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The problem of misidentification between edible and poisonous wild plants: Reports from the Mediterranean area

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ABSTRACT

Today, in many European countries, people are looking for wild edible plants to experience new tastes and flavors, by following the new trend of being green and environmentally friendly.

Young borage and spinach leaves can be easily confused by inexpert pickers with those of other plants, including poisonous ones, such as *Mandragora autumnalis* Bertol. (mandrake) or *Digitalis purpurea* L. (foxglove), common in southern and northern Italy respectively. In the last twenty years, several cases of intoxication by accidental ingestion of mandrake and foxglove have been reported. The purpose of this work was to perform a pharmacognostic characterization of young leaves from borage, mandrake, foxglove and spinach, by micro-morphological, molecular and phytochemical techniques.

The results showed that each of the three techniques investigated could be sufficient alone to provide useful information for the identification of poisonous species helping the medical staff to manage quickly the poisoned patients. However, the multi-disciplinary approach proposed could be very useful to asses the presence of poisonous plants in complex matrices, to build a database containing morphological, molecular and phyto-chemical data for the identification of poisonous species or in forensic toxicology, given their increasingly frequent use due to their low cost and relatively common availability.

1. Introduction

Nowadays in many European countries a new trend is spreading among people: the search for wild edible plants aimed at experiencing new tastes and flavors, but also to be green (Colombo et al., 2010). However, the cases of poisoning due to plant ingestion are growing worldwide, as reported by emergency rooms and poison control centers, and one of the main causes of this phenomenon is plant misidentification (Mezzasalma et al., 2017). In the Mediterranean region, different wild and semi-cultivated plant species, as well as species escaped from cultivation, are traditionally collected for culinary uses. Two common examples are *Borago officinalis* L. (borage) and *Spinacia oleracea* L. (spinach). However, the young leaves of borage and spinach may be easily confused by inexpert pickers with those of poisonous plants.

Borage is an annual herb originating in the Mediterranean region, but naturalized and widely cultivated throughout most of Europe. This is traditionally used for culinary and medicinal purposes and has a commercial value as oilseed. Due to a cucumber-like taste, borage leaves are also mixed in salads and used as vegetable in several European countries, such as Germany, Spanish (Aragón and Navarra), and Greece (Crete). In Italy, especially in the region of Liguria, borage is commonly used as filling of the traditional pasta named *ravioli* and *pansoti*, and as an ingredient of soups and vegetable pies (Cornara et al., 2009). Young leaves of borage are sometimes confused with those of other plants, such as the very poisonous *Mandragora autumnalis* Bertol. (mandrake) in Southern Italy and in Sicily, and *Digitalis purpurea* L. (foxglove) in Northern Italy. Patients who unintentionally eat leaves of

Abbreviations: LM, Light microscopy; SEM, Scanning electron microscopy; GC-MS, Gas chromatography-mass spectrometry; FID, Flame ionization detector; EDS, Energy dispersive system; BLAST, Basic Local Alignment Search Tool

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Table 1
List of analysed samples and sampling details.

Specimen	Species	Collection site	Collection year	A.N. rbcL	A.N. matK
FEM_001_BO	*Borago officinalis* L.	Agrigento, Sicilia	2017	LT992827	LT992716
FEM_002_BO	*Borago officinalis* L.	Botanic garden, Genova, Liguria	2017	LT992828	LT992715
FEM_003_BO	*Borago officinalis* L.	Botanic garden, Struppa Genova, Liguria	2017	LT992829	LT992717
FEM_004_BO	*Borago officinalis* L.	Carpi, Genova, Liguria	2017	LT992830	LT992718
FEM_005_BO	*Borago officinalis* L.	Botanic garden, Genova, Liguria	2016	LT992831	LT992719
FEM_006_DP	*Digitalis purpurea* L.	Agrigento, Sicilia	2017	LT992832	LT992615
FEM_007_DP	*Digitalis purpurea* L.	Botanic garden, Genova, Liguria	2017	LT992833	LT992616
FEM_008_DP	*Digitalis purpurea* L.	Botanic garden, Genova, Liguria	2016	LT992834	LT992617
FEM_009_MA	*Mandragora autumnalis* Bertol.	Agrigento, Sicilia	2017	LT992819	LT992812
FEM_010_MA	*Mandragora autumnalis* Bertol.	Natural reserve, Trapani, Sicilia	2017	LT992820	LT992811
FEM_011_MA	*Mandragora autumnalis* Bertol.	Botanic garden, Genova, Liguria	2017	LT992821	LT992813
FEM_012_MA	*Mandragora autumnalis* Bertol.	Favignana, Sicilia	2017	LT992822	LT992814
FEM_013_MA	*Mandragora autumnalis* Bertol.	Favignana, Sicilia	2017	LT992823	LT992815
FEM_014_MA	*Mandragora autumnalis* Bertol.	Botanic garden, Genova, Liguria	2016	LT992824	LT992816
FEM_015_SO	*Spinacia oleracea* L.	Agrigento, Sicilia	2017	LT992825	LT992817
FEM_016_SO	*Spinacia oleracea* L.	Market, Genova, Liguria	2017	LT992826	LT992818

mandrake (the most common case), or foxglove (less frequently), mistaking them for the edible *B. officinalis*, turn to the Italian Poison Control Centers or Hospital Emergency Service showing anticholinergic symptoms. In the period 1995–2007, 50 cases of intoxication by accidental ingestion of mandrake, and 6 cases due to an accidental ingestion of foxglove, were reported in Italy (Colombo et al., 2009). Other cases were also reported in the island of Crete, where two patients consumed accidentally mandrake instead of the eatable borago (Tsiligianni et al., 2009).

By the end of 2017, cases of poisoning, due to the presence of mandrake leaves mixed with spinach in commercial frozen vegetable, were reported in Italy. In one case, the hallucinogen-tainted frozen spinach caused the hospitalization of 4 family members in Milan, but the presence in the batch of the poisonous mandrake, containing tropane alkaloids, was not proven (http://www.ansa.it/). In another circumstance, 7 people were hospitalized with symptoms of mental confusion, amnesia and nausea, after eating a vegetable soup (*minestrone*). Also in this case, the suspect of vegetables contaminated with madrake leaves was advanced (http://genova.repubblica.it/). Analyses performed on soup samples by a Public Health Service laboratory confirmed the presence of three hallucinogenic substances, i.e. atropine, scopolamine and norscopolamine. The same substances were also detected in biological samples from the same patients analysed at the Anti-Poison Center (Pavia, Italy). In the light of these findings, food poisoning was attributed to leaves of mandrake or of some other infesting plant (http://www.lastampa.it/).

In this study, we show pharmacognostic characterizations of borage, spinach, mandrake and foxglove, in their young stage of development, based on micro-morphological, phytochemical and molecular analyses. This study provides a multidisciplinary approach to the problem of misidentification among edible and poisonous wild plants. The reported protocols provide an integrate and reliable identification systems to identify poison plant species in complex matrices, which could be useful to different stakeholder categories involved in the diagnostics of poisonous plants, thus allowing a quick management of patients.

2. Material and methods

2.1. Plant material

Representative samples of young leaves from *Borago officinalis* L. (Boraginaceae), *Digitalis purpurea* L. (Schrophulariaceae), *Mandragora autumnalis* Bertol. (Solanaceae), and *Spinacia oleracea* L. (Chenopodiaceae), were obtained from plants growing in two different Italian regions (Liguria and Sicily), collected directly in the field or in botanical gardens. One of us (LC) taxonomically identified plant specimens collected in the field, following standard analytical keys (Pignatti, 1982). In the case of borage and spinach, samples from the Municipal Market of Genova (Italy) were also examined.

2.2. Macro- and micromorphologycal analyses

2.2.1. Light microscopy (LM)

Unprocessed plant material was observed by a Leica M205C stereomicroscope, coupled with EC3 camera and LAS EZ V1.6.0 image analysis software. A sample-clearing process was carried out on small leaf portions by using 5% aqueous hypochlorite for 15–30 min, followed by rinsing in distilled water and immediate microscopic examination. Sections mounted on glass slides were then observed by a Leica DM 2000 transmission-light microscope, coupled with a computer-driven DFC 320 camera (Leica Microsystems, Wetzlar, Germany).

2.2.2. Scanning electron microscopy (SEM)

Small pieces of approximately 1 cm of leaves from each species, representative of the median portions, were sectioned with a razor blade. Samples were fixed in FineFIX working solution (Milestone s.r.l., Bergamo, Italy) with 70% ethanol, left overnight at 4 °C (Chieco et al., 2013), dehydrated for 1 h through a graded series of ethanol: 80, 90, 95 and 100%, and finally dehydrated in CO_2 using a Critical Point Drier processor (K850 CPD 2M Strumenti S.r.l., Roma, Italy). Dried specimens were mounted on aluminium stubs using double stick tape, and coated with 10 nm gold. SEM analysis was carried out using a Vega3 Tescan-type LMU microscopy equipped with the X-ray energy dispersive system (EDS) Apollo XSD (Tescan USA Inc., Cranberry Twp, PA, USA) at an accelerating voltage of 20 kV.

2.3. Molecular analysis and DNA barcoding

Sixteen samples of *B. officinalis*, *D. purpurea*, *M. autumnalis* and *S. oleracea* (average number of samples per species: 4 ± 1.58, range 2–6) were collected at different Italian geographically distant sites. For each species, young leaves were collected directly in the field or in botanical gardens (Table 1), and the samples were used to identify species by DNA barcoding and evaluate genetic distances.

DNA was isolated from young leaves by using the Eurogold plant DNA Mini Kit (EuroClone s.p.a., Milan, Italy). Purified DNA concentration and quality for each sample were estimated with Eppendorf BioPhotometer®. The Consortium for the Barcode of Life-Plant Working Group (CBOL, 2009) recommended the two-locus combination of rbcL + matK (RuBisCo large subunit + maturase K) as standard plant barcodes. Each sample was analysed by sequencing these two coding plastidial regions. PCR amplification for each candidate marker was performed using Wonder taq Polymerase (EuroClone s.p.a., Milan, Italy) in a 25 μL reaction volume, according to the manufacturer's