India) was used as drying agent in grinding, extraction and clean-up processes. Alumina (Type 507c Neutral pH 7, 0.05–0.15 mm, Brockmann grade I, Switzerland) and Florisil (PR Grade 100 mesh, Switzerland) were used for clean-up. *n*-Hexane (HPLC grade 99 %, Technopharm Chem, Bahadurgarh, India) and acetone (Analytical grade 99.5 %, Sisco Research laboratory, Mumbai, India) were used for extraction. Ethyl acetate (Analytical grade 99 %–101.0 %, Central Drug House, Bombay, India) and *n*-hexane (HPLC grade 99 %, Technopharm Chem, Bahadurgarh, India) were used as eluting solvents.

The samples collected from each site were separately air-dried at room temperature in the laboratory. The extraction procedure employed for vegetables (Odhiambo et al. 2009) was used for khat samples with certain modifications. Each dried sample was first mixed with anhydrous sodium sulfate and this mixture was then ground into a fine powder and homogenized. Finally 10 g of this homogenate and 10 g of anhydrous sodium sulfate were transferred into a folded filter paper, which was used as an extraction thimble. Once the mixture was loaded into a Soxhlet extractor, extraction was performed for 16 h at a rate of 4 cycles/h with 200 mL of extraction solvent (acetone-hexane, 1:1 v/v) in a 250 mL round bottom flask. Similarly, in two additional replicate extractions, running time was scheduled so as to achieve the same number of cycles. For each sample, the resulting three replicate extracts were evaporated in a rotary evaporator to approximately 2 mL for the subsequent clean-up step.

The clean-up procedure was performed with a multilayer Florisil/alumina column packed from the bottom to top with Florisil (10 g), neutral alumina (10 g), and anhydrous sodium sulphate (5 g). The column was prewashed with 5 mL of *n*-hexane and, as the solvent reached the top layer, 2 mL of the extract was added and eluted slowly with 100 mL of hexane–ethyl acetate (4:1, v/v) in three fractions. The resulting eluted fractions were mixed together and concentrated almost to dryness using a rotary evaporator, and then reconstituted with 4 mL of *n*-hexane and kept in a freezer until GC-ECD analysis.

The analysis was carried out using an Agilent Model 7890, gas chromatograph (GC) system (Wilmington, DE USA) equipped with dual micro-cell, ^{63}Ni electron capture detector (^{63}Niμ-ECD) and Agilent Model 7693A auto sampler. The sample volume injected was 2 μL with splitless mode. The separation was performed on a RXi-5Sil MS (30 m × 0.25 mm i.d. and film thickness of 0.25 μm) capillary column (Restek Corp, Bellefonte, PA, USA) and a second RXi-5Sil MS (30 m × 0.25 mm i.d. and film thickness of 0.25 μm) column with different polarity was used as a confirmatory column. Helium (99.99 % pure) was used as a carrier gas with a flow rate of 1 mL/min, and nitrogen (99.99 % pure) was used as the make-up gas. The oven temperature was programmed as follows: Initial temperature at 120°C was retained for 0.5 min; then increased to 180°C at a rate of 20°C/min and retained for 2 min, followed by an increase to 280°C at rate of 10°C/min and held for 10 min. Injector and electron capture detector temperatures were maintained at 230 and 300°C, respectively.

All glassware used was heated at 200°C overnight prior to use. In each set of investigated samples, blanks were analyzed simultaneously to check for cross contamination and interferences. As can be seen in Fig. 1, no target compound peaks were found in the chromatograms of the blanks, particularly near the retention times of the target analytes (13–16 min.). Dual column GC-ECD having different phase polarities was used for qualitative confirmation of the target analytes.

Standard stock solutions of OCPs were prepared at concentrations of 2,000 μg/mL, and then diluted to intermediate concentrations of 20 μg/mL, followed by dilution to lower working concentrations of 1 μg/mL. Spiking and calibration standard solutions were further prepared from 1 μg/mL. Five point calibration standard solutions were prepared in the range of 0.0005–0.05 μg/mL (0.0005, 0.001, 0.005, 0.01, 0.05 μg/mL). Good linearity was found in the given concentration range, with correlation coefficients higher than 0.999 in all the cases. The target analytes in the test samples and spiked samples were all quantified using this calibration curve. The calibration curve was verified on each working day with the calibration standard.

Six blank samples were analyzed to determine the limit of detection (LOD). It was expressed as the average signal corresponding to the reagent blank plus three times the standard deviation of the blank signal ($LOD = Y_B + 3S_B$)

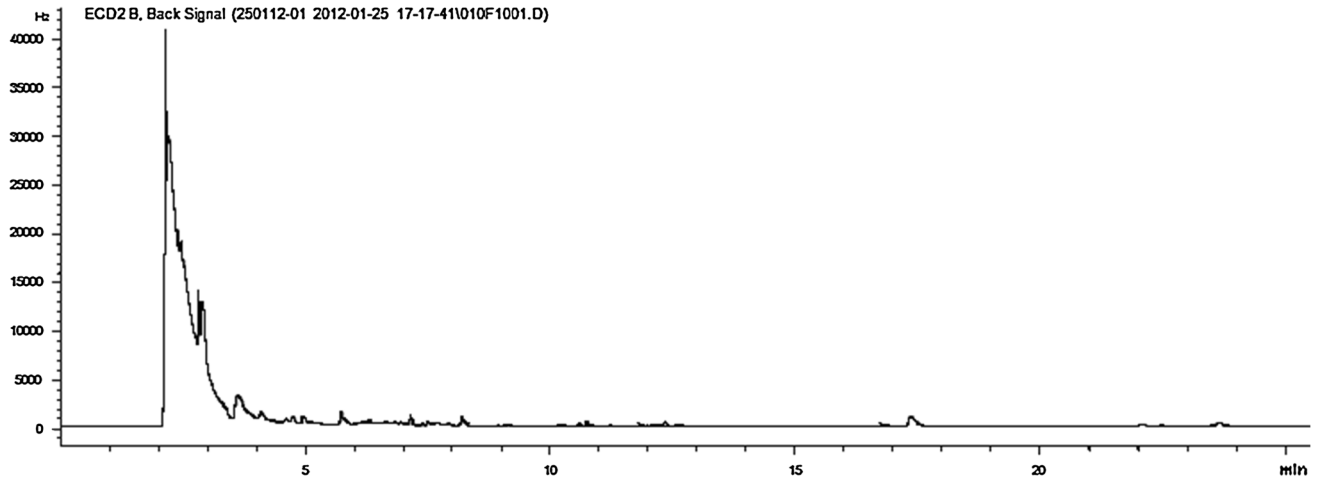

Fig. 1 GC-ECD chromatogram for a blank

(Muir and Sverko 2006). Accordingly, the LOD for the target analytes *p*,*p*′-DDT, *p*,*p*′-DDE, and *p*,*p*′-DDD were found to be 0.0046, 0.0032 and 0.0017 µg/mL, respectively. Concentrations less than these respective values were reported as less than LOD.

The repeatability of the method (intra-day precision) was tested by running five extractions of the spiked (0.025 µg/mL) test samples. Both the extraction and GC analysis were carried out under the same conditions within one day. The reproducibility of the method (inter-day precision) was also checked on three different days. The spiked test samples were extracted and analyzed at an interval of one day. As might be expected, the intra-day precision was better (having a RSD value ranging from 3.68 to 8.87) than the inter-day precision, which had a RSD range from 11.3 to 17.6. The inter-day precision (reproducibility) of the method was within the acceptable range (10 %–20 %) for pesticide residue analysis in the plant matrices (European Commission Directorate General Health and Consumer Protection 2010). This showed that the method was reasonably repeatable and reproducible under the given laboratory conditions.

Recoveries of the target analytes were 84 %, 95 % and 115 % for *p*,*p*′-DDD, *p*,*p*′-DDE and *p*,*p*′-DDT, respectively. The observed recoveries were within the acceptable range (75 %–120 %). The good recoveries obtained implied that the employed methodology resulted in exhaustive extraction of the target analytes.

Results and Discussion

The GC-ECD chromatogram of a *C. edulis* sample extract is presented in Fig. 2. The chromatogram clearly showed the target compounds without any interference, as evidenced from the blank chromatogram as well (Fig. 1). The quantitative results displayed in Table 1 showed that *p*,*p*′-DDT and *p*,*p*′-DDE were detected in all samples. Similarly, the compound *p*,*p*′-DDD was detected in samples from five of the seven districts. Lokabaya and Shebedino were the exceptions. The levels of *p*,*p*′-DDT, *p*,*p*′-DDE, *p*,*p*′-DDD investigated varied from 10.8 to 19.7, 3.5 to 18.6, 2.0 to 6.5 µg/kg, respectively. The levels of *p*,*p*′-DDT, *p*,*p*′-DDE, *p*,*p*′-DDD observed in Chuco district were 19.7 ± 2.1, 18.6 ± 1.3 and 6.5 ± 0.8 µg/kg, respectively.

The ANOVA analysis showed statistically significant differences in the mean concentrations of *p*,*p*′-DDT, *p*,*p*′-DDE and *p*,*p*′-DDD at probability 0.05 %. The least significant difference (LSD) method (Miller and Miller 2005) was applied as post hoc analysis to determine which means were different from one other. The analysis showed that all of the means were significantly different from one another with the following exceptions: Wonsho and Lokabaya for *p*,*p*′-DDT; Wonsho and Tula for *p*,*p*′-DD E; Wonsho and Tula and Dale and Boricho for *p*,*p*′-DDD. Thus, it can be stated that the concentrations of *p*,*p*′-DDT, *p*,*p*′-DDE and *p*,*p*′-DDD detected in Chuco samples are significantly greater than the concentrations observed in all other districts.

In the literature, the ratio of DDE/DDT or DDT/DDE (El-Nahhal 2004; Hussen et al. 2006) has been used to deduce whether the residues being detected are from past or recent pesticide application. In our study, the concentration of *p*,*p*′-DDT (parent compound) was higher than the concentrations of the metabolites (*p*,*p*′-DDE, *p*,*p*′-DDD) (Table 1). This implies that recent applications of DDT have occurred in the study areas, which is in agreement with a report of a survey made in 2009 (Daneil 2009).

The mean levels of total DDT determined in the present study (Table 1) did not exceed the recent maximum residue limit (MRL) of the FAO/WHO (100–200 µg/kg) for different agricultural food items (FAO/WHO 2013), or the

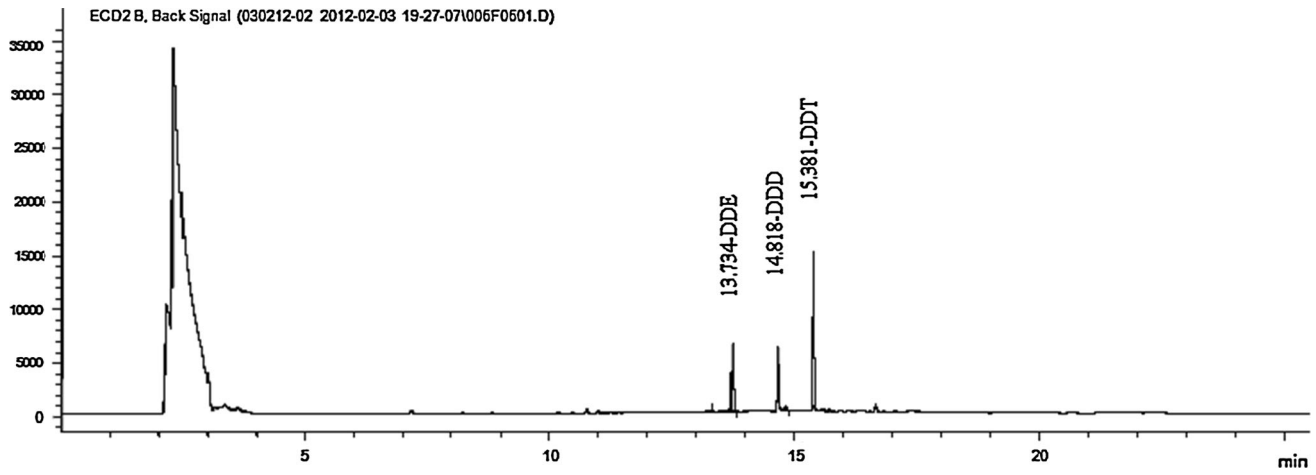

Fig. 2 GC-ECD chromatogram for an extract of a *Catha edulis* sample

Table 1 Mean concentration of DDT and its metabolites (μg/kg) in the extract of *Catha edulis* samples (mean ± SD, n = 3)

Sampling location	*p*,*p*′-DDT	*p*,*p*′-DDE	*p*,*p*′-DDD	Total DDT
Boricha	13.6 ± 4.8[a]	8.1 ± 0.4[a]	2.6 ± 0.7[a]	24.2 ± 5.0[a]
Lokabaya	12.2 ± 2.3[b]	10.2 ± 2.6[b]	<LOD	22.5 ± 5.9[b]
Dale	10.8 ± 1.4[c]	3.5 ± 0.5[c]	2.4 ± 0.6[a]	16.7 ± 4.5[c]
Shebedino	17.4 ± 8.0[d]	14.2 ± 3.5[d]	<LOD	31.7 ± 8.8[d]
Wonsho	12.2 ± 2.9[b]	11.3 ± 1.6[e]	2.0 ± 0.8[b]	25.5 ± 5.6[e]
Tula	16.6 ± 8.7[e]	11.9 ± 1.5[e]	2.1 ± 0.1[b]	30.6 ± 7.4[f]
Chuco	19.7 ± 2.1[f]	18.6 ± 1.3[f]	6.5 ± 0.8[c]	44.8 ± 7.3[g]

<LOD indicates that the concentration was below limit of detection

Superscripts with different letters denote a significant difference ($p \leq 0.05$) between sites

European Commission (EC) MRL value of 50 μg/kg (Crentsil et al. 2012) and Japanese MRL values of 200 μg/kg for different food items (WHO 2007).

In comparison to the present study, which showed only a maximum of 44.8 μg/kg of total DDT at Chuco district, very high concentrations of *p*,*p*′-DDT were reported (Daba et al. 2011) in *C. edulis* samples of Galamso (141.2–973.0 μg/kg), Aseno (194.3–999.0 μg/kg) and BadaBuna (173.9–686.9 μg/kg). On the other hand, residue levels comparable to the present study were measured in fruits and vegetables collected from a Ghanaian market (Crentsil et al. 2012). For example, residues of 12.0, 38.0 and 20.0 μg/kg of *p*,*p*′-DDT were reported in papaya, banana and mango, respectively. Similarly, concentrations ranging from 12.0 to 35.0 μg/kg of *p*,*p*′-DDT were found in tomato, cabbage and onion.

The Joint Food and Agriculture Organization of the United Nations FAO/WHO Codex Alimentarius Commission has set the acceptable daily intake (ADI) for total DDT to be 10 μg/kg (Joint FAO/WHO Codex Alimentarius Commission 2010). The ADI is expressed on a body mass basis, to which an individual in a population may be exposed daily over his or her lifetime without appreciable health risk (WHO 2007). To know the exposure of chewers to DDT and its metabolites, the estimated daily intake (EDI) of these pesticides was determined from the measured concentrations. It was calculated based on the assumption that the average *C. edulis* consumption of an adult having a body weight of 60 kg was 0.1 kg/day. The mean values for daily intakes of DDT and its metabolites were estimated using the following equation (Tomza-marciniak and Witczak 2010; Guo et al. 2007).

$$\text{Estimated daily intake (EDI)} = C_{\text{sample}} \times W_D/\text{kg body weight}$$

C_{sample} = mean concentration of pesticide in *C. edulis* sample (μg/kg); W_D = dry weight of *C. edulis* consumed (kg/day).

The EDI value of total DDT ranged from 0.0278 to 0.0747 μg/kg body weight per day for Dale and Chuco districts, respectively. The EDI value was significantly lower than the FAO/WHO ADI value. However, it should be noted that this value provides only information about DDT exposure associated with *C. edulis* chewing. Since farmers in the study area also use DDT for vegetables such as tomato and cabbage, it is likely that the actual daily intake may be higher due to additional exposure through consumption of these vegetables. It is clear that khat chewers from the Chuco district receive a higher dose of total DDT than chewers from the other six districts.

In conclusion, detectable amounts of DDT and its metabolites were found in most of the samples analyzed. This is in good agreement with a survey conducted in the same area in 2009. The concentrations determined in the *C. edulis* samples were found to be below the MRL set by FAO/WHO, Japan and the US EPA for different agricultural food items. Nevertheless, khat chewers from Chuco district

are more exposed to DDT residues from khat. Although DDT was banned in Ethiopia for cultivating agricultural products, this study revealed that the practice is still continuing. This may be due to a lack of awareness among farmers in the study area. The study revealed a compelling need for launching community-based education programs. Local health care professionals, educators, development agents, administrative bodies and researchers should collaborate in an education program for the residents of the khat-raising region of southern Ethiopia, thereby reinforcing implementation of the Stockholm convention.

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