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Ethnobotanical knowledge and nutritional properties of two edible wild plants from Central Italy: *Tordylium apulum* L. and *Urospermum dalechampii* (L.) F.W. Schmid

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Summary

Edible wild plants have provided an important source of food since time immemorial and have continued to do so until the present day. The study aimed to evaluate ethnobotanical uses and nutraceutical properties of *Tordylium apulum* L. and *Urospermum dalechampii* (L.) F.W. Schmidt. The ethnobotanical data collected showed that knowledge of these two species was not limited to alimentary use, but also included folk medicinal properties. Data obtained by nutraceutical analysis demonstrated how these species contain many of the so-called minor nutrients, such as carotenoids, tocopherol, and polyphenols. Furthermore in a comparison with some cultivated species, these species showed higher calcium, iron, and phosphorus values. *T. apulum* also showed significant vitamin A, polyphenol and ORAC values.

Introduction

In recent years many authors in Europe (Leonti et al., 2006; Pardo de Santayana et al., 2007; Herrera et al., 2014), and in Italy (Ranfa 2005a; 2005b; Nebel and Heinrich 2009; Łuczaj et al., 2013; Caneva et al., 2013; Vitalini et al., 2013; Ranfa et al., 2014; Maurizi et al., 2015), have shown renewed interest in edible wild plants (EWP), particularly for new cuisines, in folk medicine and in nutrition.

However, one of the aspects having attracted recent interest is the nutraceutical properties of EWP and the health benefits that derive from their habitual consumption (Romojaro et al., 2013). Some studies have shown that these species have significant nutraceutical value, being rich in mineral elements and bioactive compounds, with proven benefits for human health (Ranfa et al., 2011; Guarrera and Savo 2013; Ranfa et al., 2014; Maurizi et al., 2015). The nutraceutical components defined as natural ingredients are considered to be the source of health benefits over and above their nutritional contribution (Food and Culture Encyclopedia, 2010). Numerous phytochemicals (plant chemicals) that occur in fruits and vegetables are key issues of research, and evidence exists regarding their health-promoting properties, in particular anti-oxidising properties which are fundamental in the prevention of cancer and Ageing Related Diseases (ARDs) (Yang et al., 2001).

EWP play a fundamental role in the Mediterranean Diet, where anti-oxidising foods make up an important part of daily food intake (Shad et al., 2013; Ranfa et al., 2014) thanks to their high polyphenol and unsaturated fatty acid content (De Lorgeril and Salen, 2007). UNESCO recently (2010) declared the Mediterranean Diet to be part of the 'intangible cultural heritage of humanity', and in this diet EWP are habitually used in various recipes and dishes (Simopoulos, 2001).

This study analyses two of the most widely known EWPs, *T. apulum* and *U. dalechampii,* which have been collected in Umbria (Ranfa 2005a; 2005b; Ranfa et al., 2011; 2014). As research on these two species has been concentrated mainly on traditional uses in cooking and in folk medicine (Local Food Nutraceuticals Consortium, 2005; di Tizio et al., 2012; Dolina and Łuczaj, 2014), this study shows that, apart from their many ethnobotanical uses, these species also possess important nutraceutical properties, even when compared to some cultivated species.

Study area

The study was conducted in Umbria, a Region of Central Italy, situated between the Mediterranean and the Continental biogeographical regions. Umbria has a surface area of around 8,456 km^2 and a population of 895,259, and is rich in biodiversity, including many endemic species. Such diversity is reflected in the 102 Natura 2000 sites accounting for more than 15% of the regional surface (Orsomando et al., 2004; Cagiotti et al., 2010) (see Fig. 1 A).

Materials and methods

In Umbria the tradition of collecting EWPs in many of its natural and semi-natural areas is still alive. Various events such as fairs, themed exhibitions and local markets are held regularly to disseminate information and promote interest, and a wide range of publications is available on local wild vegetables and their uses (Fig. 1 B-C-D-E). Among such activities, the Department of Applied Biology of the University of Perugia has for many years now been collaborating with the Perugia Mycological and Field Naturalists'Association in organizing exhibitions of wild species, general theory courses and field trips.

Ethnobotanical analysis

Tordylium apulum L. (Umbelliferae family) and *Urospermum dalechampii* (L.) F.W. Schmidt (Compositae family) were chosen because they belong to two different families and because they are among the most widely known and collected plant species in Umbria (Ranfa, 2005a; 2005b; Ranfa et al., 2011; 2014). Ethnobotanical data were collected using an *ad hoc* semi-structured interview during the many events centred on EWPs which took place in Umbria in 2013 and 2014, for example the 'Mostra delle erbe spontanee' organized by the Perugia Mycological and Field Naturalists Association. Fifty questionnaires containing information on the two species were collected, 42 citations for *T. apulum* and 35 for *U. dalechampii* were obtained, with some interviewees completing the questionnaire for both species. The interviewees, 40 females and 10 males, average age 65 years, were asked to provide information on local names, alimentary use, folk medicine properties, parts used and traditions (see Fig. 2). They were selected from among the elderly population in rural areas who still retain knowledge of traditional uses, having spent their lives as farm workers, thus acquiring survival skills and practical knowledge by gathering, preparing and eating wild plants throughout the year. Some informants were able to provide citations

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Fig. 1: Study area and some local events on edible wild plants in Umbria, Central Italy.

on numerous wild plants. Various samples of the two species were collected, analysed with a stereomicroscope SX45 (Vision Engineering Limited), determined and classified according to the Checklist of Italian Vascular Flora (Conti et al., 2007). All the *exsiccata* of the aforementioned species are preserved in the *Erbario PERU* of the Università degli Studi di Perugia.

Nutraceutical analysis

Eight samples for each of the two species were collected, each sample consisting of at least 500 g of the edible portion of the plants, i.e. basal leaves, young leaves and stems, taken from more than 15 randomly chosen individual plants, all with a healthy external appearance. The samples were collected in various rural and semi-rural areas near Perugia, Assisi and on the slopes of Mt. Subasio (Assisi) on flat or undulating land among abandoned olive groves. The analyses were carried out in part on fresh material and in part on dry samples; in both cases the material was hand-ground.

The *moisture* content was determined by drying the leaves in an oven at 100 °C until constant weight was obtained according to the Association of Official Analytical Chemists AOAC methods (AOAC, 1990). Crude *protein* content was calculated using the conversion factor 6.25 (N×6.25) from the nitrogen content determined by the Kjeldahl method (AOAC, 1990). Total *lipid* content was evaluated using chloroform/methanol extraction based on the Bligh and Dyer procedure as described in the AOAC method (AOAC, 1990). *Ash* content was determined according to the AOAC method (AOAC, 1990) by incineration in a muffle furnace at 500 °C for 6 h. Total *dietary fibre* was determined by the enzymatic-gravimetric method according to the AOAC method (AOAC, 1995). *Carbohydrates* were calculated by difference.

According to the AOAC method (AOAC, 2006) *iron*, *calcium* and *magnesium* determination was conducted via a AA-6800 Model flame (air-acetylene) atomic absorption spectrophotometer (AAS) (Shimadzu, Kyoto, Japan) on the ashes, obtained from 4 g of each lyophilised sample, dissolved in 10 mL of 6 N of hydrochloric acid transferring the solution into a 50 mL volumetric flask and adjusting the volume with distilled water. Calcium, magnesium and iron certified stock standard solutions, 1000 ppm in HNO_3 (Carlo Erba, Milan, Italy) were used. For calcium analysis, lanthanum chloride (5%) was added to each sample to avoid interference with the phosphate. Standard working solutions were prepared according to the user manual in order to obtain the calibration curve. *Sodium* and *potassium* determination was conducted using a PFP7 Flame Photometer (Bibby Scientific, Jenway, Techne Inc., UK) on the same solution and certified stock industrial standard solutions were purchased from Jenway (Jenway, Essex, UK). Analysis accuracy was confirmed for all metals studied using certified standard reference material (NIST-SRM-1573rd, tomato leaves).

Phosphorus content was determined colorimetrically on 0.2 g of each lyophilised sample reading the absorbance at 650 nm using ammonium molybdate, hydroquinone and sodium sulphide solutions according to the AOAC methods (AOAC, 1990).

Polyphenols were determined by the Folin-Ciocalteu method with spectroscopic measurement at 760 nm (Varian UV/Vis 50 Cary Bio model, Palo Alto, CA, USA). The results were expressed as mg of gallic acid equivalent (GAE) per 100 g fresh weight (Singleton and Rossi, 1965). The assay was carried out on 250 mg of each lyophilised sample.

The values obtained were compared with the data relative to other EWPs analysed in Ranfa et al. (2014) and with some cultivated species (Inran; Ieo).

Principal components with antioxidant functions and total antioxidant capacity were assessed by Oxygen Radical Absorbance Capacity (ORAC) method (Ou et al., 2001; Usda).

α-Tocopherol (vitamin E) and β-carotene (provitamin A) were determined by a modified NP-HPLC method (Redi, 1999) operating with a Spectra Physics SP8800 Model ternary pump (Mountain View, CA, USA), and a sample-injection valve Rheodyne 7125 (Cotati, CA, USA) with a 10 L injection loop. Two detectors were joined in series in order to determine α-tocopherol and β-carotene with a single run. α-Tocopherol was detected by a fluorometric detector (FLD) (Jasco FP-920 Model, Tokyo, Japan) equipped with of a light source of 150 W xenon lamp, $\lambda_{Ex.}$ = 290 nm and $\lambda_{Em.}$ = 330 nm while β-carotene was detected by a UV/Vis detector (Shimadzu SPD-10 A VP Model, Kyoto, Japan) set at 450 nm. A personal computer with appropriate software (Varian Star Chromatograph ver. 6.00 Walnut Creek, CA, USA) for data acquisition and processing was used. Iso-

cratic separation was achieved on a Merck Hibar, LiChrosorb Si 60, 250 mm × 4 mm i.d., 5 m particle size column (Merck, Darmstadt, Germany) using a mixture of *n*-hexane and 2-propanol (98:2, v/v) as eluent at a flow-rate of 1 mL/min. The standards stock solution of α-tocopherol containing 500 mg/mL was prepared by dissolving 50 mg of α-tocopherol (Calbiochem a brand of EMD Biosciences, Inc., an affiliate of Merck KGaA, Darmstadt, Germany) in a 100 mL volumetric flask with absolute ethanol (J.T. BAKER, Mallinckrodt Baker, Milan, Italy). Solutions containing 0.25, 0.50, 1.00, 2.00, 4.00 and 8.00 mg/mL of α-tocopherol used for the calibration curve were prepared by appropriate dilution with *n*-hexane (J.T. Baker, Mallinckrodt Baker, Milan, Italy) the stock solution. The standard stock solution of β-carotene (Calbiochem a brand of EMD Biosciences, Inc., an affiliate of Merck KGaA, Darmstadt, Germany) was prepared by dissolving 25.8 mg of β-carotene in 100 mL in a volumetric flask with *n*-hexane. Solutions containing 0.10, 0.20, 0.40, 0.80, 1.60 and 3.20 mg/mL of β-carotene used for the calibration curve were prepared by appropriate dilution of the stock solution with *n*-hexane, stored at -20 °C and made freshly every week. The extraction of α-tocopherol and β-carotene from the samples was carried out by the addition 25 mL of *n*-hexane to 0.5 g of each fresh sample placed in a 50 mL polypropylene graduate conical tube Falcon 2070 (Falcon, Boston Dickinson Labware, NJ, USA). The mixture was homogenised with an Ultra Turrax (IKA TI 25, Milan, Italy) to obtain a fine suspension and then was centrifuged (Eppendorf 5810 R Model, Milan, Italy) at 4000 rpm for 5 min. Two mL of the supernatant solution were transferred to a 10 mL flask and the *n*-hexane was evaporated under a mild stream of nitrogen at 30 °C; dry residue was dissolved in 0.5 mL of mobile phase. The resulting solution was analysed by NP-HPLC. The data, collected by Workstation software (Varian Star Chromatograph software version 6.00, Walnut Creek, CA, USA), were processed to quantify α-tocopherol and β-carotene with the method of external standardisation.

Total antioxidant capacity determination was carried out by Hydrophilic Oxygen Radical Absorbance Capacity (H-ORAC). The assay was conducted with a fluorescent microplate reader FLUO star Optima (BMG LABTH GmbH, Germany), provided with a pump, set to $\lambda_{Ex.}$ =485 nm and $\lambda_{Em.}$ =520 nm and interfaced with a computer provided with a MARS Data Analysis software ver. 2.00 (BMG LABTH GmbH, Germany) for data acquisition and processing. Costar 96 well black opaque plates (Corning Costar Corporation, Cambridge, MA, USA) were used. Two hundred μL of 60 nM fluorescein (Sigma-Adrich, Steinheim, Germany) working solution was added to all experiments designated in the wells. In addition, blank wells received 20 μL of 75 mM of phosphate buffer (pH 7.2), while standard wells received 20 μL of each 10, 20, 40 and 80 mM Trolox (a vitamin E analogue hydrosoluble, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxyl acid) (Sigma Aldrich, Steinheim, Germany) standard solution in phosphate buffer and each sample well received 20 μL of solution as prepared below. Four wells for the blank, four wells for each standard solution of Trolox and four wells for the three samples for a total of 32 wells were used. The plate was then allowed to equilibrate by incubating for 30 min at 37 °C and then the fluorescence in each well was read by plate reader (2 cycles). In the next cycle, the pump was programmed to inject 60 L of 160 mM of AAPH of freshly-prepared solution into the respective wells to start the reactions. The data were collected every 2.3 min by monitoring the fluorescence. Each sample extract was obtained adding 20 mL of a CH_3COCH_3: H_2O: CH_3COOH (70:29.5:0.5 v/v/v) mixture (PRIOR et al., 2003) to 0.5 g of each fresh leaf sample in a 50 mL polypropylene graduate conical tube (Falcon 2070 Blue Max, Boston Dickinson Labware, NJ, USA). The mixture was subjected to homogenisation (IKA TI 25 Ultra Turrex, Milan, Italy) to obtain a fine suspension. After centrifuging at 4000 rpm for 5 min (Eppendorf centrifuge 5810 R Model, Eppendorf, Milan, Italy) the supernatant was diluted 1:50 with phosphate buffer and the resulting solution was used for ORAC evaluation.

Each analytical determination was replicated three times except for the H-ORAC assay, which was carried out in quadruple. Mean values and standard deviations were reported.

Results and discussion

Ethnobotanical study

From the ethnobotanical information collected, it was found that *T. apulum*, locally known as *pimpinellone*, *zampa d'oca*, *saporitella*, or *ombrellini pugliesi* is mainly used in salads or to flavour savoury pies or vegetables soups. In the past its seeds were threaded to make bracelets and necklaces. In folk medicine it was recommended to prevent hair-loss and 'nervous illnesses', as well as to bring on menstruation and as an expectorant.

The leaves of *U. dalechampii*, also known as *grugno*, *boccione maggiore*, *lattugaccio*, *cotecacchia*, *grugnole* or *radicchione selvatico*, are cooked, mixed with other herbs (e.g. *Sonchus oleraceus*, *Tragopogon pratensis*, or *Apium nodiflorum*) and used in the same way as spinach. The root, which resembles a potato, is also used. The floral buds are preserved in brine and used in the same way as capers. The plant is also used to produce an infusion to aid digestion (Fig. 2).

	scientific names	botanical family	local italian names	part(s) used	preparation	folk medicine properties	citations
	*Tordylium apulum* L.	Umbelliferae	pimpinellone, pimpinella vellutata, zampa d'oca, saporitella, ombrellini pugliesi	leaves, whole aerial parts	raw in salads or boiled	for hair loss, soothing, expectorant	42
	*Urospermum dalechampii* (L.) F.W.Schmidt.	Compositae	grugno, boccione maggiore, lattugaccio, cotecacchia, grugnole or radicchione selvatico	leaves, floral buds, roots	boiled, raw in salads, filling savoury pie, floral buds used in the same way as capers	diuretic, digestive, tonic	35

Fig. 2: The two edible wild plants analyzed. Local use, common names, parts used, preparation, some folk medicine properties and number of *ad hoc* semi-structured interview collected.

Nutraceutical aspects

It was found that *U. dalechampii* has high values of iron, calcium and potassium. *T. apulum* has higher β-carotene and vitamin E values than for example *A. graveolens, B. vulgaris, C. intybus* (see Tab. 2). Furthermore, when comparing the two species' nutraceutical values with those of other EWPs (Ranfa et al., 2014) it was found that *U. dalechampii* contains significant protein values similar to *Sanguisorba minor* and that both species contain higher phosphorus values than *Bellis perennis*, *Bunias erucago* and *Chondrilla juncea*. Particular attention needs to be drawn to antioxidant capacity calculated by means of the ORAC method. Significant values were found particularly in *S. minor* whose antioxidant capacity was superior to cultivated species such as tomatoes, peppers and carrots (source Usda). Fiber content, which plays a role in improving nutrient bioavailability, was also considerable. (Tab. 1).

T. apulum showed significant total polyphenol and ORAC values. (Tab. 2).

Conclusion

The results obtained in this research demonstrate that the two species analyzed contain considerable nutraceutical properties to supplement a balanced daily diet, this being one of the principal reasons to preserve and divulge knowledge of EWPs. Their antioxidant capacity could certainly assist nutritionists and doctors in planning more balanced diets in an effort to prevent degenerative and chronic diseases.

Indeed EWPs are being investigated as potential food supplements in various developing countries so as to increase the quality of daily food for rural populations. However, today we are witnessing a nutritional transition in the world's poorest countries whereby traditional diets based on fruit and vegetables are being replaced by calorie-rich diets based on animal fats and sugars.

Another, no less important, aspect to consider is the environmental issue: our ethnobotanical heritage must be safeguarded as it represents an opportunity to preserve biodiversity, maintain the links between town and countryside, and encourage new forms of development such as environmental tourism (Caneva et al., 2013). It would be important to retain and reinforce local knowledge of these species so as to develop new, relevant, and effective ways to revitalize languages, cultures, and ethnobotanical knowledge within contemporary contexts.

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Tab. 1: Comparison of chemical composition and mineral content (mg/100 g of edible parts) values of *T. apulum* and *U. dalechampii* with those of other cultivated species

	T. apulum	*U. dalechampii*	celery (*Apium graveolens* L.)	chard (*Beta vulgaris* L.)	cultivated cichory (*Cichorium intybus* L.)	arugula (*Eruca sativa* Hill.)	lettuce (*Lactuca sativa* L.)
Chemical composition							
water	85.5±0.91	86.1±1.03	88.3[a]	89.3[a]	95[a]	91[a]	94.3[a]
Protein	2.2±0.08	2.1±0.07	2.3[a]	1.3[a]	1.2[a]	2.6[a]	1.8[a]
Lipids	0.3±0.02	0.2±0.03	0.2[a]	0.1[a]	0.1[a]	0.3[a]	0.4[a]
Carbohydrates	5.4±0.87	5.0±0.74	2.4[a]	2.8[a]	1.7[a]	3.9[a]	2.2[a]
Dietary fiber	5.0±0.23	5.1±0.28	1.6[a]	1.2[a]	3.1[b]	0.9[a]	1.5[a]
Mineral content							
Iron	2.5±0.74	3.0±0.91	0.5[a]	1[a]	1.5[a]	5.2[a]	0.8[a]
Calcium	153.7±12.51	174.9±15.73	31[a]	67[a]	150[a]	309[a]	45[a]
Phosphorus	24.2±4.21	29.5±6.48	45[a]	29[a]	26[a]	41[a]	31[a]
Sodium	42.0±3.21	68.4±2.47	140[a]	10[a]	7[a]	27[b]	9[a]
Potassium	344.5±28.16	287.5±32.35	280[a]	196[a]	180[a]	468[a]	240[a]
Magnesium	14.3±1.46	15.3±1.89	16[a]	38[a]	-	-	-

[a]source: Tabelle di composizione degli alimenti. INRAN Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione.
[b]source: Banca Dati di Composizione degli Alimenti per Studi Epidemiologici in Italia (BDA) – IEO Istituto Europeo di Oncologia

Tab. 2: β-carotene, vitamin E, total polyphenol content and ORAC values in 100 g of the edible parts of the two wild plants analyzed

	T. apulum	*U. dalechampii*	celery (*Apium graveolens* L.)	chard (*Beta vulgaris* L.)	cultivated cichory (*Cichorium intybus* L.)	arugula (*Eruca sativa* Hill.)	lettuce (*Lactuca sativa* L.)
β-Carotene (µg/100 g)	2,614±30	2,304±25	1242[b]	1578[b]	1315[b]	1422[b]	1374[a]
Vitamin A (µg*Ret. Eq.*/100 g)	436±6	384±5	207[a]	263[a]	267[a]	742[a]	229
α-Tocopherol (Vit.E) (mg/100 g)	2.8±0.04	2.1±0.08	0.48[b]	1.05[b]	2.26[b]	0.43[b]	0.66[b]
Total Polyphenols (mg GAE/100 g)	331±7.0	143±4.8	-	-	-	-	-
ORAC (µmolTE/100 g)	493±34	437±41	-	-	-	-	-

[a]source: Tabelle di composizione degli alimenti. INRAN Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione.
[b]source: Banca Dati di Composizione degli Alimenti per Studi Epidemiologici in Italia (BDA) – IEO Istituto Europeo di Oncologia

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