

Eun-jung Lee,<sup>1</sup> Ph.D.; Sun-cheun Kim,<sup>2</sup> Phar.D.; In-kwan Hwang,<sup>1</sup> M.S.; Hee-jin Yang,<sup>2</sup> M.Pharm.;  
Youn-shin Kim,<sup>3</sup> M.D.; Myun-soo Han,<sup>4</sup> Ph.D.; Moon-sik Yang,<sup>5</sup> Ph.D.; and Yang-han Lee,<sup>4</sup> Ph.D.

## The Identification of Ingested Dandelion Juice in Gastric Contents of a Deceased Person by Direct Sequencing and GC–MS Methods\*

**ABSTRACT:** DNA and chemical analysis of gastric contents of a deceased person were handled in this work. The body of the victim was discovered in his car, submerged in a lake. We were asked to determine whether or not the gastric contents of the victim harbored drugs and dandelion material. It was suspected that the victim had been murdered by poisoning with an excess amount of sleeping medication (doxylamine), which had been homogenized with dandelion. The concentrations of 11.4 and 27.5 mg/kg of doxylamine detected from spleen and liver of the victim were far higher than the assumed therapeutic concentration. Via gas chromatography–mass spectrometry (GC–MS) analysis and direct sequencing analysis of plant genetic markers such as intergenic transcribed spacer, 18S ribosomal RNA (rRNA), *rbcL* and *trnL*, it was confirmed that the gastric contents of the victim contained taraxasterol, which is one of the marker compounds for dandelion and contained dandelion species-specific *rbcL* and *trnL-trnF* IGS (*trnLF*) sequences. The initial PCR of the genomic DNA isolated from the gastric contents showed insufficient quantity, and the second PCR, of which the template was a portion of the initial PCR products, exhibited a sufficient quantity for direct sequencing. *rbcL* and *trnLF* located in the cpDNA resulted in the successful determination of dandelion DNA in a decedent's stomach contents. GC–MS identifies the actual presence of a taraxasterol at 28.4 min. Raw dandelion was assumed to be used as a masking vehicle for excess sleeping drug (doxylamine).

**KEYWORDS:** forensic science, *rbcL*, *trnL-trnF* IGS, doxylamine, taraxasterol, GC–MS, gastric contents, dandelion

Fragmentary plant evidence tends to make traditional morphological investigation difficult, and is often ignored by many investigators for this reason. DNA analysis, as well as drug or toxin analyses of stomach contents, can constitute valuable evidence for providing investigative leads in investigations of death. Currently, the identification of ingested vegetative samples relies on microscopical and morphological examinations, but this sometimes proves problematic. DNA technology has been used extensively in human profiling (1,2) and in animal studies for forensic purposes (3), and has been gradually applied to the analysis of forensic botanical materials (4,5).

The sequencing method of specific loci or markers with different base substitution rates has been generally adopted for plant phylogenetic studies (6–14). Intergenic transcribed spacer (ITS) and 18S ribosomal RNA (rRNA) are located in the genomic nucleolar organizing region. ITS allows for the appreciation of phylogeny of a

variety of monocot and dicot plants (7,8). 18S rRNA organizes small subunits of ribosome and is used on inferring angiosperm phylogeny (9). Chloroplasts are organelles that are found in plant cells and eukaryotic algae, and are the primary organelles involved in photosynthesis. Chloroplast DNA (cpDNA) has been used extensively to infer plant phylogenies at differing taxonomic levels (10–14). Molecular markers of *rbcL* and *trnLF* are located in the DNA of chloroplasts. *rbcL* encodes for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase, which catalyzes carbon fixation in chloroplasts, and exhibits a low evolutionary rate that renders it useful for evolutionary studies at higher taxonomic levels (11,14). *trnLF* includes the 3' partial sequence of *trnL* gene and a noncoding region that is the intergenic spacer between two tRNA genes, *trnL*(UAA) and *trnF*(GAA). Noncoding regions tend to evolve more rapidly than coding regions, and have therefore proven quite useful for evolutionary studies of related species, and probably also for populations of the same species (12,14).

After 17 days of disappearance, the victim in this case was found dead in his car, which had been submerged in a lake at hot summer (missing time: 23 July 2006, body recovery date: 9 August 2006). It was suspected that the victim had been murdered by poisoning with an excess amount of sleeping medication, which had been homogenized with dandelion. As the corpse was much decomposed and blood specimens were nonexistent, we analyzed spleen and liver instead of blood for the detection of drug substances. The concentrations of doxylamine and zolpidem detected from the victim's internal organs were toxic and therapeutic levels, respectively.

The victim had cultivated dandelions, and routinely drank dandelion juice to ameliorate his liver trouble at 10 PM every night. One half-cup of dandelion juice in the morning or evening, taken on an empty stomach, is popular in Korea as an effective treatment of liver disorders and diseases. The victim had recently taken out

<sup>1</sup>DNA Analysis Sector, Western District Office of National Institute of Scientific Investigation, 111 Daeduk-Ri, Seosam-Myun, Jangseung-Gun, Chonnam 515-822, South Korea.

<sup>2</sup>Drug & Toxicology Sector, Western District Office of National Institute of Scientific Investigation, 111 Daeduk-Ri, Seosam-Myun, Jangseung-Gun, Chonnam 515-822, South Korea.

<sup>3</sup>Forensic Medicine Sector, Western District Office of National Institute of Scientific Investigation, 111 Daeduk-Ri, Seosam-Myun, Jangseung-Gun, Chonnam 515-822, South Korea.

<sup>4</sup>DNA Analysis Sector, National Institute of Scientific Investigation, 331-1 Shinwol-7Dong, Yangchun-Ku, Seoul, 158-707, South Korea.

<sup>5</sup>Department of Biological Sciences, Chonbuk National University, 664-14 Dukjin-Dong, Dukjin-Gu, Chonju City, Chonbuk, 561-756, South Korea.

\*Supported by research and development expenditures of National Institute of Scientific Investigation.

Received 27 Jan. 2008; and in revised form 14 July 2008; accepted 9 Aug. 2008.

mobile insurance when he was subjected to a traffic accident (in 2003) and when his wife became a beneficiary (US\$40,000). Since then, he was suffering from trauma and insomnia symptoms and was prescribed with a nervous sedative and a sleeping drug (zolpidem). It was assumed that the suspect had mixed homogenized dandelion and sleeping drugs (doxylamine) to avoid detection by the victim. Thus, it was speculated that the suspect had compelled the victim to ingest a great deal of sleeping medication without being noticed because homogenized dandelion had a bitter taste itself. After the patient had drunk the dandelion juice on the night in question, he might have lost consciousness. It was assumed that the suspect might have positioned him in the car, and deposited the car and victim together into the lake, in an attempt to simulate a suicide. The police asked the forensic medicine doctor to autopsy the decedent as a component of the investigation into the death. The investigator suspected the victim's wife of his death. The investigator also obtained the cellular phone records of the victim's wife, and determined that she had been in constant telephone communication with someone on the evening prior to the victim's death. When her husband died, she received 100 million won (US\$100,000) in a life insurance payout. The couple had been in fairly strained financial circumstances and had barely been able to make the life insurance payments.

For the identification of dandelion substances, the stomach contents of the victim were analyzed by chemical and DNA analysis. In the chemical analysis, taraxasterol, one of the marker compounds in dandelions, was verified. As taraxasterol exists in various plants belonging to the Asteraceae family, DNA analysis is keenly needed. In the DNA analysis, the dandelion-specific sequences of *rbcL* and *trnLF* were confirmed. Out of four genetic markers such as ITS, 5' partial region (about 411bp) of 18S rRNA, 5' partial region (446 bp) of *rbcL* and *trnLF*, two markers of *rbcL* and *trnLF* included in cpDNA supported the presence of dandelion in the stomach of the victim.

## Materials and Methods

### DNA Analysis

**Sample Collection**—During the medicolegal autopsy, gastric content samples were collected as a component of the investigation into the victim's death. Photomicrographs of the plant tissues in gastric content samples and grinded dandelion control tissues are

shown in Fig. 1 (light microscopy,  $\times 100$ ). Gastric content samples were collected in three different forms such as green granule, pellet, and supernatant. At first, the police requested three control samples of the dandelions cultivated in the victim's garden for comparison with the substances in the victim's stomach (Fig. 2). Eight supplementary leaves of dandelion controls which were randomly collected from the victim's garden are shown in Fig. 2.

**DNA Extraction**—Gastric samples were washed several times with sterilized distilled water and homogenized in a 1.5 mL tube using a plastic tool with a conical head in order to prevent sample loss. DNA was extracted from crushed green granule samples using a DNeasy Plant Mini Kit (QIAGEN Corp., GmbH, Hilden, Germany). DNA was extracted from crushed pellet samples and supernatant samples cooperatively using DNA IQ systems and Wizard Genomic DNA Purification Kit (PROMEGA Corp., Madison, WI). The isolated DNA was first quantified by agarose gel electrophoresis but the quantity of DNA could not be calculated because of its small amount. The isolated DNA might have the DNA of the epithelial cells of the victim which were collected unintentionally and might make it difficult to quantify only plant DNA by gel electrophoresis and spectrophotometer as these methods were not human-specific.

**Molecular Markers and PCR Amplification**—The universal primers for the species identification of land plants (terrestrial plants) and algae were employed in the amplification of ITS (6). Modified "e" primer (5'-AAAATCGTGAAGGTTCAAGTC-3') and modified "f" primer (5'-GATTTGAACTGGTGACACGAG-3') for the amplification of *trnLF* were used (12–14). The modified "e" primer was designed by Sang et al. (13) and was ten nucleotides farther away from the 3' end of the *trnL* 3' exon than the primer "e" of Taberlet et al. (12) in order to read sequences closer to the 5' end of the intergenic spacer. The modified "f" primer had one more nucleotide at the 5' end of the primer "f" of Taberlet et al. (12). The primer pairs were utilized in the amplification of the segment of *rbcL* (446 bp), which could be read from the translation start codon to 420 base pair. The forward primer for partial *rbcL* was 5'-AATTCATGAGTTGTAGGGAGGGA-3' (bound to -26, designed by the first author, Eun-jung Lee), the reverse primer (making 446 bp) was 5'-AATTCGCAGATCCTCCAGACGT-3' (designed by Eun-jung Lee) and other reverse primer (making 209 bp) was 5'-AGATTCCGCAGCCACTGCAGCCCCCTGCTTC-3' (*rbc19* in

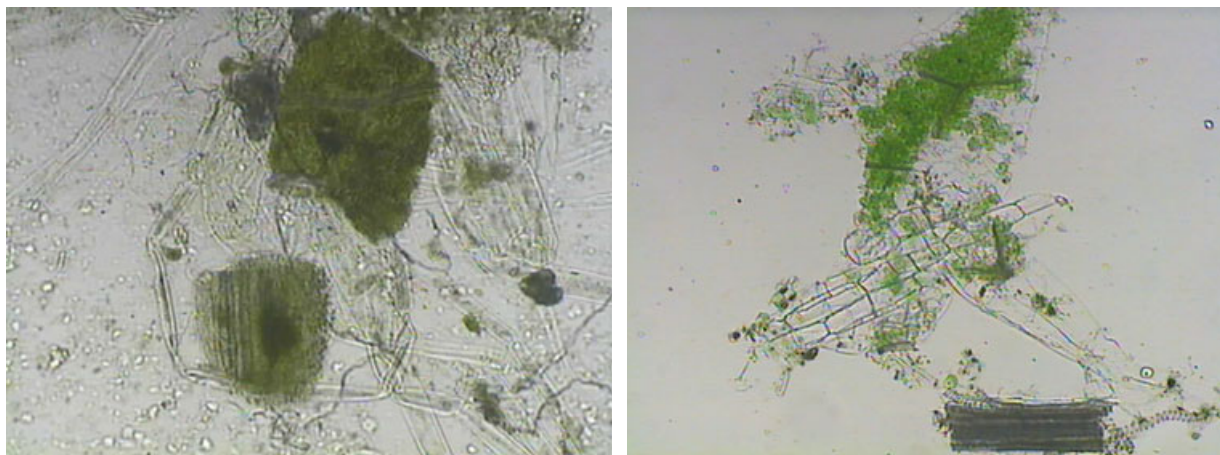


FIG. 1—Plant tissues in gastric contents ( $\times 100$ ) are shown in the left panel and dandelion control tissues ( $\times 100$ ) are shown in the right panel.



FIG. 2—First three control samples which were collected from the victim's house are shown in the left panel and supplementary eight leaves are shown in the right panel.

Ref. 15). The forward primer for 5' partial 18S rRNA was 5'-TGCAGTAAAAAGCTCGTAG-3' (Angio1F in Ref. 15), the reverse primer making about 411 bp was 5'-GGTTGAGACTAG-GACGGTATCTG-3' (designed by Eun-jung Lee) and other reverse primer making 157 bp was 5'-GCACTCTAATTTCTTCAAAG-3' (Angio2R in Ref. 15). PCR amplification was conducted in a 50  $\mu$ L total volume containing 5  $\mu$ L of Gold 10 $\times$  buffer (500 mM KCl, 100 mM Tris-HCl pH8.3, 15 mM MgCl<sub>2</sub>, 1% Triton X-100, 1600  $\mu$ g/mL BSA, 2 mM dNTP pH8.2–8.4, PROMEGA Corp.), 2  $\mu$ L of 10  $\mu$ M forward and 10  $\mu$ M reverse primers (synthesized by BIONEER Corp., Yu-sung, Daejon, South Korea), 2.5 units of AmpliTaq Gold<sup>®</sup> DNA polymerase, 2.5  $\mu$ L of template DNA isolated from the gastric contents and sterile distilled water. The PCR mixture was subjected to dwelling 11 min at 95°C and 28 cycles of 1 min each at 95°C, 1 min at 50°C, 1 min at 72°C, and 7 min at 72°C using an ABI 9700 system (manufactured by Applied Biosystems, Foster City, CA). The second PCR, in which a component of the initial PCR products was used as a template (template was 1  $\mu$ L of first PCR products), was applied. In the second amplification of *rbcL* and 18S rRNA, nested primers (15) were used for the survey of PCR specificity. The template of positive control for PCR is *Papaver somniferum* L. (opium poppy). The PCR reactions of DNA from dandelion reference leaves were conducted after the acquisition of genetic information from gastric contents.

**DNA Sequencing**—A 1/10 volume of the amplification products was assessed via electrophoresis on 2% agarose gels stained with SYBR<sup>™</sup> Green I Nucleic Acid Stain (Cambrex Corp., East Rutherford, NJ). The remnants were then purified using a QIAquick PCR Purification Kit (QIAGEN). The purified PCR products were then sequenced directly using forward and reverse primers with an ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The unincorporated fluorescent dyes were eliminated using a DyeEX<sup>™</sup> 2.0 Spin Kit (QIAGEN) and the purified sequencing products were dried approximately 1 h at 55°C. The sequencing products were then resuspended in 25  $\mu$ L of Hi-Di formamide (Applied Biosystems). DNA sequencing was conducted using an ABI 310 sequencer and analyzed with DNA Sequencing Analysis Software 3.7 (Applied Biosystems).

**Alignments and BLAST Query**—The sequences of the molecular markers obtained from the samples were arranged using BioEdit

(16), MEGA software (17) and ClustalX software (18) to determine whether they were identical or not. In case of the query of our *rbcL* segment to NCBI (National Center for Biotechnology Information), the 5' region including the forward primer-binding region and some bases prior to the ATG start codon and the 3' region including the reverse primer-binding region were excluded in order to improve the sequence specificity. A comparison was made between our secured sequences and the NCBI using BLASTn (nucleotide–nucleotide The Basic Local Alignment Search Tool) (19). For BLASTn, the Jukes-Cantor method is used to calculate the distances in NCBI. BLAST computes a pairwise alignment between a query and the database sequences and an implicit alignment between the database sequences is constructed for purposes of tree presentation of a query sequence (distance tree of results). Namely, genetic trees were simultaneously produced using BLAST pairwise alignments. Deferring to our options on BLAST of NCBI, the genetic tree method is the Neighbor-joining method, sequence label is taxonomic name, and maximum sequence difference is 0.75. Open dot represents eudicot (true dicotyledons) and black filled dot means unknown (our sequence) in the genetic tree (Figs. 3 and 4).

#### Chemical Analysis

**Extraction Procedure—Quantitative Analysis**—2 g of spleen and liver were homogenized with 5 mL H<sub>2</sub>O and dextromethorphan (I.S., 100 mg/L) was added. The sample was mixed with 3 mL 10% trichloroacetic acid for its denaturation. After centrifugation at 1150  $\times$  g for 5 min, upper layer was transferred to a new tube. The solution was basified with 0.2 mL of 6N aqueous sodium hydroxide and was extracted with 10 mL of ethylacetate. After phase separation by centrifugation, the organic layer was transferred and evaporated to dryness. The residue was injected to gas chromatography–mass spectrometry (GC–MS). The comparison for identification of taraxasterol was made with the Wiley library and the retention times of standard samples.

For target drugs, the quantitation was calculated on the ratio of peak areas of doxylamine and zolpidem to those of internal standard (dextromethorphan). Doxylamine, zolpidem, and dextromethorphan were extracted at 58, 235, and 271 m/z respectively for quantitation. Three concentration points (5, 10, and 50 mg/L) were used for the calibration. The coefficient ( $r^2$ ) of calibration determination curve was 0.99.

**Qualitative Analysis**—In case of gastric contents, except denaturation step, qualitative analysis was followed upper sequence.

**GC-MS System and Condition**—The GC-MS system consisted of an Agilent series 6890 GC interfaced with a model 5973 Agilent mass selective detector. GC-MS analysis was conducted using a HP-5 MS column (cross-linked 5% methyl phenylsilicone, 30 m long, 0.25 mm i.d., 0.25 mm film-thickness). The column oven temperature was programmed to operate at an initial temperature of 100°C for 0.5 min, increased to 280°C at a heating rate of 20°C/min, and then maintained at 280°C for 10 min. The total run time of this method was 35 min. The temperatures of the injector port and the interface were set at 270°C and 280°C, respectively. The flow rate of the carrier gas (helium) was set at 1 mL/min, the

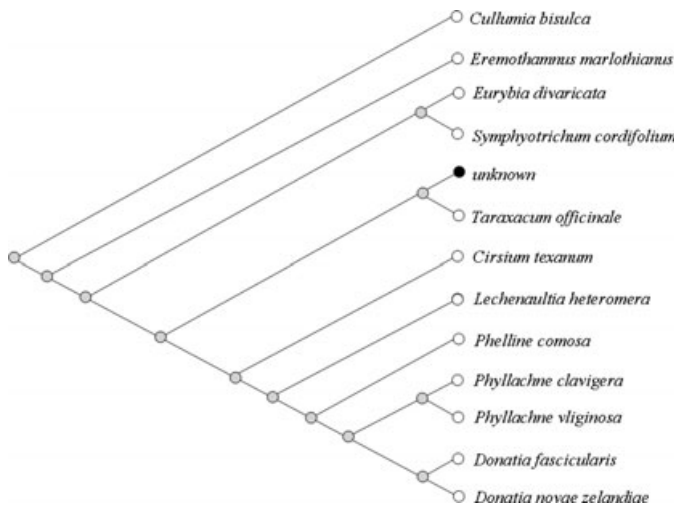


FIG. 3—Part of the genetic tree acquired from BLASTn which aligns our query data of *rbcL* (400 bp) sequences with database in NCBI. Our query data are named as “unknown” and topology is slanted type (tree method: Neighbor-joining method, sequence label: taxonomic name, maximum sequence difference: 0.75. Open dot represents eudicot and black filled dot unknown).

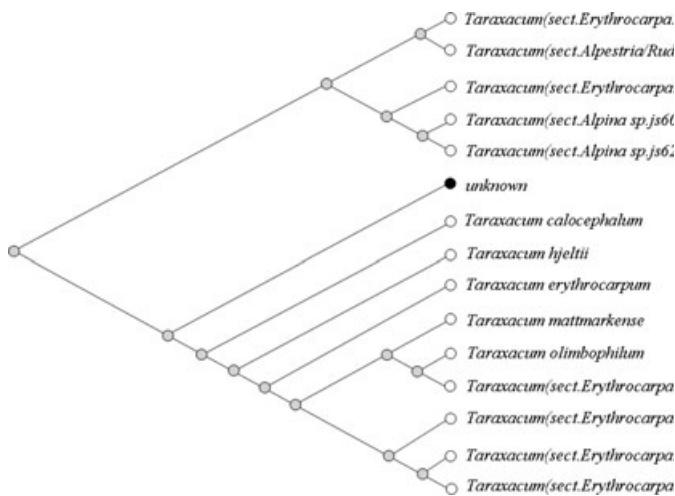


FIG. 4—Part of the genetic tree acquired from BLASTn which aligns our query data of *trnL-trnF* IGS sequences obtained from gastric contents with database in NCBI. Our query data are named as “unknown” and topology is slanted type (tree method: Neighbor-joining method, sequence label: taxonomic name, maximum sequence difference: 0.75. Open dot represents eudicot and black filled dot unknown).

ionization energy was set to 70 eV. Mass spectra were collected via scanning from *m/z* 50 to 550, at 2 sec intervals.

## Results and Discussion

The plant tissue particles were extracted from the gastric contents of the victim. Nuclear genomic DNA and chloroplast genomic DNA were prepared simultaneously from the plant tissues. The genomic DNA concentration was insufficient for a verification of its intensity value via agarose gel electrophoresis and it was difficult to discriminate plant DNA from the victim’s human DNA. There was some chance that stomach acids remained in the isolated DNA solution, and so a few template DNAs (2.5  $\mu$ L) were employed in the PCR reaction. Primer pairs of ITS, 18S rRNA, *rbcL*, and *trnLF* were utilized for the amplification of each gene, but proved to be unproductive. In order to increase the magnitude of the amplification products, the second PCR method, in which a portion of the initial PCR products was used as a template, was applied. One microliter of the initial PCR products was used as the template for the secondary PCR reaction. Nested reverse primers of *rbcL* and 18S rRNA were used for the survey of the second PCR specificity. The reverse primer referred to in a previous paper by Rollo et al. (*rbcL19* and *Angio2R* in Ref. 15), which makes an *rbcL* of 209 bp and makes an 18S rRNA of 157bp, made amplicons successfully from the first PCR product of *rbcL* and 18S rRNA (Fig. 5). First PCR products of 209 bp-sized *rbcL* and 157bp-sized 18S rRNA appeared more abundant than longer amplicons did. Sequence comparisons of  $\leq 200$  bp-sized amplicons might be inefficient in species identification, due to their small sizes because small sizes harbor little information. The results of the second PCR proved adequate for the accomplishment of the next sequencing reactions. The outcomes of the second PCR of *rbcL* from all G, P, and S samples (in Fig. 5) and that of *trnLF* from only P sample were successfully sequenced, but those of ITS and 18S rRNA were not. Sequences of *rbcL* and *trnLF* were queried as to whether NCBI possessed sequences related to them (19, Table 1).

In *rbcL*, sequences of *rbcL* from all G, P, and S samples were identical among them. Our 446 bp sequences including both primer-binding sites exhibited 98% homology with *rbcL* of *Lactuca sativa* (439/445 bp, Table 1) and 400 bp sequences exhibited 99% homology with that of *Ainsliaea acerifolia* (397/400 bp). Every

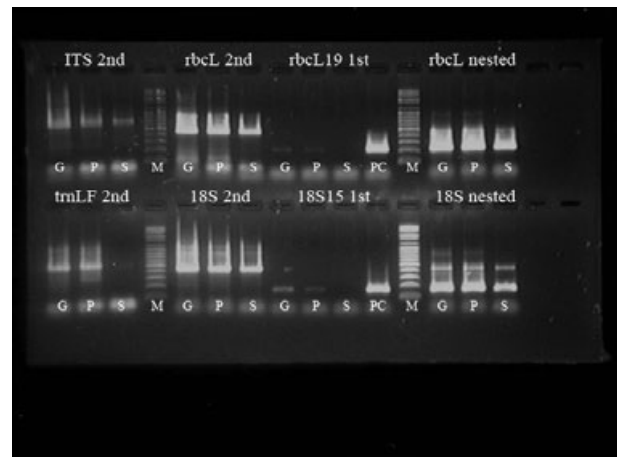


FIG. 5—The results of second PCR (ITS, *rbcL*, *trnLF* and 18S rRNA) and nested PCR (*rbcL* and 18S rRNA) of gastric samples (G: green granules, P: pellets, S: supernatant). First means the first PCR and second means the second PCR. “*rbcL19 first*” means the first PCR products of 209 bp-sized *rbcL*.

TABLE 1—NCBI-registered taxa and their accession numbers which had highest homologies with our *rbcL* sequence data of gastric content were displayed.

Gene (Segment Size)	NCBI Accession #	Taxa	Identities (Homology, %)
<i>rbcL</i> (380 bp)	AY395562.1	<i>Taraxacum officinale</i> (dandelion)	380/380 (100)
<i>rbcL</i> (400 bp)	AY874432.1	<i>Ainsliaea acerifolia</i> (Danpoong-Chwi)	397/400 (99)
<i>rbcL</i> (446 bp)	DQ383816.1	<i>Lactuca sativa</i> (lettuce)	439/445 (98)

All trimmed segments showed highest homology with different taxa mutually dependent on their compared sizes.

species exhibiting sufficiently high degrees of homology with our 400 bp *rbcL* in the NCBI were members of the Asterales order. In particular, six species such as *Cullumia bisulca*, *Eremothamnus marlothianus*, *Eurybia divaricata*, *Symphytotrichum cordifolium*, *Taraxacum officinale*, and *Cirsium texanum* were included in the Asteraceae family (Fig. 3). It has been estimated that the *rbcL* gene can be used to determine the order or family of unknown plant evidence and this study proves this assumption.

In *trnLF*, all species exhibiting higher levels of homology with our *trnLF* in the NCBI database were found to be members of the *Taraxacum* genus (Fig. 4). These facts indicate that the study of *rbcL* is not as advanced as the study of *trnLF*, or that registered sequences in NCBI as the same gene are read from different PCR primer pairs by various researchers, or possibly that *rbcL*, which

shows a low evolutionary rate, cannot be used for the discrimination of genus or species. However *trnLF*, which has a rapid evolutionary rate, was capable of discriminating between species and intraspecies in this study. Different PCR primer pairs that bind at various sites in the same gene make it difficult to compare uniform nucleotides, hence the lead researcher to obscure directions. If our 446bp *rbcL* sequence was trimmed away 46 and 20 nucleotides at 5' and 3' region, respectively, it showed 100% homology with *rbcL* of *T. officinale* [AY395562] (Table 1). The reason for these phenomena is thought to be that sequences which showed higher homologies with our queried sequences in BLAST of NCBI are ranked in due order according to their compared sequence sizes.

After securing the sequences of *rbcL* and *trnLF* from the stomach contents (about 3 days later), we conducted control experiments in order to compare the sequences of the stomach contents with the sequences of three dandelion reference samples cultivated within the victim's house. These time-differential experiments were to prevent the contamination of case samples by control samples. In *rbcL*, the sequences from stomach contents showed 100% homology with the sequences from three dandelion references, and showed no nucleotide length polymorphisms, such as insertions and/or deletions (data not shown). However, in *trnLF*, the sequences from the stomach contents did not manifest 100% homology with the sequences from the three dandelion references. Control #1 exhibited a 98.7% homology with the stomach sequences (1 bp insertion at 354 bp, 4 transitions/1 transversion, Fig. 6) but controls #2 and #3 showed differences in size (77 bp shorter than stomach sequences) as well as in sequences (1 bp

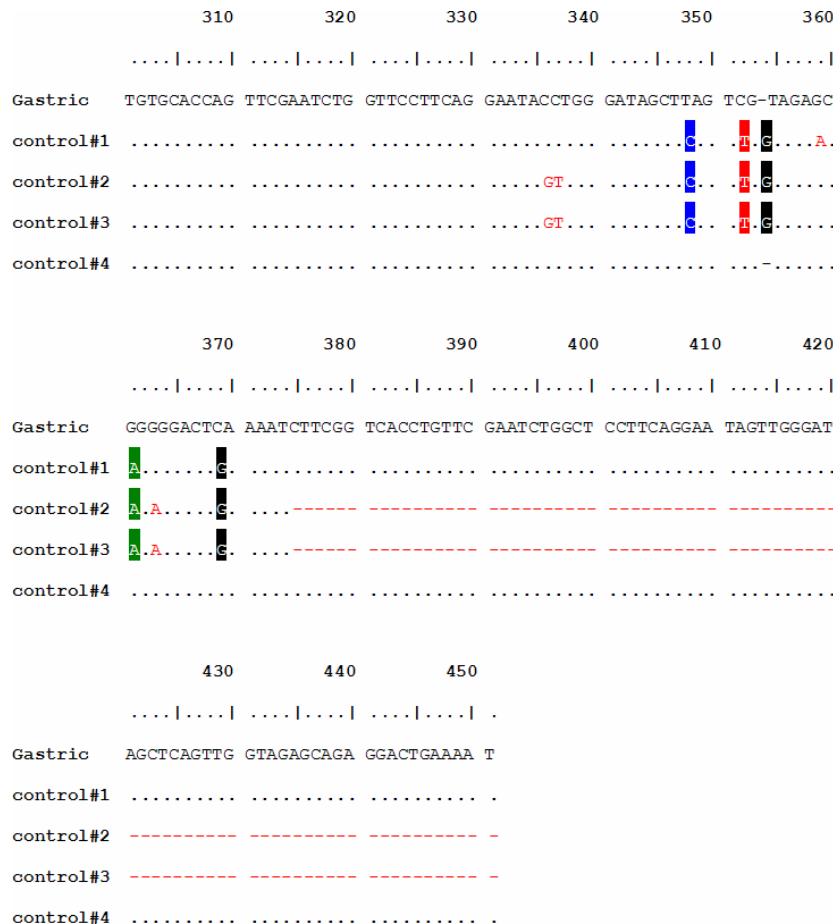


FIG. 6—Nucleotide sequences of *trnL-trnF* IGS of plant tissues from the gastric contents (named as “Gastric”) and of control dandelions.

TABLE 2—The size of each amplicon from gastric contents (GC) and control samples (Con.) are shown in base pair.

Amplicon	GC	Con. #1	Con. #2	Con. #3	Con. #4	Con. #5	Con. #6	Con. #7	Con. #8	Con. #9	Con. #10	Con. #11
<i>rbcL</i>	446	446	446	446	446	446	446	446	446	446	446	446
<i>trnL-trnF</i>	492	493	416	416	492	493	493	493	493	416	493	493

Detection frequency of 492bp *trnL-trnF* IGS is 16.7%.

insertion at 354 bp, 7 transitions/4 transversions). Seventy-three base pairs of the *trnL* 3' end regions from controls #2 and #3 were repeated next to the *trnF* gene of the stomach sequences and control #1 (Fig. 6). They differed primarily as the result of a polymorphic tRNA pseudogene located next to the *trnF* gene (20).

Looking for the same sequences with the *trnL* from the stomach contents, we asked the police to collect more dandelion samples cultivated within the victim's house. Many leaves were collected piece by piece, and eight randomly chosen leaves (Fig. 2) were subjected to the same experiments. Among eight samples, only one had the same sequence with the *trnL* from the stomach contents (Table 2, Con #4), another six samples displayed the same sequence with control #1 (Con #5, 6, 7, 8, 10, 11), and the last one (Con #9) showed the same sequence with control #2, #3. Needless to say, the *rbcL* sequences of the eight additional control samples showed no sequence variations with gastric sequences. According to the opinion of the plant morphologist, the species of dandelion cultivated within the victim's house was *T. officinale*.

The segment of *rbcL* gene can be used to determine the family or subfamily of unknown plant evidence. Discrepant results from the query of our *rbcL* (380 bp or 400 bp or 446 bp, Table 1) and from the query of our *trnL* to NCBI might be caused by the lack of international agreement about plant DNA barcodes which can standardize PCR primer pairs of the same gene. Nucleotide sequences read by different primer pairs for the same gene manage to guide us to the discrimination of the family or genus of unknown samples.

Universal markers including ITS (intergenic spacer region) and 18S rRNA are powerful with regard to the resolution of the phylogenetic relationships between plants (7–9), but were unsuitable for the DNA analysis of gastric contents, because their primers could bind to the ITS and 18S rRNA of humans (refer to NCBI accession number U13369) (21,22). A few of the gastric epithelial cells from the victim remained mixed with the plant cells, which made it

difficult to determine the ITS and 18S rRNA sequence of the plant evidence via direct sequencing. From this study, markers on cpDNA are recommended in attempts to identify gastric contents and to identify other plant evidence from human cell mixed samples.

Gastric content, spleen, and liver of the victim were submitted to analyze whether they had drug or toxic compounds. Doxylamine and zolpidem, kinds of hypnotics, were detected in gastric content (data not shown). At quantitative analysis in spleen and liver, we found that the concentrations of doxylamine were 11.4 and 27.5 mg/kg, respectively and the concentration of zolpidem was <0.10 mg/kg in the spleen and liver. It was reported that the assumed therapeutic concentration of doxylamine in liver ranged from 0.2 to 1.7 mg/kg which were calculated from postmortem specimens in nonintoxication cases (25,26). The concentration of doxylamine in victim specimens was far higher than the assumed therapeutic concentration in liver and was evaluated as a toxic level. In the case of zolpidem, it had been reported that the therapeutic concentration in blood was from 0.06 to 0.27 mg/L (27). The concentration of zolpidem in victim specimens was lower than therapeutic concentration in blood and so was evaluated as therapeutic level.

The mass spectrum from the GC–MS analysis of the gastric contents is shown in Fig. 7. The comparison for identification was made with the Wiley library and the retention times of standard samples. Sisosterol and stigmasterol substances of dandelion were also detected from the gastric juice of the victim by GC–MS analysis (data not shown). Although taraxasterol is a marker compound of dandelion, many plants such as *Daucus carota* (carrot), *Glycine max* (soybean), *Vitis vinifera* (grape), *Helianthus annuus* (sunflower), *Lactuca sativa* (lettuce), etc., have taraxasterol (23).

From these DNA and GC–MS results, it was assumed that raw dandelion had been used as a masking vehicle for excess sleeping drugs. Food remains in the stomach for approximately 2–6 h in what amounts to an acid bath, in which mechanical churning and

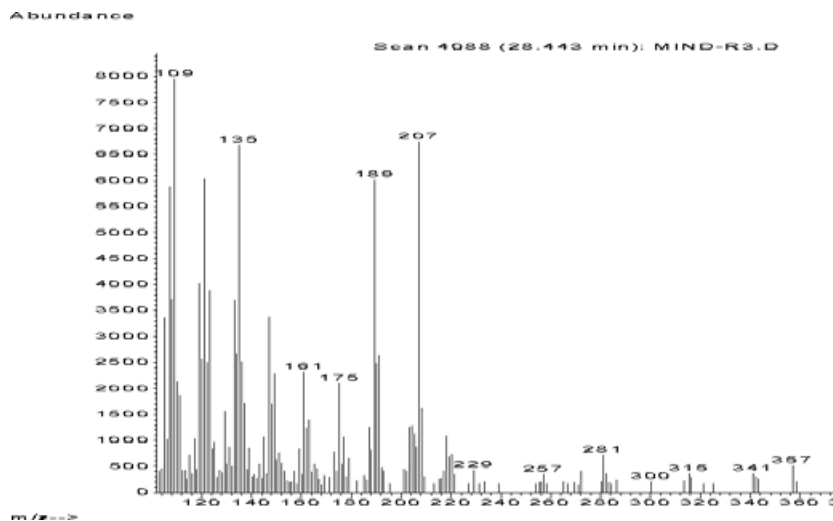


FIG. 7—Mass spectrum of taraxasterol from the gastric contents. The comparison for identification was made with the Wiley library and the retention times of standard samples.

grinding occurs. Some factors affecting the rate with which the stomach empties include caloric content, food acidity, meal volume, gender, etc. (24). The combination of the victim's empty stomach and hypochlorhydria symptom that was chronic with the victim and the unripe juice, in this case, allowed for the effective isolation of the dandelion DNA.

#### Acknowledgments

We want to thank Mu Yeol Kim (the professor in Chonbuk National University) for the morphological species identification of dandelion samples cultivated at the victim's house.

#### References

- Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC. Analysis of the VNTR locus DIS80 by the PCR followed by high-resolution PAGE. *Am J Hum Genet* 1991;48:137–44.
- Edwards A, Civitello A, Hammond HA, Caskey CT. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *Am J Hum Genet* 1991;49:746–56.
- Hsieh HM, Chiang HL, Tsai LC, Lai SY, Huang NE, Linacre A, et al. Cytochrome b gene for species identification of the conservation animals. *Forensic Sci Int* 2001;122:7–18.
- Virtanen V, Korpelainen H, Kostamo K. Forensic botany: usability of bryophyte material in forensic studies. *Forensic Sci Int* 2007;172(2–3):161–3.
- Craft KJ, Owens JD, Ashley MV. Application of plant DNA markers in forensic botany: genetic comparison of *Quercus* evidence leaves to crime scene trees using microsatellites. *Forensic Sci Int* 2007;165: 64–70.
- White TJ, Bruns T, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. *PCR protocols: a guide to methods and applications*. New York: Academic Press, Inc., 1990:315–22.
- Sun Y, Skinner DZ, Liang GH, Hulbert SH. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theor Applied Genet* 1994;89:26–32.
- Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS, Donoghue MJ. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Ann Mo Bot Gard* 1995;82(2):247–77.
- Soltis DE, Soltis PS, Nickrent DL, Johnson LA, Hahn WJ, Hoot SB, et al. Angiosperm phylogeny inferred from 18S ribosomal DNA sequence. *Ann Mo Bot Gard* 1997;84(1):1–49.
- Clegg MT. Chloroplast gene sequences and the study of plant evolution. *Proc Natl Acad Sci USA* 1993;90:363–7.
- Clegg MT, Gaut BS, Learn GH Jr, Morton BR. Rates and patterns of chloroplast DNA evolution. *Proc Natl Acad Sci USA* 1994;91:6795–801.
- Taberlet P, Gielly L, Pautou G, Bouvet J. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol Biol* 1991;17:1105–9.
- Sang T, Crawford DJ, Stuessy TF. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *Am J Bot* 1997;84:1120–36.
- Gielly L, Taberlet P. The use of chloroplast DNA to resolve plant phylogenies: noncoding versus *rbcL* sequences. *Mol Biol Evol* 1994; 11:769–77.
- Rollo F, Ubaldi M, Ermini L, Marota I. Otzi's last meals: DNA analysis of the intestinal content of the Neolithic glacier mummy from the Alps. *Proc Natl Acad Sci USA* 2002;99:12594–9.
- Tippmann HF. Analysis for free: comparing programs for sequence analysis. *Brief Bioinform* 2004;5:82–7.
- Kumar S, Tamura K, Nei M. MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* 2004;5:150–63.
- Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, et al. Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Res* 2003;31:3497–500.
- Altschul SF, Madden TL, Alejandro AS, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997;25:3389–402.
- Håkan W. Chloroplast DNA variation and reticulate evolution in sexual and apomictic sections of dandelions. *Mol Ecol* 1999;8:2023–35.
- Gonzalez IL, Chambers C, Gorski JL, Stambolian D, Schmickel RD, Sylvester JE. Sequence and structure correlation of human ribosomal transcribed spacers. *J Mol Biol* 1990;212(1):27–35.
- Gonzalez IL, Wu S, Li WM, Kuo BA, Sylvester JE. Human ribosomal RNA intergenic spacer sequence. *Nucleic Acids Res* 1992;20:5846.
- Duke JA. *Handbook of phytochemical constituents of GRAS herbs and other economic plants*. Boca Raton: CRC Press, 1992.
- Coyle HM. *Forensic botany—principles and applications to criminal casework*. Boca Raton: CRC Press, 2005.
- Levine B, Klette K, Radentz S, Smith ML, Smialek JE. Antihistamine concentrations in postmortem blood and liver specimens. *Forensic Sci Int* 1996;81:73–6.
- Baselt RC. *Disposition of toxic drugs and chemicals in man*, 5th ed. Foster City, CA: Chemical Toxicology Institute, 2000.
- Lichtenwalner M, Tully R. A fatality involving zolpidem. *J Anal Toxicol* 1997;21:567–9.

Additional information and reprint requests:

Eun-jung Lee, Ph.D.

DNA Analysis Sector

Western District Office of National Institute of Scientific Investigation

111 Daeduk-Ri

Seosam-Myun, Jangseung-Gun

Chonnam 515-822

South Korea

E-mail: ejlee36@nisi.go.kr