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TITOLO TESI

BIODIVERSITY STUDY ON WILD FOOD PLANTS TRADITIONALLY CONSUMED IN THE AREA OF BOLOGNA (EMILIA ROMAGNA REGION, ITALY) AND IN THE MIDDLE AGRI VALLEY (BASILICATA REGION, POTENZA PROVINCE, ITALY)

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INDEX

1 INTRODUCTION

1.1 ETHNOBOTANICAL STUDIES ABOUT WILD FOOD PLANTS

Before the so-called "economic boom" (1950 – 1970), Italy was mainly an agriculture-based economy and society. Poverty, dryness and wars made it difficult to meet subsistence needs (Cederna, 1980) and, therefore, edible wild plants represented an alternative food source or sometimes the only one (Targioni-Tozzetti, 1797). Wild food plant gathering practices and their way of consumption were slowly integrated into the customs of a territory, becoming part of the Traditional Local Knowledge (TLK). The process of industrialization and urbanization changed the way of living and society, which became less and less rural. The use of mechanized agriculture and the development of transport improved the availability of vegetables and, consequently, wild food plant practices and the related local knowledge, strongly connected with rural societies, almost totally disappeared. Furthermore, intensive agriculture, which generally involved extensive use of pesticides, and pollution, largely impaired wild flora biodiversity, reducing the availability of some wild plants used as food.

In the past, the majority of ethnobotanical research was preferentially focused on traditional medicinal plants (Sakarkar et al., 2011; Silva et al., 2011), giving less attention to wild food plants, however, over the last two decades, an increasing interest on this type of plants, even in modern societies, led to many local ethnobotanical studies (Paoletti et al., 1995; Pardo-de-Santayana et al., 2005; Łuczaj et al., 2013). The international political attention towards biodiversity topics and its link to nutrition and health (Convention on Biological Diversity in 1992, United Nation Decade on Biodiversity 2011-2020, Expo 2015) has surely contributed in driving forward wild food plants research.

Several researches demonstrated that many edible wild plants have nutritional or therapeutic value due to the presence of biologically active compounds (Sanchez-Mata et al., 2012; Pereira et al., 2011; The Local Food-Nutraceuticals Consortium, 2005). For example *Tamus communis* and *Humulus lupulus* contain respectively a high amount of citric and malic acids and antioxidants which are beneficial to human health due to their ability to chelate metals (Sanchez-Mata et al., 2012). *Borago officinalis* resulted to be a source of γ-linoleic acid and

other fatty acids that are precursors to mediators of the inflammatory response (Pereira et al., 2011). *Raphanus raphanistrum* showed anti-diabetic and anti-proliferation activities, while *Cynara cardunculus* demonstrated a high mood-disorder regulating activity (The Local Food-Nutraceuticals Consortium, 2005).

Wild food plants are generally characterized by having high nutritional and low energy values (with the exception of high-fat organs or in-season fat deposits) (Samson and Pretty 2006; McMichael et al., 2007). They are rich in antioxidants (Pieroni et al., 2002) and have a very high flavonoid content when compared with regular fresh vegetables, fruits and beverages commonly consumed in Europe (Table 1, Trichopoulou et al., 2000).

Table 1. Flavonoid content of Greek edible wild greens and vegetables, fruits and beverages commonly consumed in Europe (Trichopoulou et al., 2000)

	Myricetin mg/100g	Quercetin mg/100g	Kaempferol mg/100g	Luteolin mg/100g	Apigenin mg/100g
Foeniculum vulgare Mill	19.8	46.8	6.5	0.1	<0.07
Allium schoenoprasm L.	<0.03	10.4	12.5	0.3	<0.07
Sonchos oleraceus L.	3.6	16.0	3.8	6.5	3.8
Tordylium apulum	1.6	29.3	2.9	0.6	<0.08
Papaver rhoeas L.	1.1	26.3	2.3	0.1	0.1
Rumex obtusifolius L.	5.7	86.2	10.3	<0.02	<0.05
Daucus carota L.	0.4	1.1	0.2	34.1	12.6
Endive (Chicorium endiva L.)	<0.1	<0.1	4.6	<0.1	<0.2
Onion (Allium cepa L.)	<0.1	34.0-34.7	<0.2	<0.1	<0.2
Celery (Apium graveolens var. dulce Pers.)	<0.1	<0.1	<0.2	0.5-2.2	1.6-10-8
Apple (Malus pumila Mill.)	<0.1	2.0-3.6	<0.2	<0.1	<0.2
Red wine	0.9	1.1	<0.1	<0.05	<0.1
Black tea	0.3	1.4-1.7	1.4-1.6	<0.05	<0.1

Apart from the characteristic of higher yields and ecological adaptation, cultivated species are selected for having more palatable and sweeter taste and they contain less fiber and secondary compounds (Johns, 1990), respect to the relative wild species. These secondary compounds are chemicals with a wide range of potentially physiological and pharmacological interactions including toxic, anti-microbial, anti-inflammatory, astringent,

hypoglycemic, appetizing, diuretic, stomachic, carminative, laxative, aphrodisiac and more properties (Rivera et al., 2005).

Many wild food species were also proven to have important beneficial effects in preventing several chronic diseases of modern society, such as age-related and heart pathologies, diabetes and some types of cancer (The Local Food-Nutraceuticals Consortium, 2005; Trichopoulou et al., 2000; Finkel et al., 2000; Maritim et al., 2003). Besides, the proportion of medicinal plants among gathered food species was found to be twice or more than compared to the cultivated food species. The pharmacological properties of wild food plants are widely recognized in many cultures so much to include them deliberately them in the daily diet (Johns, 1990). These species fit into the modern concept of functional food.

Around 400 BC, Hippocrates firstly said that medicine may be food and food may be medicine. Nowadays this concept is well established (Etkin, 1996; Moerman, 1994; Rivera et al., 2005) and people, that traditionally gather wild greens, know about additional health beneficial properties of these plants.

In the Mediterranean area, the use of wild food plants was thoroughly investigated during the years 2003-2005 by the European Union-funded RUBIA Project (Pieroni et al., 2006). The selected study sites were Albania, Cyprus, Egypt, Greece, Italy, Morocco and Spain, countries in which the way of using wild plants, closely related to traditions, environment and cultural heritage, varied greatly. Although the most reported species were sometimes the same (e.g. belonging to Asteraceae and Lamiaceae families), their cultural importance changed among the different areas. The habit of using wild food plants played an important role in the life of Mediterranean rural people, however, the spread of plant folk uses has been progressively decreasing over the last generations, and is particularly evident in urban areas (Pieroni et al., 2006; Hadjichambis et al., 2008).

In Italy, a comparative ethnobotanical study on wild food plants analysing twenty-one communities located throughout the Italian peninsula including the islands of Sardinia and Sicily, produced a comprehensive picture of the country (Ghirardini et al., 2007). This survey showed that gathering, processing and consuming wild food plants are still important activities in all selected areas. Even if the wild plants are differently used among the studied areas, some ethnobotanical contact points exist among the various Italian regions especially represented by few wild species known and consumed by the majority of the considered

communities. These botanicals are *Asparagus acutifolius, Reichardia picroides, Cichorium intybus, Foeniculum vulgare, Sambucus nigra, Silene vulgare, Taraxacum officinalis, Urtica dioica, Sonchus* and *Valerianella* spp. In particular, *Borago officinalis* was reported to be used in all Southern and Northern Italian sites. In general, the results showed that in Southern Italy the erosion of wild TLK plants was happening at a slower rate than in Northern Italy and probably Southern Italians have a higher appreciation of wild vegetables, which have often a strong or bitter taste (Ghirardini et al., 2007).

Changes in the contemporary use of wild food plants in Italy and other European countries have also been recently studied (Łuczaj et al., 2012). The results confirmed that the traditional use of wild edibles is largely decreasing due to socioeconomic and ecological changes. The use of wild plants in Europe is often associated with famine periods as a mean of basic survival. In 19th and 20th centuries, several famine outbreaks occurred in different Europe regions (Łuczaj et al., 2012): the potato blight (1844-1849) that affected many potato-dependent countries, Athens famine (1941-1942), the Dutch winter of hunger (1944-1945) and the Balkans conflicts (1991-1995) that was hopefully the last European food crisis period.

The gradual decrease of the necessity of use from Ireland to Poland, a Russian famine (1892), World War I (1914-1918), the revolution and the establishment of Soviet Union (1921-1922), Ukraine famine, known as *holodomor* (1932-1933), the Spanish Civil War (1936-1939), World Was II, the siege of Leningrad (1941-1944), the of wild food plants associated with lifestyle changes, urbanization, large-scale farming brought to a decrease of plant knowledge and contact with nature. In large parts of Northern and Eastern Europe, people only collect wild fruits and mushrooms, while in Southern Europe some wild greens, such as *Taraxacum spp., Asparagus acutifolius L., Scolymus hispanicus L.* and *Silene vulgaris* are relatively popular (Łuczaj et al., 2012). Nonetheless, even in those communities where the gathering of some wild vegetables is still practiced, the knowledge is becoming fragmented and the practise is restricted almost exclusively to older people. Moreover, it has to be mentioned a new phenomena associated with wild plant use: ethnic minorities that retain their traditions during the immigration in new countries (Łuczaj et al., 2012).

Despite this, wild plants are becoming a part of the new thinking about food: they are very important as health food, and in food security and Slow Food movements. The interest in

wild food plants is actually gaining media attention. Numerous field guides and books were recently issued, workshops organized and web sites opened. The use of wild greens was also promoted by agritourism and local restaurants as a part of the local traditional heritage, as well avant-garde restaurants even tough, in this case, without any connection with the local culinary traditions. (Łuczaj et al., 2012).

Ultimately, it is possible to note that the use of wild food plants is strictly linked to man and his history and so characterised by a dynamic process.

1.2 METABOLIC SCREENING OF WILD FOOD PLANTS

1.2.1 BIOACTIVE COMPOUNDS

Food plants, defined as crops that grow wild or are cultivated and gathered or harvested to be consumed, are a very important source of metabolites which are provided to humans through the everyday diet.

Other than carbohydrates, fats, proteins and essential micronutrients as vitamins and mineral salts, the plant world provides many different phytochemicals such as phenols, terpenes, alcaloids, pigments (such as carotenoids chlorophylls. These include tens of thousands of compounds that belong to several chemical classes sometimes very different among botanical families. All these compounds are peculiar to the plant kingdom and not synthesized by human beings, usually have a low molecular weight, show a defence or vessillary functions in plants (secondary metabolites), have complementary and overlapping mechanisms of action. In general many of these compounds have protective effects on human health when their dietary intake is significant, showing biological properties as antioxidant activity, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation, modulation of hormone metabolism, reduction of blood pressure, antibacterial and antiviral activities.

Wild species may have a great potential as a source of bioactive compounds, dietary supplements or as ingredients for the production of functional foods. Moreover, as the nutritional scientific studies suggest, a good everyday diet is linked to diversification of the food base, therefore wild species may represent a valid alternative (while preserving local traditions) among the variety of already available vegetables. For all those reasons, the advance of knowledge about the chemical composition of wild food plants is regarded as an

important issue in nutritional and phytotherapy research (Salvatore et al., 2005; Ansari et al., 2005).

1.2.2 PHENOLIC COMPOUNDS

Polyphenols are naturally occurring compounds found largely in fruits, vegetables, cereals and beverages (Scalbert et al., 2005; Spencer et al., 2008). Polyphenols are secondary metabolites generally involved in plants in defense against ultraviolet radiation or aggression by pathogens or herbivores. More than 8,000 polyphenolic compounds have been identified in the different plant species. In food, polyphenols may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability. All plant phenolic compounds arise from a common intermediate, phenylalanine, or a close precursor, shikimic acid (Tsao, 2010). They may occur both in free and conjugated forms linked to one or more sugar residues via hydroxyl groups, although direct linkages of the sugar (polysaccharide or monosaccharide) to an aromatic carbon also exist. Association with other compounds, like carboxylic and organic acids, amines, lipids and linkage with other phenols is also common. Polyphenols may be classified into different groups as a function of the number of phenolic rings that they contain and on the basis of the structural elements that bind these rings to one another (Tsao, 2010). The main polyphenol classes include phenolic acids, flavonoids, stilbenes and lignans (Figure 1). The most abundant in vegetables are phenolic acids and flavonoids that represent respectively 30% and 60% of total polyphenols eaten within Mediterranean diet (Prakash and Gupta, 2009).

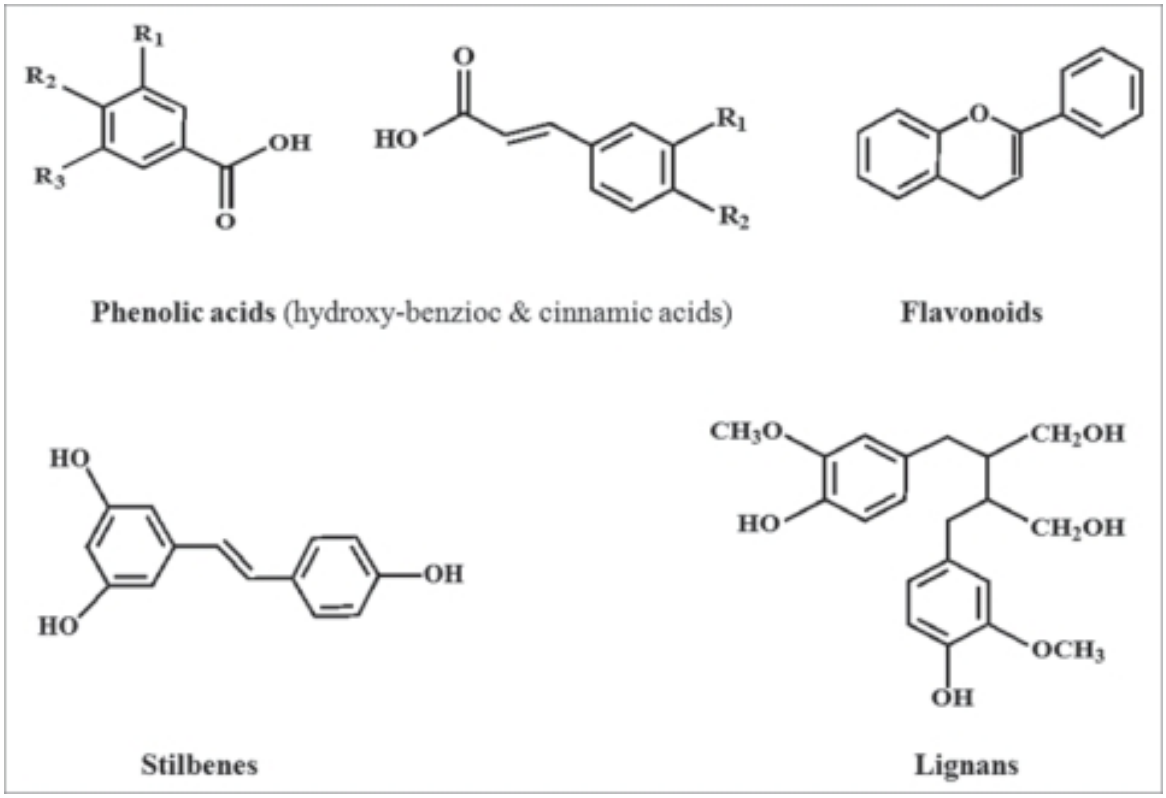

Figure 1. Chemical structures of the different classes of polyphenols.

1.2.3 FLAVONOIDS

Flavonoids are the largest class of polyphenols present in nature, of which over 5,000 different type of molecules, have been identified. Flavonoids are also the most studied group of polyphenols. This group has a common basic structure consisting of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle. Many of them are responsible for the attractive colours of the flowers, fruits and leaves. Based on the variation of the type of heterocycle ring involved, flavonoids may be divided into six subclasses: flavonols, flavones, flavanones, flavanols, anthocyanins and isoflavones (Figure 2).

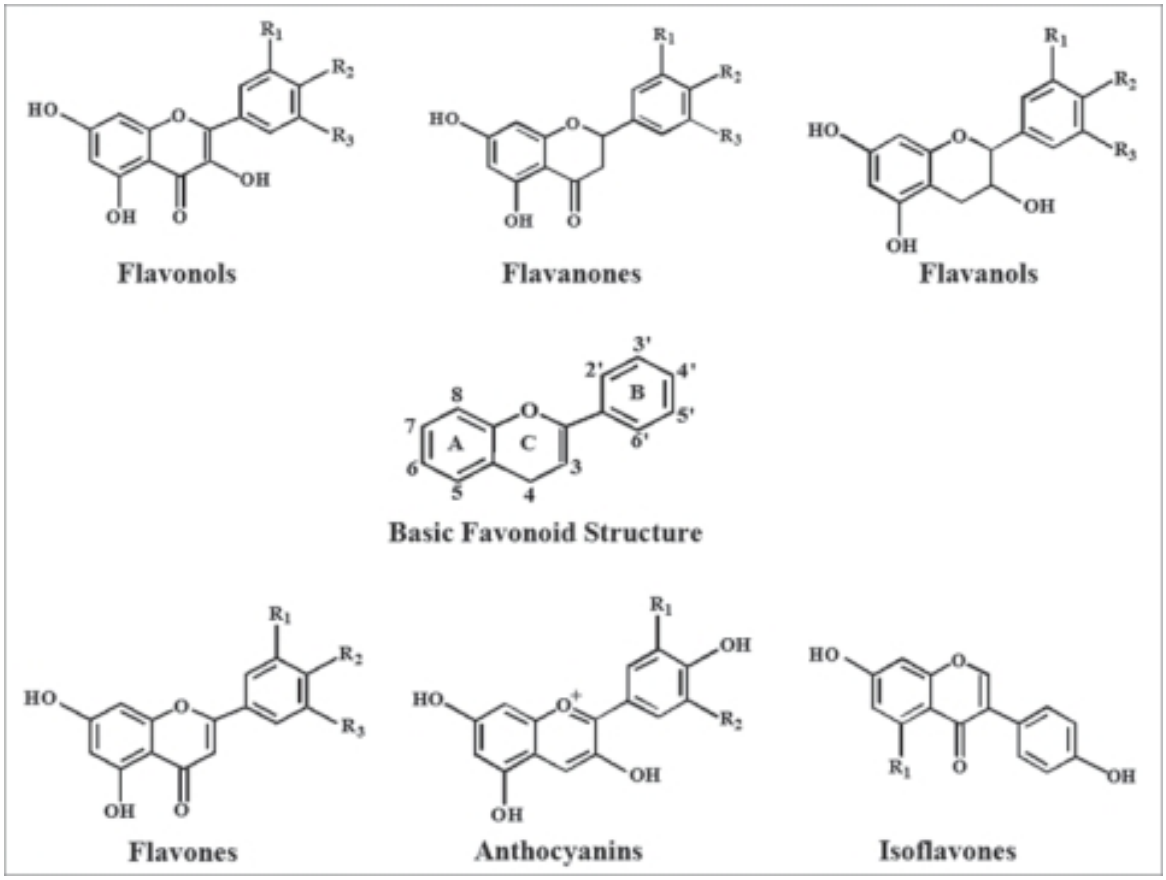

Figure 2. Chemical structures of sub-classes of flavonoids.

1.2.4 POLYPHENOL ANTIOXIDANT CAPACITY

Many of the plant-origin compounds and particularly secondary metabolites show biological activities, among which one of the most important both for plant and human life, is the antioxidant capacity.

In living organisms, antioxidant compounds are needed to prevent the formation and contrast the effect of reactive oxygen and nitrogen species, which are generated *in vivo* and cause damage to DNA, lipids, proteins, and other biomolecules. Endogenous antioxidant defences (superoxide dismutases, H_2O_2-removing enzymes, metal binding proteins) are inadequate to completely prevent damage, so diet-derived antioxidants are important in maintaining health. Several dietary compounds have been shown to be important antioxidants and in particular plant phenolics, among which especially flavonoids. There are increasing evidences that as antioxidants, polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated

with oxidative stress (Luqman et al., 2006; Pandey et al., 2009; Pandey et al., 2010. Polyphenols can accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components (Clifford et al., 2000) and increasing plasma antioxidant capacity. The plasma antioxidative capacity increases following the consumption of polyphenol-rich food and this may be explained by the presence of reducing polyphenols and their metabolites in plasma, by their effects upon concentrations of other reducing agents, or by their effect on the absorption of pro-oxidative food components, such as iron (Scalbert et al., 2005). Besides, epidemiological studies and associated meta-analyses, strongly suggested that long term consumption of diets rich in plant polyphenols offered some protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Graf et al., 2005; Arts et al., 2005).

1.2.5 CHLOROPHYLL AND CAROTENOIDS

Chlorophylls are the most abundant pigments in plants, cyanobacteria and algae and responsible of their green color. They are extremely important biomolecules, critical in photosynthesis allowing plants to absorb energy from light. Plants contain two different forms of chlorophylls: chlorophyll a that has a $-CH_3$ residue linked to the C7 of the porphyrin ring, and chlorophyll b that instead has a –CHO residue (Figure 3).

Chlorophylls in green foods are credited with numerous interventions in human health maintenance including reduction of blood pressure, blood sugar control, brain activation, antimutagenic and anticlastogenic effects, and more (Bailey, 2003). The antimutagenic properties of chlorophylls have been demonstrated in various assays, and clearly chlorophyll has potential to act as a chemopreventive compound in humans (Sarkar et al., 1996).

The dark green vegetables are simultaneously excellent sources of carotenoids which contribute together with the chlorophylls to the health-protective properties (Davies, 2004). Carotenoids (pigments from yellow to red color) are a ubiquitous component of all photosynthetic organisms as they are required for assembly and function of the photosynthetic apparatus. More than 600 carotenoids have been found in plants, subdivided into two main classes carotenes and xanthophylls, of which the most studied are beta-carotene, lycopene and lutein (Figure 4). Carotenoids are also a vital part of human diet as

antioxidants and precursors to vitamin A.

Several research have recognized carotenoids as playing an important role in the prevention of human diseases (Rao and Rao, 2007).

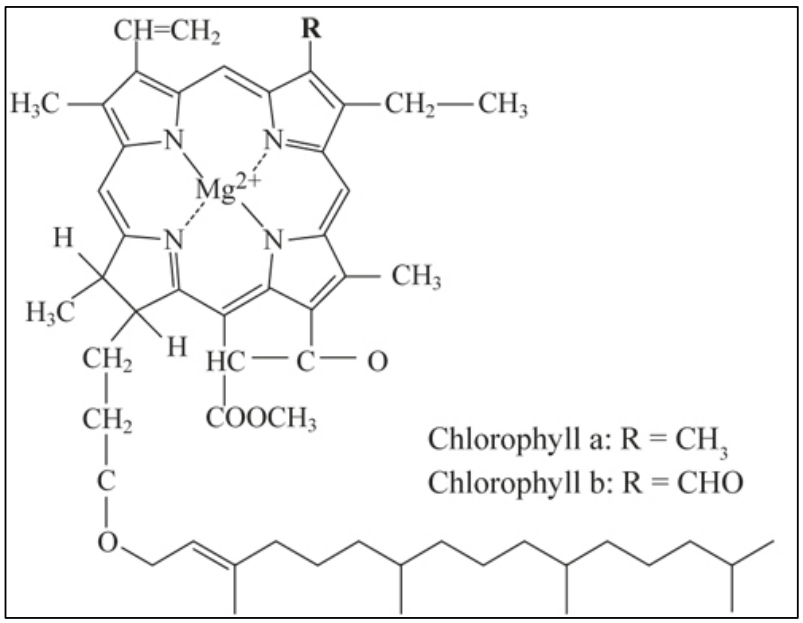

Figure 3. Chlorophyll a and chlorophyll b

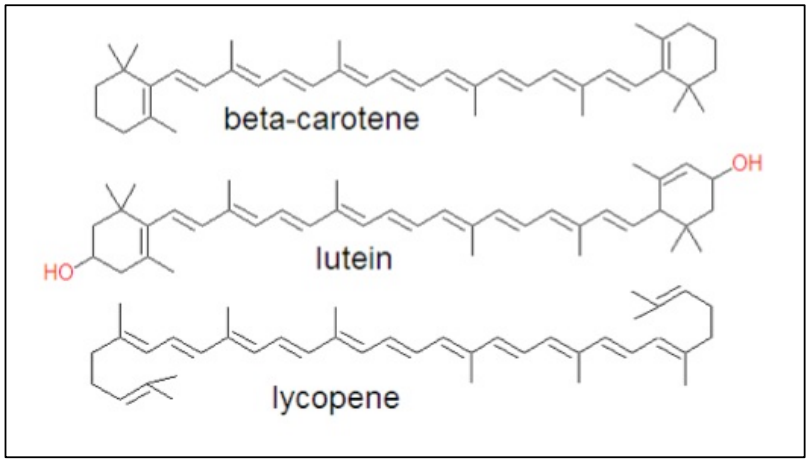

Figure 4. The most studied carotenoid molecules

1.2.6 PROTEINS

Plants are an important source of proteins and the need for new source of proteins, both for the growing human population and for domestic animal feeding, is rapidly increasing. In Italy proteins are included in the diet mainly through cereals (29%) and meat (27%) with only a small contribution by vegetables and fruits (8%) (investigation INRAN-SCAI 2005-2006) that usually have a low protein content. In countries with poverty and high birth rates, wild plants are considered the major source of dietary protein.

Proteins from plant (or animal) origin have been found to be physiologically active, either in a direct manner through their presence in the unprocessed food itself or after the release of their peptides from the original proteins by hydrolysis *in vivo* or *in vitro* which may occur during industrial food processing and fermentation and also during food digestion. A wide range of peptide activities were described (Hartmann and Meisel, 2007) including antimicrobial properties, blood pressure-lowering (ACE inhibitory) effects, cholesterol-lowering ability, antithrombotic and antioxidant activities, enhancement of mineral absorption/bioavailability, cyto- or immunomodulatory effects, and opioid activities. Moreover, some peptides are multifunctional and can exert more than one of the effects mentioned (Meisel, 2004).

1.3 AIM OF THE STUDY

The aim of this project was to record the Traditional Local Knowledge (TLK) concerning the traditional uses of wild food plants together with all the practices linked as gathering, processing, cooking, including the therapeutic uses, to re-discover plant species often under-utilized or neglected and to identify those with new or underestimated healthy effects for human people.

This research was performed in two areas belonging to the province of the city of Bologna (Emilia-Romagna region, Northern Italy) and in the Middle Agri Valley (Potenza province, Basilicata region, Southern Italy).

Up to now no research has been carried out on the use of wild food plants in these territories and, therefore, this study represents the first attempt to collect and save from oblivion an important part of the cultural heritage preserved by these populations.

Using an ethnobotanical approach, people still retaining TLK about wild food plants were interviewed recording the edible species and related practices used. The results of the two different investigated areas were compared.

The ethnobotanical study was followed by a metabolic screening of the samples of the wild food plant species most cited by the informants.

Finally, the most considerable and cited species in the area of Bologna, *Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell, was analysed by an *untargeted* metabolomics approach.

2 MATERIALS AND METHOD

2.1 ETHNOBOTANICAL STUDY

2.1.1 METHODS

Ethnobotanical information was collected by standard ethnobotanical tools (Alexiades and Sheldon, 1996), such as participant observation, as well as open and semi-structured interviews. The respondents were selected by a snowball sampling technique, which is asking an informant to suggest another informant. Local associations for elderly people were also contacted to identify the first informants. A questionnaire form, used as a guideline for the ethnobotanical interviews was used and is reported below. All participants as well as their parents had to be born and to have always lived in the study area. In fact, the origin of the family home is of extreme importance as Traditional Local Knowledge is formed and handed down mainly within the family. Conversations and discussions were also held with people working in vegetarian restaurants, organic farms and botanical gardens to obtain information on the actual use and knowledge of wild food plants. The purpose, method and nature of the research were previously explained and informed consent was obtained from all informants (verbal informed consent used for the ethnobotanical survey as shown below).

Interviews were carried out both individually and in groups. When conducted in groups, the respondents were stimulated to express their personal experience. During the first phase of the interview, the informants were asked to freely recall all the wild plant species that they had used in the past and/or are presently using for food purposes. For each mentioned plant species, the informants were asked to state the folk name, the parts of plant used, the period of harvesting, culinary and other possible uses, the frequency of use and whether they had used the plant in the past or are still using it. Processing and cooking activities were also precisely described. The respondents were able to speak freely but sometimes it was necessary to encourage them by providing some suggestions (e.g. “have you ever seen/used this plant?”) just to help them to recall the information.

Particular attention was paid to the therapeutic effects that may have been perceived after ingestion of some particular wild food plants. Moreover, the medicinal use of plants, with specific procedures of preparation and application, were always addressed. The perception

of wild species in relation to their cultivated analogues and the possible impacts, benefits or risks on human nutrition and health, were also investigated. The taste and level of appreciation of the consumed plant species, were described.

For each mentioned plant species a Relative Frequency of Citation index (RFC) was calculated. The RFC index expresses the number of informants who cited a specific wild food plant divided by the total number of informants. It was used to assess the local importance of each species and may vary from 0, when nobody refers to the plant as useful, to 1, when all informants mention the use of a species (Tardìo et al., 2008).

The informants who were particularly knowledgeable on wild food plants and are still using them, were chosen as *key informants* and their interviews were implemented to better understand the way of plant collection, food preparation, gender relation and mode of passing down local knowledge.

The key informants were also helpful and active in gathering the mentioned plant species, which they called by the relative folk or Italian common names. The collected wild plants specimens were successively identified by expert botanists (Dr. Mossetti Umberto and Dr. Managlia Annalisa of the University of Bologna), and renamed following standard botanical nomenclature (Pignatti, 1982).

Questionnaire form for ethnobotanical research
Bologna University
Department of Biological, Geological and Environmental Sciences, University of Bologna,
Via Irnerio 42, 40126 Bologna, Italy

Identity of interviewed person
Name:
Surname:
Residence:
Gender: female □ male □
Age:

Place of formation of traditional knowledge
Place of birth:

Childhood place:
Teens age place:
Adult age place:

Wild food plants
Do you use wild plants as food? Yes ♩ No □
In the past □ Nowadays □

Which ones? è *freely recall all the wild food plants used*

For which wild food plant mentioned:
Folk name

In which period of the year do you collect this plant?

Which parts of plant do you use?

How do you cook it?

During the seasonal period, how often do you eat it?
Once a day □
Once a week □
Once a month □
Once a year □
Now never, only in the past □
I've never eaten it, I have only heard about it □

Taste
What does it taste like?
Sweet □ Bitter □ Acid □ Salty □ Astringent □
Looks like _____

Does it like to your sons? Yes □ No □
Does it like to your grandchildren? Yes □ No □

Functional foods/ Medical foods
Do you use any wild food plants mentioned as medicine?

Do the wild food plants used have some beneficial effects on your health?
If yes, describe these effects in details.

Do the wild food plants used have adverse effects?
If yes, which ones?

Do you think they can have any impact on your life and your health?

Some of these plants can relieve the symptoms of any diseases?
If yes, which ones?

CONSENSO VERBALE INFORMATO ALLA PARTECIPAZIONE AD UNO STUDIO DI RICERCA DAL TITOLO

STUDIO DELLA BIODIVERSITÀ DELLE PIANTE ALIMURGICHE NEL TERRITORIO CHE CIRCONDA LA CITTÀ DI BOLOGNA

Buongiorno, il mio nome è Sabrina Sansanelli e sono una dottoranda in Biodiversità ed Evoluzione dell'Università di Bologna.
Sei stato scelto per partecipare ad uno studio sulle piante selvatiche utilizzate in cucina nel territorio che circonda la città di Bologna.
Lo scopo di questo studio è quello di ricercare e non perdere le tradizioni popolari bolognesi sulle erbe spontanee utilizzate in cucina.
La partecipazione in questo studio ti occuperà il tempo che vorrai dedicarmi per una o più interviste.
Se accetti di far parte di questa ricerca, ti chiederò le seguenti cose:

1. *Ti farò delle domande sull'uso delle piante selvatiche che utilizzi o utilizzavi in passato come alimento.*

2. *Durante l'intervista, registrerò l'audio, se me lo consentirai.*

Non ci sono rischi né benefici nel partecipare a questo studio.
Non ci sono costi o pagamenti.
Resterai anonimo. Solo il contenuto del video e le foto potranno essere divulgate per scopi documentari.
Se hai delle domande, per favore fammele ora.
La tua partecipazione a questa ricerca è volontaria e potrai rifiutare di partecipare o smettere di collaborare in qualsiasi momento.

CONSENSO VERBALE INFORMATO ALLA PARTECIPAZIONE AD UNO STUDIO DI RICERCA DAL TITOLO

STUDIO DELLA BIODIVERSITÀ DELLE PIANTE ALIMURGICHE PER LA CARATTERIZZAZIONE DI NUOVI PRINCIPI ATTIVI NUTRACEUTICI E FARMACEUTICI

Buongiorno, il mio nome è Sabrina Sansanelli e sono una dottoranda in Biodiversità ed Evoluzione dell'Università di Bologna.
Sei stato scelto per partecipare ad uno studio sulle piante selvatiche utilizzate in cucina nella Val D'Agri, in provincia di Potenza.
Lo scopo di questo studio è quello di ricercare e non perdere le tradizioni popolari lucane del territorio della Val D'Agri legate alle erbe spontanee utilizzate in cucina. Se decidi di partecipare a questo studio, sarai una delle 100 persone di questa ricerca. La partecipazione a questo studio ti occuperà il tempo che vorrai dedicarmi per una o più interviste.
Se accetti di far parte di questa ricerca, ti chiederò le seguenti cose:

3. *Ti farò delle domande sull'uso delle piante selvatiche che utilizzi o utilizzavi in passato come alimento.*

4. *Durante l'intervista, registrerò l'audio e il video, se me lo consentirai.*

5. *Solo se vorrai, ti farò una foto.*

Non ci sono rischi né benefici nel partecipare a questo studio.
Non ci sono costi o pagamenti.
Resterai anonimo. Solo il contenuto del video e le foto potranno essere divulgate per scopi documentari.
Se hai delle domande, per favore fammele ora.
La tua partecipazione a questa ricerca è volontaria e potrai rifiutare di partecipare o smettere di collaborare in qualsiasi momento.

2.2 METABOLIC SCREENING OF WILD FOOD PLANTS

2.2.1 MATERIALS

The gathering activity of the wild food plants mentioned by the informants was performed in both areas together with *key informants* (people interviewed who particularly retain TLK and that are still using wild food plants). The collected parts of plants were those traditionally used as well as the sampling period (early spring for the majority of wild food plant species). Thirty-four samples of wild food plants were totally collected: 13 in Bologna area and 21 in Middle Agri Valley corresponding to 27 plant species.

The plant folk taxa were identified by expert botanists (Dr. Mossetti Umberto and Dr. Managlia Annalisa) of the University of Bologna, followed the standard botanical nomenclature (Pignatti, 1982).

The collected samples were frozen with liquid nitrogen, grinded and stored at -80°C until further analysis.

2.2.2 TOTAL POLYPHENOL QUANTIFICATION

Wild food plant samples (0.5 gFW) were extracted by overnight shaking at 4°C with 5 ml of 95% (v/v) methanol and centrifuged 5000 rpm for 15 min at 4°C (Beckman J2-HS centrifuge, JA 20 rotor, Fullerton, CA, USA). Obtained methanolic extracts were used to assess the antioxidant activity and to quantify the amount of polyphenols, flavonoids and antioxidant activity.

Total polyphenols were determined by using the Folin-Ciocalteu method (Singleton et al., 1999). A suitable volume of methanolic extract was diluted to 1.6 ml with water and 100 μL of Folin-Ciocalteu reagent were added. After 5 min the reaction was stopped with 300 μL of 20% (w/v) sodium carbonate. The mixture was vortex for 15 sec and incubated at 40°C for 30 min in the dark, before measuring the absorbance at 765 nm (spectrophotometer Jasco V-530, Jasco Europe S.r.l., Carpi, Italy). The results were expressed as gallic acid (GA) equivalents by means of a dose-response calibration curve.

2.2.3 TOTAL FLAVONOID QUANTIFICATION

Total flavonoid content of methanolic extracts was quantified by using the method described by Zhishen et al. (1999). Different sample aliquots were added to 400 µL of water and 30 µL of 5 % (w/v) $NaNO_2$ and left to stand for 5 min. Successively, 30 µL of 10% (w/v) $AlCl_3$ were added and the mixture was incubated for another 6 min before stopping the reaction by the addition of 200 µL of 1M NaOH. The mixture volume was finally taken up to 1 mL with water and the absorbance at 510 nm was determined. The results were expressed as catechin (CAT) equivalents by means of a dose-response calibration curve.

2.2.4 DETERMINATION OF ANTIOXIDANT ACTIVITY

Antioxidant activity was measured by using the 20-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method (Re et al., 1999) with minor modifications (Ferri et al., 2013). One mL of the ABTS working solution was added to different aliquots of methanolic extracts. After incubation for 30 min at 30°C in the dark, absorbance at 734 nm was measured. The results were expressed as ascorbic acid (AA) equivalents by means of the dose–response calibration curve.

2.2.5 CHLOROPHYLL AND CAROTENOID QUANTIFICATIONS

Chlorophyll and carotenoid amounts were measured using the method described by Radwan et al. (2007). Samples (0.2 gFW) were added with 1.5 ml of 85% (v/v) acetone, then vortex twice for 30 sec respectively. The mixtures were centrifuged 2500 rpm for 5 min at 4°C and and the supernatants were used to measure absorbance at different wavelengths:

- 644 nm for chlophyll-b
- 663 nm for chlophyll-a
- 452.5 nm for carotenoids

The absorbances obtained were elaborated with the following equations to obtain pigments concentration (µg/ml):

chlorophyll-a = $10.3 \times A_{663} - 0.98 \times A_{644}$

chlorophyll-b = $19.7 \times A_{644} - 3.87 \times A_{663}$

carotenoids = $4.2 \times A_{452.2} - [(0.0264 \times \text{chlor-a}) + (0.426 \times \text{chlor-b})]$

2.2.6 DETERMINATION OF PROTEIN AMOUNTS

The amount of protein was determined following the method of Lowry et al. (1951). The plant samples (0.2 gFW) were added with 1 ml of extraction buffer (Tris-HCl 50 mM, pH 8, 2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride (PMSF)). After agitation and centrifugation for 20 min 14000 rpm at 4°C, the supernatants were collected. A suitable volume of extracts was added to 200 µL NaOH 1N, taken to 1 mL with water. 5 ml of a solution made by 50 ml of Na_2CO_3 2% (w/v) in NaOH 0.1N and 1 mL of $CuSO_4$ 0.5% (w/v) in NaK tartrate 0.1% (w/v) were added to the mixture and left to stand for 10 min at environment temperature. Successively, 0.5 mL of Folin reagent (diluted 1:2 with water) was added, vortex, left to stand 30 min at environment temperature and absorbance at 750 nm was measured. A calibration curve was prepared by using different amounts of BSA (Bovine Serum Albumin).

2.3 METABOLOMICS STUDY OF CREPIS VESICARIA SUBSP. TARAXACIFOLIA (THUILL) THELL

Ultra High Performance Liquid Chromatography-Electrospray-Time of Flight Mass Spectrometry (UPLC-ESI-TOF MS) was used with a not targeted approach to analyse the metabolomics profile of *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill.) Thell., that is the plant species more recognized and more appreciated in the area of Bologna and also used sometimes as medicine. The metabolomics study was performed at the Laboratory of Foodomics at the Institute of Food Science Research (CIAL) belonging to the National Research Council (CSIC) in Madrid (Spain) during the period from 1 February 2013 to 1 July 2013.

2.3.1 PLANT SAMPLES PRE-TREATMENT AND EXTRACTION

Crepis vesicaria L. subsp. *taraxacifolia* (Thuill.) Thell. samples were ground with liquid nitrogen, lyophilized and cry milled (Cryomill Retsch Gmbt). Plant sample (0.1 gFW) were extracted using methanol/water 75/25 v/v + 0.1% v/v formic acid (FA), vortexed for 30 seconds, sonicated 15 min at room termperature (Ultrasons Selecta), centrifuged 5000

rpm/10min/4°C (Centrifuge 5804 R-eppendorf) and the supernatant was filtered with 0.2 μm nylon filter (Symta/F263-2). The reference procedure for the sample extraction was reported by De Vos et al., 2007, and was optimised on the basis of the UPLC-ESI-TOF MS results obtained.

2.3.2 UPLC-ESI-TOF MS SETUP

LC–MS parameters (UPLC Agilent Technologies 1290 Infinity and Agilent Technologies 6540 UHD Accurate-Mass Q-TOF LC/MS) were based on Godzien et al. (2010) and optimized after testing many different factors as column, mobile phase, gradient, flow rate. The different procedures were compared in terms of metabolic information (number and intensity of chromatographic peaks).

Extracted plant samples were injected (2 μL sample volume) in the UPLC equipped with a reverse-phase C8-bonded silica column at 30 °C (Zorbax Eclipse Plus C8 2.1x100mm, 1.8 micron, Agilent). The system was operated with a flow rate of 0.5 mL/min of mobile phase (solvent A: water with 0.01% formic acid (v/v); organic solvent B: acetonitrile with 0.01% v/v formic acid). The total analysis time per sample was 23 min. The gradient started with 0% of B during the first 2 min, and increased to 50% in 12 min, and reached 100% in 14 min. The gradient was held at 100% B until 18 min and returned to starting conditions in 0.5 min, keeping the re-equilibration until 23 min. Data were collected in negative and positive ESI mode in separate runs on a Q-TOF operated in full scan mode from m/z 50 to 1100. The capillary voltage was 4000 V with a scan rate of 1.5 scans per second, the nebulizer gas flow rate was 10 L /min, the pressure was maintained at 40 psi and the temperature at 350 °C. During the analyses, two reference masses were used: m/z 112.9856 and m/z 966.0007. These masses were continuously infused to the system to allow constant mass correction. All samples, during the analyses, were kept in the LC auto-sampler maintained at 4°C.

2.3.3 ACIDIC AND ENZIMATIC HYDROLYSIS

Acidic and enzymatic hydrolyses of *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill.) Thell. samples were performed in order to widen the information amount at metabolic level.

The acid hydrolysis was conducted using hydrochloric acid at two different concentrations (1.2 M and 2 M) and by using two incubation times (2 and 4 hours). The Viscozyme multi-

enzymes complex (purchased by Novozyme) was used for the enzymatic hydrolysis. The key enzyme activity is provided by endo-b-glucanase that hydrolyses (1,3)- or (1,4)-linkages in b-D-glucans. The product contains also activity of xylanase, cellulase, hemicellulase. The protocol adopted was the following: 50 mg dry plant sample were added to 5 ml of acetate buffer (CH3COONa/CH3COOH, 4.5 pH, 0,1 M solution), then to 5 µL (1 mg/µL) of Viscozyme and kept for 2 hours at 50 °C and 350 rpm.

The extracted sample were then directly injected into UPLC and analysed as previously described.

2.3.4 METABOLIC PROFILES COMPARISON OF THE THREE CONSUMING WAYS OF CREPIS VESICARIA SUBSP. TARAXACIFOLIA (THUILL) THELL BASAL LEAVES

In order to compare the metabolomics profiles, three different type of *Crepis vesicaria* plant extracts were performed simulating the three most popular traditional ways of preparation.

At first *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill.) Thell. samples were placed in boiling water for 10 minutes and water and pellet were separated. The pellet was extracted using the above mentioned extraction solvent (methanol/water 75/25 v/v + 0.1% v/v FA) obtaining the *pellet extract* (cooked *Crepis* leaves, alimentary cooked use). The water fraction was not further processed and used as *water extract* (*Crepis* leaves cooking water, medicinal use). *Pellet and water extracts* were afterwards analysed according to the LC-MS untargeted metabolomics protocol. The third extract is represented by the *methanol-water* extract of the raw sample (raw *Crepis* leaves, alimentary raw use).

2.3.5 DATA TREATMENT AND COMPOUNDS IDENTIFICATION

The resulting data files were processed by subtracting background noise and unrelated ions by the Molecular Feature Extraction tool in the MassHunter Qualitative Analysis Software (Agilent Technologies). This tool was then used to create a list of all possible components.

Standards were purchased by Sigma-Aldrich and Extrasynthèse and were used for identification compounds (Table 2).

All experiments were repeated with identical chromatographic conditions and results were compared and used to confirm or refuse the identification of compounds supposed.

Table 2. Standards used with their formula, monoisotopic mass and mass charge ratio

NAME	FORMULA	MONOISOTOPHIC MASS	m/z (M-H)
HYDROSSIBENZOIC ACID			
4-hydroxybenzoic acid	C7H6O3	138,0316941	137,0238691
Gallic acid	C7H6O5	170,0215233	169,0136983
Vanillic acid	C8H8O4	168,0422587	167,0344337
Syringic acid	C9H10O5	198,0528234	197,0449984
Salicilic acid	C7H6O3	138,0316941	137,0238691
HYDROSSICINNAMIC ACID			
Caffeic acid	C9H8O4	180,0422587	179,0344337
Ferulic acid	C10H10O4	194,0579	193,050075
P-Coumaric acid	C9H8O3	164,0473441	163,0395191
Rosmarinic acid	C18H16O8	360,0845175	359,0766925
Sinapic acid	C11H12O5	224,0685	223,0612
Cholorogenic acid	C16H18O9	354,0950822	353,0872572
CATECHINS			
Catechin (C)	C15H14O6	290,0790382	289,0712132
Epicatechin (EC)	C15H14O6	290,0790382	289,0712132
Epicatechin monogallate (ECG)	C22H18O10	442,0899968	441,0827
FLAVONOIDS			
FLAVONES			
Luteolin	C15H10O6	286,0477381	285,0399131
Luteolin-7-glucoside	C21H20O11	448,1005615	447,0927365
Diosmetin	C16H12O6	300,0633881	299,0555631
Apigenin	C15H10O5	270,0528234	269,0449984
Apigenin-7-O-glucoside	C21H20O10	432,1056469	431,0978219
FLAVONOLS			
Quercetin	C15H10O7	302,0426527	301,0348277
Quercetin-3-O-glucoside	C21H20O12	464,0954761	463,0876511
Quercetin-3-ramnoside (Quercitrin)	C21H20O11	448,1005615	447,0927365
Quercetin-3-D-galactoside	C21H20O12	464,0954761	463,0876511
Quercetina-3-rutinoside (Rutina)	C27H30O16	610,1533849	609,1455599
Kaempferol	C15H10O6	286,0477381	285,0399131
Kaempferol-3-O-glucoside	C21H20O11	448,1005615	447,0927365
Isorhamnetin-3-O-glucoside	C22H22O12	478,1111	477,1039
FLAVANONE			
Hesperidin	C28H34O15	610,1897704	609,1819454

Accurate masses of features were firstly searched against METLIN database (http://metlin.scripps.edu), that is a freely accessible web-based data repository. METLIN includes an annotated list of known metabolite structural information that is easily cross-correlated with its catalogue of high-resolution Fourier transform mass spectrometry (FTMS) spectra, tandem mass spectrometry (MS/MS) spectra, and LC/MS data.

Accurate mass data and isotopic distributions for the precursor and product ions were studied and compared to spectral data of reference compounds, if available, obtained under identical conditions for final confirmation (METLIN).

3 RESULTS AND DISCUSSION

3.1 ETHNOBOTANICAL STUDY

3.1.1 STUDY AREAS

The ethnobotanical survey was carried out in two different areas of the Italian peninsula: the territory surrounding the city of Bologna (Emilia-Romagna region) in Northern Italy and the middle part of Agri Valley (Basilicata region) in Southern Italy.

3.1.1.1 Area of Bologna

The area of Bologna is comprised between the Panaro river (to the North-West), the Santerno river (to the South-East), the Ferrara province (to the North-East), the Apennine mountains (to the South-West) (Figure 5). This area is included in Bologna province that is the most populated province of Emilia Romagna region. The territory is very diversified including valleys, hills and mountains going from 11 m.a.s.l. (San Pietro in Casale) to 347 m.a.s.l. (Monghidoro).

The survey was performed during the following periods: March - April 2012 and September - October 2013.

Figure 5. The location of the study area located in Bologna's province (North of Italy).

3.1.1.2 Agri Valley

The Agri Valley is located in the South-West part of Basilicata, within the province of Potenza, and takes its name from the Agri River. Its shape is that of a wide varix, characterised by different environments and surrounded by Volturino (1,856 m) and Viggiano (1,725 m) mountains. The Agri Valley may be subdivided geographically and socio-economically in three distinct parts: Upper, Middle and Lower (Basso et al., 1998). The present survey was focused on the Middle Agri Valley that consists by the following municipalities: Sant'Arcangelo, Roccanova, Castronuovo, Aliano, Gallicchio, Missanello, San Chirico Raparo, San Martino D'Agri, Guardia Perticara, Montemurro, Spinoso, Castelsaraceno (Figure 6).

The interviews were performed during the months of May and August 2012 and January 2013.

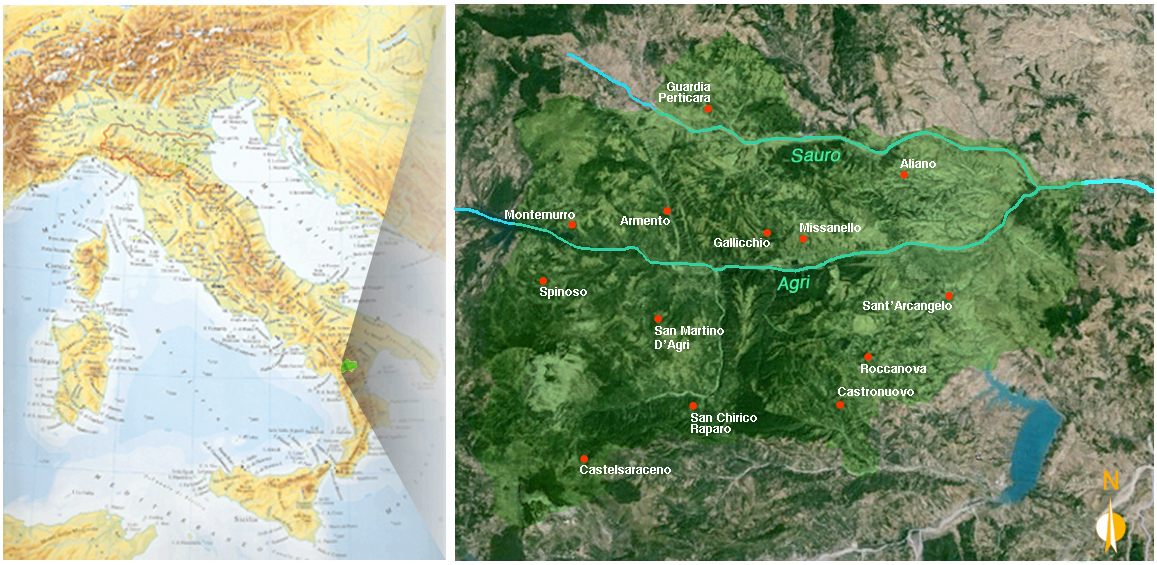

Figure 6. The location of the study area located in the Middle Agri Valley (Potenza's province, South of Italy).

3.1.2 STUDY AREAS COMPARISON

The two investigated areas are very different from various point of view: geographically, historically, socially and economically.

The area contiguous the city of Bologna is one of the more economically developed regions of Italy. In this area, after the end of Second World War, many socio-economic changes occurred bringing economic well-being, industrial activities and the development of new transport infrastructures that well connected people, houses, services and workplaces. These changes inevitably mutated the lifestyle, the family system and the nature of communities. The local knowledge, shared among family and community members, was thus less and less passed down to the following generations.

Moreover, the growth of industrial activities and new businesses mainly located in the city of Bologna, caused the incoming of human resources toward the city area and the depopulation of nearby small towns, especially those in the Apennines.

Besides, staring from the 50's following the economic development, the city of Bologna and its hinterland became a catalyst for people mostly coming from Southern Italy and in the

last decade also from Romania, Moldova, Bangladesh, Ucraina and Pakistan (Table 3, Figure 7 and 8).

Table 3. Number of foreign citizens in the various Italian regions (ISTAT data, January 2015)

REGIONS	Total foreign citizens	Percentage of foreigners on the total population	Percentage change of previous year
1. Lombardia	1,152,320	11.52%	2.00%
2. Lazio	636,524	10.80%	3.30%
3. Emilia-Romagna	536,747	12.06%	0.50%
4. Veneto	511,558	10.38%	-0.60%
5. Piemonte	425,448	9.62%	0.00%
6. Toscana	395,573	10.54%	2.10%
7. Campania	217,503	3.71%	6.70%
8. Sicilia	174,116	3.42%	7.20%
9. Marche	145,130	9.36%	-0.70%
10. Liguria	138,697	8.76%	0.20%
11. Puglia	117,732	2.88%	6.70%
12. Friuli Venezia Giulia	107,559	8.77%	-0.30%
13. Umbria	98,618	11.02%	-1.30%
14. Trentino-Alto Adige	96,149	9.11%	-0.20%
15. Calabria	91,354	4.62%	5.60%
16. Abruzzo	86,245	6.48%	2.30%
17. Sardegna	45,079	2.71%	6.90%
18. Basilicata	18,210	3.16%	7.30%
19. Molise	10,800	3.45%	5.20%
20. Valle d'Aosta	9,075	7.07%	-2.80%

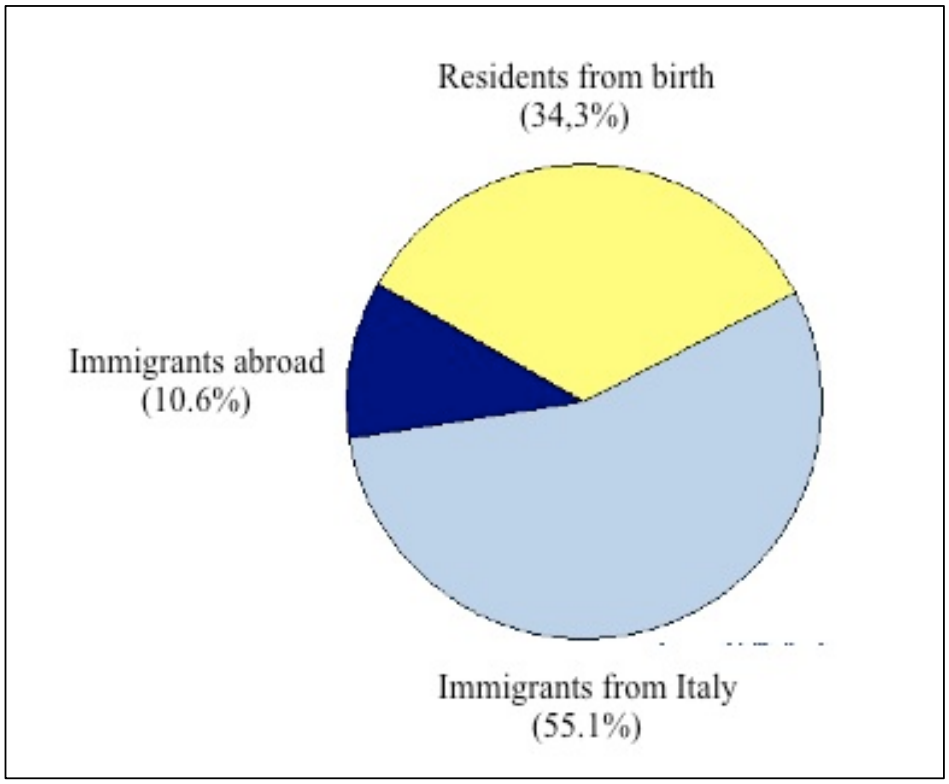

Figure 7. The origin of residents in Bologna's area, data of 2011 (I flussi migratori a Bologna, Comune di Bologna, Dipartimento programmazione settore statistica, 2012).

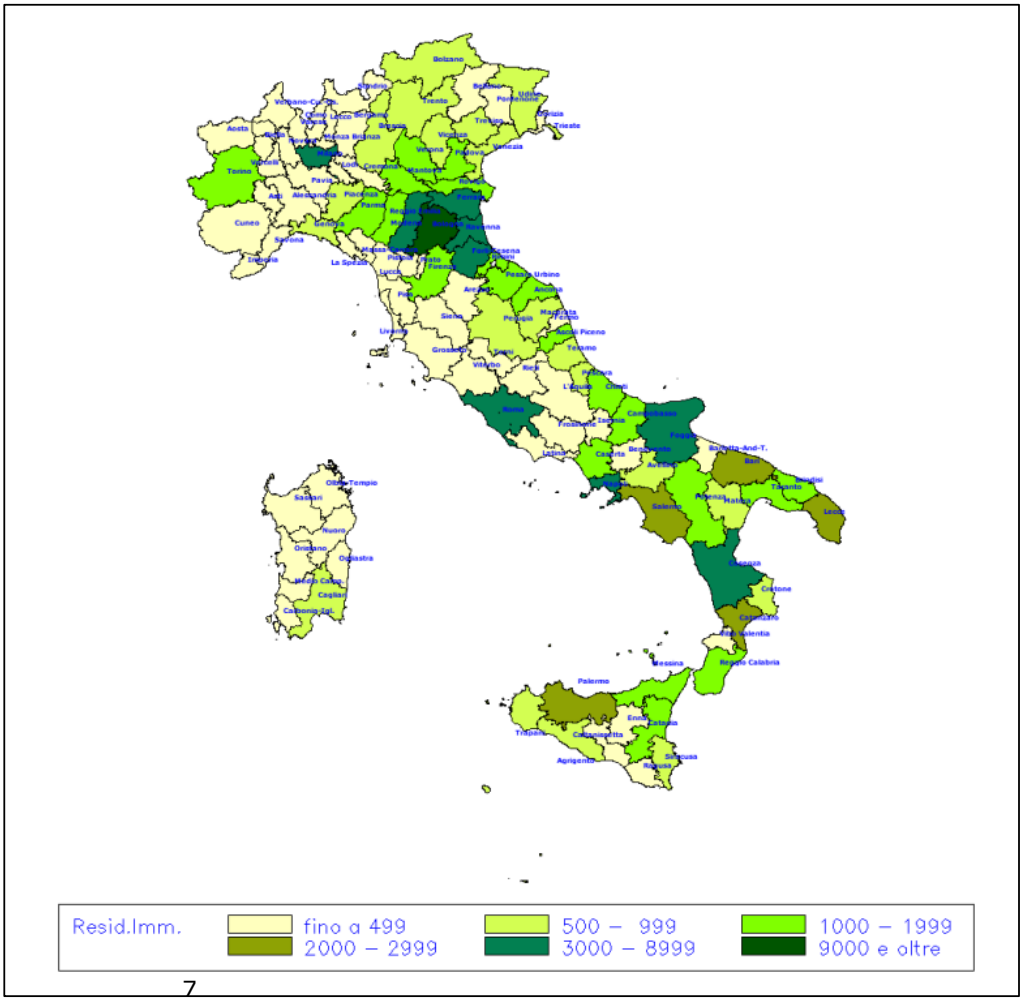

Figure 8. Italian immigrants resident in Bologna divided for province of origin in December 31, 2011 (I flussi migratori a Bologna, Comune di Bologna, Dipartimento programmazione settore statistica, 2012).

On the contrary to Bologna's area, the territory of Middle Agri Valley is characterised by a few villages sustained primarily by agriculture. Durum wheat, olives, vineyards and fruit trees are the predominant crops. The lack of industries and its geographical isolation from the commercial and touristic traffic have caused most of the young people to leave the area and emigrate abroad or to the North of Italy. Even the discovery of many rich oil fields still in operation and in expansion phase did not bring the hoped economic well-being, so that the emigration wave was never arrested and is actually still continuing (Figure 9). For this reason, the area has actually a low population density equal to 34.5 inhabitants/km^2 and is

mainly inhabited by the elderly (Contributo al piano strategico intercomunale della Val D'Agri, 2014).

Figure 9. Trend of residence population in Potenza's province as an example of the emigration wave between 2001 and 2014.

3.1.3 ETHNOBOTANICAL STUDY CONDUCTED IN THE AREA OF BOLOGNA

3.1.3.1 Informants

Thirty-nine people, 25 women (64%) and 14 men (36%), were interviewed. The age of the informants ranged between 48 and 92, with a mean of 71 and a median of 75. Nine informants were younger than 60, 10 were aged between 60 and 75, and 20 were older than 75. Finding persons who still retained Traditional Local Knowledge (TLK) about wild food plants uses was particularly difficult. This shows that much of the local knowledge has already been lost and that it is necessary and urgent to carry out this kind of research in this territory. The results showed that TLK was almost equally shared between the two genders (the average number of species quoted per gender was: 10.2 for women and 9.1 for men), however, the women gave much more details and information on the used traditional wild food plants. Women had a better preserved memory, probably because they were almost exclusively in charge of processing and cooking wild plants, while gathering activities were carried out by both genders. These data are partially in agreement with several studies performed in the Mediterranean area (Forbes et al., 1976; Nebel et al., 2006; Pieroni et al., 2005) that showed that women are the major depositaries of wild plant local knowledge.

Many conversations with people working in vegetarian restaurants, organic farms and botanical gardens led to understand that the traditions about wild food plants were very little known and shared among local population.

At present time, there is a part of society that is very careful about having a healthy and genuine diet and therefore very interested toward wild food plants (independently from their popular traditions) as they are considered rich of healthy components and, as study Bologna's area is a highly anthropomorphized environment, a way to get closer to nature.

3.1.3.2 Wild food plant data

The informants mentioned a total of 66 wild food plants (Table 4), including greens (leafy plants eaten as vegetables), fruits and semi-wild plants. The mean number of species quoted per informant was 9.8. Wild plants used for making liqueurs (in particular digestive liqueurs) were also taken into consideration, because these still are traditionally drunk at the end of a meal. The mentioned wild edible plants are reported in Table 4 which lists the botanical species and family name, English and Italian common names, Italian folk names (when available), the parts of the plant used, the culinary and medicinal usage and the Relative Frequency of Citation Index (RFC). Most of the recorded species are commonly used in the Mediterranean area, such as *Cichorium intybus* L., *Sonchus asper* L., *Borago officinalis* Weber, *Papaver rhoeas* L. (Leonti et al., 2006) (Table 4), whereas others are mainly eaten in Northern and Central Italy, such as *Bellis perennis* L., and *Capsella bursa-pastoris* (L.) Med. (Ghirardini et al., 2007).

The RFC ethnobotanical index indicates, for a given folk species and analysed area, the degree of knowledge shared among the informants. The RFC may vary from 0 to 1, consequently, a RFC value close to 1 indicates that a species is very important from a cultural and traditional point of view. The highest RFC index (0.77) was found both for *Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell (Figure 10) and *Taraxacum officinale* Weber (Figure 11). *C. vesicaria* subsp. *taraxacifolia* (Thuill) Thell is known by the folk name "*strecapugno"*, while *T. officinale* Weber is known as "*piscialét"*. *C. vesicaria* subsp. *taraxacifolia* (Thuill) Thell is more appreciated than *T. officinale* Weber because of its bitter, slightly crisp flavour, the memory of which is well preserved even in people who do not consume it anymore. Moreover, *T. officinale* Weber is a species widely known in Italy

and abroad, not only as an edible plant but also for its therapeutic properties used for depurative and digestive purposes and for mitigating hepatic diseases. Its high availability and characteristic ripe fruits, make it easy to find and collect the plant. On the contrary, as revealed by the interviewed people, *C, vesicaria* subsp. *taraxacifolia* (Thuill) Thell, is not easy to recognise and quite difficult to find. The subspecies *taraxacifolia* of *C. vesicaria* is also consumed in other areas throughout Central Italy, often as substitute of *C. vesicaria L. subspecies vesicaria* and *Crepis biennis L.* (Picchi and Pieroni, 2005). *C. vesicaria L. subspecies vesicaria* is also present in the study area but people identify, collect and consume only the subspecies *taraxacifolia*. No information is available on knowledge and collection of *C. biennis L.*

Other wild plants which scored a high RFC index value were: *Urtica* spp. (0.74), *Clematis vitalba* L. (0.51), *Valerianella locusta* L. (Laterrade) (0.41), *C. intybus* L. and *Diplotaxis tenuifolia* L. (DC) (0.38) and *Sonchus* spp. (0.33) (Table 4, Figure 12).

In particular, *Urtica* spp. resulted to be the most consumed species and is much more valued today than it was in the past. This plant is in fact well integrated in homemade local cooking (e.g. to make green pasta, or to fill and season hand-made pasta) and dishes containing *Urtica* are proposed by several local restaurants. The wide use of *Urtica* may also depend on the fact that is a ruderal plant, characterized by a rapid growth close to people's residences, thus being readily available for collection and consumption.

In the present survey, herbs used to make hot beverages (decoctions or infusions) such as *Malva sylvestris* L. and *Matricaria chamomilla* L. were not considered as foods (not included in Table 4), but classified as medicinal plants having therapeutic effects.

A large group of listed plant species (19), were mentioned by a single informant for this reason they may be considered as uncertain data (Table 4). These results point out how strongly eroded is actually the knowledge about wild food plants in the study area of Bologna. The obtained data were compared with an ethnobotanical survey conducted around Lake Vrana (Northern Dalmatia, Croatia) (Luczaj et al., 2013) which was based on a similar number of informants (43) that mentioned around 57 different wild food plants. The survey reported that, wild vegetables were still widely used in the Lake Vrana area. In fact, although less popular among young people, old and middle aged people still retained wide knowledge and collected them. The average number of species quoted was higher in Lake

Vrana area (12.4) than in Bologna's territory (9.8) on the contrary to the percentage of plant species mentioned by a single informant (14% and 29% respectively for Lake Vrana and the present studies), thus confirming the highly eroded nature of wild food plants knowledge in the area of Bologna.

Figure 10. Plant sample and flowers of *Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell

Figure 11. Basal leaves, flower and infructescence of *Taraxacum officinalis* Weber

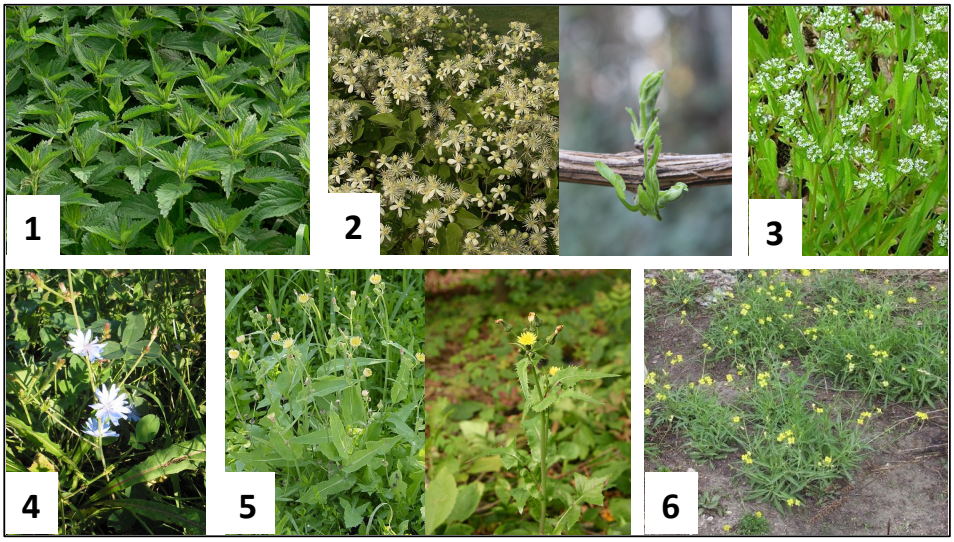

Figure 12. Some of the most cited wild food plants species by Bologna's area informants. 1: *Urtica* spp.; 2: *Clematis vitalba* L.; 3: *Valerianella locusta* L.; 4: *Cychorium intybus* L.; 5: *Sonchus* spp.; 6: *Diplotaxis tenuifolia* L. (Laterrade).

Table 4 List of the wild food plants used in the area of Bologna

Botanical name	RFC	Botanical family	English common name	Italian common and/or folk names	Parts of the plant used	Culinary use	Medicinal use (preparation and administration)
Achillea ptarmica L. *	0.03	Asteraceae	sneezewort	achillea	flowers	salads	
Allium schoenoprasum L. *	0.03	Liliaceae	chives	erba cipollina	leaves	flavouring	
Allium ursinum L. *	0.03	Liliaceae	wild garlic	aglio selvatico	bulbs	flavouring	
Asparagus acutifolius L.	0.15	Asparagaceae	wild asparagus	asparago selvatico/ *asparagina*	shoots	salads, pan-fried	
Bellis perennis L. *	0.03	Asteraceae	common daisy	margheritina	young leaves	salads	
Borago officinalis L.	0.08	Boraginaceae	starflower	borragine	leaves	salads, pancakes, pies	
Calamintha nepeta L. *	0.03	Lamiaceae	lesser calamint	nepetella	leaves	salads	
Calendula officinalis L. *	0.03	Asteraceae	common marigold	calendula	flowers	salads	
Capsella bursa pastoris L. *	0.03	Brassicaceae	shepherd's-purse	borsa del pastore	young leaves	salads	
Castanea sativa Mill.	0.10	Fagaceae	chestnut	castagno	fruits	fresh fruits	
Cichorium intybus L.	0.38	Asteraceae	wild chicory	cicoria selvatica/ *radećć, radećć cavdagn*	young leaves	salads, pan-fried	
Clematis vitalba L.	0.51	Ranuncolaceae	traveller's joy	vitalba/*vizeibra*	shoots	salads, pan-fried, omelettes, mixed vegetables	
Cornus mas L.	0.05	Cornaceae	cornelian cherry	corniolo	fruits	rural snack, liqueurs	
Corylus avellana L.	0.05	Corylaceae	common hazel	nocciolo	fruits	fresh fruits	
Crataegus azarolus L. *	0.03	Rosaceae	azarole	azzeruolo/*lazaren*	fruits	rural snack	
Crataegus monogyna Jacq.	0.15	Rosaceae	common hawthorn	biancospino/*spen bianc*	fruits, shoots, leaves	rural snack	Relaxing, insomnia and heart problems (flowers infusion)
Crepis sancta (L.) Babc.	0.15	Asteraceae	hawk's-beard	radicchiella/*ciocapi at*	young leaves	salads, pan-fried	Diuretic and laxative (food)
Crepis vesicaria subsp. taraxacifolia (Thuill.) Thell.	0.77	Asteraceae	beaked hawk's beard	radicchiella/*strecap ugno*	young leaves	salads, pan-fried, omelettes, pasta dough	Depurative, refreshing, blood cleaning, diuretic, laxative (cooking water, food)

Diplotaxis tenuifolia L. (DC)	0.38	Brassicaceae	wall rocket	rucola selvatica	leaves	salads	
Fagus sylvatica L. *	0.03	Fagaceae	common beech	*al fasoli (fruits)*	fruits	rural snack	
Ficus carica L.	0.05	Moraceae	common fig	fico	fruits	fresh fruits	
Foeniculum vulgare Mill.	0.13	Apiaceae	wild fennel	finocchio selvatico	stems (I), leaves (II), seeds (III)	liqueurs (I), flavouring (II, III), mixed vegetables (I)	
Gentiana lutea L. *	0.03	Gentianaceae	great yellow gentian	genziana	roots	liqueurs	
Humulus lupulus L. *	0.03	Cannaboideae	common hop	luppolo	shoots	pasta sauce	
Juglans regia L.	0.10	Juglandaceae	walnut	noce	fruits	liqueurs, fresh fruits	
Juniperus communis L.	0.15	Juniperoideae	common juniper	ginepro	fruits	flavouring, liqueurs	
Laurus nobilis L.	0.10	Lauraceae	bay laurel	alloro	leaves	flavouring, liqueurs	
Lippia citriodora Kuntze	0.08	Verbenaceae	lemon verbena	erba luigia	leaves	liqueurs	Swelling trauma (decoction)
Lonicera caprifolium L.	0.08	Caprifoliaceae	sweet honeysuckle	caprifoglio/*ligabôsc*	shoots	salads	
Malus sylvestris (L.) Mill *	0.03	Rosaceae	European crab apple	melo selvatico	fruits	fresh fruits	
Medicago sativa L. *	0.03	Fabaceae	alfalfa	erba medica/*spagna*	leaves	salads, mixed vegetables	
Melissa officinalis L. *	0.03	Lamiaceae	lemon balm	melissa, erba limone	leaves	flavouring	
Mentha spp.	0.13	Lamiaceae	mint	menta	leaves	flavouring, liqueurs	Digestive (decoction)
Mespilus germanica L.	0.05	Rosaceae	medlar	nespolo	fruits	fresh fruits	
Morus spp.	0.05	Moraceae	mulberry	mora	fruits	fresh fruits	
Papaver rhoeas L.	0.05	Papaveraceae	field poppy	papavero/*rosetta*	young leaves	salads, pan-fried, mixed vegetables	
Portulaca oleracea L.	0.05	Portulacaceae	purslane	portulaca	leaves	salads, liqueurs	
Primula spp.	0.10	Primulaceae	primrose	primula	flowers (I), leaves (II)	salads (I), rural snacks (I), pasta stuffing (II)	
Prunus avium L.	0.05	Rosaceae	wild cherry	ciliegio selvatico	fruits	fresh fruits	
Prunus cerasifera Ehrh.	0.10	Rosaceae	myrobalan plum	mirabolano/*rustican*	fruits	rural snacks	
Prunus cerasus L.	0.05	Rosaceae	sour cherry	amareno/*visciole*	fruits (I), leaves (II)	fresh fruits (I), liqueurs (II)	
Prunus laurocerasus L.	0.05	Rosaceae	lauroceraso	lauroceraso/*lauro*	fruits	liqueurs	
Prunus spinosa L.	0.28	Rosaceae	sloe	prugnolo selvatico/*prugnól,*	fruits	rural snacks, liqueurs	

				spini, strozchi			
Punica granatum L. *	0.03	Punicaceae	pomegranate	melograno	fruits	fresh fruits	
Pyrus pyraster Burgsd	0.05	Rosaceae	wild pear	pero selvatico	fruits	fresh fruits	
Robinia pseudoacacia L.	0.21	Fabaceae	black locust	acacia/*acâg*	flowers	pancakes, rural snacks	
Rosa spp.	0.28	Rosaceae	dog rose	rosa selvatica/*pizzincul (fruits)*	shoots (I), fruits (II), flowers (III)	rural snack (I, II), jams (II, III)	
Rosmarinus officinalis L.	0.13	Lamiaceae	rosemary	rosmarino	leaves	flavouring, liqueurs	Digestive (decoction); decongestant (fumigations)
Rubus spp.	0.31	Rosaceae	wild blackberry	rovo/*râza*	fruits (I), shoots (II)	fresh fruits (I), liqueurs (I), jams (I), rural snacks (II)	
Rumex acetosa L.	0.15	Polygonaceae	sorrel	acetosa/*êrba brossca*	leaves	rural snacks	
Ruscus aculeatus L. *	0.03	Ruscaceae	butcher's broom	pungitopo	shoots	pan-fried	
Salvia pratensis L.	0.10	Lamiaceae	meadow clary	salvia selvatica	leaves	salads, flavouring, omelettes, liqueurs	Digestive (decoction); female genital problems (infusion); toothpaste (fresh leaves)
Sambucus nigra L.	0.21	Adoxaceae	elderberry	sambuco	fruits (I), flowers (II)	jams (I, II), pancakes (II), liqueurs (II)	Antirheumatic (food: jam)
Sanguisorba minor Scop.	0.05	Rosaceae	salad burnet	pimpinella/*pampinela*	leaves	salads	
Satureja hortensis L. *	0.03	Lamiaceae	summer savory	santoreggia	leaves	flavouring	
Silene vulgaris (Moench) Garcke	0.26	Caryophyllaceae	bladder campion	silene rigonfia/*strìdel, ciucchett*	young leaves	salads, pan-fried, pasta sauce, pasta dough, omelettes	
Sonchus asper L. (Hill)/ Sonchus arvensis L.	0.33	Asteraceae	sow thistles	grespino/*frabbs*	young leaves	pan-fried, salads	Depurative, diuretic, laxative (food)
Sorbus domestica L.	0.13	Rosaceae	seviceberry	sorbo	fruits	fresh fruits	
Tanacetum balsamita L. *	0.03	Asteraceae	costmary	erba di santa Maria	leaves	liqueurs	
Taraxacum officinale Weber	0.77	Asteraceae	swines snout	tarassaco/*piscialet*	young leaves	salads, pan-fried, omelettes, mixed vegetables	Depurative, refreshing, draining, diuretic (food, cooking water)

Thymus spp.	0.08	Lamiaceae	thyme	timo	leaves	flavouring	
Trifolium pratense L. *	0.03	Fabaceae	red clover	trifoglio dei prati	flowers	rural snacks	
Urtica spp. (dioica, urens)	0.74	Urticaceae	nettle	ortica	leaves	pasta stuffing and dough, salads, omelettes, mixed vegetables	Refreshing, kidney problems, mineralizing (food, cooking water); hair strength, shine, dandruff (cooking water); anti- arthritic (fresh leaves rubbed on the body); insecticide (leaves macerated in water)
Valerianella locusta L. (Laterrade)	0.41	Valerianaceae	lamb's lettuce	valerianella/ *grassagallina*	leaves	salads	
Viola spp.	0.08	Violaceae	violet	viola	flowers	salads	
Vitis vinifera L. subsp. Sylvestris (Gmelin) Hegi	0.13	Vitaceae	wild grape	vite selvatica	fruits (I), shoots (II), leaves (III)	fresh fruits (I), rural snacks (II), mixed vegetables (III)	

Folk Italian names are written in italics.
Roman numbers indicate the correlation between the traditional culinary use and a specific part of the plant.
* indicates plant species mentioned by a single informant.
RFC: Relative Frequency of Citation Index;
Medicinal use: in brackets the way plants or parts of it are prepared and administered to give the mentioned therapeutic effect.

The folk plant species mentioned by the people interviewed in the present study belonged to 33 different botanical families (Table 5). The most representative families were Rosaceae (14 plants) and Asteraceae (9 plants). The parts of the plants used and recorded for each mentioned species were represented in Figure 13. In general, leaves were most frequently used (33), followed by fruits (24) and shoots (9). The ways of consumption of wild food plants (Figure 14) indicated that plants were most often consumed raw, in salads prepared with the tender young leaves (25) collected in the early vegetative rosetta stage when they have a less bitter taste, or boiled. They were also frequently used as liqueur ingredients (17), a habit still in use, or eaten as fresh fruits (14). In the past, some wild plant parts were extemporaneously eaten raw as a rural snack (13, Figure 14) by kids and collecting them was often experienced as a competing game. Rural snacks consisted mainly of berries, but also of young shoots, leaves and flowers, such as those of *Primula* spp. L., *Trifolium pratense* L and *Robinia pseudoacacia* L., from which, in particular in case of the latter, the sweet nectar was sucked. In addition to flowers and ripe fruits, kids were often attracted by the sour taste of unripe wild fruits and young shoots. The only rural snack consumed as leaves was *Rumex acetosa* L. popularly known as "*erba brossca*" which means in fact sour grass. In general, the Asteraceae wild greens were cooked by pan-frying or consumed together with other wild plants as mixed vegetables (Figure 14 and Table 4).

The plant species of the present study (area of Bologna, Northern Italy) were compared with those listed by two Italian ethnobotanical surveys focused on wild food plants traditions and carried out in Castelmezzano village and in the Graecanic area (Lucania and Calabria region, southern Italy) (Nebel et al., 2006; Pieroni et al., 2005). From a general point of view, most of the recorded plant species are common among the three study areas, like most of the wild fruits and some Asteraceae plants. On the other hand, some specific differences could be pointed out such as for thistles (*Carlina acaulis* L., *Cynara cardunculus* L. ssp. *cardunculus*, *Silybum marianum* L., *Scolymus hispanicus* L.) which were collected and consumed by both people of Castelmezzano and Graecanic area, but not in Bologna. Conversely, species like *Sanguisorba minor* Scop., widely known as *"pampinela"*, and *Urtica* spp., are still very popular and used in Bologna's territory, but were not present in the other two surveys (Nebel et al., 2006; Pieroni et al., 2005).

Table 5 Botanical families of wild food plants traditionally consumed in the area of Bologna

Botanical family	**N° of wild food plant species**
Rosaceae	14
Asteraceae	9
Lamiaceae	7
Fabaceae	3
Brassicaceae	2
Fagaceae	2
Liliaceae	2
Moraceae	2
Adoxaceae	1
Apiaceae	1
Asparagaceae	1
Boraginaceae	1
Cannaboideae	1
Caprifoliaceae	1
Caryophyllaceae	1
Cornaceae	1
Corylaceae	1
Juniperoideae	1
Gentianaceae	1
Juglandaceae	1
Lauraceae	1
Papaveraceae	1
Polygonaceae	1
Portulacaceae	1
Primulaceae	1
Punicaceae	1
Ranuncolaceae	1
Ruscaceae	1
Urticaceae	1
Valerianaceae	1
Verbenaceae	1
Violaceae	1
Vitaceae	1

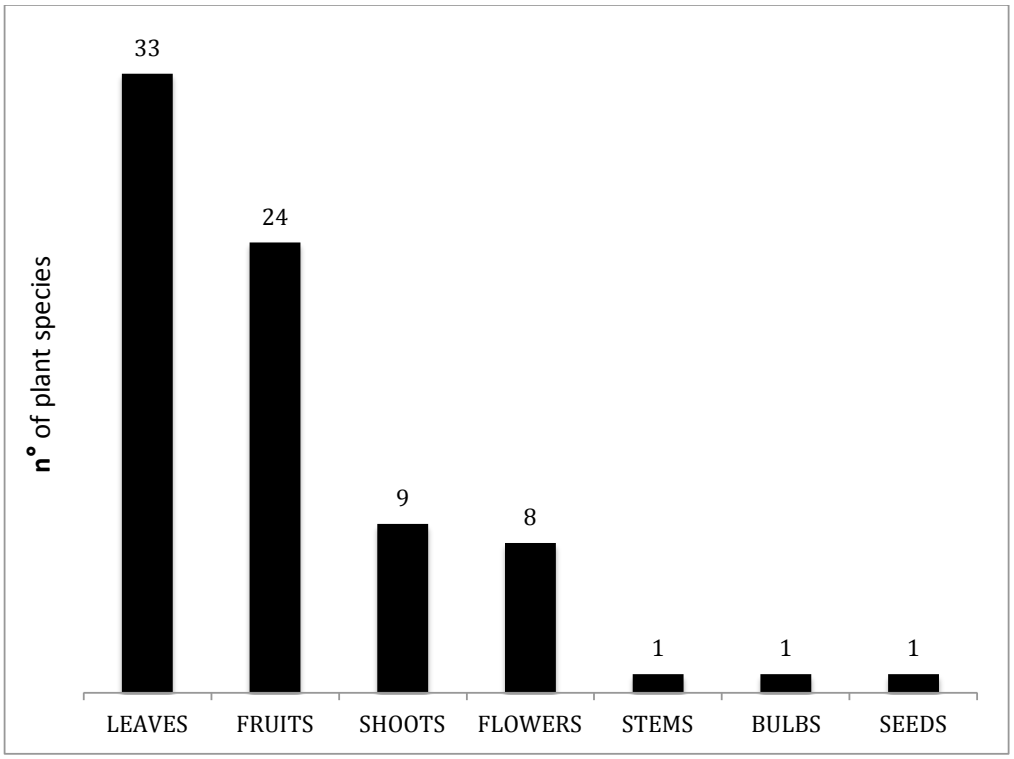

Figure 13. Parts of the wild food plants traditionally consumed in the area of Bologna. The number above each bar indicates the total number of species used in each category.

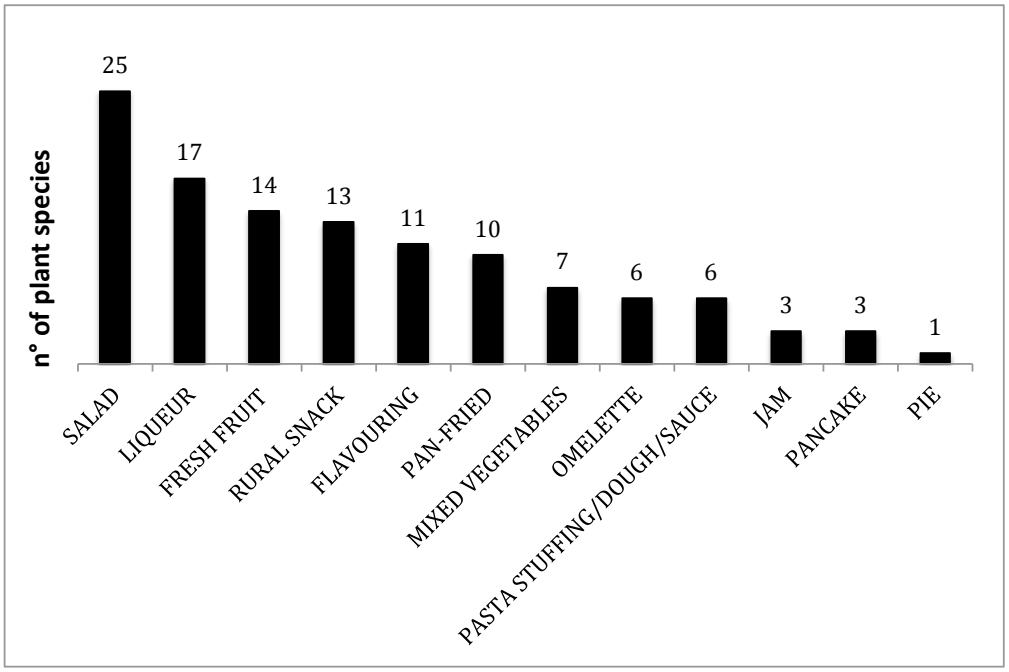

Figure 14. Culinary uses of the wild food plants traditionally consumed in the area of Bologna. The number above each bar indicates the total number of species used in each category.

3.1.3.2 Folk plant classification and folk names

Folk plant names were mentioned by the informants according to their own plant classification (folk systematic) in which the elementary unit is represented by a folk generic, also called ethnospecies (as defined by Signorini et al., 2007), recognizable on the basis of differences in macro-morphology, habitat and use of the plant (Berlin et al., 1973). The present survey evidenced that, in several cases, more than one related plant species, that cannot be distinguished by a non-expert, were assimilated and identified as a single ethnospecies (under differentiation). It should be noted that wild food plants were usually collected at the rosette stage or as young shoots, when the plant lacks a flower, the most important botanical identification character. This is the case for folk plants commonly named "*frabbs*", a term that equally refers to two species, *Sonchus asper L.* and *Sonchus arvensis L.,* that are morphologically related but have a different leaf shape (Table 4, Figure 15 and 16). As these two species have a similar taste, they were indiscriminately used and, therefore, shared the same folk name. Analogously, the word "*radećć*" was equally assigned to *Cichorium intybus* L., *Sonchus* spp. L., *Taraxacum officinale* Weber and *Crepis* spp., a large group of plants of which the basal leaves were collected in the same period of the year (end of winter - beginning of spring, sometimes early autumn), eaten raw (tender leaves of young plants) or boiled (bigger older leaves collected in the late vegetative stage), and cooked in a similar way (Figure 17). In Dalmatia (Southern Croatia), an analogous group of species (mainly *C. intybus* L. and *Crepis* spp.), belonging to Cichorioideae (Asteraceae family), are similarly called "*radić*" and collected as rosette basal leaves (Łuczaj et al., 2013).

In general, plant names of folk systematic are not useful for botanical identification but rather associated to practical purposes and final use. In addition, plant folk names may be related to botanical characteristics, habitat, taste or the relationship between man and those plants. The present study pointed out several folk names related to a botanical character, such as "*strecapugno*", which means "clenched in a fist", referring to *C. vesicaria* subsp. *taraxacifolia* (Thuill) Thell (Table 4, Figure 18). In fact, local people knew that this plant, after being cut from the ground, was going to rapidly close on itself and so they cleaned it immediately after collection. "*Piscialét*" instead referred to the diuretic property of *T. officinale* Weber (Figure 11). As earlier mentioned, *C. intybus* L. was commonly indicated

as "*radećć*" but also more specifically as "*radećć cavdagn*", suggesting that this species belonging to the radećć category was mostly found along small country roads (cavdagn). Other examples of meaningful folk names are the above-mentioned "*erba brossca*", which referred to the acid taste of *Rumex acetosa* L. leaves, and "*ciucchett*", a onomatopoeic word that matched the popping sound produced when *Silene vulgaris* (Moench) Garcke flowers, which have a balloon-like capsule, were squeezed (Figure 18).

Figure 15. *Sonchus asper* L.: basal leaves and flowers.

Figure 16. *Sonchus arvensis* L.: basal leaves and flowers.

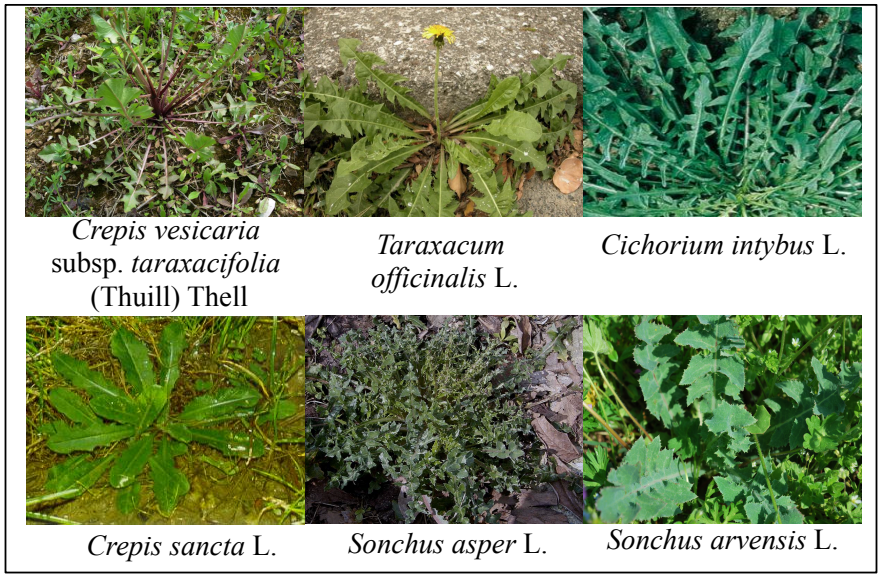

Figure 17. "Radećć" species: group of plants of which the basal leaves were collected in the same period of the year and consumed in the same way.

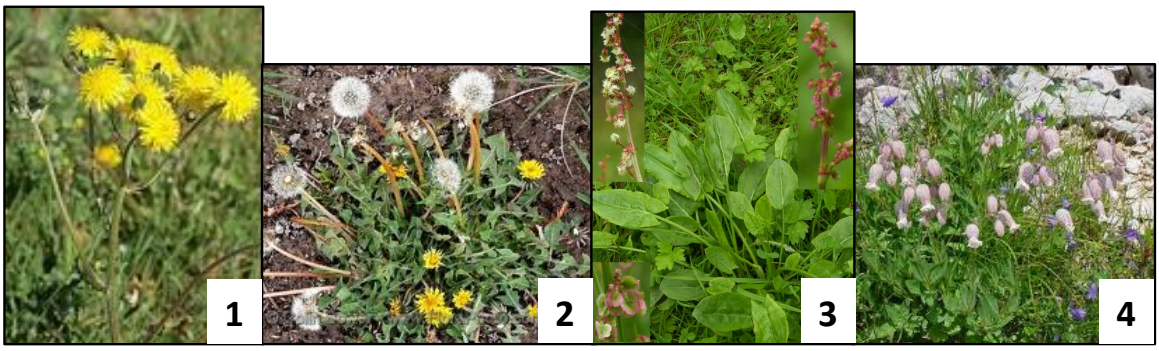

Figure 18. Folk names of wild food plants consumed in Bologna's area.
1: Strecapugno (*Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell); 2: piscalét (*Taraxacum officinale* Webwe); 3: erba brossca (*Rumex acetosa* L.); 4: ciucchett (*Silene vulgaris* (Moench) Garcke).

3.1.3.3 Medicinal use of wild food plants

Eleven plant species of the area of Bologna, were also mentioned as having therapeutic effects (Table 4). A few of these, after specific therapeutic preparation, were used as medicine without any direct relation to their alimentary use. *Lippia citriodora* Kuntze, *Salvia pratensis* L., *Mentha* spp., *Rosmarinus officinalis* L., all belonging to the Lamiaceae family, were used to make a decoction for digestive purposes. In addition, *L. citriodora* Kuntze leaf decoction was applied to treat muscular and articular pains after a trauma, *S.*

pratensis L. was used for female genital problems (infusion) and as toothpaste (fresh leaves), while *R. officinalis* L. was utilized as a decongestant (fumigations). The flower infusion of *Crataegus monogyna* Jacq. was reported to be relaxing, to facilitate sleep and to be useful for heart problems. Other folk plants were reported to have therapeutic effects when part of the everyday diet. The wild species with the highest number of cited medicinal uses was *Urtica* spp., which if consumed within the diet or as beverage (cooking water), was reported to be refreshing, mineralizing and active against kidney problems. Moreover, *Urtica* cooking water was often used as shampoo to improve hair strength and shine and to eliminate dandruff. Other reported applications were as a remedy for arthritis (by rubbing fresh leaves on the aching areas of the body) and as insecticide (using macerated leaves). Some plants, in particular *T. officinale* Weber, *C. vesicaria* subsp. *taraxacifolia* (Thuill) Thell and *Sonchus* spp. were defined as functional foods (Pieroni and Quave, 2006) having depurative, blood cleaning and refreshing effects. Besides, *T. officinale* Weber, *C. vesicaria* subsp. *taraxacifolia* (Thuill) Thell (of which informants reported to also drink the cooking water) and *Crepis sancta* (L.) Babc. were in general reported to have diuretic and laxative actions so that these plants may all be considered medicinal foods (Pieroni and Quave, 2006). Finally, *Sambucus nigra* L., consumed as jam, was mentioned to relieve bone problems like rheumatisms.

3.1.3.4 Perception and health impact of wild food plants

During the interviews, the informant's perceptions regarding the impact of wild food plants on human nutrition and health, as well as the differences between wild, self-cultivated and large-scale cultivated edible plants, were also investigated. All interviewed people perceived wild plants as being the healthiest for humans because they grow naturally without man's intervention and, consequently, they were likely to contain the highest amounts of nutrients and beneficial substances. The respondents also perceived self-cultivated plants as better than those produced on a large-scale and purchased in stores, because, in the latter case, the exact production process was unknown.

3.1.3.5 Taste of collected wild food plants

Among the secondary metabolites produced by plants, phenolics, terpenes and alkaloids (Bourgaud et al., 2011) are those that mainly contribute to the bitter, sour or astringent tastes (Bravo, 1998; Rouseff, 1990; Drewnowski and Rock, 1995; Ames et al., 1990). These substances mostly accumulate in leaves and shoots, but also in flowers and roots and, among other effects, provide a defence against herbivorous predators by making the plant unpalatable (Bravo, 1998). Although potentially beneficial to human health in small doses, many of such compounds are, in fact, toxic (Ames et al., 1990). Among the previously listed wild plants (Table 4), those reported to be the most bitter were *Clematis vitalba* L., *T. officinale* Weber and *C. vesicaria* subsp. *taraxacifolia* (Thuill) Thell, with a different degree of bitterness depending on individual perception. A wild edible plant with a particular strong flavour similar to arugula is *Diplotaxis tenuifolia* L. (DC) that, for this reason, was always used in combination with other vegetables. Instead, a delicate sweet flavour, also appreciated by children (often reluctant to eat wild greens because of their bitterness), was reported for *Silene vulgaris* (Moench) Garcke and for *Valerianella locusta* L. (Laterrade) which, therefore, were also eaten raw in salads (Table 4, Figure 11).

3.1.4 ETHNOBOTANICAL STUDY CONDUCTED IN MIDDLE AGRI VALLEY

3.1.4.1 Informants

Fifty-eight people still retaining traditional local knowledge were interviewed, 43 women (74%) and 15 men (26%). The age of informants ranged between 33 and 96 years, with a mean of 70 and a median of 73.

In general, it was simpler to find women available to speak about wild food plants, respect to than men, probably because the women live longer and more women are present among the elderly. (Figure 19; Contributo al piano strategico intercomunale della Val D'Agri, 2014). In addition, for many years in the past, in area of the Val D'agri, the men head of the family used to emigrate elsewhere for work reasons, while wives and children remained at the villages. Besides the preparation of food was considered a "female affair".

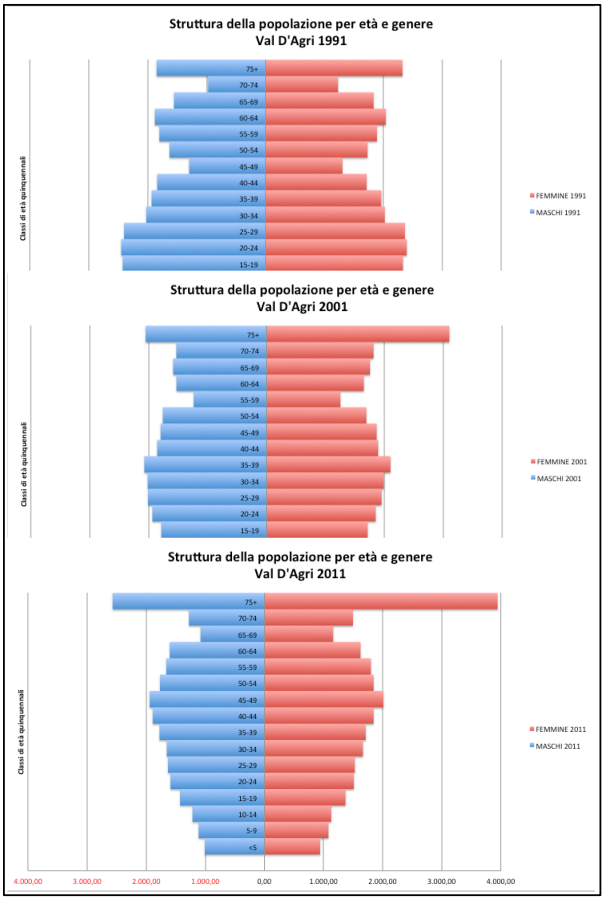

Figure 19. The composition of the population of Agri Valley shown by age and gender detected by census 1991, 2001 and 2011 (ISTAT data).

3.1.4.2 Wild food plant data

Interviewed informants cited 45 different wild plant species that were listed in Table 6 together with the relative botanical family, RFC index, parts of plant used, common and folk name, culinary and medicinal uses.

Some mentioned plant species are still under study in order to definitively correlate the folk with the botanical names and therefore they were not reported in Table 6. In particular, it was not possible to identify a group of wild plants belonging to the "artichoke family" as they were very similar and almost not anymore used in the area. However, based on other ethnobotanical researches conducted in neighbouring areas and on the collected information, it was possible to speculate that the mentioned species could have been: *Cynara cardunculus* L. spp. *Cardunculus* L., *Carduus pycnocephalus* L., *Onopordum illyricum* L., *Scolymus hispanicus* L., *Silybum marianum* (L.) Gaertner, *Cnicus benedictus*

L., *Galactites tormentosa* Moench, *Cirsium arvense* (L.) Scop. For these reasons the number of wild food plant species was underestimated.

Similarly to the Bologna's area, in the Agri Valley survey, the most representative botanical families were Rosaceae (7 plant species cited of 45 = 15%) and secondly Asteraceae (6 plant species cited of 45 = 13%) (Table 7). The parts of the plants mostly used were leaves (22 plant species of 45 = 49%) and fruits (12 plant species of 45 = 27%) (Figure 20).

Twelve of the mentioned wild fruits are actually less and less spread and almost forgotten (*Arbutus unedu* L., *Cornus mas* L., *Sorbus domestica* L., *Ziziphus jujuba* Mill.); on the contrary, other species, although they only grow in certain habitats of the study area, are still largely collected and appreciated (for example *Ficus carica* L., *Fragaria vesca* L., *Rubus ulmifolius* Scott.).

Overall, the wild food species most known and consumed was *Cychorium intybus* L. (RFC 0.95), followed by *Sonchus* spp. (*oleraceus* L., *asper* L., *arvensis* L.) (RFC 0.76), *Foeniculum vulgare* Mill. (RFC 0.6) and *Beta vulgaris* L. (RFC 0.52) which were also recognized, used and appreciated (Table 6, Figure 21).

The ethnobotanical research on wild food plants traditionally consumed in the Basilicata region dates back to the late 80s of the last century (Caneva et al., 1997). The areas close to the city of Potenza, in the North of the region, were those most studied. This research pointed out 230 plants used for food or aromatic purposes, among which several belonged to the Asteraceae family. Noteworthy, it was the use of *Cardueae* species (prickly adult plants belonging to the Asteraceae family) that could be considered a poverty index of those populations as these species are full of thorns and requires a long procedure to make them edible. The study described the alimentary use of stems of *Carduus pycnocephalus* L., *Cnicus benedictus* L., *Eringium campestre* L., *Galactites tormentosa* Moench, *Cynara cardunculus* L., *Onopordum* sp. pl. (cut into small pieces and fried); leaves of *Scolymus hispanicus* L. (boiled or fried as omelette); flowers and leaves of *Silybum marianum* (L.) (boiled or cooked in a soup). On the contrary, in the present study in the area of Middle Agri Valley, the informants also refereed the use of the above mentioned plant species but only as a past habit.

Other researches regarding wild food plants were carried on on the Tyrrhenian sector of Basilicata (Guarrera and Salerno, 2003, Guarrera et al., 2006) and on the Arbereshë population that came in the region as immigrants and since some centuries lived there as

quite a closed community (Pieroni et al., 2001, Giusti et al., 2002, Pieroni, 2003, Pieroni and Quave, 2006). The only previously published ethnobotanical study in Agri Valley area was carried out by Capasso et al. (1982), focused on the traditional phytotherapy and represented the first study made in Basilicata region on ethnomedicine. Some plant species reported as phytoremedies (Capasso et al., 1982), were also mentioned in the present study to be used as food, but without any relation to possible therapeutic properties, indicating the loss during the years of such knowledge. The only wild species reported as medicinal plant in both studies was *Foeniculum vulgare* Mill., whose seeds were used in infusion together with other plants to promote the ejection of intestinal gas (Capasso et al., 1982) and to improve digestion (present survey) so confirming the use as gastrointestinal remedy. Besides, Capasso et al. (1982) reported the use of *Foeniculum vulgare* Mill. seeds as bacteriostatic so explaining the reason why they were traditionally added to salami in this area.

Table 6. List of the wild food plants used in the Middle Agri Valley

Botanical name	RFC	Botanical family	Part of plant used	Common name	Folk name
Allium schoenoprasum L.	0.03	Amaryllidaceae	leaves	erba cipollina	
Arbutus unedo L.	0.05	Ericaceae	fruits	corbezzolo	gan'l
Apium nodiflorum (L.) Lag.	0.02	Apiaceae	leaves	crescione	crescione
Asparagus acutifolius L.	0.48	Asparagaceae	shoots	asparago selvatico	sparasc'
Beta vulgaris L.	0.52	Chenopodiaceae	leaves	bietola comune	iet'
Borago officinalis L.	0.43	Boraginaceae	leaves	borragine	burraccia
Capparis spinosa L.	0.14	Capparaceae	buds	capperi selvatici	
Cichorium intybus L.	0.95	Asteraceae	leaves	cicoria selvatica	cicoria
Clematis vitalba L.	0.34	Ranuncolaceae	shoots	vitalba	grambullin', vitacchia
Cornus mas L.	0.05	Cornaaceae	fruits	corniolo	curnal'
Diplotaxis tenuifolia L. (DC)	0.12	Brassicaceae	leaves	ruchetta selvatica	
Equisetum arvense L.	0.02	Equisetaceae	shoots	coda di cavallo	stoccagnung
Ficus carica L.	0.02	Moraceae	fruits	fico	fic'
Foeniculum vulgare Mill.	0.60	Apiaceae	seeds/leaves/stems	finocchio selvatico	finuch'
Fragaria vesca L.	0.19	Rosaceae	fruits	fragola di bosco	
Glycyrrhiza glabra L.	0.21	Fabacecae	roots	liquirizia	rrarc
Humulus lupulus L.	0.19	Cannabaceae	shoots	luppolo	gupl'
Lactuca serriola L.	0.10	Asteraceae	leaves	lattuga selvatica	scarola
Lactuca virosa L.	0.03	Asteraceae	leaves	lattuga selvatica	scarola
Laurus nobilis L.	0.14	Lauraceae	leaves	alloro	laur'
Leopoldia comosa (L.) Parl.	0.47	Asparagaceae	bulbs	lampascione	cipullun',
Malus sylvestris Mill.	0.02	Rosaceae	fruits	melo selvatico	pomo silvestr'
Mentha pulegium L.	0.14	Lamiaceae	leaves	menta poleggio	piliesc'
Mentha spicata L.	0.14	Lamiaceae	leaves	menta selvatica	
Mespilus germanica L.	0.03	Rosaceae	fruits	mespolo selvatico	nespulë
Morus sp.	0.03	Moraceae	fruits	gelso	cieusi
Origanum vulgare L.	0.19	Lamiaceae	leaves	origano comune	arigan'
Papaver rhoeas L.	0.29	Papaveraceae	leaves	papavero	paparina
Pastinaca sativa L.	0.22	Apiaceae	roots	pastinaca	rrarc pastanacc'

Portulaca oleracea L.	0.31	Portulaceae	leaves	porcellana comune	purchiazz'
Picris hieracioides L.	0.17	Asteraceae	leaves	aspraggine comune	spruin'
Prunus spinosa L.	0.07	Rosaceae	fruits	prugno selvatico	
Pyrus pyraster (L). Du Roi	0.07	Rosaceae	fruits	pero selvatico	
Robinia pseudoacacia L.	0.09	Fabacecae	flowers	acacia	cagg'
Rosmarinus officinalis L.	0.07	Lamiaceae	leaves	rosmarino	
Rubus ulmifolius Schott	0.22	Rosaceae	fruits	rovo comune	rivital'
Ruscus aculeatus L.	0.10	Liliaceae	shoots	pungitopo	
Sambucus nigra L.	0.10	Adoxaceae	flowers	sambuco	
Sinapis alba L.	0.03	Brassicaceae	leaves	senape bianca	sinap'
Sinapis arvensis L.	0.38	Brassicaceae	leaves	senape selvatica	ass'n
Sonchus spp. (oleraceus L., asper L., arvensis L.)	0.76	Asteraceae	leaves	grespino comune	sivon'
Sorbus domestica L.	0.10	Rosaceae	fruits	sorbo comune	sur'v
Taraxacum officinalis Weber	0.09	Asteraceae	leaves	tarassaco	pasc' percor'
Urtica spp.	0.29	Urticaceae	leaves	ortica	lurdicul'
Ziziphus jujuba Mill.	0.05	Rhamnaceae	fruits	giuggiolo comune	scesc'l

Table 7 Botanical families of wild food plants traditionally consumed in the Middle Agri Valley.

Botanical family	**N° of wild food plant species**
Rosaceae	7
Asteraceae	6
Lamiaceae	4
Apiaceae	3
Brassicaceae	3
Asparagaceae	2
Fabaceae	2
Moraceae	2
Adoxaceae	1
Amaryllidaceae	1
Boraginaceae	1
Cannabaceae	1
Capparaceae	1
Chenopodiaceae	1
Cornaceae	1
Equisetaceae	1
Ericaceae	1
Lauraceae	1
Liliaceae	1
Papaveraceae	1
Portulacaceae	1
Ranuncolaceae	1
Rhamnaceae	1
Urticaceae	1

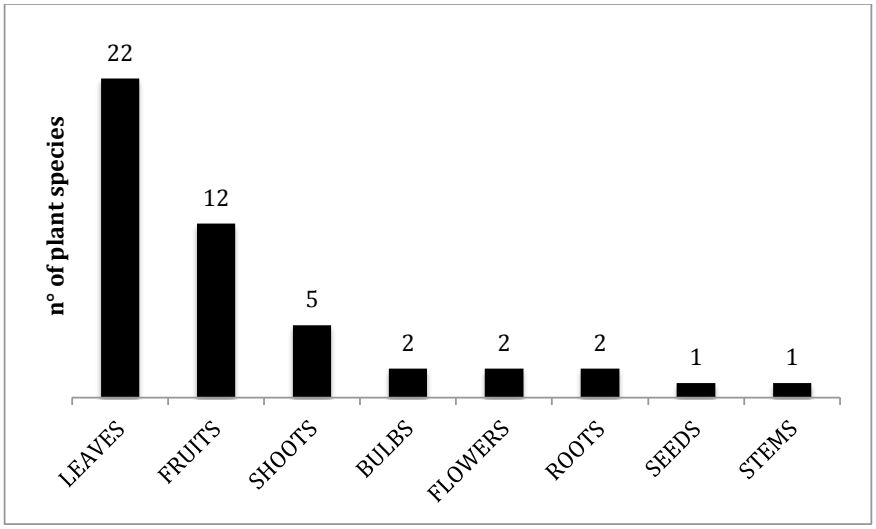

Figure 20. Parts of the wild food plants traditionally consumed in the Middle Agri Valley. The number above each bar indicates the total number of species used in each category.

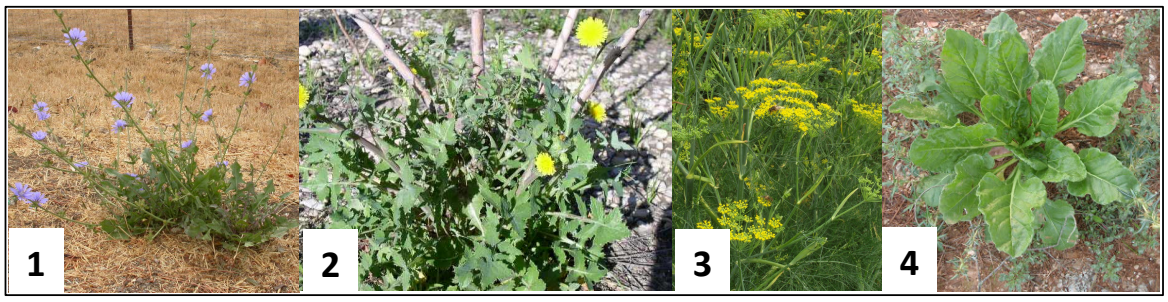

Figure 21. Most consumed wild food plants species in the Middle Agri Valley.
1: *Cichorium intybus* L.; 2: *Sonchus oleraceus* L.; 3: *Foeniculum vulgare* Mill.; 4: *Beta vulgaris* L.

3.1.4.3 Traditional foods and dishes using wild plants

Born because of hunger, wars, drought and poorness and grown up in rural societies, the practice of culinary use of wild plants slowly stratified in a territory becoming part of its habits and protagonist of some traditional foods and dishes.

Middle Agri Valley local cuisine is poor and simple but racy, tasty and savoury.

The most frequently mentioned culinary use of greens was as mixed soup (14 plant species). The vegetable soups were generally prepared with beans together with *Beta vulgaris* L., *Borago officinalis* L., *Cichorium intybus* L. (Figure 22). Another wide culinary greens use was as pan-fried (12 plant species) especially with eggs and local homemade salami.

Two species were mentioned as rural snacks: the peeled roots of *Glycirrhiza glabra* L. and the flowers of *Robinia pseudoacacia* L. that were both sucked as their sweet flavour was particularly appreciated by children. In today's society children have few opportunities to be in contact with nature in a free and independent way and they also have available ready-to-use sweet products at their homes. For these reasons, the rural snacks mentioned by the informants have always been described as something belonging to the memories and the past. Similarly to the past, in Middle Agri Valley, wild fennel seeds (*Foeniculum vulgare* Mill.) are nowadays still commonly used to aromatize salami giving them a recognizable taste. Wild asparagus spears (*Asparagus acutifolius* L.) are boiled and fried with olive oil, garlic, eggs and homemade salami. Bulbs of "*lampascioni*" (*Leopoldia comosa* (L.) Parl.) are particularly relished and are macerated in cold water or boiled and prepared in different ways: in oil, pickled, seasoned with olive oil, garlic and chilli or fried with garlic, tomatoes and dried peppers. *Beta vulgaris* L. is used to make stuffed pizza "*calzoni"* with raisin.

After summer rains, it is use to collect escargots that are cooked using a particular wild dried herb called in dialect '*piliesc*' *(Mentha pulegium* L.).

To celebrate Carnival, it's widely cooked the "*rafanata*", a thick omelette made with "*rafano*" (radish), the grated root of *Armoracia rusticana* Gaertn., Mey. et Scherb., pecorino cheese, eggs and parsley. "*Rafano"* is also grated on pasta, especially local homemade "*fusilli"*. This radish gives to these dishes an intense and very hot flavour. *Armoracia rusticana* Gaertn., Mey. et Scherb. was not listed in Table 6 because is a semi-wild plant species, increasingly rare in the wild, but very important as traditional food of this area and largely consumed especially during the winter.

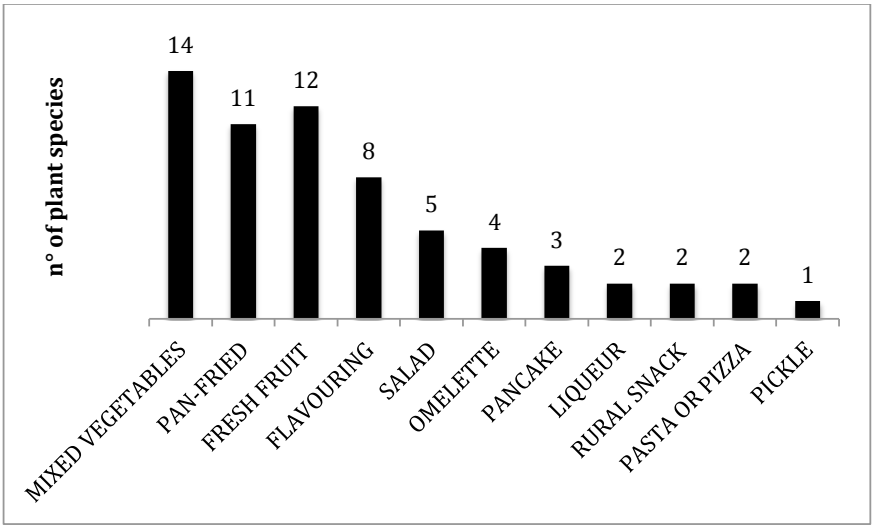

Figure 22. Culinary uses of the wild food plants traditionally consumed in the Middle Agri Valley. The number above each bar indicates the total number of species used in each category.

3.1.4.4 Medicinal use of wild food plants

In the Middle Agri Valley, only few wild food species were mentioned to be used for medicinal purposes.

Foeniculum vulgare Mill. was reported to improve digestion and for this reason it was added to beans soup. The aerial part of *Glycyrrizha glabra* L. was mentioned to be used for feet sweating and the bulbs of *Leopolda comosa* (L.) Parl. to be rubbed at the temples to soothe burning eyes. Flowers of *Sambucus nigra* L. were used together with those of chamomile to make decoction in case of stomachache. The cooking water of some wild greens was also used for therapeutic purposes like *Asparagus acutifolius* L. and *Cichorium intybus* L. respectively for kidneys and liver wellbeing.

3.1.4.5 Folk names

During the study, difficulties were encountered when trying to link folk and botanical names. This was mainly due to the use, in different municipalities sometimes only a few kilometres far from each other, of different folk names related to the same plant species. This is the case of *Clematis vitalba* L. called "*vitacchia"* (municipality of Aliano) and "*grambullin"* (Castronuovo, S. Andrea and Roccanova); *Sonchus* spp. called "*sivun"*

(Sant'Arcangelo), "*cardelle"* (Roccanova, San Martino) and "*iung"* (Aliano); *Sinapis arvensis* L. called "*marogna"* (Roccanova) and "*ass'n"* (Sant'Arcangelo).

It should be noted that none of the cited names had a meaning related to a botanical characteristic of the plant or to its use. When asked regarding the meaning of given folk names, the informants always answered that those were just the proper names of wild plants.

3.1.4.6 Perceived health impact and taste of wild food plants

The bitter taste frequently peculiar of many greens, overall mostly present in plants belonging to the Asteraceae family, was often partially neutralized by boiling or by leaving the plant soaking in water for many hours. Anyway the preference of the bitter or sweet taste was reported to be very variable among the informants. Rarely a single wild green was cooked alone making a soup but most frequently many vegetables were mixed to reach a better final taste. The wild food plants were perceived as healthier, more genuine and tastier than the relative cultivated plant species. A woman living in Sant'Arcangelo declared: "*in the past wild greens were eaten by necessity, now they're eaten for pleasure*".

3.1.5 COMPARISON OF ETHNOBOTANICAL DATA OF TWO AREAS

The ethnobotanical data obtained from the two investigated areas were compared (Table 8).

The most representative botanical family in both areas was that of Rosaceae (14 plant species out of 66 = 21% in Bologna area; 6 plant species out of 45 = 13% in Middle Agri Valley) (Tables 5 and 7). The parts of the plants mostly quoted and used in both areas were the leaves followed by the fruits (Figures 13 and 20).

Crepis vesicaria L. subsp. *taraxacifolia* (Thuill) Thell (the species mostly mentioned in the area of Bologna) was completely unknown to the Middle Agri Valley people even if it was reported to be present in the regional flora (Conti et al., 2005), while *Cychorium intybus* L. (the species mostly mentioned in the Middle Agri Valley area) was used from Bologna people but less frequently respect to the local population of Middle Agri Valley. As regards *Urtica* spp., this plant was and still is very used in the area of Bologna, while in the Middle Agri Valley, was principally used as animal feed and not as people food.

RFC values of the most frequently mentioned plant species in the two study areas, were analysed and compared (Table 8). This ethnobotanical index indicates (for a given folk taxon and a study area) the degree of a shared knowledge possessed by the informants, even if the plant is not anymore consumed. Therefore a high RFC value indicates a wild food plant species very important from a cultural point of view and RFC mean values indicate the local knowledge level of sharing and preserving. Notably, the wild food local knowledge seemed to be more shared and preserved among Middle Agri Valley people than among Bologna's people. In fact, the RFC mean value of Middle Agri Valley was 0.2 in comparison to a RFC of 0.14 in the area of Bologna. Interestingly, *Cychorium intybus* L. RFC value obtained from Middle Agri Valley data was very high (0.95) meaning that this plant was mentioned by almost all respondents. The mean wild food plant species number cited from Middle Agri Valley and Bologna area respondents resulted to be respectively 10.38 and 9.97 (Table 8), indicating (even if not at a significative level) that Middle Agri Valley people knew and used a larger number of wild edibles.

The social and economic situation of the two areas necessarily affected the use of traditional wild food plants by the local population. The economic development and the immigration phenomenon of Bologna area determined on one side the early abandonment of traditional practices, on the other side an overlap and blend of this kind of traditional local knowledge with that of people immigrated from South of Italy and abroad. In the Middle Agri Valley the emigration, depopulation and isolation led to the survival of old traditions but, since they were predominant heritage of older people, the TLK is actually handed down less and less to children and grandchildren mainly given due to people emigration and modernization of lifestyle with a substantial abandonment of the rural lifestyle.

Table 8. Comparison of the most relevant ethnobotanical data regarding collected in the area of Bologna and in the Middle Agri Valley

Data	**Area of Bologna**	**Middle Agri Valley**
N° of informants	39	58
Women (% over total)	25 (64%)	43 (74%)
Men (% over total)	14 (36%)	15 (26%)
Mean age	71	70
Most representative botanical family (% over total species)	Rosaceae (21%)	Rosaceae (13%)
Mostly used part of the plants	leaves	leaves
Most representative species (highest RFC)	*Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell/*Taraxacum officinalis* L. (0.77)	*Cychorium intybus* L. (0.95)
Mean RFC	0.14	0.2
Mean of number of wild food plant species cited from informants	9.97	10.38

3.2 METABOLIC SCREENING OF WILD FOOD PLANTS

3.2.1 WILD FOOD PLANT SPECIES

Thirty-four wild food plant samples were gathered of which 13 in Bologna's territory and 21 in Middle Agri Valley. Table 9 shows the botanical name of plant species, the common name, the part collected for each plant and the area of collection.

Table 9. Collected wild food plant species.

Plant species	Common name	Collected part of the plant	Collection area
Apium nodiflorum (L.) Lag.	Crescione	Leaves	Middle Agri Valley
Asparagus acutifolius L.	Asparago selvatico	Shoots	Bologna & Middle Agri Valley
Bellis perennis L.	Margherita comune	Young leaves	Bologna
Beta vulgaris L.	Bietola commune	Leaves	Middle Agri Valley
Cichorium intybus L.	Cicoria selvatica	Young leaves	Bologna & Middle Agri Valley
Clematis vitalba L.	Vitalba	Shoots	Bologna & Middle Agri Valley
Crepis vesicaria subp. taraxacifolia (Thuill) Thell	Radicchiella a foglia di tarassaco	Young leaves	Bologna
Diplotaxis tenuifolia L. (DC)	Rucola selvatica	Young leaves	Bologna
Foeniculum vulgare Mill.	Finocchio selvatico	Leaves and stems	Middle Agri Valley
Glycyrrhiza glabra L.	Liquirizia	Roots	Middle Agri Valley
Humulus lupulus L.	Luppolo	Shoots	Middle Agri Valley
Lactuca serriola L.	Lattuga selvatica	Leaves	Middle Agri Valley
Leopoldia comosa L. (Parl.)	Lampascione	Bulbs	Middle Agri Valley
Mentha pulegium L.	Menta poleggio	Leaves	Middle Agri Valley
Mentha spicata L.	Menta selvatica	Leaves	Middle Agri Valley
Papaver rhoeas L.	Papavero	Young leaves	Middle Agri Valley
Pastinaca sativa L.	Pastinaca	Roots	Middle Agri Valley
Picris hieracioides L.	Aspraggine commune	Young leaves	Middle Agri Valley
Ruscus aculeatus L.	Pungitopo	Shoots	Bologna & Middle Agri Valley
Salvia pratensis L.	Salvia selvatica	Leaves	Bologna
Sambucus nigra L.	Sambuco	Flowers	Middle Agri Valley
Sanguisorba minor Scop.	Pimpinella	Leaves	Bologna
Silene vulgaris (Moench) Garcke	Strigoli	Leaves	Bologna
Sinapis arvensis L.	Senape selvatica	Leaves	Middle Agri Valley

Sonchus spp.	Grespino commune	Young leaves	Bologna & Middle Agri Valley
Taraxacum officinalis Weber	Tarassaco	Young leaves	Bologna
Urtica spp.	Ortica	Leaves	Bologna & Middle Agri Valley

3.2.2 QUANTIFICATION OF TOTAL POLYPHENOLS, TOTAL FLAVONOIDS AND OF ANTIOXIDANT ACTIVITY

Total polyphenol, total flavonoid and antioxidant activity contents were quantified by spectrophotometric analyses, starting from methanolic extracts of collected plant species.

Among the wild food plant samples gathered in the area of Bologna, the highest total polyphenol content measured in *Sanguisorba minor* Scop. (17.16 mg GA eq/gFW), followed by *Clematis vitalba* L. (7.18 mg GA eq/gFW) and *Salvia pratense* L. (3.87 mg GA eq/gFW) (Figure 23). Regarding the plant samples collected in Middle Agri Valley, *Clematis vitalba* L. (12.65 GA eq/gFW) exhibited the highest total polyphenol content, followed by *Mentha spicata* L. (8.48 GA eq/gFW) and *Mentha pulegium* L. (5.22 GA eq/gFW) (Figure 24). If we consider all edible plants analysed, regardless from the collection area, the highest total polyphenol amount was measured in *Sanguisorba minor* Scop.

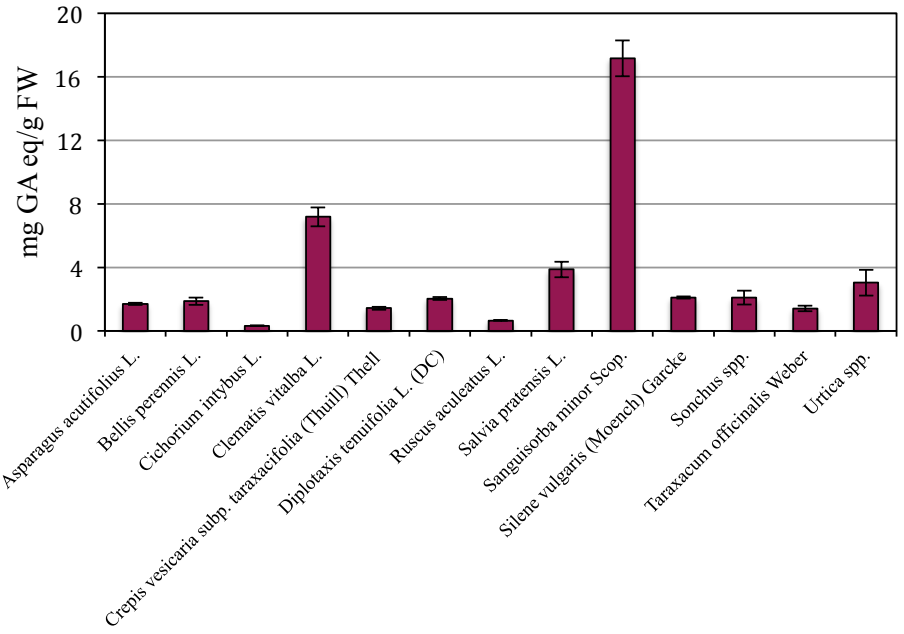

Figure 23. Quantification of total polyphenols in wild food plant samples collected in Bologna's area. Total polyphenol data are expressed as mg of gallic acid (GA) equivalent per g of fresh weight of plant sample ± SD (mg GA eq/FW).

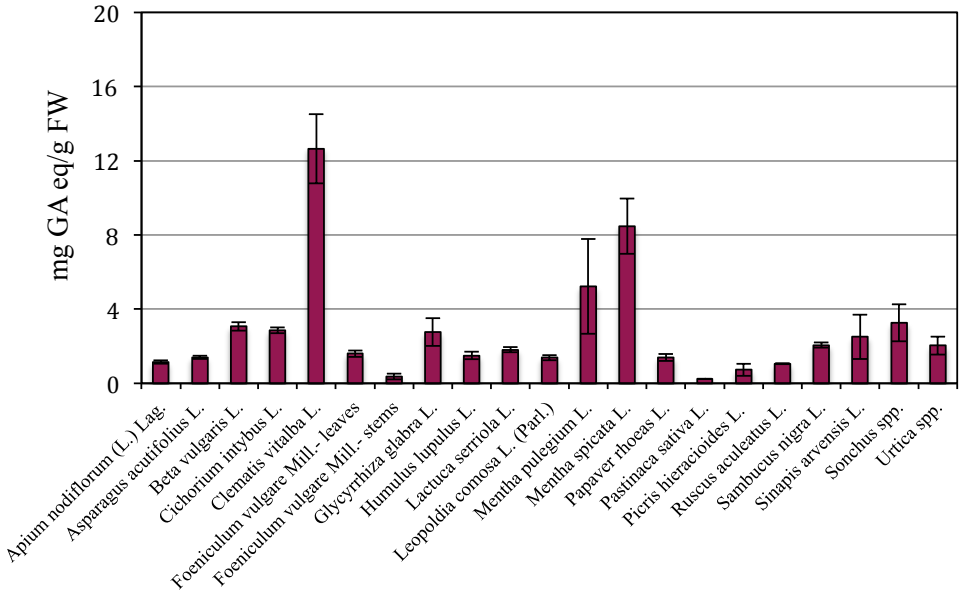

Figure 24. Quantification of total polyphenols in wild food plant samples collected in Middle Agri Valley. Total polyphenol data are expressed as mg of gallic acid (GA) equivalent per g of fresh weight of plant sample ± SD (mg GA eq/FW).

In the area of Bologna, total flavonoid quantification and antioxidant activity results are in agreement with previous data regarding total polyphenol content. In fact, *Sanguisorba minor* Scop. (5.62 mg CAT eq/gFW and 30.06 mg AA eq/gFW), *Clematis vitalba* L. (5.09 mg CAT eq/gFW and 8.20 mgAAeq/gFW) and *Salvia pratense* L. (4.15 mg CAT eq/gFW and 7.43 mgAAeq/gFW) showed the highest values (Figure 25 and Figure 27). Instead, in the Middle Agri Valley, the highest values for both flavonoid content and antioxidant activity were shown by *Mentha spicata* L. (8.33 mg CAT eq/gFW and 14.71 mgAAeq/gFW) followed by *Clematis vitalba* L. (6.77 mgCATeq/gFW and 13.93 mgAAeq/gFW) and *Mentha pulegium* L. (4.78 mgCATeq/gFW and 11.54 mgAAeq/gFW) (Figure 26 and Figure 28).

As expected, a positive correlation could be pointed out among total flavonoid content and the level of antioxidant activity since this activity in plants is largely due to polyphenolic compounds of which flavonoids are one of the most abundant classes.

Among all wild food plant analysed, *Sanguisorba minor* Scop. showed the highest total polyphenol content and the highest antioxidant activity while *Menta spicata* L. evidenced the highest flavonoid content.

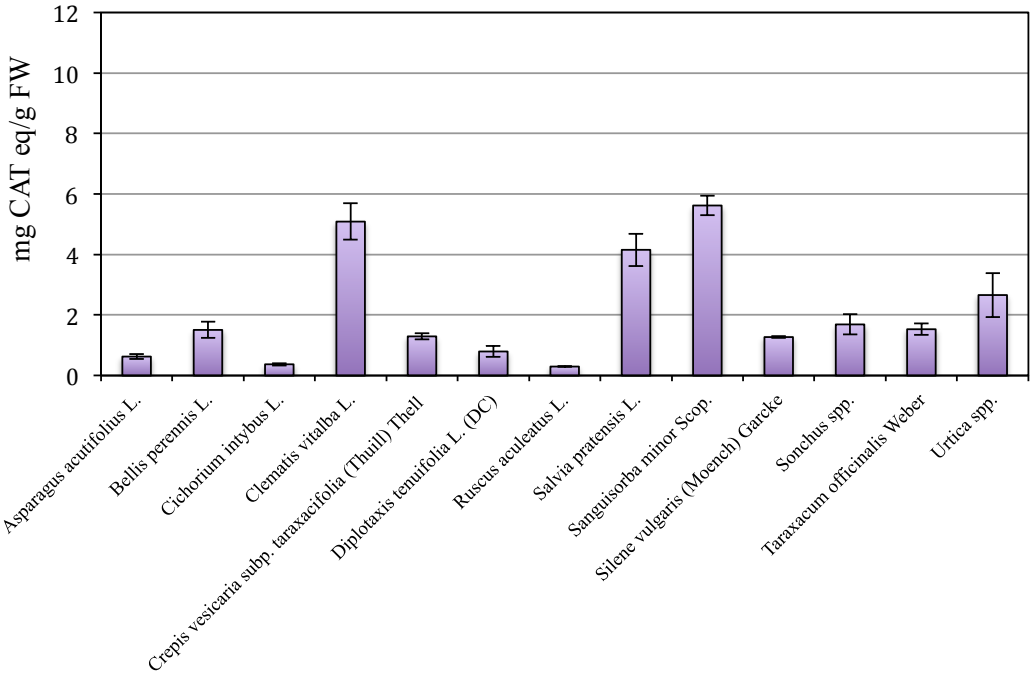

Figure 25. Quantification of total flavonoids in wild food plant samples collected in Bologna's area. Total flavonoid data are expressed as mg of catechin (CAT) equivalent per g of fresh weight of plant sample ± SD (mg CAT eq/gFW).

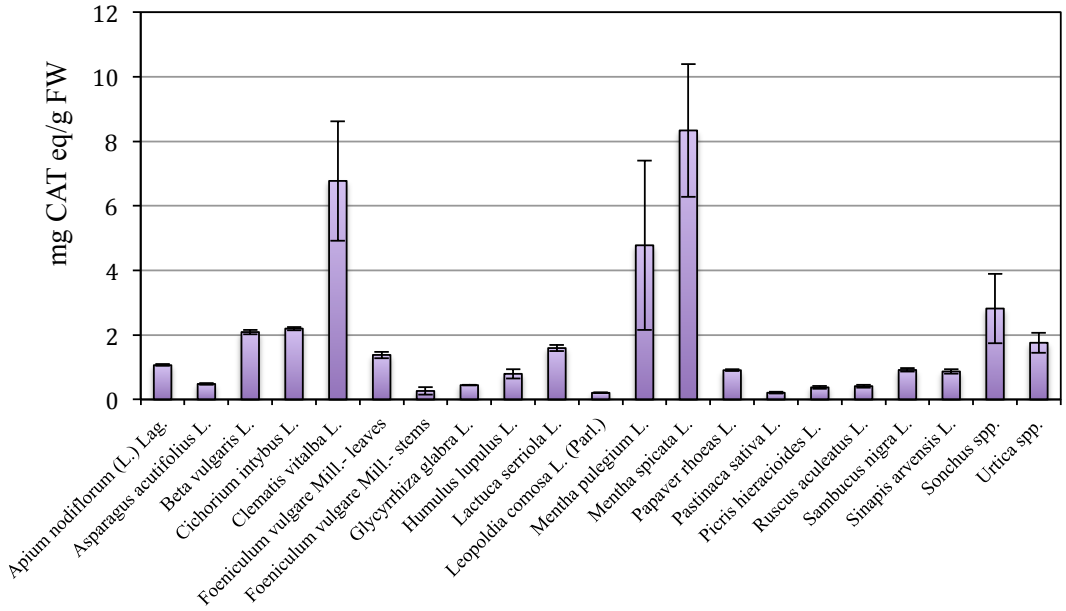

Figure 26. Quantification of total flavonoids in wild food plant samples collected in Middle Agri Valley. Total flavonoid data are expressed as mg of catechin (CAT) equivalent per g of fresh weight of plant sample ± SD (mg CAT eq/gFW).

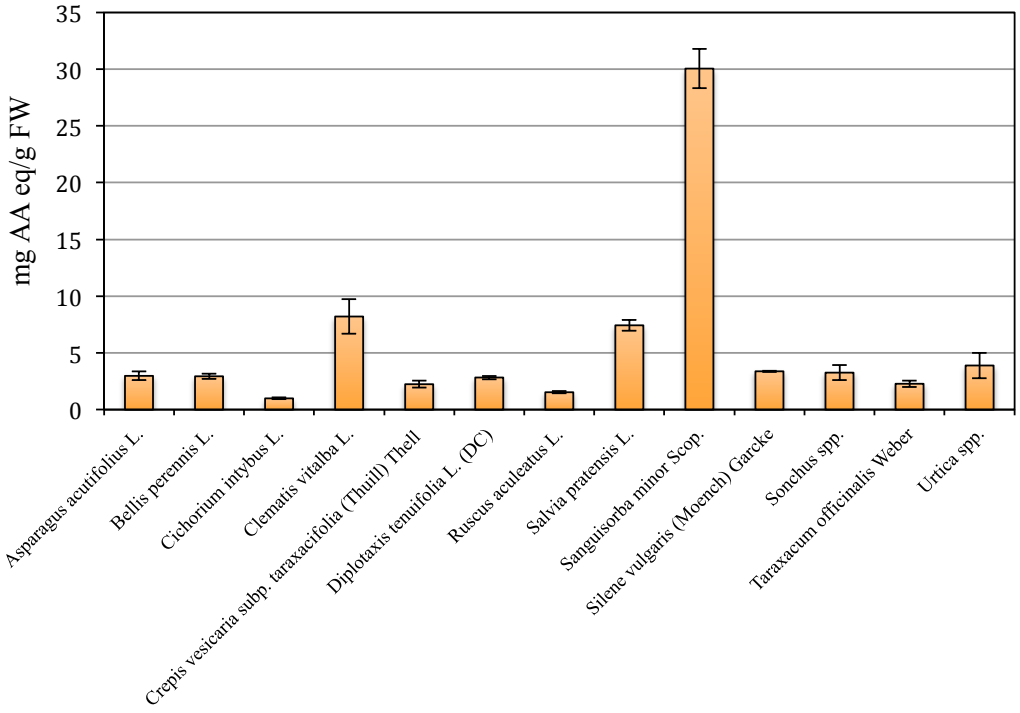

Figure 27. Antioxidant activity of wild food plant samples collected in Bologna's area. Antioxidant activity is expressed as mg of ascorbic acid (AA) equivalent per g of fresh weight of plant sample ± SD (mg AA eq/gFW).

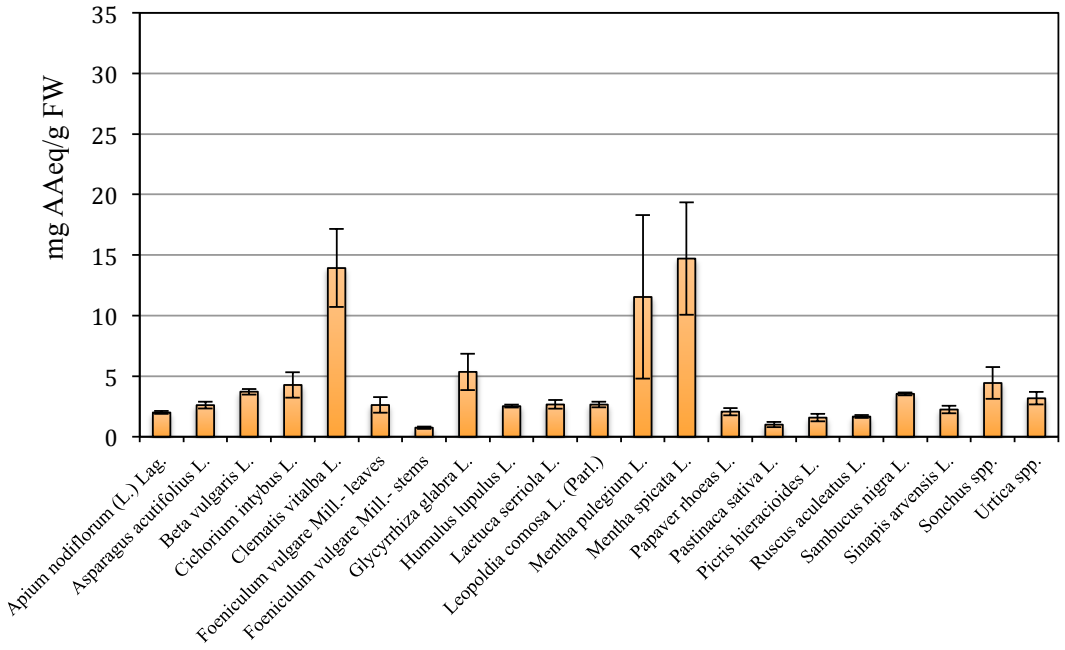

Figure 28. Antioxidant activity of wild food plant samples collected in Middle Agri Valley. Antioxidant activity is expressed as mg of ascorbic acid (AA) equivalent per g of fresh weight of plant sample ± SD (mg AA eq/gFW).

To better understand the health value of the analysed wild food plant samples, we can compare their total polyphenol and flavonoid contents and antioxidant activity with previous data obtained in commonly eaten and well-known beneficial foods such as grapes and wines (Tassoni et al., 2013) and wheat flower (Ferri et al., 2013).

Total polyphenols were determined in Sangiovese red berries and wines by using the same Folin-Ciocalteu method used in the present study. *Sanguisorba minor* Scop. collected in Bologna's area and *Clematis vitalba* L. collected in Bologna and Agri Valley areas resulted to be 3.24folds, 1.35-folds and 2.39 folds respectively higher in polyphenols than Sangiovese berries (on average 5.3 mgGAeq/gFW, Tassoni et al., 2013).

Sangiovese wine resulted to have about 4.0 gGA eq /L so to obtain 280 mg of GA equivalent for a cup of glass (70 ml). The same polyphenols intake of a cup of Sangiovese wine can be obtained with only 16.4 gFW of *Sanguisorba minor* Scop. or 22.1 gFW of *Clematis vitalba* L. collected in Middle Agri Valley.

Comparing the polyphenols content between *Sanguisorba minor* Scop. and wheat flower (methanol extract pH8, Folin-Ciocalteu method, Ferri et al., 2013), *Sanguisorba minor* Scop. resulted to be richer in polyphenols 63.32-fold than wheat flower (0.271 mg GA eq /gFW).

Menta spicata L. proved to have a content of flavonoids 9.41-fold higher than wheat flower (0.885 mg CAT eq/gFW, methanol extract pH8, method described by Zhishen et al., 1999, Ferri et al., 2013).

Sanguisorba minor Scop. revealed to exhibit an antioxidant activity 1.1-fold higher than Sangiovese red berries (on average 27.4 AA eq /gFW, Tassoni et al., 2013) and 15.77-fold higher than wheat flower (1.906 mg AA eq /gFW, 95% methanol extract, ABTS method, Ferri et al., 2013).

3.2.3 QUANTIFICATION OF CHLOROPHYLLS AND CAROTENOIDS

Chlorophylls and carotenoid contents were quantified by spectrophotometric analyses, starting from acetone 85% (v/v) extracts of collected plant species.

Urtica spp. and *Sinapis arvensis* L., collected respectively in Bologna's area and in Middle Agri Valley, showed the highest content of chlorophyll-a, chlorophyll-b and carotenoids (Figure 29 and 30). Among all tested the wild food plant samples, those having the highest

chlorophyll-a, chlorophyll-b and carotenoids levels were *Sinapis arvensis* L., *Mentha spicata* L. and *Lactuga serriola* L. (Figure 30), all collected in the Middle Agri Valley.

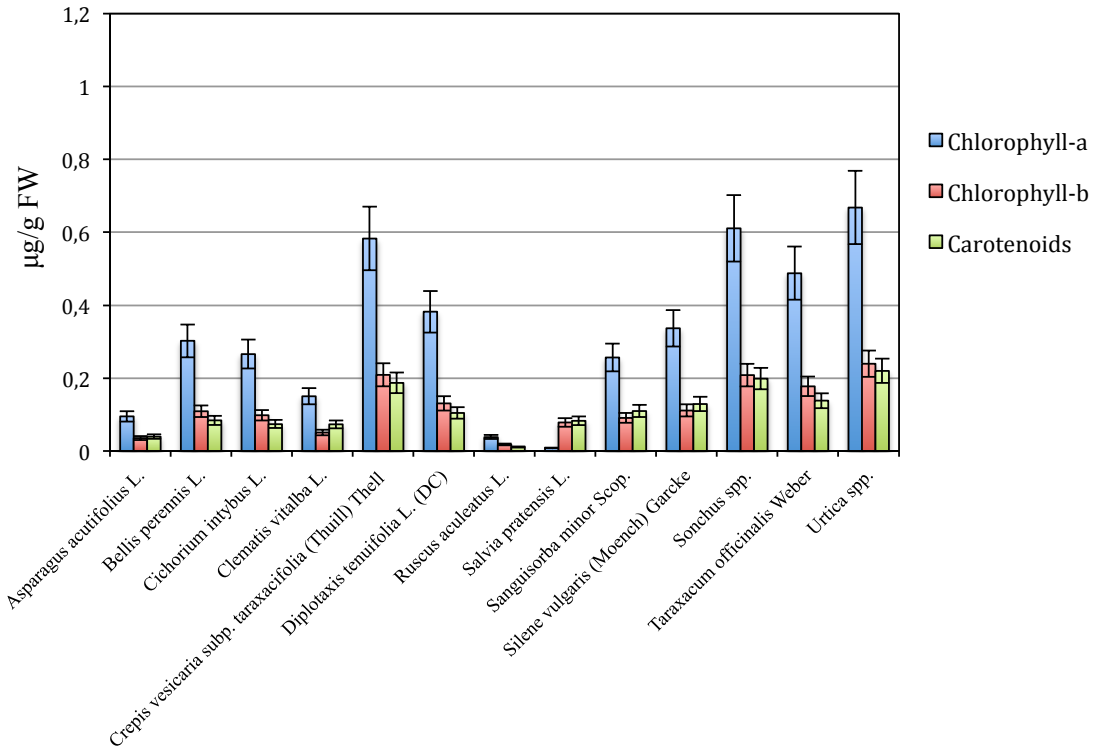

Figure 29. Chlorophyll and carotenoid contents in wild food samples collected in Bologna's area. Data are expressed as µg per g of fresh weight of plant sample ± SD (µg/gFW).

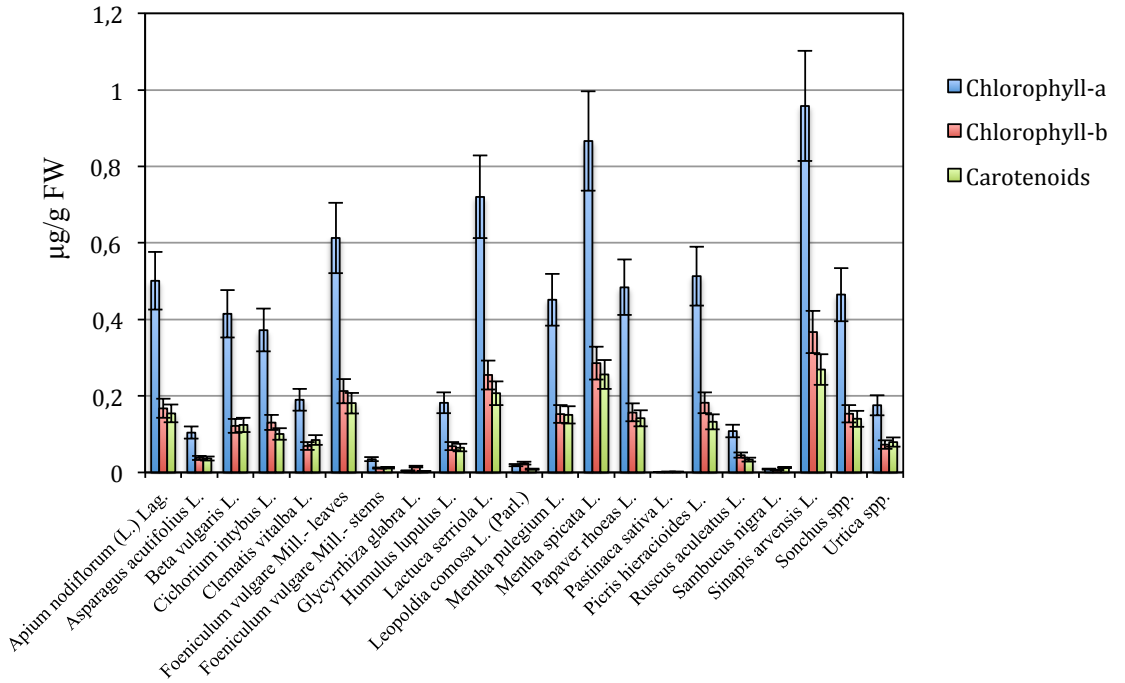

Figure 30. Chlorophyll and carotenoid contents in wild food samples collected in Middle Agri Valley. Data are expressed as µg per g of fresh weight of plant sample ± SD (µg/gFW).

3.2.4 PROTEIN QUANTIFICATION

Protein content was quantified by spectrophotometric analyses, starting from Tris-HCl buffer extracts of collected plant species.

Among the plant samples collected in Bologna's area (Figure 31) and Middle Agri Valley (Figure 32), *Urtica* spp. (29.58 mg/gFW) and *Clematis vitalba* L. (50.46 mg/gFW) showed respectively the highest protein content. Comparing all tested samples, *Clematis vitalba* L. (50.46 mg/gFW), *Glycyrrhiza glabra* L. (42.12 mg/gFW) and *Mentha spicata* L. (31.37 mg/gFW), all collected in Middle Agri Valley, proved to be those richest in proteins.

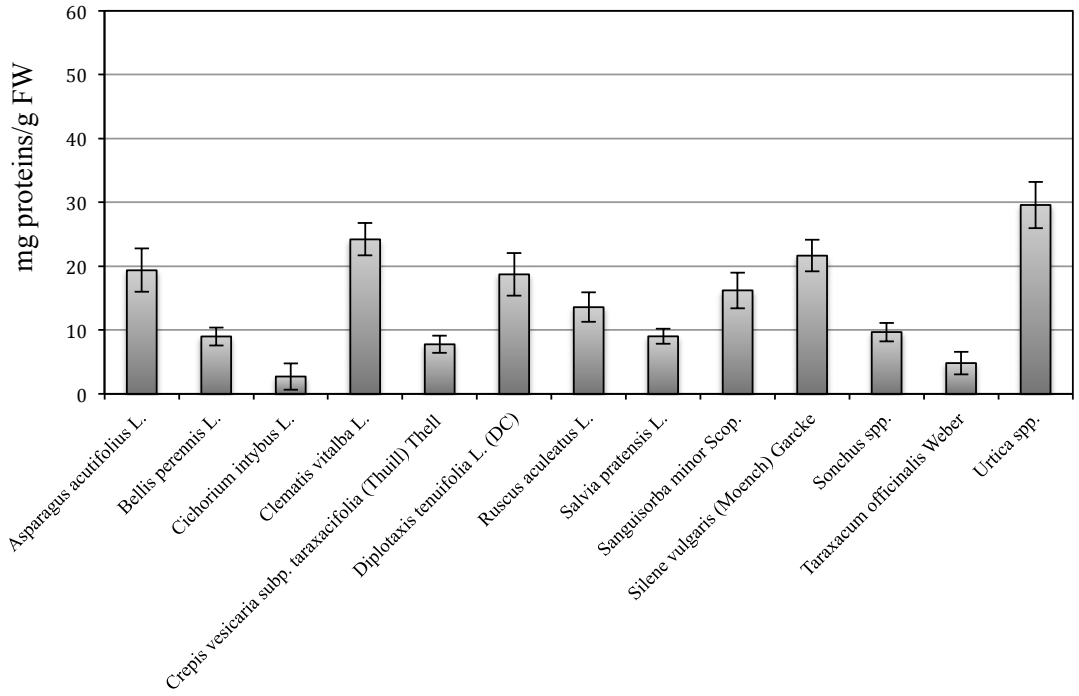

Figure 31. Total protein quantification of wild food plant samples collected in Bologna's area. Data are expressed as mg of proteins per g of sample fresh weight ± SD (mg/gFW).

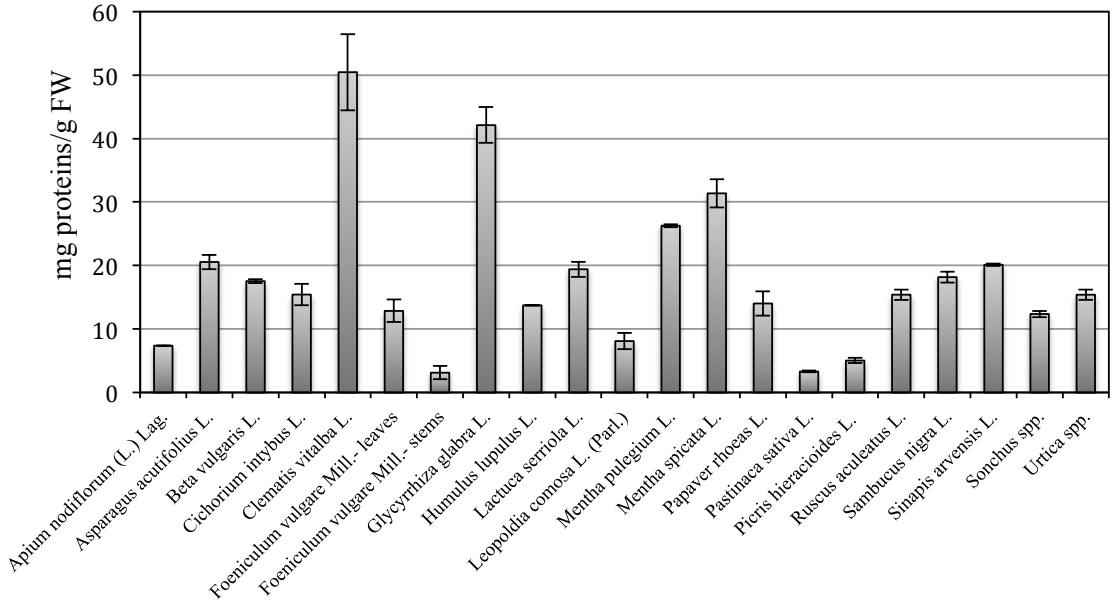

Figure 32. Total protein quantification of wild food plant samples collected in Middle Agri Valley. Data are expressed as mg of proteins detected per g of sample fresh weight ± SD (mg/gFW).

3.2.5 COMPARISON OF WILD FOOD PLANT SAMPLES COLLECTED IN BOTH STUDY AREAS

Various factors can influence plant growth and, consequently, the biosynthesis and accumulation of metabolites. Soil composition, amount of water, intensity and quantity of light, and photoperiod and temperature may positively or negatively influence biochemical biosynthetic pathways.

Data regarding five wild edible plants, *Clematis vitalba* L., *Cichorium intybus* L., *Sonchus* spp., *Urtica* spp. and *Asparagus acutifolius* L., *Ruscus aculeatus* L., collected in both study areas, were directly compared and significant differences were observed. In particular, the amount of total polyphenols, total flavonoids, antioxidant activity (Figure 33), proteins (Figure 34) and chlorophyll/carotenoids (Figure 35), were compared.

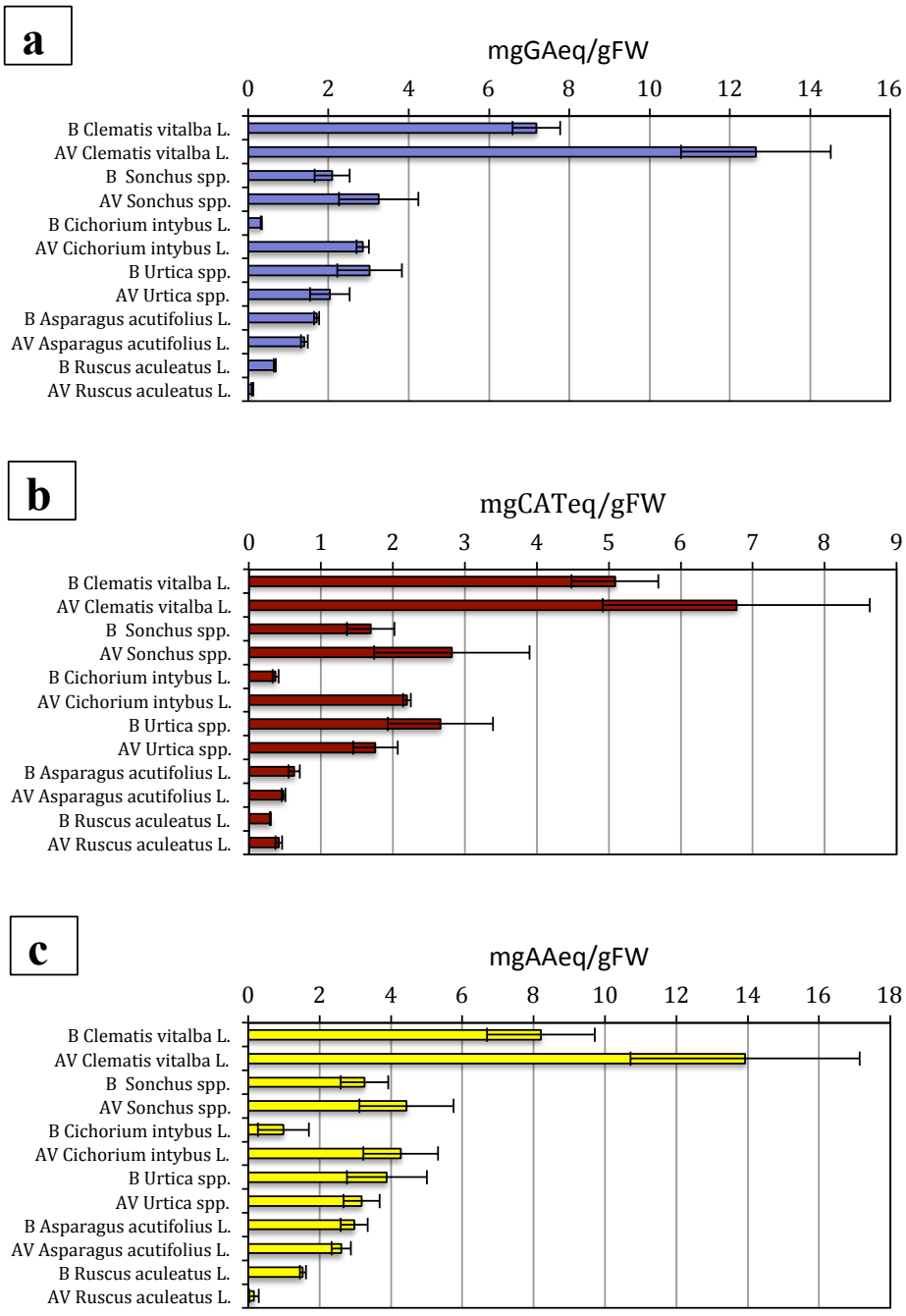

Figure 33. Comparison of total polyphenols, total flavonoids and antioxidant activity levels of wild food plant species collected in both study areas.
Total polyphenol data (a) are expressed as mg of gallic acid (GA) equivalent per g of fresh weight (mg GA eq/gFW), total flavonoid data (b) are expressed as mg of catechin (CAT) equivalent per g of fresh weight (mg CAT eq/gFW) and antioxidant activity (c) is expressed as mg of ascorbic acid (AA) equivalent per g of fresh weight (mg AA eq/gFW). All data are the mean ± SD.
Plant samples collected in Bologna's area are indicated with B, while those collected in Middle Agri Valley are indicated with AV.

As shown in Figure 33 a, b and c, *Clematis vitalba* L., *Cichorium intybus* L. and *Sonchus* spp. showed higher values of polyphenol, flavonoid and antioxidant activity in the samples collected in Middle Agri Valley (indicated with AV) respect to samples collected in Bologna's area. On the contrary, *Urtica* spp., *Asparagus acutifolius* L. and *Ruscus aculeatus* L. showed highest values in samples gathered in Bologna's area.

Urtica spp. collected in Bologna's area and *Clematis vitalba* L. collected in Middle Agri Valley showed the highest values respectively for protein (Figure 34) and chlorophyll/carotenoid contents (Figure 35).

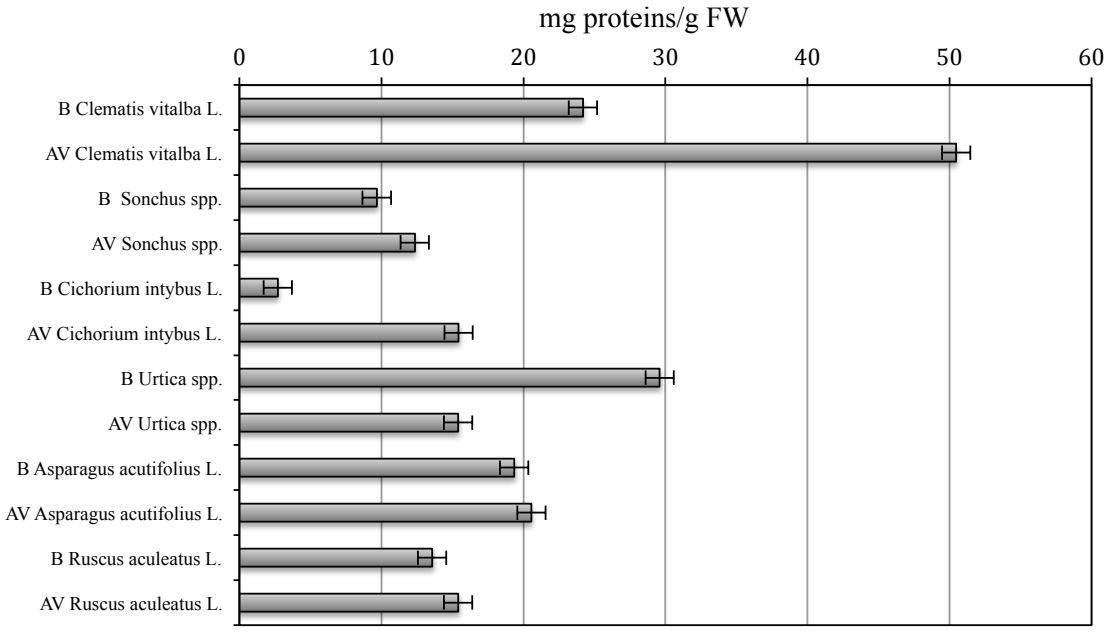

Figure 34. Comparison of protein amounts of wild food plant species collected in both study areas.
Data are expressed as mg of proteins per g of sample fresh weight ± SD (mg /gFW). Plant samples collected Bologna's area are indicated with B, while those collected in Middle Agri Valley are indicated with AV.

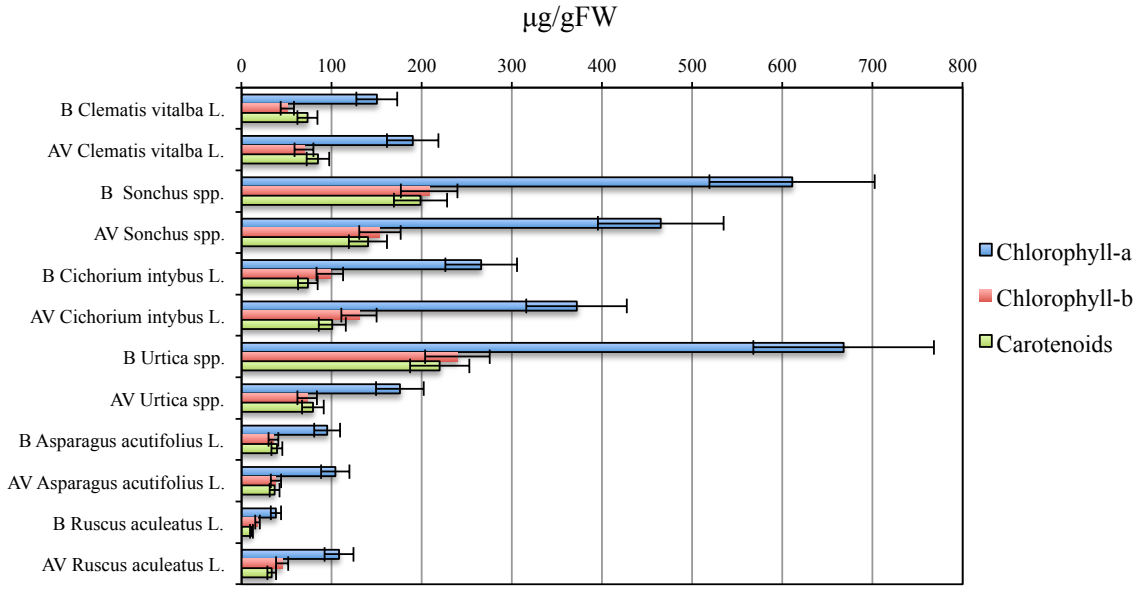

Figure 35. Comparison of chlorophyll-a, chlorophyll-b and carotenoid amounts of wild food plant species collected in both study areas.
Data are expressed as µg per g of fresh weight ± SD (ug / gFW). Plant samples collected Bologna's area are indicated with B, while those collected in Middle Agri Valley are indicated with AV.

It has however to be pointed out that these data only allow a very preliminary comparison of some wild plant samples collected in both study areas. In order to have reliable, repeatable and statistically representative data, other biological samples, multiple specimens, gathered in different seasons and in different collection years have to be tested. The obtained data will subsequently lead to a significative comparison of the differences in the metabolite composition and levels of the wild food plants collected in the two study areas.

3.3 METABOLOMICS STUDY OF CREPIS VESICARIA SUBSP. TARAXACIFOLIA (THUILL) THELL

3.3.1 PLANT METABOLOMICS

As the genome is all the genetic information in a plant, and the proteome is all the proteins, the "metabolome" is all the metabolites: metabolomics is the study of plant small-molecules metabolites profiles. Such small metabolites represent the outcome of gene expression and define the biochemical phenotype. The small metabolites include the intermediates and end products of metabolism, covering both primary metabolites (e.g. sugars, amino acids, fatty acids and organic acids) and secondary metabolites (e.g. phenylpropanoids and alkaloids) (Patti et al., 2012). The composition of herbal products is largely unknown and difficult to determine. Moreover, plants respond to their environment by changing the metabolome, so the composition of plant material can vary depending on the plant growth conditions. In this perspective metabolomics is a useful approach for the simultaneous analysis of many compounds in herbal products making a sort of "metabolites photography".

The most advantageous methodology in plant metabolomics is untargeted, also known as global metabolite profiling. Untargeted metabolomic methods are global in scope and have the aim of simultaneously measuring as many metabolites as possible from biological samples without bias (De Vos et al., 2007).

The metabolomics holistic perspective based on the profiling of a multitude of biochemical components opens up a unique and novel opportunity to reinvestigate natural products. In the study of their bioactivity, the necessary reductionistic approach on single active component, has been successful in the discovery of new medicines, but, at the same time, the synergetic effects of components were lost. Metabolomics allows up the possibility of studying the effect of complex mixtures, such as phytoremedies used in popular traditions.

3.3.2 CREPIS VESICARIA SUBSP. TARAXACIFOLIA THUILL THELL

Crepis vesicaria L. subsp. *taraxacifolia* (Thuill.) Thell. is a plant subspecies belonging to the Asteraceae family. The plant is annual or biennial, herbaceous, with leaves in a basal rosette that are long about half of the stem. Generally, it grows along the edges of country roads, along the ditches, on fat meadows and uncultivated areas but nowadays is in strong expansion along sidewalks and in industrial areas. *Crepis vesicaria* can be found at an altitude ranging from 0 and 1,200 meters above sea level. The Italian most used name is

"*radicchiella a foglia di tarassaco*". Its presence in Italy was reported in North Central area, Campania, Basilicata and Sardegna regions (Conti et al., 2005). In particular, *Crepis vesicaria* was the plant species most mentioned and appreciated by the local people of Bologna's area, which were used to consume it traditionally as food-medicine by eating the basal leaves raw, boiled or as omelettes and salad (see paragraph *Ethnobotanical study conducted in the area of Bologna* and Table 4), while the cooking water was used as depurative and diuretic, often related to liver and kidneys issues.

The metabolomic profile of *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell samples collected in Bologna's area during the ethnobotanical survey, was analysed through a MS-based untargeted metabolomic approach (UPLC-QTOF-ESI-MS) at the Foodomics laboratory, CSIC, Madrid, Spain.

The analysis was performed on this plant species for many reasons:

1. *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell is an unusual wild subspecies never used as traditional food in Northern Italy
2. no metabolic and metabolomics studies were previously performed on this species
3. to better understand its possible therapeutic properties as this plant is used also as a medicinal.

3.3.3 SELECTION OF THE EXTRACTION SOLVENT

In order to maximize the *Crepis* metabolite extraction efficiency, three extraction solvents were tested: methanol 100% (v/v), methanol 100% (v/v) plus 0.1% (v/v) of formic acid (FA) and methanol-water (75:25, v/v) plus 0.1% (v/v) of FA.

The *Crepis* samples extracted with the three solvents were compared in terms of metabolite peak number and peak intensity. Figure 36 shows the pattern obtained with the Total Ions Chromatogram tool (Agilent Technologies, MassHunter Workstation, Qualitative Analysis Software, version B.03.01). Clearly, the methanol-water extraction led to a larger number of peaks having a higher intensity meaning a greater degree of metabolite extraction. To evaluate the metabolite extraction efficiency of the three solvents, the intensity of the peaks of two compounds (chlorogenic acid and luteoin 7-O-glucoside), were compared. The two compounds were identified in *Crepis* extracts by analysing their retention time and spectral data respect to those of the relative pure standards (Figure 37). The results, obtained by Extracted Ion Chromatogram tool, confirmed that the solvent methanol-water (75:25, v/v)

plus 0.1% (v/v) FA, gave the highest peak intensity. In addition, by using the Molecular Feature Extraction tool (Agilent Technologies, MassHunter Workstation, Qualitative Analysis Software, version B.03.01), 849 different metabolites could be detected, in a run of about 18 min, in *Crepis* sample extracted with methanol-water (75:25, v/v) with 0.1% (v/v) FA solvent respect to 566 compounds in methanol 100% and 632 compounds in methanol + FA 0.1% (Figure 38).

In conclusion, the solvent methanol-water (75:25, v/v) with 0.1% (v/v) FA, was selected for further experiments.

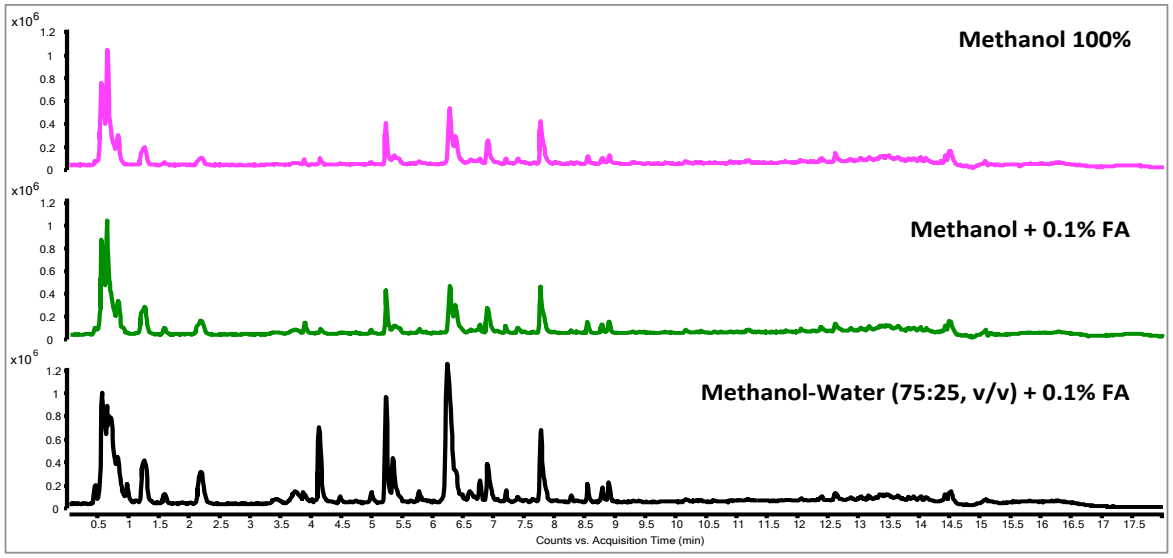

Figure 36. Total Ion Chromatograms (MassHunter Qualitative Analysis Software version B.03.01) of *Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell sample extracted by methanol 100%, methanol with 0.1% (v/v) formic acid (FA) and methanol-water (75:25, v/v) with 0.1% (v/v) FA.

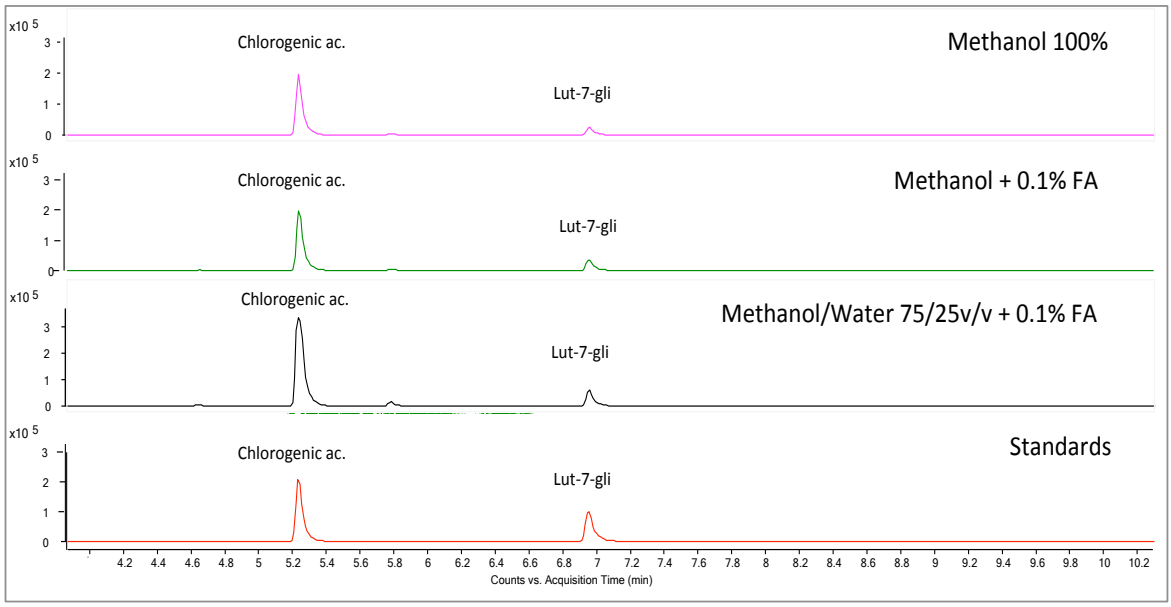

Figure 37. Extracted Ion Chromatograms (MassHunter Qualitative Analysis Software version B.03.01) of chlorogenic acid and luteolin-7-O-glucoside obtained by using the three different extraction solvents compared with their standards.

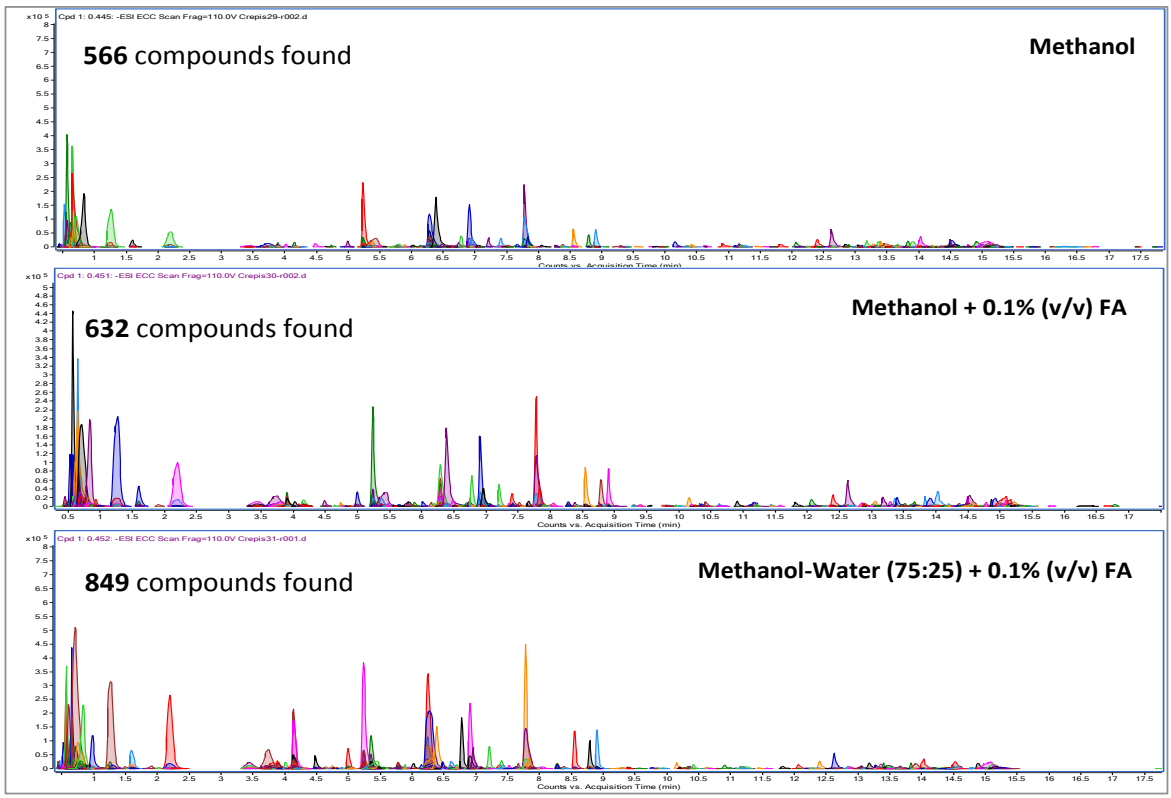

Figure 38. *Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell samples extracted by methanol 100%, methanol with 0.1% (v/v) FA and methanol-water (75:25, v/v) with 0.1% (v/v) FA. Chromatograms visualised by Molecular Feature Extraction tool (MassHunter Qualitative Analysis Software version B.03.01)

3.3.4 ACIDIC AND ENZYMATIC HYDROLYSIS

Acidic and enzymatic hydrolysis of the *Crepis* samples were performed in order to fine-tune the information regarding some specific metabolites.

The acidic hydrolysis performed with hydrochloric acid, had the ability to break the glycoside bonds and to release the respective aglycones. The reaction was performed using 2 different acid concentrations (1.2 M and 4.0 M) and 2 incubation times (2h and 4h), leading to identify the peaks, generated by the acidic hydrolysis, which showed an increase of peak area corresponding to an increase of the released aglycone concentration.

The compounds analysed by using this method were in particular the flavones luteolin and apigenin and the flavonols quercetin and kaempferol that generally present in vegetables in different glycosylated forms.

Acidic hydrolysis showed that luteolin glycosylated forms were present at high levels in *Crepis* samples, apigenin derivatives were detected at lower levels while quercetin and kaempferol derivatives were absent (Figure 39 and 40). In particular, quercetin seemed to give a peak at the retention time similar to the reference compound, but the increase of this peak was not correlated to the increase of the strength of the acid hydrolysis, indicating that quercetin is only present in the not glycosylated form (Figure 40).

The enzymatic hydrolysis of *Crepis* samples was carried out with Viscozyme which is reported to have a cinnamic acid esterase activity leading to the release of caffeic, ferulic and p-coumaric acids from their conjugated forms (Zheng et al., 2009, Bartolomé and Gómez-Córdoves, 1999; Begoña et al., 1999). In comparison to reference compounds, after enzymatic hydrolysis, the Extracted Ion chromatograms of released caffeic, p-coumaric and ferulic acids, were analysed. *Crepis* samples were rich in caffeic acid conjugated forms, presented a lower level of p-coumaric acid conjugates while ferulic acid conjugates were present only in traces amount (Figure 41).

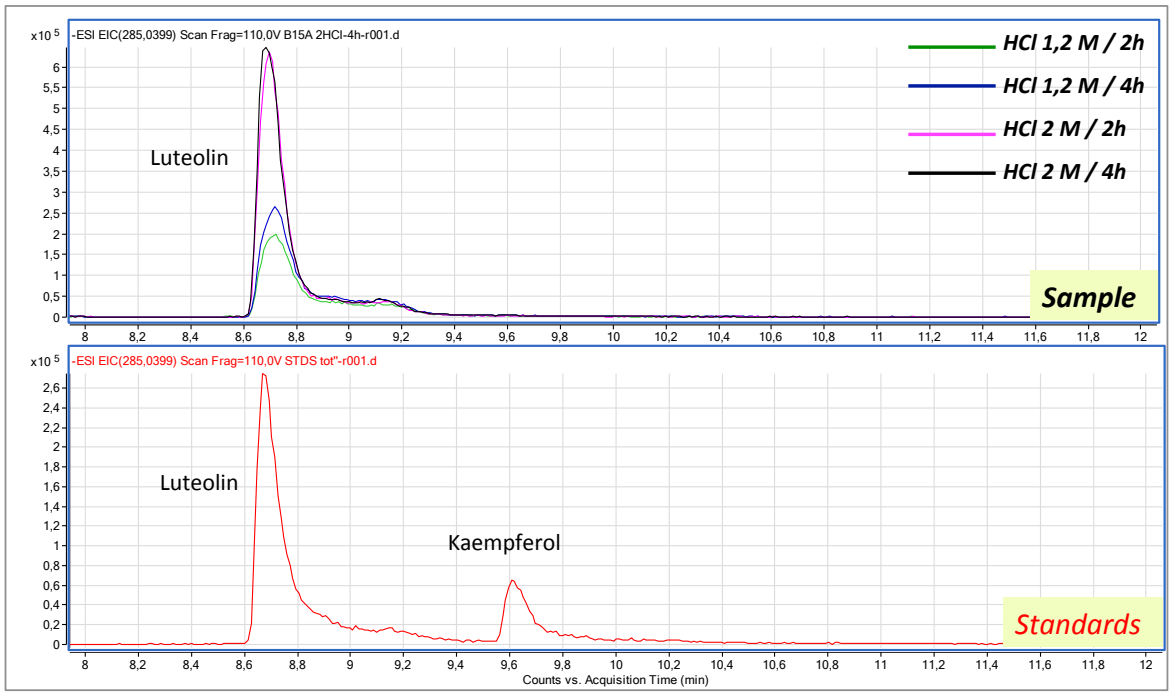

Figure 39. Extracted Ion chromatograms (MassHunter Qualitative Analysis Software version B.03.01) of luteolin and kaempferol of acidic hydrolysis of *Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell sample, in comparison to the relative standard compounds.

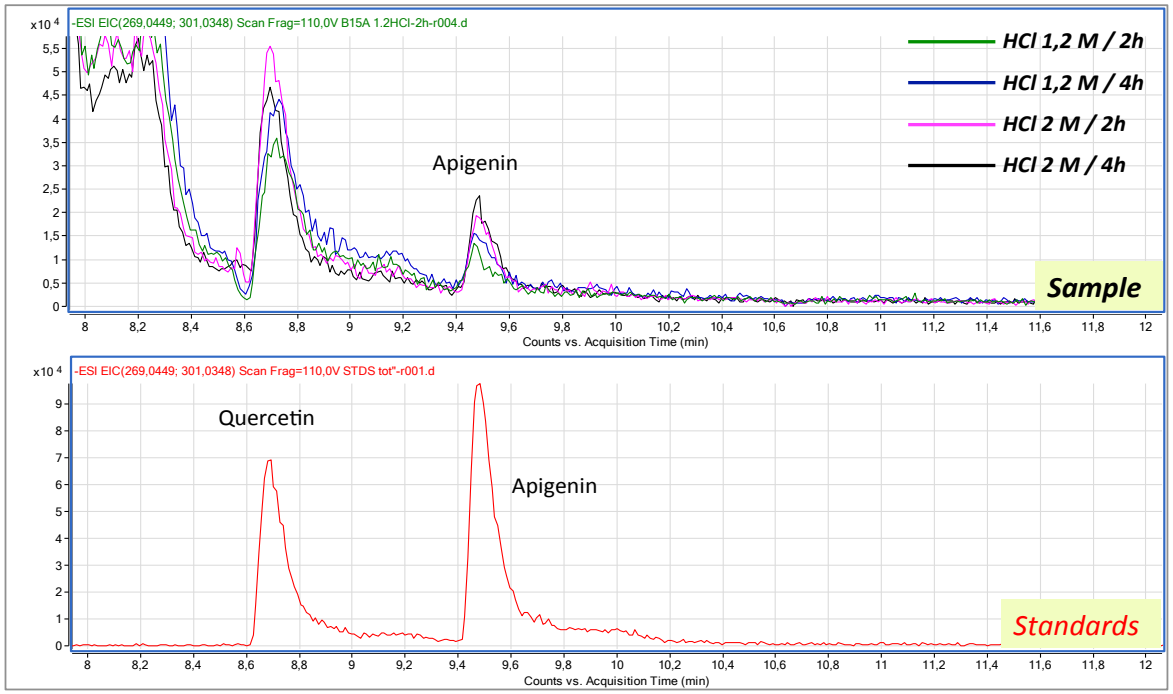

Figure 40. Extracted Ion chromatograms (MassHunter Qualitative Analysis Software version B.03.01) of quercetin and apigenin of acidic hydrolysis of *Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell sample, in comparison to the relative standard compounds.

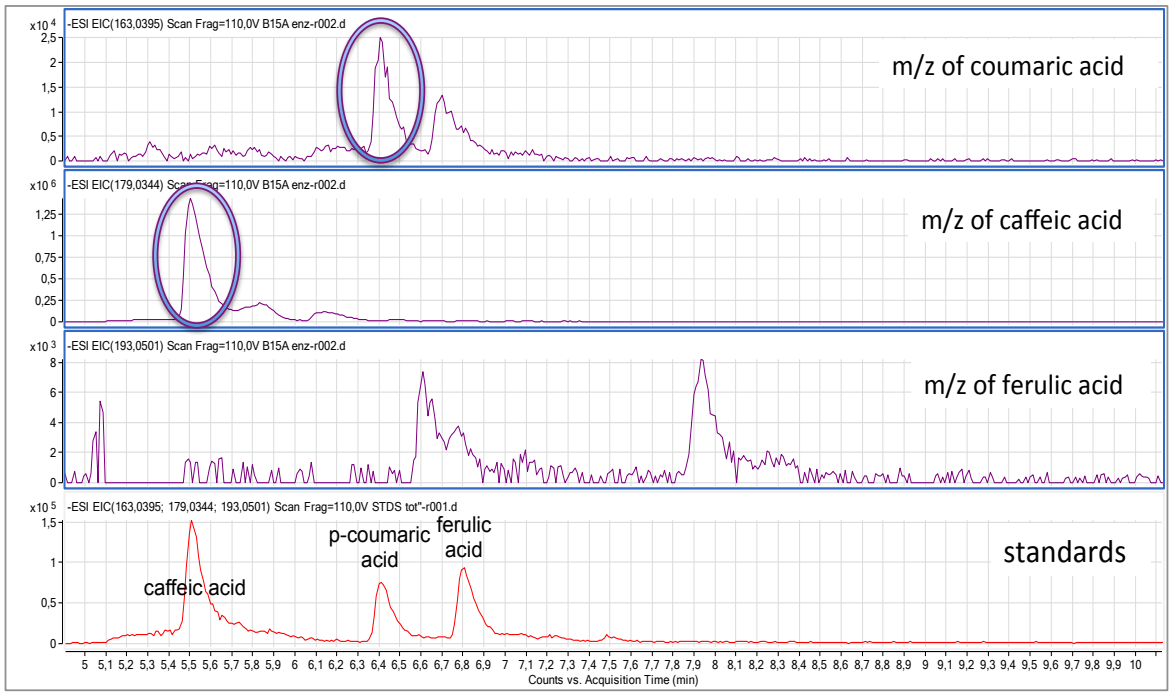

Figure 41. Extracted Ion chromatograms (MassHunter Qualitative Analysis Software version B.03.01) of coumaric, caffeic and ferulic acids released after of enzymatic hydrolysis of *Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell sample, in comparison to the relative standard compounds.

3.3.5 TENTATIVE IDENTIFICATION OF COMPOUNDS

Accurate m/z value and retention time for each metabolite peak was annotated, and tentative compound identification, based on the obtained theoretical molecular formula, METLIN metabolites database, standards and hydrolysis data, was carried out.

Theoretical compound molecular formulas were extrapolated from all the obtained information and a tentative identification of 15 metabolites was performed (Table 4).

Table 4. Compounds tentatively identified in *Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell samples through UPLC-QTOF-ESI-MS method.

N°	RT	species	m/z exp	m/z calc	ppm	Massa	Formula	Name
1	0,60	M-H	191,0566	191,0561	-2,7479	192,0634	C7H12O6	**Quinic acid**
2	0,61	M-H	149,0095	149,0092	-2,0133	150,0164	C4H6O6	**Tartaric acid**
3	3,91	M-H	177,0194	177,0193	-0,5649	178,0266	C9H6O4	**Esculetin (6,7-dihydroxycoumarin)**
4	3,91	M-H	311,0410	311,0409	-0,2411	312,0481	C13H12O9	**Caftaric acid (caffeoyl tartaric acid)**
5	4,88	M-H	339,0725	339,0722	-0,9585	340,0794	C15H16O9	**6,7-dihydroxycoumarin glucoside**
6	5,12	M-H	353,0881	353,0878	-0,8496	354,0951	C16H18O9	**Chlorogenic acid (caffeoylquinic acid)**
7	5,97	M-H	609,1464	609,1461	-0,4925	610,1534	C27H30O16	**Luteolin diglucoside**
8	6,21	M-H	473,0709	473,0726	3,5407	474,0798	C22H18O12	**Cichoric acid (dicaffeoyltartaric acid)**
9	6,31	M-H	609,1463	609,1461	-0,3283	610,1534	C27H30O16	**Luteolin diglucoside**
10	6,80	M-H	461,0729	461,0725	-0,8675	462,0799	C21H18O12	**Luteolin derivative (lut-7-glucuroside)**
11	6,87	M-H	447,0940	447,0927	-2,8518	448,1022	C21H20O11	**Luteolin 7-O-glucoside**
12	7,25	M-H	515,1201	515,1195	-1,0677	516,1268	C25H24O12	**Cynarin (dicaffeoylquinic acid)**
13	7,44	M-H	447,0937	447,0927	-2,2367	448,1022	C21H20O11	**Luteolin glucoside**
14	7,49	M-H	431,0986	431,0984	-0,3479	432,1056	C21H20O10	**Apigenin 7-O-glu**
15	8,68	M-H	285,0407	285,0399	-2,8066	286,0477	C15H10O6	**Luteolin**

Table 4 shows the name of the metabolites tentatively identified listed along the elution order. For each compound the relative retention time (RT), the species of charged ion measured (M-H: deprotonated ion), the theoretical and calculated mass/charge ratio (m/z exp and m/z calc), the ppm error between the two m/z values, the molecular weight and brute formula, are reported.

Among the detected compounds chlorogenic acid was detected at a higher level (Figure 42). Also quinic, caftaric and cichoric acids were detected at high amounts (Figure 43).

An indication on the metabolite concentration in the plant sample could be obtained by

measuring the intensity of the peak.

Ions Chromatograms of the other detected metabolites are shown in Figure 43 and 44.

Overall, the most abundant compounds were caffeic acid derived molecules, as evidenced by data from enzymatic hydrolysis, such as chlorogenic acid, caftaric acid, cichoric acid and cynarin.

Many luteolin conjugated forms were detected, as confirmed by the acidic hydrolysis, but at lower levels than caffeic acid conjugates Free luteolin was found at a very small amount (peak intensity of $0.7x10^4$), while the most abundant luteolin-conjugated was luteolin-7-O-glucoside (peak intensity of $6.9x10^5$), which were both identified by comparison to the relative reference compounds. Other tentatively identified luteolin derivatives were a luteolin glucoside, a luteolin glucuronide and two luteolin diglucoside. In these cases it was not possible to exactly locate the luteolin carbon number of the glycosidic bond and to better understand their structures a MS/MS fragmentation study should be performed.

Two luteolin diglucoside with the same molecular formula (same molecular ion, m/z and fragmentation patterns) but different RT (5.97 min and 6.31min), were detected. It was hypothesised that these two luteolin species could have the glucoside residues attached in different sites or they could be stereoisomers, explaining the different retention time with a different steric encumbrance.

Two coumarins, esculin and its glucoside (6,7-dihydroxycoumarin glucoside), were also found. The only apigenin glucoside detected was apigenin 7-O-glucoside.

In contrast to the huge variety of sesquiterpene lactones reported in the literature (Zidorn, 2008), only a limited number of flavonoids and phenolic acids are known to be present in the genus *Crepis* and no one in *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell.

Zidorn (2008) investigated flavonoids and hydroxycinnamic acids in the flowering heads of plants of the genus *Crepis* founding luteolin, luteolin 7-O-glucoside, luteolin 7-O-gentiobioside, luteolin 7-O- glucuronide, and luteolin 4'-O-glucoside and caffeic acid conjugated forms as chlorogenic acid, 3,5-dicaffeoylquinic acid, caffeoyltartaric acid and cichoric acid. Free luteolin, luteolin-7-O-glucoside, chlorogenic acid and dicaffeoylquinic acid were detected in all the fifteen *Crepis* species as well as their presence was confirmed in *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell leaves. The aerial parts of *Crepis vesicaria* L. were studied by Mañez et al. (1992) reporting the presence of luteolin, luteolin-4-O-glucoside and luteolin-7-O-glucoside. Consequently, it seems most probable that the

luteolin glucoside peak shown at 7.44 min RT detected in *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell leaves could correspond to luteolin-4-O-glucoside. The comparison with a specific reference standard if available, could confirm this hypothesis.

Many of the detected compounds have proven to have beneficial biological activities (Jiang et al., 2005, Matsuta et al., 2011, Srivastava and Gupta, 2009).

On the basis of the present results, the most abundant metabolite in *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell was chlorogenic acid (peak intensity of $3.6x10^6$). Chlorogenic acid is a hydroxycinnamic acid, derived from the conjugation of caffeic acid and quinic acid. It is commonly found in coffee, tea, fruits and miscellaneous vegetables (Clifford, 1999).

Chlorogenic acid was studied for its antioxidant (Kweon et al., 2001), antibacterial activity (Lou et al., 2011) and for its potentially preventive action against cancer (Feng et al., 2005), cardiovascular diseases (Olthof et al., 2001) and diabetes (McCarthy, 2005). Besides *in vitro* tests demonstrated that chlorogenic acid can inhibit the reaction of nitrosamine formation that is highly carcinogenic to the liver and kidneys (Kono et al., 1995). Nitrosamine formation can take place both in food and, *in vivo*, in the stomach and the chlorogenic acid inhibition action is present in both situations.

Since the basal leaves of *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell are rich of chlorogenic acid, having the biological activities above mentioned, we can consider the popular use of this wild species as a healthy alimentary practice.

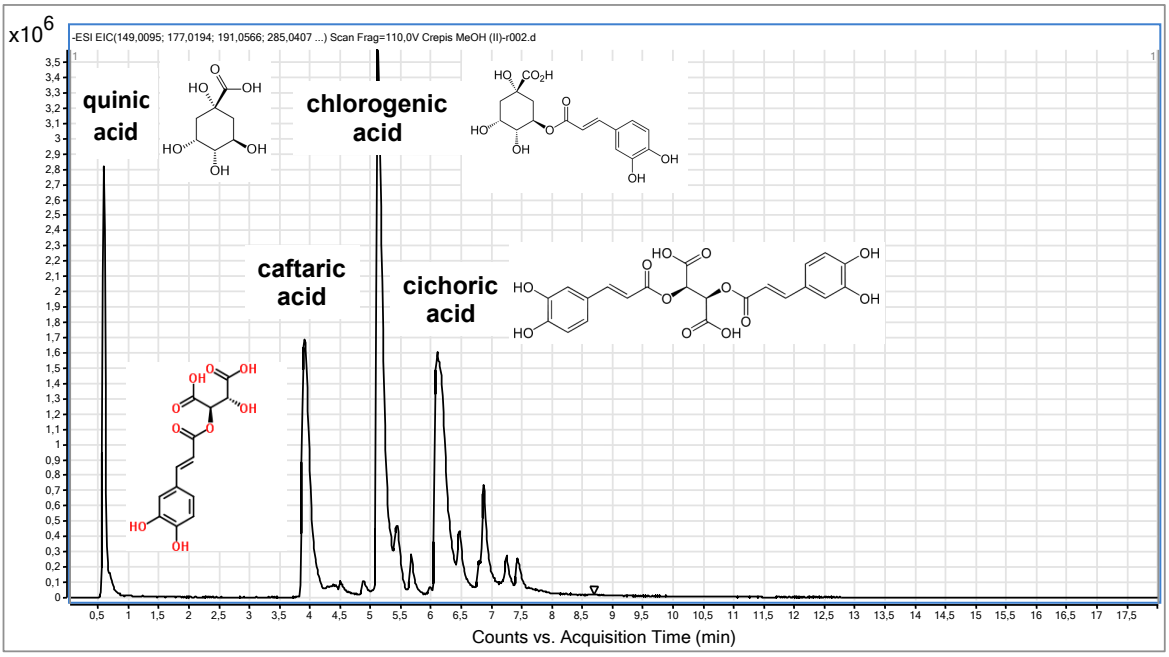

Figure 42. Extracted Ion chromatograms (MassHunter Qualitative Analysis Software version B.03.01) of quinic acid, caftaric acid, chlorogenic acid, cichoric acid

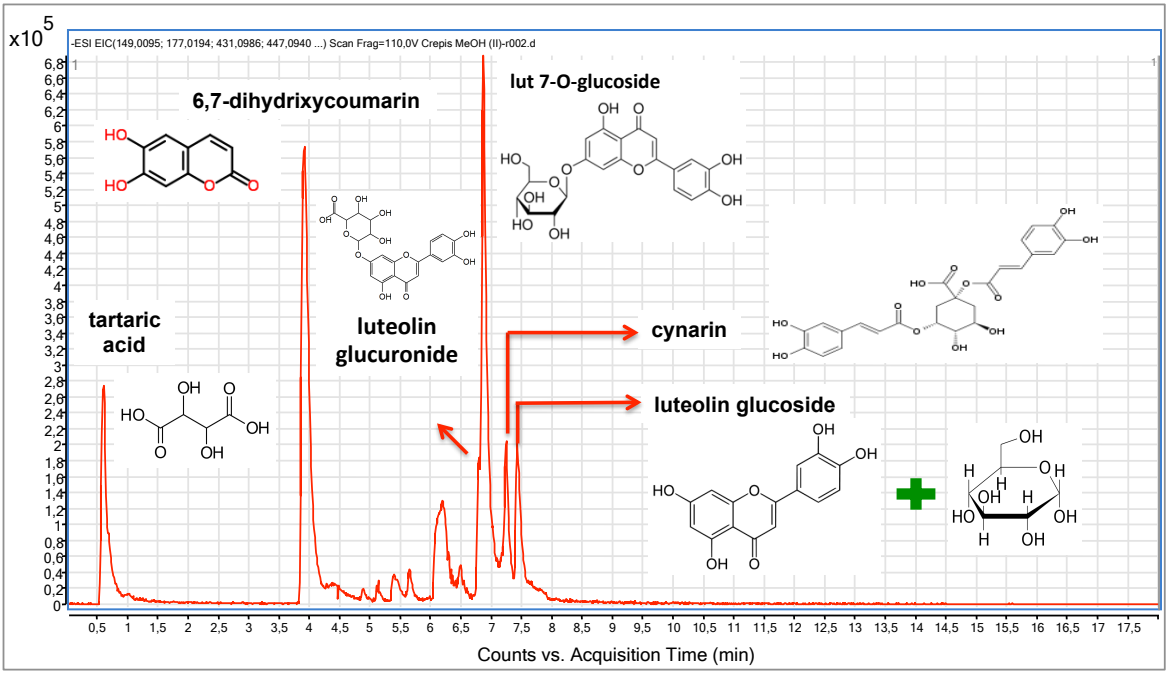

Figure 43. Extracted Ion chromatograms (MassHunter Qualitative Analysis Software version B.03.01) of tartaric acid, 6,7-dihydroxycoumarin, luteolin glucuronide, luteolin 7-O-glucoside, cynarin, luteolin glucoside

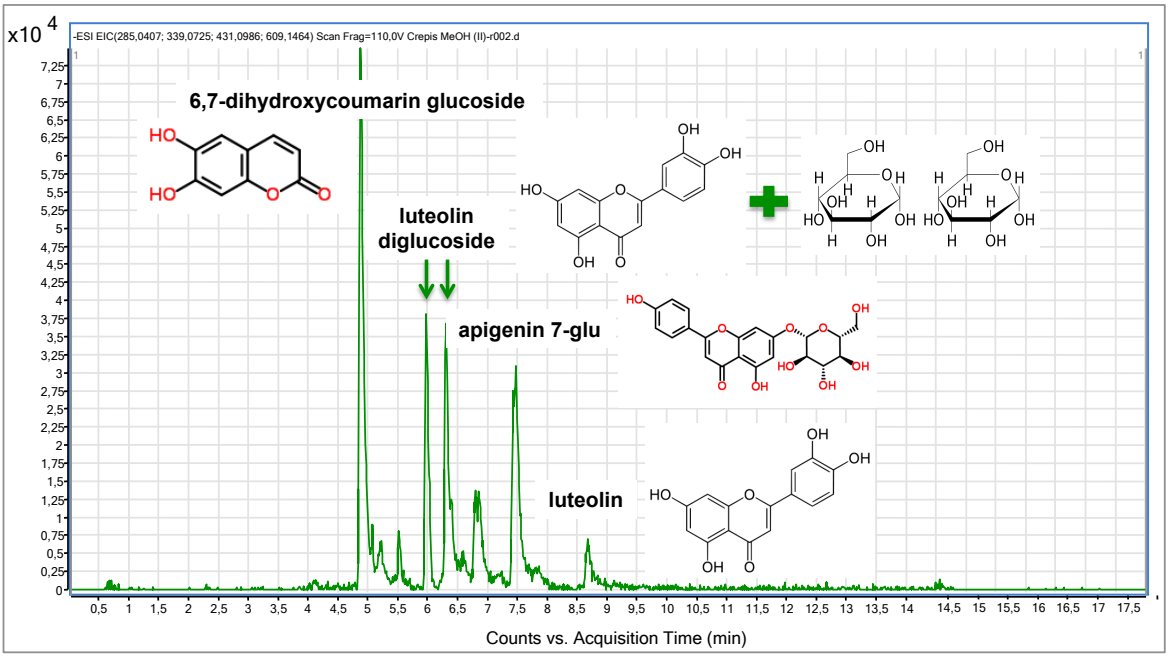

Figure 44. Extracted Ion chromatograms (MassHunter Qualitative Analysis Software version B.03.01) of 6,7-dihydroxycoumarin glucoside, luteolin diglucoside, apigenin 7-O-glucoside, luteolin

3.3.6 METABOLIC PROFILES COMPARISON OF THE THREE CONSUMING WAYS OF CREPIS VESICARIA SUBSP. TARAXACIFOLIA (THUILL) THELL BASAL LEAVES

In order to better understand the therapeutic benefits/risks of dietary consumption of *Crepis vesicaria*, the metabolomic profile of plant samples processed following the traditional cooking ways of the people of Bologna's area, were acquired.

When *Crepis* basal rosette leaves were gathered in an early vegetative stage, so to have a tenderer and less bitter vegetable, Bologna's people used to consume it raw as salad.

The metabolomic profile of the raw rosette leaves (alimentary raw use) was obtained following the *methanol-water plus 0.1% (v/v) FA* extraction extensively described above.

Bologna's people used also to boil *Crepis* leaves and, in addition, they could drink the cooking water to obtain a depurative, diuretic and hepatoprotective effects. To simulate the home cooking procedure, the plant sample was put in boiling water for 10 min and then pellet and water were separated.

The water, called hereinafter *water extract*, is equivalent to the *Crepis* leaves cooking water, traditionally drunk for therapeutic purposes (medicinal use). The pellet, that represents the

cooked *Crepis* leaves, was extracted with the methanol-water solvent (methanol-water 75:25 v/v plus 0.1% v/v FA) thus obtaining the *pellet extract* (alimentary cooked use).

Summarizing, in the following results, the alimentary raw use is represented by the *methanol-water extract*, the alimentary cooked use is represented by the *pellet extract* and the cooking water, used as medicinal remedy, by the *water extract*.

The metabolic profiles of the three *Crepis* extracts (*methanol-water extract*, *pellet extract, water extract*) were analyzed by the UPLC-QTOF-ESI-MS procedure and compared.

The extracted ion chromatograms of caffeic acid conjugates of the three extracts are shown in Figure 45. Notably, in comparison with the raw leaves extract (*methanol-water extract*), after boiling procedure, the *pellet extract* profile indicates a high depletion of all caffeic acid related compounds that are instead present in the *methanol-water extract.* In addition, caftaric acid (caffeoyil tartaric acid), cichoric acid (dicaffeoyltartaric acid), both conjugates of caffeic acid and tartaric acid, were completely transferred into the water extract and they seem not to be degraded by thermal treatment. In fact, the same compounds were found in lower amount in the *pellet extract* (Figura 45).

After boiling, most of the chlorogenic acid, that is a conjugate of caffeic acid and quinic acid, was detected in the *water extract*. Comparing the relative peak intensities, about one third of the chlorogenic acid (respect to the level detected in the *methanol-water extract*) can still be detected in the treated sample (*pellet extract*) indicating that this compound is not subjected to thermal degradation. This is in agreement with Friedman and Jurgens, (2000), who demonstrated that chlorogenic acid molecule is quite stable when heated.

Always comparing the relative peak intensities and in comparison to the level detected in the *methanol-water extract*, cynarin (1,5-dicaffeoyl quinic acid) was detected for about one fourth of its total amount in the *water extract* while the remaining was only partially present in the *pellet extract* so to suppose its possible thermal degradation.

Caffeic acid was not detected either in *water extract* or in *pellet extract* proving that the caffeic acid conjugates present in *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell. sample and detected after enzymatic digestion, were not hydrolysed after a 10 min treatment in boiling water. A similar result was reported by Pistón et al. (2014) which, analysing phenolic compounds in artichoke hydroalcoholic and decoction extracts (comparable to *methanol-water* and *water extracts*), detected chlorogenic acid (hydroalcoholic: 43±2 mg/g extract; decoction: 40±3 mg/g extract) and cynarin (hydroalcoholic: 14±1 mg/g extract;

decoction: 6.5±0,5 mg/g extract) prevalently in the hydroalcoholic extract respect to the decoction extract while caffeic acid was not revealed. Caffeic acid was anyway reported in literature as a possible product of cynarin hydrolysis (Pérez-García et al., 2000).

The comparison of coumarin derivatives (6,7-dihydroxycoumarin and 6,7-dihydroxycoumarin-glucoside) among the three extracts is shown in Figure 46. In particular, 6,7-dihydroxycoumarin was only measured in *water extracts* and was absent in the *pellet extract,* while its glucoside (6,7-dihydroxycoumarin-glucoside) was partially retained into the treated sample (*pellet extract*) and/or partially hydrolysed by the boiling procedure.

Figures 47 and 48 show the chromatographic profiles of luteolin glucosides and of apigenin-7-O-glucoside that is the only apigenin conjugates detected in *Crepis* sample. These molecules were only partially extracted by boiling procedure and detected both in water and pellet extracts; they also seemed to be partially degraded by hydrolysis reactions.

A completely different trend was evidenced for luteolin whose concentration increased steeply in *pellet extract* (Figure 47). Also apigenin was only detected in the *pellet extract*, while it was not identified in *methanol-water* and *water extracts* (Figure 48). These results could be explained assuming that the heat treatment (10 min at 100 °C), to which the plant sample was subjected, caused the break of the glycosidic bonds with a consequent release of the aglycones, in this case luteolin and apigenin. Since the aglycones are less polar than the relative glycosides it is possible to hypothesise that, during boiling, these compounds were not extracted by water and were mainly retained in the pellet.

In conclusion, it is possible to state that, in comparison with raw leaves extract, the boiling process depleted, sometimes even totally, *Crepis* leaves of caffeic acid, coumarin, luteolin and apigenin derivatives but enriches them of luteolin and apigenin. The cooking water, in turn, was partially impoverished of all metabolites analysed, except for caftaric acid and cichoric acid, in comparison to the not treated plant sample (*methanol-water* extract).

As many studies proved that all the metabolites identified in these analyses, may exhibit beneficial biological activities (Jiang et al., 2005, Oliver et al., 1999, Matsuta et al., 2011, Srivastava and Gupta, 2009), the most healthy way to consume *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell seems to be raw. The only nutritional reason to consume the basal leaves of the wild plant as boiled is to intake luteolin and apigenin (the latter being anyway present at a very low level), compounds that have demonstrated antioxidant, anti-inflammatory, antimicrobial, anti-allergic and anticancer activities (Lopez-Lazaro, 2009,

Seelinger et al., 2008).

However, if the bitter taste of *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell leaves makes it impossible to consume them raw but only after boiling, the traditional use of drinking the cooking water still remains of course a healthy habit since it allows not to lose the portion of beneficial metabolites solubilized in the water. In fact, as confirmed by the interviews to the people of Bologna's area, the cooking water was drunken for depurative and diuretic purposes, often referring to liver and kidneys issues.

The wide-range of therapeutic indications cannot be attributed to a single compound, but to the action of several beneficial compounds that together generate additive or synergistic pharmacologic effects.

To confirm or refuse the traditional therapeutic use of *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell leaves, it would be necessary to make *in vivo* pharmacological tests of leaf extracts, similarly to what was previously done for artichoke (*Cynara cardunculus* L. subsp. *scolymus* (L.) Hayek) leaf extracts that exhibited hepatoprotective, bile-expelling and diuretic activities as well as the ability to inhibit cholesterol biosynthesis and LDL oxidation (Lattanzio et al., 2009). Since artichoke leaves are rich of mono- and dicaffeoylquinic acids, and of flavonoids, such as luteolin and its 7-O-glucoside, as well as *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell, it is plausible to suppose that *Crepis* could show the same properties found for artichoke, so confirming the depurative, diuretic and hepatoprotective effects traditionally reported.

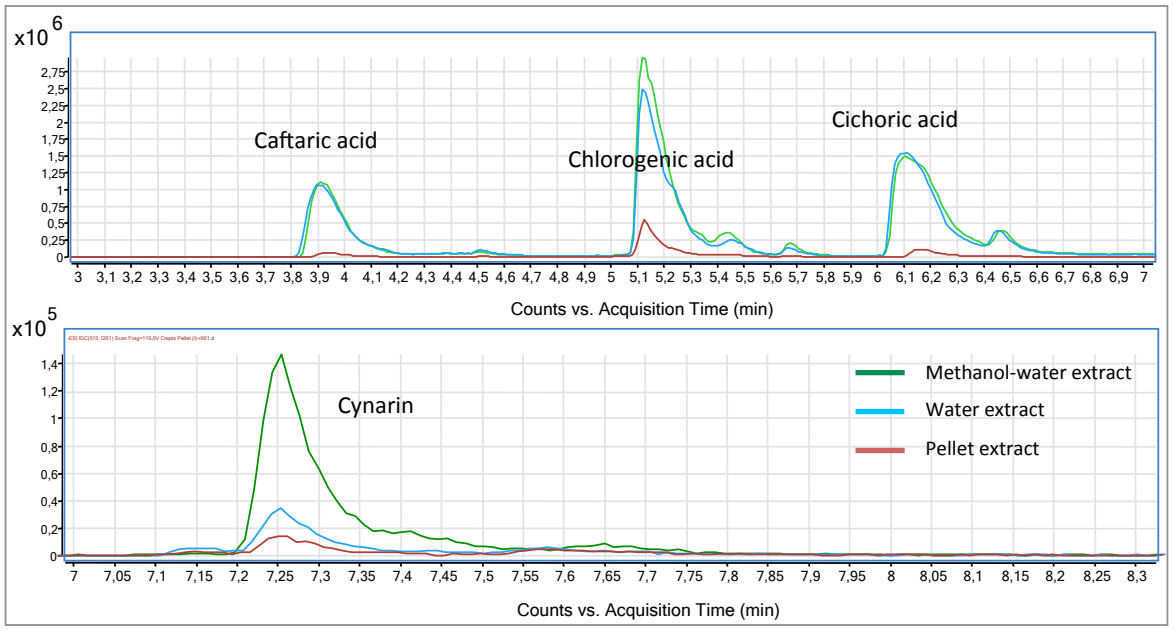

Figure 45. Caffeic acid conjugated forms Extracted Ion chromatograms ((MassHunter Qualitative Analysis Software version B.03.01)) comparison in methanol-water, water and pellet extracts of *Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell samples.

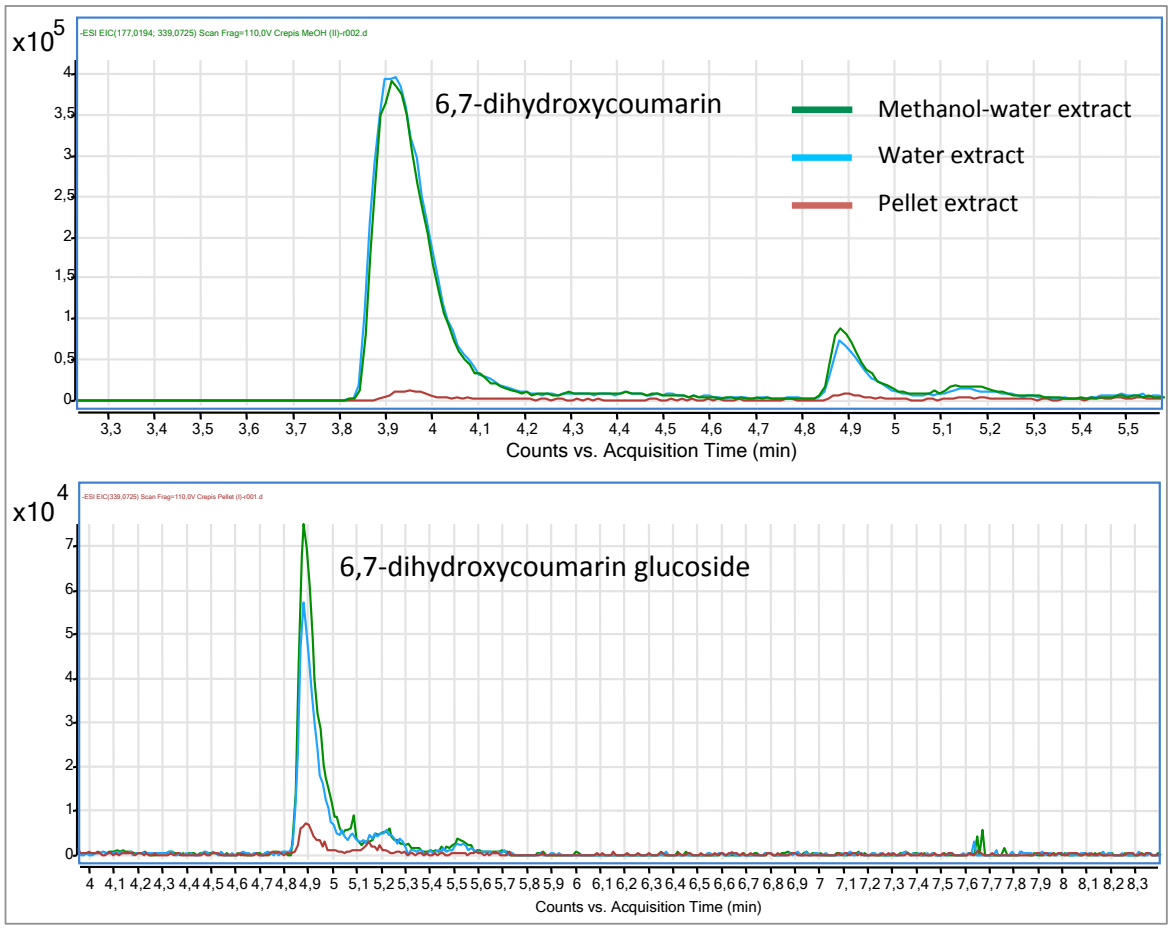

Figure 46. Coumarins Extracted Ion chromatograms (MassHunter Qualitative Analysis Software version B.03.01) comparison in methanol-water, water and pellet extracts of *Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell samples.

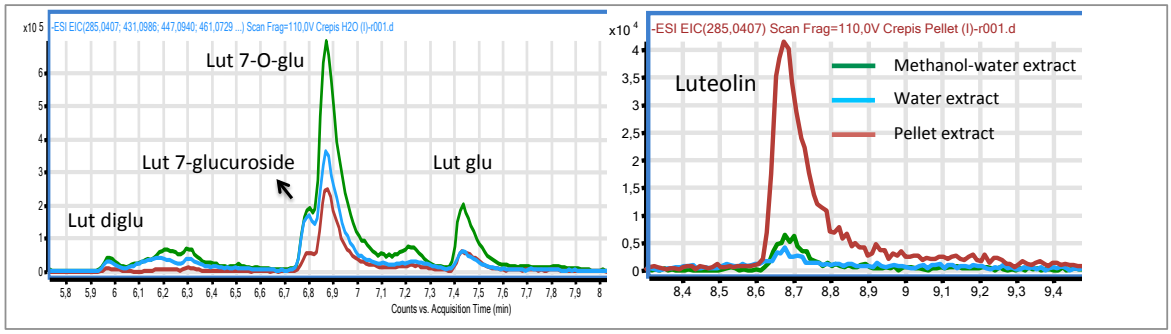

Figure 47. Luteolin conjugated forms Extracted Ion chromatograms (MassHunter Qualitative Analysis Software version B.03.01) comparison in methanol-water, water and pellet extracts of *Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell samples .

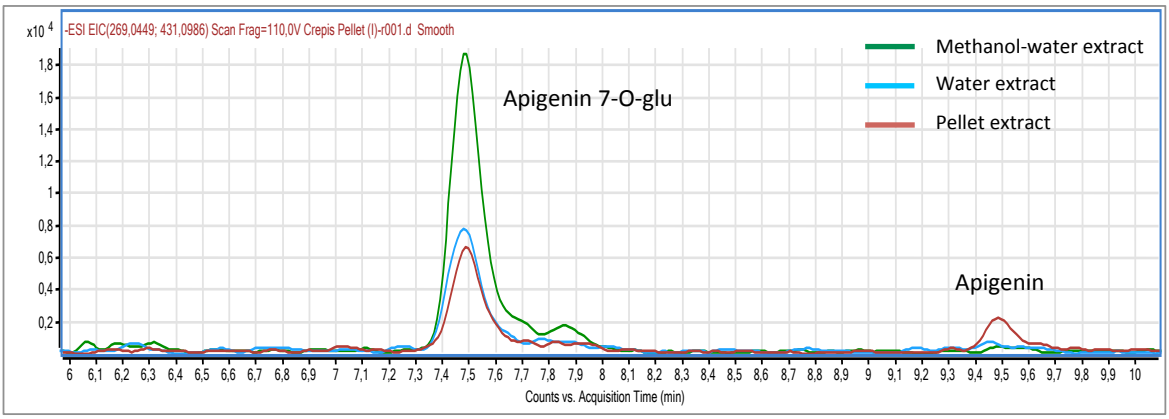

Figure 48. Apigenin derivatives Extracted Ion chromatograms (MassHunter Qualitative Analysis Software version B.03.01) comparison in methanol-water, water and pellet extracts of *Crepis vesicaria* subsp. *taraxacifolia* (Thuill) Thell samples.

4 CONCLUSIONS

4.1 ETHNOBOTANICAL STUDY

The present study was aimed at documenting the traditional local knowledge (TLK) on wild food plants in two study areas, one belonging to the province of Bologna (Emilia-Romagna region, North of Italy) (Figure 5) and one to the Middle Agri Valley (Basilicata region, Potenza province, South of Italy) (Figure 6). These areas were not previously investigated on the traditional use of the wild food plants by any other ethnobotanical research performed in Italy. The obtained results may allow preserving part of the local cultural heritage that seems to be quickly disappearing along with the people, some of them very elderly, who still retain this type of knowledge. The popular traditions regarding wild food plants in both areas, in fact, are being progressively lost because they are not anymore handed down to new generations, so that today young people do not acquire any information regarding wild edibles that characterized the diet of their forebears. The present survey revealed that, in spite of the loss of TLK, the use of wild food plants is being revaluated today as these plants are perceived as healthy and represent the preservation of biodiversity, as well as a way of getting back to the nature, old traditions and own roots.

In Bologna's area the revaluation of wild food plants mainly follows ecological reasons that fall within a personal search for a healthier life, while in the Middle Agri Valley this positive revaluation of wild herbs is closely related to the local habits and traditions.

In the present era, characterised by large-scale food distribution, which has generally led to a decrease in food quality, the interest in wild edibles is conversely generally increased.

The contribution of the present study in preserving local knowledge and traditions may hopefully reinforce this new growing trend to become a habit, leading to enrich the local diet with new "old traditional" foods beneficial for human health.

4.2 METABOLIC SCREENING OF WILD FOOD PLANTS

Following the ethnobotanical study, 34 wild food plant samples were collected of which 13 in Bologna's territory and 21 in Middle Agri Valley, for a total of 27 plant species. The plant samples (Table 9) were analysed by means of different spectrophotometrical techniques in order to identify the wild food plant species richest in bioactive substances

with beneficial health effects such as polyphenols, flavonoids, carotenoids, chlorophylls and proteins.

Among the analysed 27 wild food plant species, all coming from the Mediterranean area, *Sanguisorba minor* Scop. was richest in polyphenols and showed the highest antioxidant activity, *Mentha spicata* L. was richest in flavonoids, *Clematis vitalba* L. (collected in Middle Agri Valley) contained the highest protein content and *Sinapis arvensis* L. the highest amount of chlorophylls and carotenoids.

Sanguisorba minor Scop. and *Clematis vitalba* L. (collected in Middle Agri Valley) resulted to be 3.24 folds and 2.39 folds respectively higher in polyphenols than Sangiovese berries (on average 5.3 mgGAeq/gFW, Tassoni et al., 2013)

Besides the same polyphenols intake of a cup of Sangiovese wine can be obtained with only 16.4 gFW of *Sanguisorba minor* Scop. or 22.1 gFW of *Clematis vitalba* L. (collected in Middle Agri Valley).

As all know the beneficial effects of red grapes and wine, these results evidenced that wild plants can be considered healthy foods and some of them can have surprising beneficial properties that deserve to be further investigated.

4.3 METABOLOMICS STUDY OF CREPIS VESICARIA SUBSP. TARAXACIFOLIA (THUILL) THELL

Metabolomic techniques give the opportunity to investigate natural products taking into account the entire phytocomplex constituted by several compounds that play synergic healthy effects. The untargeted metabolomic approach is very useful for studying traditional wild food plants and traditional medicine having the potential to enhance the understanding of the correlation between biochemical composition and therapeutic effects of a certain food or plant extract.

The LC-MS analyses performed in the present study gave a preliminary qualitative profile of the metabolites of *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell basal leaves.

This plant species was chosen to be further investigated because it is an unusual wild subspecies never used as traditional food in Northern Italy and no metabolic and metabolomics studies were previously performed on this species. Another reason was to better understand its possible therapeutic properties as this plant is used also as a folk medicine in the area of Bologna.

The metabolomic analyses mainly focused on some polyphenol groups (hydrossicinnamic acids, flavonoids and coumarins), and even tough it was not possible to quantify the detected metabolites, a general preliminary overview of the metabolite profile of Crepis could be drawn allowing the correlation with reported healthy and therapeutic effects.
The results obtained reported that this species, regarding the polyphenols groups analysed, resulted to be similar to artichoke (*Cynara cardunculus* L. subsp. *scolymus* (L.) Hayek) as they are both rich of hydrossicinnamic acids as mono- and dicaffeoylquinic acids and flavonoids as luteolin and its 7-O-glucoside. It is so possible to hypnotize that the hepatoprotective, bile-expelling and diuretic activities showed by artichoke (Lattanzio et al., 2009) could be exhibited also by *Crepis vesicaria* L. subsp. *taraxacifolia* (Thuill) Thell so confirming the depurative, diuretic and hepatoprotective effects traditionally reported.

4.4 FINAL CONCLUSION

The inclusion of these wild vegetables in our daily diet could contribute to enhance the food biodiversity as well as its nutritional and healthy quality. The identification of wild food plant species, containing high levels of bioactive molecules, may therefore improve human health and potentially help to prevent many diseases very common in the modern society. In the past, in fact, in Italy and other Western countries, many wild greens were used as food as well as played an important role in domestic animal feeding. Nowadays we can consider wild plants as under-exploited or neglected sources of food and bioactive compounds.
The general health advice to eat more fruits and vegetables is no longer sufficient and out-of-date. We need good guidance on which fruits and vegetables to eat.

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