



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DOTTORATO DI RICERCA IN
SCIENZE DELLA TERRA, DELLA VITA E DELL'AMBIENTE

Ciclo 36

Settore Concorsuale: 05/A1 - BOTANICA

Settore Scientifico Disciplinare: BIO/01 - BOTANICA GENERALE

ETHNOBOTANICAL, METABOLOMIC AND BIOACTIVITY STUDY OF ITALIAN
ALIMURGIC PLANT SPECIES TRADITIONALLY USED IN FOLK MEDICINE

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Esame finale anno 2024

*“Let food be thy medicine
and medicine be thy food”*

Hippocrates of Cos

(400 B.C.)

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LIST OF ABBREVIATIONS

AA: ascorbic acid

Antiox: antioxidant activity

Antiox_Norm: normalized antioxidant activity

API: apigenin

ba: bark

BL: bioluminescent

BO: *Borago officinalis* L.

BSA: bovine serum albumin

CAFA: caffeic acid

CAT: catechin

CFUs: colony forming units

CHLORA: chlorogenic acid

CI: citation index

CINA: trans-cinnamic acid

COUMA: p-coumaric acid

D₂O: deuterium oxide

DNS: 3,5-dinitrosalicylic acid

EC: epicatechin

ECG: epicatechin gallate

EGC: epigallocatechin

EGCG: epigallocatechin-gallate

FERA: trans-ferulic acid

fl: flowers

FV: *Foeniculum vulgare* Mill.

GA: gallic acid

GI: growth inhibition

GLU: D-glucose

HP: *Hypericum perforatum* L.

HPLC-DAD: high performance liquid chromatography - diode array detector

KA: kojic acid

KAE: kaempferol
L-DOPA: 3,4-dihydroxy-L-phenylalanine
le: leaves
Luc: luciferase
LUT: luteolin
LUT-7-glu: luteolin-7-glucoside
mg AA eq/gDW: milligram of ascorbic acid equivalent per gram of dry weigh
mg BSA eq/gDW: milligram of bovine serum albumin equivalent per gram of dry weigh
mg GA eq/gDW: milligram of gallic acid equivalent per gram of dry weigh
mg GLU eq/gDW: milligram of D-glucose equivalent per gram of dry weigh
mg KA eq/gDW: milligram of kojic acid equivalent per gram of dry weigh
MHB: Mueller-Hinton broth
MIC: minimal inhibitory concentration
MTT: thiazolyl blue tetrazolium bromide
MYR: myricetin
NAR: naringenin
NF-κB: nuclear factor κB
NFκB-RE: NFκB response element
NMR: nuclear magnetic resonance
Nrf2: nuclear factor erythroid 2
P: Apulia
PCA: principal component analysis
PICEAT: piceatannol
PMs: primary metabolites
Poly: polyphenols
Poly_Norm: normalized polyphenols
Prot: proteins
Prot_Norm: normalized proteins
PROTA: protocatechuic acid
QUERC: quercetin
r: region
RedSug: reducing sugars

RedSug_Norm: normalized reducing sugars

ro: roots

RUT: rutin

S/L: solid – liquid ratio

SD: standard deviation

SDS: sodium dodecyl sulphate

SGF: simulated gastric fluid

SIF: simulated intestinal fluid

SINA: sinapic acid

SIRA: syringic acid

SMs: secondary metabolites

SN: *Sambucus nigra* L.

sp: species

SSF: simulated salivary fluid

st: stems

T: Tuscany

TNF- α : tumour necrosis factor

UI: use index

v/v: volume/volume

VAN: vanillin

VANA: vanillic acid

AIM AND OUTLINE OF THE THESIS

Alimurgic plants are wild plants which grow spontaneously in the environment. It is well known that many of these plants, due to the presence of biologically active compounds, have both nutritional and therapeutic values so that they can be considered as food-medicines. Over the centuries, mainly in rural communities, alimurgic plants constituted the main source of food and medicine ingredients.

The aim of this thesis was to study, analyse and compare, both for their phytochemical profile and biological activities, several Italian wild food plant species traditionally consumed and used also as medicinal plants given their therapeutic properties, in order to validate their widely uses in folk medicine.

Following the outline of the present thesis and of the work performed. Chapter 1: short introduction on alimurgic plants, ethnobotanical studies and plant metabolites. The experimental work was started with a wide and detailed bibliography search of published papers (from 1980 to March 2022) about Italian alimurgic species already studied from an ethnobotanical point of view (Chapter 2) to identify the wild food plants mostly reported by informants to be traditionally used for their healing and beneficial health effects. Among the most cited species, seven plants were selected for the following studies, and were collected in three different Italian regions (Apulia, Liguria and Tuscany). An initial biochemical characterisation of the extracts of the different plant organs (flowers, leaves, stems, roots, bark, depending on the species), was performed with spectrophotometric techniques (Chapter 3). Successively, an *in vitro* oro-gastric-duodenal digestion of medicinal preparations (i.e., infusion and decoction) made with selected plant organs was performed (Chapter 4), in order to compare the phytochemical composition of herbal remedies before and after a simulated human digestion. Some selected extracts (flowers, leaves and stems of *B. officinalis* and *H. perforatum*) were then subjected to a baseline characterization with NMR (determination of their molecular structure) and evaluated for antimicrobial activity during a short period abroad at ITQB NOVA, Oeiras, Portugal (Chapter 4).

The last part of the work (still in progress) consisted in the evaluation of anti-tyrosinase and anti-inflammatory activity of selected extracts, and of possible hormetic effects, by means of enzymatic and cell-based assays (Chapter 5). The discussion of all achieved results and the final conclusions are then included in Chapter 6.

This study, set out to determine the validation of medicinal uses of wild plant preparations and to improve the current knowledge of Italian alimurgic species widely used in folk medicine reconfirming that the selected species are rich in phytochemicals with important biological activities, and can justify their traditionally reported medical uses for external and internal applications.

Chapter 1

Introduction

1.1 Alimurgic Plants

Alimurgic plants, are wild plant species growing spontaneously in the environment without being cultivated. Since ancient time, these species played an important role in human life [1], as they were widely used not only for food but also for feed, medicines, cosmetics, as repellents, recreational activities, production of clothes and for cultural or religious rituals.

The term “alimurgic” was officially coined in the 1766 by the Tuscan naturalist Giovanni Targioni Tozzetti [2], who published in the following year a book dealing with the use of wild plants to increase the quantity of food in case of famine or wars, when obtaining food products and nutrients was difficult [3]. The use of alimurgic plants for culinary purposes has always been widespread throughout the world [4, 5], mainly by elder people experienced with the collection, taste, texture and odour of wild species, and able to orally hand down the traditional knowledge to the following generations [6]. For example, *Foeniculum vulgare* Mill. was used to flavour bread, soup and meat, *Melissa officinalis* L. to aromatize salads and omelettes, *Origanum vulgare* L. to season poultry, fish and sauces [7]. Since ancient times plants were gathered from the wild, especially in rural communities, to provide food in times of wars and famines, and, together with culinary uses, wild species were also widely used as natural medicines and drugs [8].

Even though in the second half of the XX century a loss of traditional local knowledge caused by the urbanisation and industrialisation of those years, was recorded, in the last decades there has been a revaluation of the use of wild plants both for food and therapeutic purposes. Nowadays, the use of wild plants in everyday care is a common study subject, and numerous ethnobotanical studies were published collecting information on wild plant traditional medicinal uses, the so-called folk medicine [9–12]. The aim is to preserve this traditional knowledge and at teaching new generations to sustainably exploit nature biological resources. More recently, alimurgic plants were often assimilated to *functional food* which was defined by Bigliardi and Galati [13] as food that improves general conditions of the body, decreases the risk of some diseases, and may have some therapeutic uses. This definition was related to their

content of minerals, vitamins, dietary fibers, essential fatty acids and secondary metabolites in general higher than cultivated species [14, 15]. A single species can have different uses depending on the region or country, local cultural traditions, availability in the environment [16]. For example for medicinal purposes, *Citrus aurantium* L. raw fruits are used against cough in Indonesia [17] and decoction and infusion of the aerial parts are taken orally against diabetes in Morocco [18], the whole plant of *Eucalyptus globulus* Labill. is used raw for stomachache and cold in Africa [19] and a leaves decoction for urinary problems or raw leaves for topic use in China [20]. Decoction and infusion of leaves of *Borago officinalis* L. are used against depression and loss of memory in Algeria [21], and flowers infusion as diuretic and refrigerant in Spain [22].

1.2 Ethnobotanical Surveys

As previously mentioned, most of the knowledge on alimurgic species was collected by means of ethnobotanical studies that were, and still are, important to avoid the loss of information on traditional local knowledge and cultural diversity in modern age, and to recover data regarding the use of wild species with healthy effects.

The term *ethnobotany* defines the research field concerned with the relationship between traditional customs of people concerning plants and their environment. Ethnobotanical surveys indeed, involve knowledge and use of plants in the context of cultural, social and economic significance [23].

These studies share common objectives: to explore the local knowledge of food and medicinal plants in rural contexts, to record uses, applications and conservations of wild plants, to preserve and to make all these information available for further phytochemical researches in order to provide the basis for the creation of new drugs [7].

In general, ethnobotanical surveys follow the same work line: i) interviews to local people on their traditional use of wild or cultivated plants as food, medicine or other uses, ii) identification of the cited plant species, iii) data analyses by means of different ethnobotanical indexes.

- i. People native of the investigated area, mainly farmers, shepherds, elder people, or people who belong to families which have strong link with local traditional activities, are interviewed on their traditions about the use of plants as food, medicine, etc. Informants are generally chosen by applying the purposive technique or the snowball sampling technique, of which the first consists in the researchers' own choice of the most suitable

subjects for the study, while in the second the existing informants recruit other subjects experienced in traditional plant use [24]. Most of the interviews are conducted by following guidelines established for ethnobotanical data collection, outlined by various published papers over the years [25, 26], in a unstructured (with open questions), semi-structured (with both a questionnaire and free conversation) or structured way (by using a questionnaire to be filled with specific questions) [27]. Informants are requested to provide specific information for each cited plant species, for instance folk use, preparation and parts used, way of administration, gathering period and recipes.

- ii. The second step consists in the identification and collection of the quoted species. Plant taxonomical identifications are carried out with the auxilium of scientific botanical books (e.g., in Italy Flora d'Italia by Pignatti [28]) or compared to other dry species conserved in herbaria. After collection, a voucher specimen of each plant is deposited in a public herbarium (e.g., University collections).
- iii. Ethnobotanical information is often elaborated with specific indices, different depending on the study. The most-commonly used indices are:
 - Informant Consensus Factor (ICF): to test the homogeneity of knowledge of the informants about the use of plants for example to treat a specific human disease [29]. The value of this index ranges from 0 to 1. A high value (close to 1) indicates that almost all informants agree in using that species for a specific use, while a low value (close to 0) indicates that the informants disagree on the use of the taxa and the choice of the species was random.
 - Relative Frequency of Citation (RFC): to calculate the local importance of each species [30]; this index varies from 0, if the species was not cited, to 1, if the species was cited by all informants.
 - Ethnobotanicity Index (EI): to estimate the importance of the cited plants in the studied area [30], expressed in percentage (higher is the value, higher is the knowledge of useful plants in the studied area).
 - Cultural Importance Index (CI): to estimate the cultural significance of each species [29]; value between 0 and 1, is used to verify to what extent each species is present in the popular culture and in the memory of the inhabitants; the closer the value is to 1, the more the species is mentioned and used by informants.

- Use Value (UV): to demonstrate the relative importance of species known locally [31]; is useful in determining the use reliability to support the pharmacological properties of the plant (higher is the value, higher is the importance of the species known locally).

The previous indices are useful for describing the results of ethnobotanical and ethnopharmacological studies, focusing on the importance and value of a single species [32]. These kinds of surveys are therefore important not only in preserving traditional knowledge about the uses of plants, but also to identify species which can be natural substitutes of chemical drugs.

1.3. Primary and Secondary Metabolites in Alimurgic Plants

Plants synthesize hundreds of phytochemical compounds to control essential life functions such as growth, morphogenesis, reproduction, adaptation to environmental changes and defence.

Plant metabolites can be generally divided into primary and secondary metabolites according to their main biological functions. Primary metabolites (PMs), proteins, carbohydrates, lipids and nucleic acids, are common to all living organisms including plants, and are associated with fundamental processes such as growth, development and reproduction of living cells and energy production. On the other hand, secondary metabolites (SMs), such as phenolics, alkaloids, terpenes, steroids, lignans, are specifically synthesized to increase the ability of plants to deal with biotic and abiotic environmental challenges and as defence (e.g. against animal herbivores, other plants, pathogen bacteria, fungi, etc) [33]. In contrast with PMs, common to all plants, SMs evolve dynamically and are different depending on the plant species, taxonomic group, or different plant habitats [34].

Alimurgic plants contain many different phytochemicals and especially their SMs proved to have biological activities, such as antioxidant, antimicrobial, anti-inflammatory, anticancer, that may improve human health and exert healthy and curative effects, when plants or their extracts are consumed [35].

Given that the pharmacological activity of a plant preparation depends not only on a single compound, but on the combined activities of several metabolites, most of the phytochemical research on plants having medicinal applications dealt with the screening of primary and secondary metabolites and their biological activities.



Figure 1.1. Species studied in the PhD project.

In Table 1.1 several examples of phytochemical studies related to the alimurgic plant species taken into consideration in this thesis (Fig. 1.1) are listed including the classes of analysed metabolites.

Table 1.1. Primary and secondary metabolites screened by phytochemical studies related to the species taken into consideration in the project.

Species	Metabolites	References
<i>Borago officinalis</i> L.	Phenols, flavonoids, proteins, carbohydrates, tannins, terpenoids, lipids, alkaloids, vitamins	[36–40]
<i>Cynodon dactylon</i> (L.) Pers.	Alkaloids, flavonoids, phenols, tannins, proteins, carbohydrates	[41, 42]
<i>Foeniculum vulgare</i> Mill.	Phenols, flavonoids, vitamins, proteins, carbohydrates	[43–45]
<i>Hypericum perforatum</i> L.	Phenols, flavonoids, proteins, reducing sugars, alkaloids, steroids	[46–49]
<i>Malva sylvestris</i> L.	Phenols, flavonoids, tannins, alkaloids, vitamins, carbohydrates	[40, 50, 51]
<i>Sambucus nigra</i> L.	Phenols, flavonoids, sugars, fatty acids, proteins	[52–56]
<i>Urtica dioica</i> L.	Phenols, flavonoids, carbohydrates, alkaloids, proteins, amino acids, tannins	[57–60]

The classes of metabolites considered and analysed in the present thesis are very briefly introduced below.

1.3.1. Proteins

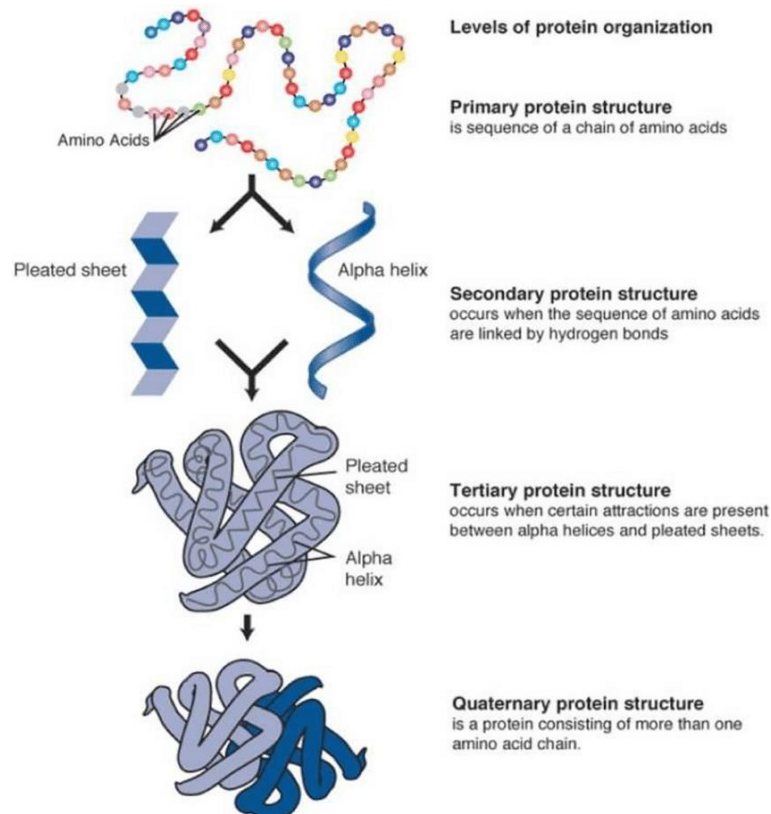


Figure 1.2. The four classes of protein structure [61].

Proteins are complex molecules composed by 20 different amino acids, which are connected through peptide bonds. Protein structure is divided into four levels: primary (linear sequence of amino acids), secondary (spatial disposition of amino acid sequence), tertiary (three-dimensional form) and quaternary (spatial disposition of proteins with more than one peptide chain) structures (Fig. 1.2). The functional differences of proteins depend on their structural characteristics, amino acid sequence, size and type of the peptide chain [62], as well as on their physicochemical characteristics, such as surface hydrophobicity, net charge, and presence of reactive groups [63]. Proteins are essential macronutrients for human nutrition, and nowadays, the demand of sustainable and high-nutritional and healthy alternative food protein sources is increasing. To this extent, plant proteins represent a good alternative to animal proteins (i.e., dairy, egg and meat) for several respects such as lower environmental impact, lower production

costs, more availability and lower animal exploitation. For all these reasons people are becoming more interested in replacing meat proteins with vegetable proteins, and the search of new sources of proteins alternative to animal proteins is growing [64]. In addition to their high nutritional value, a wide range of biological activities can be ascribed to protein peptide sequences (typically consisting of 2-20 amino acid chains, also called bioactive peptides) encoded in the native sequence of the precursor dietary protein and released after enzymatic proteolysis occurring during human oro-gastrointestinal digestion [65]. Antihypertensive, antioxidant, anti-inflammatory, anticancer, hypocholesterolemic, antimicrobial and antifungal activities are among the bioactive peptide biological activities promoting human health [65–67]. Examples of vegetable sources of bioactive peptides are soybeans [68], cereals [69] and oilseed plants [62] but also many alimurgic plants such as *Allium ursinum* L. and *Capsella bursa-pastoris* Medik. [6].

1.3.2. Carbohydrates

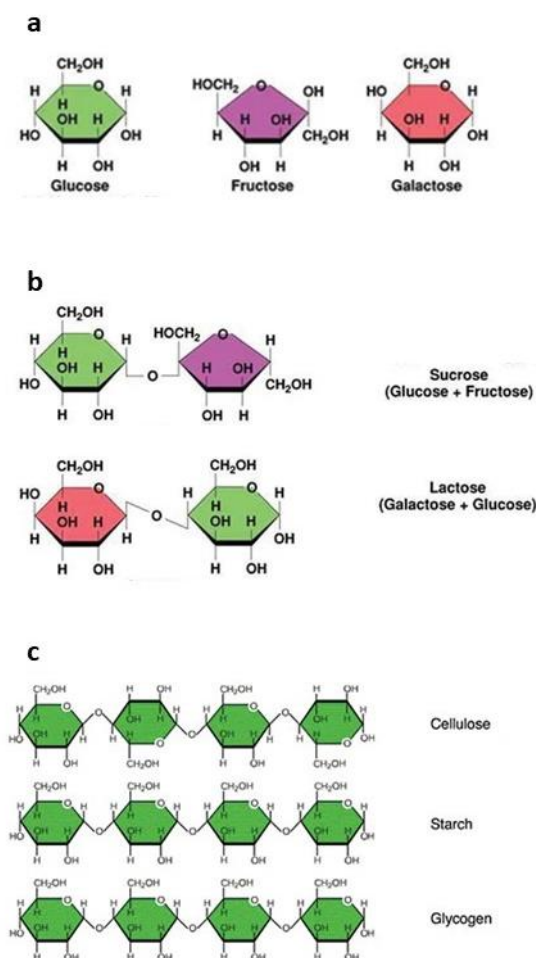


Figure 1.3. Chemical structure of carbohydrates. a) monosaccharides b) disaccharides c) polysaccharides. Image source [70].

Carbohydrates are classified into four chemical groups, monosaccharides (C_3 - C_7 compounds, such as glucose and fructose), disaccharides (two monosaccharide units, such as sucrose and lactose), oligosaccharides (5-6 monosaccharides units) and polysaccharides (several monosaccharide units, such as starch, cellulose and glycogen) (Fig. 1.3).

Carbohydrates are synthesized by plants during photosynthesis, represent source of energy, carbon skeleton for organic compounds and they act as signalling molecules for abiotic and biotic stress [71, 72]. In particular, sugars play a key role in regulation and signalling in plant life, in fact they control metabolism, growth, stress responses and development in plants [73]. The most abundant sugars in plants are the disaccharides sucrose and maltose, and the monosaccharides glucose and fructose. These two monosaccharides can act as reducing agents thanks to the presence of an aldehyde or a ketone group, being therefore called reducing sugars [74]. Reducing sugars exhibit antioxidant activity due to their reducing power and their radical scavenging ability [75].

1.3.3. Phenolics

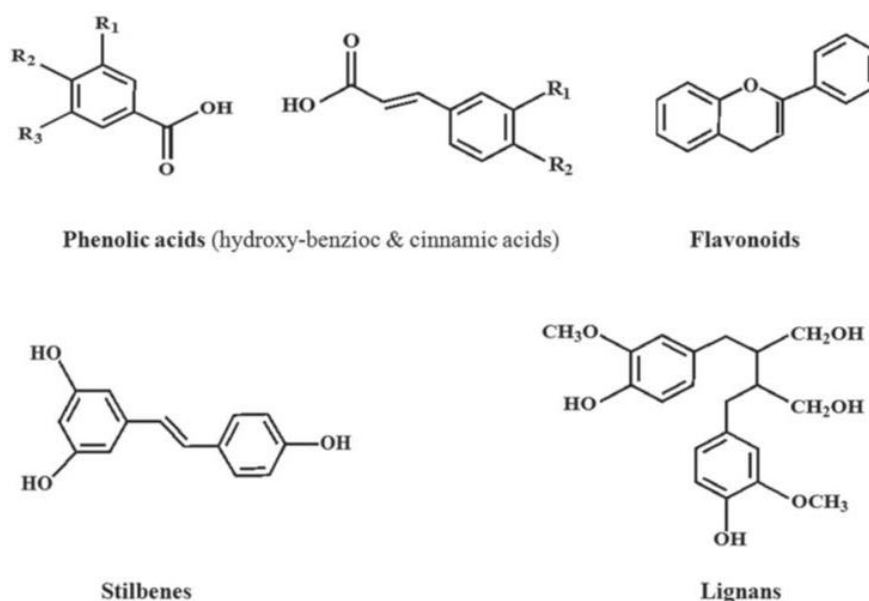


Figure 1.4. Chemical structures of the different classes of phenolic compounds. Image source [76].

Phenolics are a large group of more than 10,000 different compounds composed by one or more aromatic rings with one or more hydroxyl groups. They constitute a heterogeneous group of molecules that, according to their chemical structure and to the number of hydroxyl groups, show a more or less strong ability to scavenge free radicals and consequent antioxidant capacity [77]. Phenolics can be divided into different groups based on the number of phenolic rings and on other structural elements that bind these rings together:

- Phenolic acids: monomeric structure with one carboxylic acid group, one benzenic ring and hydroxyl or methoxyl groups [78]. They are subdivided into two classes, hydroxycinnamic (i.e., caffeic acid and ferulic acid) and hydroxybenzoic acids (i.e., gallic acid and vanillic acid).
- Flavonoids: the widest group of polyphenols, representing one of the oldest and most conserved classes of SMs. They have a basic structure consisting of two aromatic rings bounded by three carbons forming an oxygenated heterocycle, and are divided into six subclasses based on the type of heterocycle involved [76]: flavonols, flavones, flavanones, flavanols, anthocyanins and isoflavones.
- Stilbenes: compounds with a basic skeleton containing 14 carbons (C₆–C₂–C₆) in which a double-bonded ethylene bridge links the two phenolic rings [79]. The main representative molecule of this class of polyphenols is resveratrol, which is well known for its beneficial effects on human health, such as antihypertensive, antioxidant, anti-inflammatory, immunomodulatory, antimicrobial and anticancer properties [80].
- Lignans: compounds derived from the oxidative dimerization of two or more phenylpropanoid units (C₆C₃ phenylpropane skeleton). They can be classified in different groups according to their structure: lignans, neolignans, norlignans, hybrid lignans, and oligomeric lignans [81].

Polyphenols are plant secondary metabolites generally involved in defence against pathogens, UV radiation and act in adaptation and signalling of plants to environmental changes and between plants or plants and other organisms.

Many of these compounds are present in plant-derived foods and when assumed through daily diet, these molecules represent bioactive compounds involved in the protection and maintenance of human health, also preventing various degenerative diseases associated with oxidative stress (i.e., cancer, cardiovascular and neurodegenerative diseases) [78]. Most of the antioxidants present in human diet are polyphenols such as rutin, typical flavonoid of fruit,

vegetables, tea and wine [82], and caffeic acid, an hydroxycinnamic acid present in many food sources (coffee drinks, blueberries, apples and cider) [83].

Plants grown under mild stress conditions (e.g., severe temperature, dehydration, lack of nutrients, UV irradiation, herbivore attack, fungal and bacterial infection) produce higher levels of phytochemicals compared to those being cultivated under ideal conditions [15]. Therefore alimurgic species have typically higher concentrations of metabolites than cultivated plants, providing them with important medicinal properties [84], and so the focus of this thesis is the analyses of health benefits of these species and their phytochemicals, which are differently distributed in all plant organs [85].

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Chapter 2

Ethnobotanical Review and Dataset Compiling on Wild and Cultivated Plants Traditionally Used as Medicinal Remedies in Italy

Published as: Monari, S.; Ferri, M.; Salinitro, M.; Tassoni, A. (2022). Ethnobotanical Review and Dataset Compiling on Wild and Cultivated Plants Traditionally Used as Medicinal Remedies in Italy. *Plants*, 11, 2041. <https://doi.org/10.3390/plants11152041>

Abstract

Over the centuries, wild plants have constituted the main food ingredients and traditional medicine in rural communities. In the last decades, thousands of ethnobotanical studies have been conducted, with the aim of documenting the traditional knowledge on wild and cultivated plants both for food and therapeutic purposes. In the present work, 75 published papers related to Italian ethnobotanical knowledge on wild and cultivated plants traditionally used for medical purposes were analysed and data on 1117 different species organized in the first dataset to target medicinal applications only. For each plant species, the Italian region of use, plant organs, mode of preparation, specific pathological group of application, citation index, and use index were listed. The different therapeutic applications were subdivided into nine main pathological groups according to the targeted human apparatus. Overall, the cited species with highest number of uses were related to the treatment of the digestive system and of skin-ears-eyes-hair diseases, followed by diseases of the genito-urinary and respiratory systems. The 13 most relevant species were identified on the basis of their citation and use indexes. The present review on Italian medicinal flora aims to provide valuable information on wild and cultivated species, which are potential sources of plant-based therapeutic remedies, to preserve and reevaluate endangered traditional folk knowledge.

2.1. Introduction

Plants play an important role in human nutrition and health, due to the high nutritional value given by their well-known bioactive compounds (proteins, fibers, polyphenols, vitamins, etc.). Over the centuries in rural communities worldwide, wild edible plants, growing spontaneously in their natural habitat without being cultivated, have constituted the main food and medicine ingredients.

In the last decades, thousands of ethnobotanical studies have been conducted to document the traditional knowledge on wild and cultivated plants perceived as healthy in cultural, social, and economic contexts [1–4]. Ethnobotany has applications in many fields, and studies the relationships between people and plants and conservation of biodiversity (with special regard to the documentation and maintenance of indigenous and local botanical knowledge), and records information on locally available species, which are potential sources of new natural drugs as alternatives to synthetic ones [5–7].

In general, nowadays, such traditional local knowledge is mainly preserved by elderly people, even though in the last years, the interest in these plant species has again been increasing [8, 9]. Traditional medicine is still practiced in many territories and in developed countries, both by people who are involved and not involved in agricultural practices [10, 11]. In addition, wild plants were used in the past and are still used today for many different practices, such as feed and veterinary, agricultural, cosmetic, domestic, and magic and ritual uses [3, 12–15].

The Mediterranean Basin is a territory with a wide plant diversification due to its mild climate. This area has been populated since millennia and much traditional knowledge of the use of plants has thus been passed through the generations [16–18]. In particular, the common daily Mediterranean diet is characterized by significant intake of vegetables, fruits, and spices [19] and both cultivated and wild food species may represent an essential part of this diet, not only for their nutritional value but also for their medicinal properties [4, 11, 20].

Italy is located in the middle of the Mediterranean Sea in southern Europe. Because of the north–south extension of the Italian Peninsula and the mountainous hinterland, the climate of Italy shows different patterns. In the Northern Alps, the climate is strongly dependent on the elevation, with mild to cool summers and mostly cold winters. Northern and central plain regions mostly show a humid subtropical climate with cool winters and hot summers while coastal areas and a large part of the south are characterized by mild winters, rainy autumns and springs, and hot and dry summers. The heterogeneous climate has led to the diversification of a rich endemic

flora and to the diversification of the use of spontaneous flora among different regions. Up to now, ethnobotanical reviews conducted on Italian species have focused on the use of wild plants as food [3, 9] or on medicinal species from specific geographic areas [13, 21].

In this panorama, it seemed of primary importance to compile a complete checklist of Italian wild and cultivated plants with medicinal effects to interconnect this knowledge with their known benefits on human health and to complement previous studies targeting mostly food applications (e.g., [3, 9]) or specific geographic areas (e.g., [13, 21]). The present study represents a wide and detailed bibliographic search of all Italian wild and cultivated plant species already studied from ethnobotanical and phytochemical points of view. The aim of this review was the creation of the first dataset in which all the information on wild and cultivated medicinal plants traditionally used in Italy was collected and standardized. This study focused only on medicinal uses while culinary and veterinary uses were not considered.

2.2. Methods

Data collection was carried out by means of an extensive bibliographic search of all the published papers related to Italian ethnobotanical studies. The search was conducted through the Scopus (<https://www.scopus.com>, last access on 24 March 2022) and Google Scholar (<https://scholar.google.com>, last access on 24 March 2022) websites. The combination of exact keywords “ethnobotanical study” AND “medicinal plant” AND “Italy” was used. The search produced an output of 489 papers, published from 1980, related to Italian alimurgic plants. After reading all 489 papers, only 75 (2 of which were in the Italian language), which dealt with Italian species with therapeutic uses, were finally selected and analysed in detail (last update March 2022). All papers or information referring only to the use of plants as food and those related to veterinary or agronomic uses were excluded. The botanical names of plant species were checked and updated according to the website The World Flora Online (<http://www.worldfloraonline.org>, last access on 26 July 2022).

A complete dataset was created in an Excel worksheet (showed at the end of the thesis, Appendix 1) including the following information for each plant species (listed by genus alphabetic order): botanical name of the species/subspecies, botanical family, wild or cultivated status, number of citations, references, pathological groups, citation index (CI), and use index (UI).

The therapeutic uses were classified into 9 different pathological groups: (1) digestive system diseases, (2) skin-ears-eyes-hair diseases and wounds, (3) systemic disorders, (4) genito-urinary system diseases, (5) respiratory system diseases, (6) nervous system diseases, (7) cardio-circulatory system diseases, (8) muscular-skeletal diseases, and (9) metabolic diseases.

The ethnobotanical information collected was evaluated and compared by calculating two different indexes: citation index (CI) and use index (UI). CI was calculated by dividing the number of citations of a given species by the total number of analysed papers (75). UI was obtained by dividing the number of pathological groups for which a species was used by the total number of pathological groups (9).

Graphical elaborations were carried out using R 4.0.2 software (<https://cran.r-project.org>, last access on 14 April 2022), Microsoft Excel®, and Paint® 11.2201.22.0 for Microsoft 365. To avoid overlapping, points were randomly scattered on the y-axis (± 0.1) using the R function *geometry jitter*.

2.3. Results and Discussion

2.3.1. Italian literature data

A total of 75 papers were selected and analysed (Table 2.1), of which 72 were original research papers and 3 were reviews, describing ethnobotanical surveys on the traditional uses of plants for human diseases in specific Italian regions. A complete dataset about the wild and cultivated plant species traditionally used in Italy for medicinal purposes was produced (Appendix 1).

Table 2.1. Reviewed papers according to the distribution in Italian regions.

Italian regions	Papers
Abruzzo	[21–24]
Basilicata	[21, 25–31]
Calabria	[21, 32–35]
Campania	[19, 36–45]
Emilia-Romagna	[46–48]
Friuli-Venezia Giulia	[49, 50]
Lazio	[21, 23, 51, 52]

Liguria	[53–55]
Lombardia	[5, 56–60]
Marche	[21, 61–63]
Molise	[21, 23, 24, 64, 65]
Piemonte	[66–69]
Puglia	[21]
Sardegna	[70–77]
Sicilia	[20, 76, 78–82]
Toscana	[47, 83–86]
Trentino-Alto Adige	[13]
Umbria	[21, 87, 88]
Valle d’Aosta	[14]
Whole Italy	[89, 90]

All studies were carried out by interviewing people (mostly elderly) born or living in the studied area for a long time.

Figure 2.1a shows the number of ethnobotanical studies that included medicinal applications for each region. The data showed that a total of 19 regions (out of 20) were analysed in these studies, with only the Veneto region missing from any paper. Campania was the most quoted (in 11 papers), followed by Basilicata and Sardegna (8 papers) and Sicilia (7 papers). On the other hand, considering the total number of different medicinal species recorded in each region (Figure 2.1b), Italian regions with the highest number of analysed plants were Campania (520 species), Trentino Alto-Adige (269 species), and Lombardia (251 species).

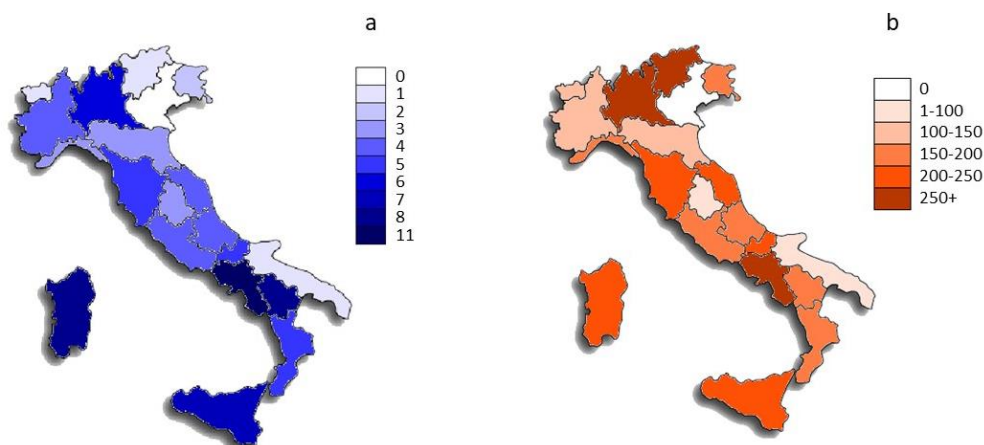


Figure 2.1. Maps showing the number of ethnobotanical studies that included medicinal applications (a) and the number of wild and cultivated plant species used for medicinal purposes (b) at the Italian regional level.

2.3.2. Medicinal Plants

Data were collected on 1117 species, including 42 subspecies, belonging to 125 botanical families. In 69 cases, only the genus was indicated while the species was unspecified (sp.pl., species plures). The families mostly quoted were Compositae with 145 species (12.97%), followed by Lamiaceae (86, 7.69%), Rosaceae (66, 5.90%), and Fabaceae (64, 5.72%) (detailed data in Appendix 1). Conversely, by analysing only the food uses, Paura et al. [9] found that the most quoted families of wild plants were Asteraceae and Brassicaceae (20.22% and 6.98%, respectively).

The plant organs that were used most for the medicinal preparations (Figure 2.2a) were the leaves (34%); entire plant (16%), generally referring only to the aerial parts, including buds and branches; inflorescences (14%) (whole or specific parts, such as petals, stamen, etc.); infructescences (12%); and roots (including tubers, rhizomes, bulbs) (12%). Seeds and their processed parts, such as flour, were used for 5% of the preparations while the remaining 7% of medicinal remedies were based on bark, bulbs, stems, latex, or other plant parts (Figure 2.2a).

The spectrum of preparation methods was more diversified (Figure 2.2b). One-third of the medicinal remedies were prepared as a decoction (33%) (the plant is added to cold water, heated and boiled for a few minutes, and the mixture is left to stand at room temperature and filtered) while 22% as an infusion (the plant is added to boiling water and left to stand at room temperature for a few minutes). These data are in agreement with Paura et al. [9], who claimed that infusion and decoction methodologies are mainly associated with medicinal properties and

excluded them from their study based on the food use of wild plants. In addition, the plant was directly spread on the part to be treated (as poultices, including bandage, cataplasm, and cream) (14%). A wide range of preparation methods have been indicated in the literature among which raw (5%), maceration (4%), compress made with plant (3%), juice extraction (2%), syrup, oil, tincture, dried fumigation (all 1%), and others (7%) were identified (Figure 2.2b).

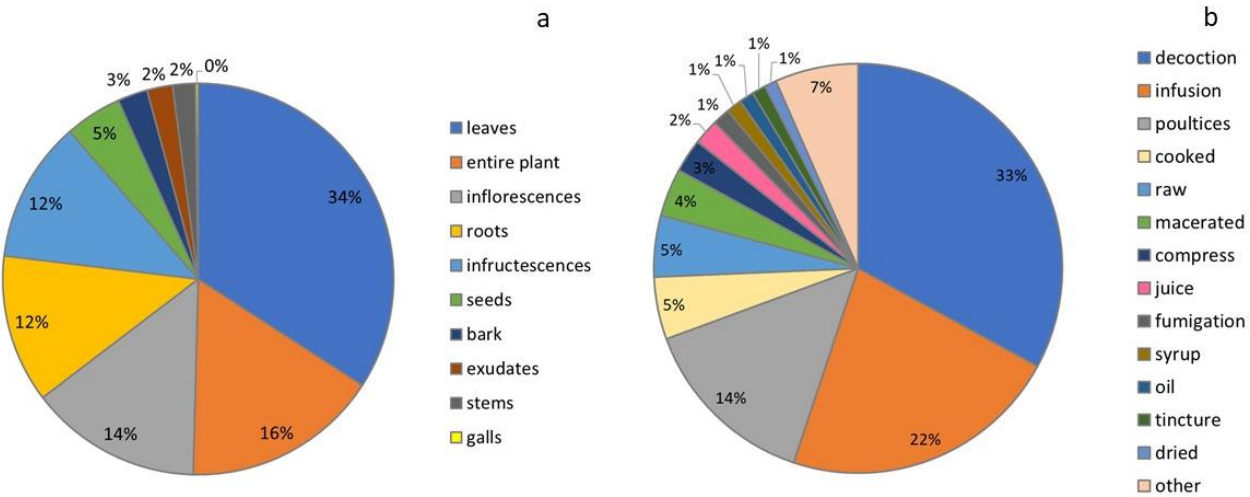


Figure 2.2. Plant parts used for medicinal preparations. (a) Type of remedy preparations and applications. (b) Other preparations include liqueur, ointment, crushed, milled, chewed, jam, eaten, cooking water, powdered, oleolite, washes, rubbed, extract, pulped, distilled, smoked, sniffed, and kept in pockets. The classifications used were based on standardized descriptors as reported by Cook [91].

2.3.3. Pathological Groups

Given the very large spectrum of disorders and diseases addressed by medicinal remedies made with wild and cultivated plants, the therapeutic uses quoted in the reviewed literature were classified into nine different pathological groups (Table 2.2, Appendix 1), which fit best with the data collected in the present study and also partially taking into consideration previous standardized categorizations [91]. Table 2.2 shows the resulting nine pathological groups and resumes for each of the uses and treated diseases quoted by the informants.

Table 2.2. *Pathological groups and principal therapeutic applications of cited plant species. The classification used was based on the standardized descriptors reported by Cook [91].*

Pathological Groups	Principal Therapeutic Uses and Treated Diseases	n. of Plant Species	n. of Papers
1—Digestive system diseases	Astringent, anti-abdominal pain, anti-diarrheic, carminative, eupeptic, cholagogue, anti-parasitic and mycotic diseases, lenitive of teeth and mouth inflammations, oral disinfectants	705	74
2—Skin-ears-eyes-hair diseases and wounds	Lenitive of insect stings, emollient, cicatrizing, curative of dermatitis, skin infections, aesthetic problems, ear infections, ophthalmic inflammations, chilblains, wounds, burns, bruises	661	72
3—Systemic diseases	Antimicrobial, anti-phlogistic, anti-pyretic, tonic, reconstituent, depurative, analgesic, coadjuvant, cleansing, diaphoretic	531	70
4—Genito-urinary system diseases	Menstruation regulator, abortifacient, galactagogue, oxytocic, lenitive of urinary diseases and cystitis, lithotripter, depurative	522	75
5—Respiratory system diseases	Anti-asthma, cold, cough, bronchitis, expectorant, pulmonary diseases, inflammation of the respiratory system	499	69
6—Nervous system diseases	Sedative, calming effects, drowsiness, antidepressive, memory booster, headache, insomnia, epilepsy	348	70
7—Cardio-circulatory system diseases	Action on the cardiac rhythm, vasoconstrictor/vasodilatory, pressure regulation, blood depurative, cardiopathies, varicose veins	333	72
8—Muscular-skeletal diseases	Lumbago, arthritis pains, effect on calcium and bone metabolism, sprains, rheumatism, swollen feet and legs	301	68
9—Metabolic diseases	Mineral integrator, vitamin deficiency, diabetes, antigout, cytotoxic, hypo/hypercholesterolemic	223	46

Overall, the detected plants were mostly used for the treatment of the digestive system and skin-ears-eyes-hair diseases and wounds groups (with more than 600 plant species each), which also showed the highest number of targeted diseases. This observation may be explained by the fact that most of these ailments are generally mild and easy to cure with plants [47]. About 500 species were used for diseases of the genito-urinary and respiratory systems. A similar number of species were utilized for the more general systemic disorders category. These results are in agreement with the findings of several other ethnobotanical studies [45, 54, 63].

Brief descriptions of the pathological groups with a few examples of plant species applications are presented in the following sections.

2.3.3.1. Digestive System Diseases

In total, 74 papers listing 705 species described different ways to treat digestive system ailments (Table 2.2, Appendix 1). This pathological group is composed of digestive system diseases, parasitic and helminthic infections, and teeth and mouth problems.

A total of 190 species were cited for their digestive properties, principally after drinking an infusion or decoction or after alcoholic maceration. The seeds of *Foeniculum vulgare* Mill., leaves of *Laurus nobilis* L., flowers and leaves of *Rosmarinus officinalis* L., and leaves of *Salvia officinalis* L. were the most utilized plant organs [14, 60, 73, 78].

Gastric ailments (total of 62 species), such as gastritis and nausea, were treated mostly by drinking a decoction or infusion of specific plant organs, for instance, a decoction of *Hordeum vulgare* L. fruits [45] or *Cynara cardunculus* L. leaves [81] and an infusion of *Citrus limon* (L.) Osbeck fruits or *Ocimum basilicum* L. aerial parts [23]. However, the consumption of raw or cooked seeds of *Vicia faba* L. [44], fruits of *Capsicum annuum* L. [43], or leaves of *Oxalis acetosella* L. [59] was also used against heartburn.

Regarding intestinal disorders (219 species), the most common symptoms treated with plants were the simplest ones, such as diarrhoea, belly pains, and constipation, which are easy to solve with immediate administration of the treatment. The leaf decoction or raw fruits of *Arbutus unedo* L. [43, 44] and raw fruits of *Sorbus domestica* L. [28, 52] were the most cited remedies to solve diarrhoea. Moreover, several plants were used against constipation and for their carminative properties such as *Mercurialis annua* L., *Prunus* sp.pl., *Daucus carota* L., *Petroselinum crispum* (Mill.) Fuss, and *Carum carvi* L. [37, 46]. Other plants were used in the case

of anorexia and inappetence. *Gentiana* sp.pl. was the most representative genus utilized for this remedy (*G. acaulis* L., *G. dinarica* Beck, *G. lutea* L., *G. punctata* L., *G. verna* L.) [14, 23, 65].

Several liver protectors (90 species) were also mentioned by the interviewed people. A decoction of the flowers and leaves and an infusion of the roots of *Cichorium intybus* L. showed purifying and hepato-protective effects [59], and cooking water and cooked leaves of the same plant were eaten for liver wellbeing [26]. A leaf and root decoction of *Taraxacum campylodes* G.E.Haglund was used as a depurative for the liver and the boiled leaves were eaten as a depurative [14, 54] in addition to the boiled leaves or decoction of *Silybum marianum* (L.) Gaertn. [67]. The liver-protecting characteristic of these species is essentially due to their cholagogue, cholaretic, and depurative activities [90].

Here, parasitic and helminthic infections (73 species) were considered part of the intestinal tract pathological group because the plant treatments cited were mostly against enteric parasites. *Allium sativum* L. was the most widely used plant for vermifuge and anti-helminthic action, with many different uses and preparations for this purpose, for example, drinking bulb juice [50] or crushed bulb decoction [37, 39], eating raw crushed bulbs [65, 84], bulb necklace, or vapor inhalation [35]. Other species used to treat parasitic diseases were *Artemisia absinthium* L. (aerial part infusion or maceration in alcohol) [14, 22, 67], *Ruta chalepensis* L. (leaf infusion or aerial part maceration in oil) [33, 90], and *Ricinus communis* L. (seed oil) [40].

Several species (179) were reported for their anti-inflammatory properties for the mouth (72), teeth (137), and gums (18). The leaves of *Lactuca sativa* L. were widely reported in ethnobotanical studies as anti-odontalgic and for the treatment of toothache: a decoction of the leaves was used for washes against mouth inflammations and the boiled leaves were used on inflamed teeth [39, 43]. The whole plant of *Malva sylvestris* L. was the most reported species for the treatment of oral cavity ailments (50 citations), with a decoction of the aerial parts used as a mouthwash for toothache [52] or the flowering parts as an antiseptic against caries [65], pills obtained after boiling and pressing the plant used to treat gum inflammation [61], and chewing of leaves [63] and washes with a decoction of the roots [39] used in toothache.

2.3.3.2. Skin-Ears-Eyes-Hair Diseases and Wounds

In total, 72 papers quoted 661 different species (Table 2.2, Appendix 1) as cicatrizing of skin injuries (wounds, cuts), emollients (rash, erythema, sunburns, dermatitis), lenitive (bruises, ecchymosis, insect stings, chilblains), and for cosmetic issues such as furunculosis, corns, hair

loss, varicose veins, chapped hands, or wound healing. Treatments for ear and eye inflammation were mainly topic preparations.

Many species (144) were used as emollients in the case of dermatitis, rash, and other skin irritations. Tubers of *Solanum tuberosum* L. were used in various ways, for example, raw slices were used on skin burns [39, 44], boiled potato slices were used on the affected area [37], fresh pulp was externally applied to minor burns and reddened skin [89], and cataplasm made with raw tuber was applied as a paste [72]. Decoctions with *Malva sylvestris* L., both with the roots [74] and the flowering aerial parts [30], were used to relieve dermatitis, in addition to the peeled stems of *Opuntia ficus-indica* (L.) Mill [39, 76] and the whole flowering plant of *Viola odorata* L. [40]. Seed oil of *Prunus dulcis* (Mill.) D.A.Webb [72] and a decoction of the flowers or leaves of *Malva neglecta* Wallr. and *Urtica* sp.pl. leaves [90] were used against eczema and sunburns.

Folk medicine remedies against bruises, chilblains, and similar skin problems were also widespread (118 species). The crushed aerial parts of *Parietaria judaica* L. (together with the seeds of *Linum usitatissimum* L. and egg albumen) [37, 44], aerial part infusion of *Artemisia absinthium* L. [68], and flower oil of *Hypericum perforatum* L. and *H. perforatum* L. [81] were utilized in poultice recipes that were topically applied with lenitive properties against bruises. To relieve itching and swelling due to chilblains, known remedies were directly applied leaves or fruits of *Citrus sinensis* (L.) Osbeck [43], resin of *Larix decidua* Mill. (sometimes cooked in oil or butter) spread on the affected area [14], and grated and boiled rhizome of *Iris germanica* L. directly placed on the skin [36].

Plants (81) with skin lenitive properties were also used to heal insect bites and stings. Among them, the most cited were fresh bulbs of *Allium cepa* L. and *A. sativum* L. rubbed on the skin [45, 63], fresh leaves of *Plantago lanceolata* L. and *Plantago major* L. applied to bee and mosquito bites [52, 54], cataplasm made with the aerial parts of *Clinopodium nepeta* (L.) Kuntze [81, 88], and directly applied fruit latex of *Ficus carica* L. [30, 34].

Concerning plants involved in dermatological problems, a considerable set of species (108) were used for several cosmetic issues. Furunculosis benefitted from the uses of several plants such as topically applied fresh leaves and fruits of *Rubus ulmifolius* Schott [81], fresh leaves of *Ocimum basilicum* L. [37], flower infusion of *Borago officinalis* L. [68], compress with the aerial parts of *Malva sylvestris* L. [61], and root decoction of *Arctium lappa* L. [14]. Poultice with the aerial parts of *Sedum maximum* (L.) Suter. [57], latex from the stems of *Chelidonium majus* L. [55], crushed

leaves of *Rumex* sp.pl. [54], and cataplasm with the tubers of *Asphodelus microcarpus* Saltzm. & Viv. [90] were applied to treat corns and warts.

Inflammation of the eyes and ears was treated with topical applications of several preparations (90 species). Compress made with a flower infusion or decoction of *Matricaria chamomilla* L. [49, 65], leaf decoction [57] or flower infusion [14] of *Sambucus nigra* L., and shoot sap of *Vitis vinifera* L. used as a collyrium [40, 44] are examples of good remedies in the treatment of conjunctivitis and inflamed eyes. Topically applied oil from the fruits of *Olea europaea* L. [40, 44] and fumigations with the leaves of *Arundo donax* L. [90] were considered effective in the case of otitis.

Hair loss and dandruff were also treated with medicinal plants (16): a leaf decoction of *Urtica dioica* L. was widely drunk or more often externally applied in washes to strengthen hair and prevent hair loss [48, 61, 69] and a decoction of the aerial parts of *Parietaria officinalis* L., leaves of *Origanum vulgare* L., or *Urtica* sp.pl. was considered effective against dandruff [23, 45, 48]. A bandage and poultice made with some plant organs were used as a moisturizing cream for chapped skin (e.g., flowering tops of *Achillea millefolium* L. [61] or inflorescences of *Calendula officinalis* L. [55]).

Several plants (196) were used for healing wounds: a poultice made with the leaves of *Plantago major* L. [53, 57], warmed leaves of *Malva sylvestris* L. [32, 79], cataplasm with the crushed seeds of *Linum usitatissimum* L. [41, 57], direct application of the resin of *Pinus* sp.pl. [68, 90], and rubbing with the flower oil of *Hypericum perforatum* L. [69, 82] were some of the most popular vulnerary remedies.

2.3.3.3. Systemic Diseases

In total, 531 plant species were reported in 70 papers (Table 2.2, Appendix 1) as useful in the case of systemic disorders to improve the general state of health. These plants were not effective against a specific affection but in curing and strengthening the whole organism.

Some of these plants (166) were defined as a reconstituent tonic, depurative, or detoxicant to help in the recovery from different diseases by purifying the blood and inducing perspiration. A leaf decoction of *Cichorium intybus* L. was drunk before meals as a depurative of the intestine and blood [19, 54, 58] while a leaf or flower decoction of *Taraxacum campylodes* G.E. Haglund was used as a kidney depurative [14, 56]. Infusions or decoctions drunk as detoxifiers were made

with the roots of *Cynodon dactylon* (L.) Pers, aerial parts of *Melissa officinalis* L., aerial parts of *Urtica dioica* L., and seeds of *Foeniculum vulgare* Mill. [49, 83].

Other plants (48) were identified as being analgesic and lenitive and effective in relieving different types of pain due to their general soothing action. The aerial parts of *Parietaria judaica* L. were topically applied for their analgesic effects [43], a poultice made with the macerated petals of *Rosa canina* L. was a lenitive for the skin [90], and flowers and other aerial parts of *Hypericum perforatum* L. macerated in olive oil were locally applied as a lenitive in a wide range of inflammation [77].

Other species were considered as fortifiers due to their corroborant properties and their capacity of contrasting weakness. The fresh aerial parts of *Artemisia genipi* Weber ex Stechm. and *Artemisia umbelliformis* Lam. were chewed during exercise as a tonic and energizer [59], fresh fruits of *Juglans regia* L. were eaten as mineralizing and energizing snacks [57], and raw seeds of *Prunus dulcis* (Mill.) D.A. Webb were eaten in the case of weakness [22].

Some species were also used for their antipyretic and immunostimulant action. *Centaureum erythraea* Rafn is well known for the febrifuge activity of a decoction made with the aerial parts [31] or whole plant [55] or a flower infusion [90]. Many species have shown generic antipyretic properties (e.g., *Ajuga iva* (L.) Schreb., *Marrubium vulgare* L., *Juniperus communis* L.) while others have been used specifically for malarial or typhoid fevers, such as *Gentiana lutea* L. (root infusion) [23], *Centaurea calcitrapa* L. (decoction with the aerial parts) [75], *Rhamnus alpina* L. (decoction with fruits) [65], and *Apium nodiflorum* (L.) Lag. (leaf infusion) [74]. A small number of species had immunostimulant effects, including the flowers of *Rosa canina* L. [68] and bulbs of *Allium sativum* L. [44].

2.3.3.4. Genito-Urinary System Diseases

All 75 papers that were examined cited plant species (in total 522) (Table 2.2, Appendix 1) with properties to treat diseases of the urinary tract and genital-reproductive system.

Treatment of urinary tract diseases included remedies for cystitis, enuresis, gall stones, and various renal troubles from 343 species. A widespread remedy for kidney and urinary issues was a decoction of *Cynodon dactylon* (L.) Pers. rhizomes, which was drunk in the morning on an empty stomach [51] and after 2 h of maceration in water [33]. *Asparagus* sp.pl. (particularly *A. acutifolius* L. and *A. officinalis* L.) were well known for their strong diuretic and depurative action due to their high mineral content [44]. In particular, a decoction of *A. acutifolius* L. shoots or *A.*

officinalis L. roots was employed and *A. tenuifolius* Lam. turions were eaten for their depurative and diuretic effects [56]. The most cited species known to be useful against kidney stones and renal colic were *Ceterach officinarum* Willd. and *Cynodon dactylon* (L.) Pers. In addition, a decoction of the leaves, rhizomes, or whole plant of *C. officinarum* Willd was drunk to expel renal calculi [78] and a decoction of the rhizomes or whole plant of *C. dactylon* (L.) Pers. was taken orally against cystitis and kidney stones [37].

Most of the species (136) identified for healing problems in the genital apparatus act on women's diseases such as menstrual pains (i.e., amenorrhea, dysmenorrhea, irregular menses), delivery problems (i.e., miscarriage, galactagogue or stopping the secretion of milk, uterine bleeding, uterotonic), and hormonal stimulation (i.e., fertility or antifertility effects, estrogenic). The flowers of *Matricaria chamomilla* L. were widely used for menstrual pains as a decoction of fresh flowers [37], infusion of dried flowers [57], or direct application of flowers as a hot unguent [54]. Additionally, the leaves of *Laurus nobilis* L. were useful to treat dysmenorrhea by preparing an infusion with honey [45] or a decoction [37]. Regarding delivery problems, some species were used as partum enhancers: examples include *Rubus idaeus* L. (dried leaf infusion), *Malva sylvestris* L. (bath in leaf infusion), and *Linum usitatissimum* L. (seed oil to drink) [57]. Other species were active in preventing uterine bleeding during or after labor, such as *Punica granatum* L. (decoction of fruit peel), *Capsella bursa-pastoris* (L.) Medik. (infusion of the dried plant), and *Bellis perennis* L. (flower infusion) [40].

Geraci et al. [20], also in accordance with other studies, focused their ethnobotanical survey on several wild plants with galactagogue properties traditionally used by women during breastfeeding to increase milk production. Several species (48) were quoted for this purpose such as the boiled aerial parts of *Borago officinalis* L. [28], the boiled leaves of *Cichorium intybus* L. [43], a seed and leaf decoction of *Foeniculum vulgare* Mill. [37, 73], a seed infusion of *Nigella damascena* L. directly applied on the breast [78], and a leaf infusion [14] or the aerial parts of *Urtica dioica* L. rubbed topically [42].

The use of plants to aid miscarriage in unwanted pregnancies is no longer practiced [37], but in the past, a high dosage of active toxic compounds of some species (31) was used as abortifacients. *Petroselinum crispum* (Mill.) Fuss. was a well-known species with abortive effects, for example, via ingestion of either large doses of raw leaves [14, 55] or an aerial part decoction [30] while a concentrated preparation of the seeds excited uterine muscle fibers, causing miscarriage [40]. Other abortive preparations were a decoction of the aerial parts of *Adiantum*

capillus-veneris L. [41], a flower infusion of *Artemisia absinthium* L. [50], and a vegetative part decoction of *Ruta graveolens* L. [79].

Male genito-urinary diseases treated with plant remedies (from 29 species) mainly include prostate affections. To prevent prostate cancer, a bath of the leaves of *Plantago lanceolata* L. and *P. major* L. [68] and a leaf decoction of *Beta vulgaris* L. [44] were used. To cure prostatitis, a fruit and leaf infusion of *Vaccinium myrtillus* L. [69] and a flower infusion or herbal tea with the aerial parts of *Epilobium angustifolium* L. [14, 24] were employed. *Ficus carica* L. latex of unripe fruits [54] and *Euphorbia helioscopia* L. latex [27, 28] were used as penis vasodilators.

2.3.3.5. Respiratory System Diseases

According to 69 scientific papers, 499 species were used to treat respiratory system ailments (Table 2.2, Appendix 1). The most frequent uses (specifically from 282 species) were against cold, cough, and inflammation (such as laryngitis, bronchitis, and pneumonia). For example, the leaves of *Salvia officinalis* L. [34] and roots of *Foeniculum vulgare* Mill. [43] were used to make a fumigation against cold and cough, in addition to a flower infusion of *Sambucus nigra* L. [56] and a leaf decoction of *Laurus nobilis* L. [39]. Other species were cited for their action against pneumonia and bronchitis, such as *Opuntia ficus-indica* (L.) Mill. (decoction of cladodes) [41] and *Triticum aestivum* L. (bran poultice topically applied on the chest and back) [54].

A typical south Italian remedy is the so-called ricotto, considered a panacea for several respiratory diseases, and consisting of a decoction prepared with several different plant ingredients, boiled in water for 30–60 min, filtered, and cooled down before drinking. This preparation is currently still used for its bechic, expectorant, and anti-asthmatic properties, and the recipe may vary from one person to another. For example, Scherrer et al. [39] provided a recipe composed of *Ficus carica* L., *Prunus spinosa* L., *Pyrus communis* L., *Malus domestica* Borkh., *Prunus armeniaca* L., *Hordeum vulgare* L., *Citrus limon* (L.) Osbeck, *Malva sylvestris* L., *Foeniculum vulgare* Mill., *Cynodon dactylon* (L.) Pers., and *Laurus nobilis* L. as the main ingredients. An alternative is provided by Motti and Motti [37], where the informants mentioned the use of *Plantago lanceolata* L., *Plantago major* L., *Ceratonia siliqua* L., *Prunus dulcis* (Mill.) D.A.Webb., *Citrus reticulata* Blanco, *Clinopodium nepeta* (L.) Kuntze, and *Urtica membranacea* Poir. ex Savigny. Overall, a total of 47 different plant species were cited as the ingredients of ricotto in the considered studies.

A peculiar application of plants against asthma was smoking of the dried leaves of three different species: *Tussilago farfara* L. [31], *Rosmarinus officinalis* L. [51], and *Datura stramonium* L. [44].

2.3.3.6. Nervous System Diseases

A total of 348 species from 70 papers were cited as being beneficial for nervous system diseases (Table 2.2, Appendix 1). These plants were reported to act as sedative and calming agents (including treatment against insomnia, anxiety, and epilepsy), as remedies against depression and headache, and as nervous stimulants.

Papaver rhoeas L. and *P. somniferum* L. were widely used. A decoction of seeds was used as a sleeping drug [36], flower or fruit infusions were administered to children to help against insomnia [32, 87], a tea obtained from petals was used as a sedative [54], a fruit decoction was used against neuralgia [44], and young shoots were added to other recipes to prevent anxious or nervous states [39]. Flowers of *Matricaria chamomilla* L. were also largely used for neurological applications. In particular, a tea made with flower heads was drunk as a tranquilizer and to treat headache [49, 69], a flower infusion was used for its mild sedative and hypnotic properties [5, 83], and an inflorescence decoction and a bath with the stems were used as remedies for irritability [44].

The fruit juice of *Citrus limon* (L.) Osbeck [35], leaf decoction of *Rosmarinus officinalis* L. [73], leaf infusion of *Salvia officinalis* L. [32], tincture made with the flowers of *Melissa officinalis* L. [57], and root decoction of *Valeriana officinalis* L. [45] were preparations reported for the treatment of headache.

Depression and Parkinson's disease were other important neurological affections treated with plant remedies from 12 species. In particular, a flower infusion or tincture of *Hypericum perforatum* L. [24, 56], aerial part infusion of *Leonurus cardiaca* L., and aerial part tincture of *Heracleum sphondylium* L. [89] were used as anti-depressives. In Guarino et al. [40], *Atropa belladonna* L., *Datura stramonium* L., and *Hyoscyamus niger* L. were cited for their use in Parkinson's disease due to their antiepileptic and antispasmodic effects.

2.3.3.7. Cardio-Circulatory System Diseases

In total, 72 references reported the use of 333 species for the treatment of cardio-circulatory system diseases (Table 2.2, Appendix 1). This group includes actions on blood circulation

(haemostatic effects, anti-atherosclerosis, against varicose veins), and blood pressure (against hypertension), and remedies for cardiac troubles.

Various species (16) were used to improve blood circulation. A bandage with a flower infusion of *Achillea millefolium* L. [89] or drinking a leaf infusion of *Fagopyrum esculentum* Moench [57] were known as stimulants of blood circulation in the Lombardia region, a flower infusion of *Crataegus monogyna* Jacq. Was used in Basilicata [27, 28], and drinking fruit juice of *Citrus limon* (L.) Osbeck or eating raw fruits of *Capsicum annuum* L. [37] were remedies adopted in Campania. A bandage and poultice made with some plant organs (e.g., leaves of *Brassica oleracea* L. [59], leaves of *Arctium lappa* L., and fruits of *Aesculus hippocastanum* L. [57]) were also frequently topically applied on varicose veins.

Some plants (9) were also used to prevent severe vascular pathologies such as atherosclerosis, including *Arbutus unedo* L. (fruit decoction) [71] and *Crataegus laevigata* (Poir.) DC. (flower infusion) [89].

Different species (59) were utilized to regulate blood pressure in the case of hypertension such as a leaf infusion of *Olea europaea* L. [32, 75, 86]; chewing of the bulbs, a tincture [45], or raw consumption [30, 37] of *Allium sativum* L. [14]; and a leaf decoction of *Cichorium intybus* L. [51, 75].

With regard to cardiac diseases, folk medicine mainly concerns cardio-tonic substances or remedies against tachycardia. Species with cardiotonic effects include *Crataegus monogyna* Jacq. (flower infusion [56]), *Nerium oleander* L. (very diluted leaf infusion), and *Marrubium vulgare* L. (cataplasm from the boiled aerial parts) [90].

2.3.3.8. Muscular-Skeletal Diseases

In total, 68 papers cited 301 plant species known to be useful against muscular-skeletal pathologies, including remedies for sprains, little fractures and weak bones, backache and stiff-neck, arthritis, rheumatisms, and muscular pains (Table 2.2, Appendix 1).

The majority of citations (161 species) dealt with the treatment of rheumatic pains. Different preparations of *Urtica dioica* L. showed anti-rheumatic properties: a leaf decoction [74], topical use with vigorous rubbing on the skin of the topical part [38], 40 days of drinking an infusion of the aerial parts [33], the leaves applied as a poultice [5], and a root decoction [31]. *Dioscorea communis* (L.) Caddick & Wilkin (mainly a cataplasm or ointment with the fruits) [70, 72],

Rosmarinus officinalis L. (leaf infusion) [33, 57], and *Juniperus communis* L. (berry infusion in grappa or fruit infusion) [49, 54] were also often reported for this kind of treatment.

The number of species reported against arthritis and other articular pains was also relevant (79). To treat arthrosis, topical used was common, for example, the flowers of *Hypericum perforatum* L. in an infusion or macerated in oil [5, 32], marc of *Vitis vinifera* L. [35, 90], a compress made with a bark decoction of *Sambucus nigra* L. [57], crushed leaves of *Dryopteris filix-mas* (L.) Schott. [40], and resin of *Abies alba* Mill. [68]. The same was applied for sprain medications, mainly consisting of a cataplasm, infusion, decoction, or directly application of crushed plants to the affected part. Among the most used plants, the resin of *Picea abies* (L.) H.Karst. or *Abies alba* L. smeared on the interested area [58], the aerial parts of *Centaurea calcitrapa* L. used to make a cataplasm [81], an alcoholic oil maceration of the dried flowers of *Arnica montana* L. used during relieving massages [55], the crushed aerial parts of *Parietaria judaica* L., and a whole plant decoction of *Matricaria chamomilla* L. [44] were identified.

Beneficial effects on backache and stiff-neck were reported after the use of various plants (19), for instance, *Brassica oleracea* L. (roasted leaves applied topically) [30], *Urtica dioica* L. (stems or leaves applied locally) [32], and *Alnus incana* (L.) Moench (compress of dried leaves) [69]. Other preparations (from 18 different species) were used to relieve pain due to sore and swollen legs or feet, such as the use of powdered *Myrtus communis* L. leaves in shoes [25], a cataplasm with the fresh leaves of *Brassica oleracea* L. [34], or soaking feet in a seed decoction of *Sinapis alba* L. [51].

2.3.3.9. Metabolic Diseases

In total, 223 species (listed in 46 papers) were identified as being active in healing metabolic diseases, such as those showing antidiabetic and antigout activities, cytotoxic action against cancer cell proliferation, and mineral and vitamin integrators (Table 2.2, Appendix 1).

Several plants (35) were used for their hypoglycaemic and anti-diabetic activities, including *Salvia officinalis* L. (leaf decoction in wine drunk after meals [39]) and *Urtica dioica* L. (cooked leaves [21] or decoction/infusion of the whole plant [75]). Moreover, a jam of the fresh fruits or a leaf infusion of *Vaccinium myrtillus* L. [59] and the leaves of *Morus nigra* L. and *M. alba* L. [40] were reported to stabilize blood sugar levels in diabetic patients. Gout was treated with the bark of *Sambucus nigra* L. (crushed and applied on painful body parts [40] or as a compress or decoction

[57]). Other remedies against gout were a fresh root juice or seed decoction of *Apium graveolens* L. [62] and a whole plant decoction of *Polygonum aviculare* L. [44].

To regularize cholesterol metabolism, oral preparations were consumed (drunk or eaten), for instance, a leaf decoction from the basal rosette of *Taraxacum campylodes* G.E.Haglund [77], boiled receptacles of *Cynara scolymus* L. [37], fruit juice of *Citrus limon* (L.) Osbeck [41], and fruits of *Atropa bella-donna* L. macerated in water [23].

A very small number of plant remedies (12) were used as a preventive action against cancer and not as a curative action. A bath with the leaves of *Plantago lanceolata* L. and *P. major* L. was used to prevent prostate cancer in addition to a bath with the whole plant of *Potentilla reptans* L. [68]. *Equisetum arvense* L. (wrapping with plant boiling water) [59] and *Colchicum neapolitanum* (Ten.) Ten. [40] were also cited for their antitumor properties.

With regard to vitaminic and remineralizing properties, 26 wild and cultivated plants were added to recipes as a mineral salt supplement. Some examples were the aerial parts of *Urtica dioica* L., which are cooked and added to salads [45]; the seeds of *Prunus dulcis* (Mill.) D.A.Webb., which are very rich in minerals [22]; and the fresh leaves of *Nasturtium officinale* R.Br., which are used as ingredients in salads, soups, and omelettes [56]. Other plants were used to improve the vitamin intake, such as the pulp or juice of *Ribes nigrum* L. fruits [89]; the fresh aerial parts of *Cardamine amara* L. eaten as a snack, used as an ingredient in salads, or cooked [57]; and the raw roots of *Daucus carota* L. eaten as a source of provitamin A [90].

2.3.4. Most Relevant Italian Medicinal Plant Species

For each species considered in the present study (Appendix 1), the citation index (CI, number of citations of the species/75 (total number of analysed papers)) and the use index (UI, number of pathological groups for which the species was used/9 (total number of pathological groups)) were calculated to analyse the collected ethnobotanical information in order to identify the most relevant wild and cultivated Italian plant species used for medicinal purposes. A graphical representation of all 1117 cited species according to their CI and UI (see the detailed data in Appendix 1) is shown in Figure 2.3. The 13 plant species highlighted in green (Figure 2.3) represent those showing both the highest number of citations (CI above 0.6) and the highest number of therapeutic applications (UI above 0.8) distributed in different pathological groups. Interestingly, in all interested Italian regions, all highly cited species were classified as spontaneously growing wild or both wild and cultivated. None of them were only cultivated.

A short summary of the preparation methods and the therapeutic use of each of the most relevant 13 species is shown below (see more details and references in Appendix 1).

Malva sylvestris L. (CI 0.84, UI 1.00). Mallow was reported to be an excellent medicinal plant with many different therapeutical uses. The flowers and leaves were used to make decoctions and infusions useful for diverse skin and mouth inflammations, gastrointestinal diseases, vaginal and urogenital system inflammations, and respiratory ailments. A decoction made with the roots was an excellent remedy to treat many affections such as cough, sore throat, stomachache, toothache, menstrual pain, hypertension, dermatitis, and weakness. In other countries, such as the Iberian Peninsula and Turkey, mallow was also used as an antipyretic [92] and for abdominal pain [93].

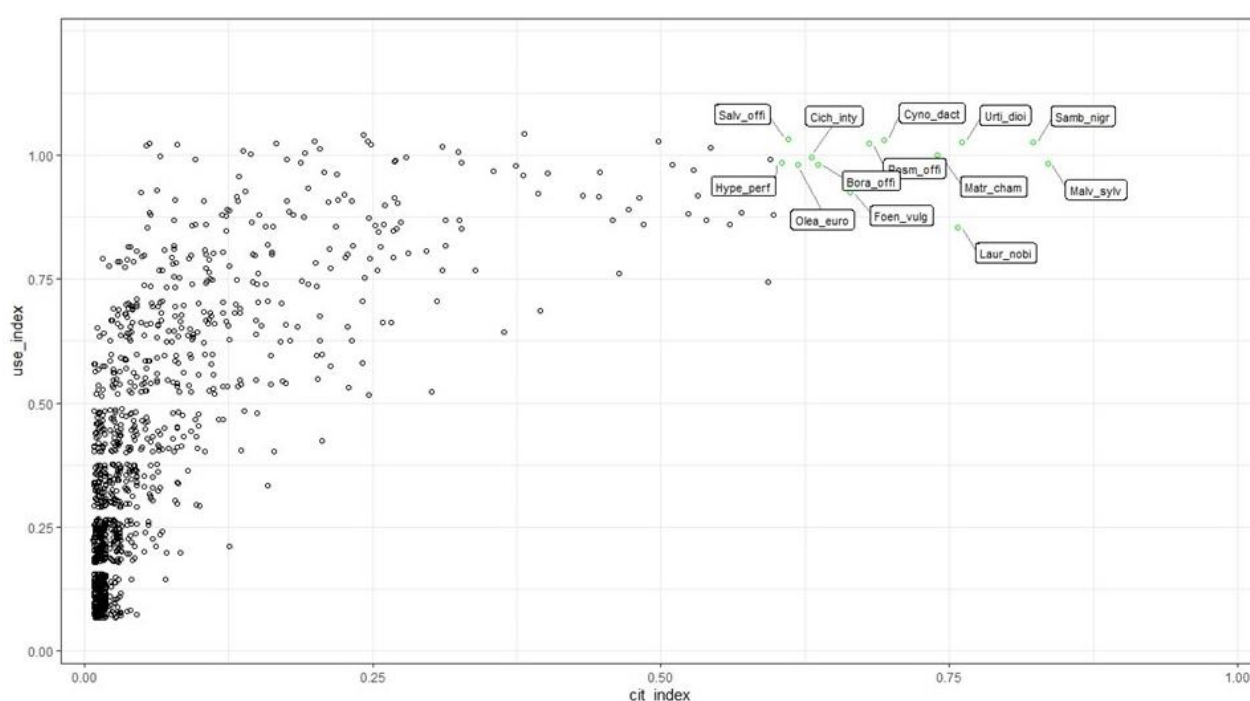


Figure 2.3. Graphic distribution of the cited medicinal species according to the citation (CI) and use (UI) indexes. To avoid overlapping, dots above 0.8 UI were randomly scattered on the y-axis (± 0.1) using the R function *geometry_jitter*. In green, most relevant species with a CI above 0.6 and UI above 0.8 are shown. *Malv_sylv*, *Malva sylvestris* L.; *Samb_nigr*, *Sambucus nigra* L.; *Urti_dioi*, *Urtica dioica* L.; *Matr_cham*, *Matricaria chamomilla* L.; *Cyno_dact*, *Cynodon dactylon* (L.) Pers.; *Rosm_offi*, *Rosmarinus officinalis* L.; *Bora_offi*, *Borago officinalis* L.; *Cich_inty*, *Cichorium intybus* L.; *Olea_euro*, *Olea europaea* L.; *Hype_perf*, *Hypericum perforatum* L.; *Salv_offi*, *Salvia officinalis* L.; *Laur_nobi*, *Laurus nobilis* L.; *Foen_vulg*, *Foeniculum vulgare* Mill.

Sambucus nigra L. (CI 0.82, UI 1.00). Many different preparations made with the flowers and bark were used against a wide range of affections. A decoction, infusion, or syrup made with the

flowers were drunk to treat cough, cold, sore throat, bronchitis, fever, headache, toothache, hypertension, and abdominal pain. A cataplasm, oleolite, decoction, infusion, or boiled flowers were topically applied on bruises, rheumatisms, dislocated bones, eye irritation (infusion as a collyrium), and metabolic and skin diseases (i.e., gout, skin redness, hematomas, wounds). The bark also has other applications: topically on burns and sores as a lenitive, to cure arthritis, as a laxative and diuretic, and used as a systemic anti-inflammatory. The same uses were also reported in many other Mediterranean countries such as Greece [94], Spain [92], and Portugal [95].

Urtica dioica L. (CI 0.76, UI 1.00). Nettle was another widely recognized medicinal plant. The leaves were used both internally as an expectorant, diuretic, depurative, digestive, and galactagogue and to treat anaemia, cold, and cough; and externally to treat dandruff and hair loss, dermatitis, painful joints, rheumatisms, chilblains, and wounds. The roots were used internally as an antirheumatic, to treat abdominal pain and colitis, and to fight gastric and duodenal ulcers. The aerial parts were used for liver disorders, intestinal inflammation, water retention, and to treat epistaxis and toothache. These Italian methods of preparation are in agreement with those cited, among others, in Spain, Serbia, and Morocco [92, 96, 97].

Matricaria chamomilla L. (CI 0.74, UI 1.00). The flower heads of chamomile were widely used to treat cold and respiratory system diseases, digestive system disorders, and neurological problems (against irritability and to promote sleep). It was also used against menstrual and muscular pains and as a collyrium for tired eyes and conjunctivitis. Similar applications have also been identified worldwide (e.g., Mexico, Serbia, Bulgaria, and Pakistan [96, 98–100]).

Cynodon dactylon (L.) Pers. (CI 0.69, UI 1.00). The roots and rhizomes were used for a wide variety of diseases (also as an ingredient of a ricotto decoction), including cystitis, hepatitis, renal stones, cough and flu, fever, rheumatisms, dysmenorrhea, abscesses, and prostatitis. Conversely, an extract of the whole plant was used both orally and topically against respiratory disorders and skin diseases in Pakistan [98] and India [101].

Rosmarinus officinalis L. (CI 0.68, UI 1.00). Rosemary is a typical Mediterranean plant with similar therapeutic applications in many worldwide countries [92, 94, 102]. The aerial parts, mainly the leaves, had many phytotherapeutic uses, including a local analgesic, anti-septic, sedative, spasmolytic, anti-pyretic, and energetic, and to treat cough, constipation, bronchitis, and asthma. It was an ingredient of the Italian ricotto decoction.

Borago officinalis L. (CI 0.64, UI 1.00). Borage has been widely reported in ethnobotanical research for its diuretic, emollient, expectorant, galactagogue, diaphoretic, and anti-inflammatory properties. Among the various affections treated with borage preparations, some examples are cold, cough, lung diseases, stomachache, intestinal regulation, abdominal pains, reddened skin, pimples, eczema, burns, toothache, and gout (topical use). Its use is also reported in other countries, such as in Algeria [103] to treat depression and other neurological disorders, and in the Spanish Balearic Islands [104] as an antipyretic and anticatarrh.

Cichorium intybus L. (CI 0.64, UI 1.00). The leaves and aerial parts were used mostly for their diuretic, laxative, and depurative properties, in accordance with other countries' reports [2, 98], and the roots were used to protect and purify the liver, to treat hypertension, and as an anti-acne agent.

Olea europaea L. (CI 0.62, UI 1.00). Preparations with the leaves and fruit oil of the olive tree were used for a wide range of affections. A leaf decoction was used both internally, drunk as a diuretic, hypotensive, and febrifuge and to treat gastrointestinal disorders, and externally to heal gout, wounds, and other dermatological problems. The fruit oil was used for its antiseptic, anti-inflammatory, and vulnerary properties against otitis, burns, haemorrhoids, sun rash, acne, rheumatisms, and sprains. When the oil is drunk, it has laxative and hypotensive properties. The leaves were widely used in Turkey as a herbal tea infusion to reduce blood pressure and diabetes or were chewed to heal oral wounds [105].

Hypericum perforatum L. (CI 0.61, UI 1.00). Hypericum flowers were widely used in phytotherapy, not only in Italy but all over the world [93, 96, 100, 101]. An infusion to aid digestion, as a cholagogue and anti-depressant, to treat constipation and sleep disorders, with relaxing properties is used. A maceration in olive oil is applied to burns, sores, skin rash, wounds, contusions, and erythema. A decoction (also an ingredient of ricotta) was drunk to treat gastrointestinal and hepatic colic as a digestive and blood depurative. Moreover, the leaves and other aerial parts were used for menstrual pains, arthrosis, joint pain, and rheumatisms.

Salvia officinalis L. (CI 0.61, UI 1.00). The plant organs mostly utilized were the leaves, which have many therapeutical uses, including toothache and other mouth problems, stomachache, respiratory ailments, headache, and febrifuge. In some North African states, this plant was also used as an appetizer, anti-diarrheic, and anti-diabetic [103, 106].

Laurus nobilis L. (CI 0.76, UI 0.89). In addition to being used in food recipes, laurel is also a medicinal plant widely present in Italian popular medicine and worldwide [93, 103, 105]. Most

of the preparations for curative purposes utilized a laurel leaf decoction or infusion for external use (rheumatisms, arthritis, insect bites, contusions) and internal use (gastrointestinal pains, abdominal colic, cold, cough, stimulant of blood circulation, menstrual pain, and galactagogue action).

Foeniculum vulgare Mill. (CI 0.66, UI 0.89). Fennel was widely consumed in cooking and largely used in folk medicine. It was used for gastrointestinal disorders (poor digestion, aerophagia, stomachache, colic, gastric acidity, etc.), respiratory issues (sore throat, cold, cough, bronchitis), female ailments (galactagogue and emmenagogue action, to fight dysmenorrhea), and irritability and swelling and as an anti-pyretic, anti-rheumatic, and detoxifier. Similar therapeutic applications were also reported in many other countries [95, 107].

2.4. Conclusions and perspectives

The purpose of the present study was to, for the first time, collect and organize a standardized dataset containing detailed information available from the literature about Italian folk medicinal remedies for human health produced using wild and cultivated plants. Based on the large amount of literature data collected from 75 analysed scientific papers, the present study confirmed that traditional medicine was widely diffused in the past all along the peninsula and it is still practiced in many territories by people involved and not involved in agricultural practices. The most cited species (citation index close to 1) were diffused and used in many Italian regions while others had only local application. Thirteen plant species were identified as being the most relevant in Italy for medicinal use: *Malva sylvestris* L., *Sambucus nigra* L., *Urtica dioica* L., *Matricaria chamomilla* L., *Cynodon dactylon* (L.) Pers., *Rosmarinus officinalis* L., *Borago officinalis* L., *Cichorium intybus* L., *Olea europaea* L., *Hypericum perforatum* L., *Salvia officinalis* L., *Laurus nobilis* L., and *Foeniculum vulgare* Mill.

A wide range of phytotherapeutic uses were recorded, mainly concerning disorders of the digestive system, skin-ears-eyes-hair, systemic system, and genito-urinary system. In general, the most common traditional medicinal remedies were prepared by simple and easy methods, such as an infusion or decoction of the plant parts, followed by ingestion or direct external topic application. Most of the identified plants with medicinal applications were also used as food ingredients [9], therefore proving the tight relation between daily diet and human health present in the traditional folk knowledge of the different Italian regions.

This review, by collecting and organizing the knowledge of Italian wild and cultivated medicinal flora, is, therefore, a starting point for further specific bioactivity and bioprospecting studies aimed at the formulation of therapeutic drugs that are more environmentally sustainable and respectful of plant biodiversity.

Author Contributions

Conceptualization, S.M. and A.T.; Methodology, S.M.; Formal Analysis, M.S.; Investigation, S.M.; Writing—Original Draft Preparation, S.M.; Writing—Review and Editing, M.F. and A.T.; Supervision, A.T. All authors have read and agreed to the published version of the manuscript.

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Chapter 3

New Insights on Primary and Secondary Metabolite Contents of Seven Italian Wild Food Plants with Medicinal Applications: A Comparative Study

Published as: Monari, S.; Ferri, M.; Salinitro, M.; Tassoni, A. (2023). New Insights on Primary and Secondary Metabolite Contents of Seven Italian Wild Food Plants with Medicinal Applications: A Comparative Study. *Plants*, 12, 3180. <https://doi.org/10.3390/plants12183180>

Abstract

Wild food plants are widely consumed all over the world and many have both nutritional and therapeutic value due to the presence of biologically active compounds. The present research, for the first time, aims to compare primary and secondary metabolite levels among different plant organs (flower, leaf, stem, root, bark) of seven species (*Borago officinalis* L., *Cynodon dactylon* (L.) Pers., *Foeniculum vulgare* Mill., *Hypericum perforatum* L., *Malva sylvestris* L., *Sambucus nigra* L., *Urtica dioica* L.) collected in three different Italian regions (Liguria, Tuscany, Apulia). Plant organ samples were extracted with water or 95% (v/v) methanol and liquid fractions were analysed using spectrophotometric assays. The best results were obtained for *Hypericum perforatum* L., followed by *Sambucus nigra* L. and *Borago officinalis* L. As also confirmed via PCA analysis on normalized data, flower and leaf extracts of all species exhibited higher levels of polyphenols (up to 105.7 mg GA eq/gDW), reducing sugars (up to 389.2 mg GLU eq/gDW), proteins (up to 675.7 mg BSA eq/gDW) and of antioxidant capacity (up to 263.5 mg AA eq/gDW). No differences among the regions of gathering were detected after spectrophotometric assays, which was confirmed via PCA analysis. These data contribute to further validate the traditionally reported healing effects of these species on human health.

3.1. Introduction

Wild food plants are native species which grow spontaneously in the environment without being cultivated [1]. These edible species have been widely consumed all over the world and constitute a substantial part of the human diet [2]. In the past, their importance increased during famine periods, but after industrialization from 1950 onward, this kind of traditional knowledge has been progressively disappearing [3]. In the last few decades, these plant species have been revalued and are receiving considerable increasing attention in general by ethnobotanists and nutritionists [4–6].

Nowadays, wild plants are used in many food recipes, feed, production of drugs (for human and veterinary medicine), household items and ritual uses [7]. Focusing on human health applications, several ethnobotanical papers have recorded the worldwide use of spontaneous edible plants for medicinal purposes [6, 8]. Due to the presence of several biologically active compounds [9], these species have been reported to have both nutritional and therapeutic value, and are therefore being considered as food–medicines. Plants are in fact rich in a wide variety of phytochemicals [10], and herbal preparations for medicinal usage contain various types of primary and secondary metabolites, such as, among others, sugars, phenolic acids, flavonoids, tannins, proteins [11]. Among secondary metabolites, polyphenols have been proven to have biological activities in humans like antioxidant, anti-inflammatory and antimicrobial capacities [12–14], and properties against chronic or degenerative diseases [15], while primary metabolites, such as sugars and proteins, show a considerable nutritional value [16]. Unrefined sugars show higher nutritional quality and antioxidant capacity with respect to refined ones and are composed in large amounts of reducing sugars (e.g., glucose and fructose) [17] that have beneficial effects, especially when combined with secondary metabolites such as polyphenols and flavonoids.

The synthesis and accumulation of different plant metabolites not only depends on the species but also on the environmental conditions in which the plant itself is growing. External factors such as light, temperature, moisture, and soil characteristics can affect the phytochemical profile of a plant organism [18]. A comprehensive plant metabolomic analysis needs to consider the habitat, and a comparison of the effects of different environmental factors is mandatory. Over the last few years, several phytochemical studies were published on Italian wild edible plants with therapeutic uses [6]. These studies reported data on specific plants in a restricted sampling area [19], on two or more species gathered in various locations of the same region [20], or

cultivated plants [21]. To the best of our knowledge, the present paper is the first comparing different organs of diverse wild edible/medicinal plants across different Italian regions.

Recently, the Italian ethnobotanical studies published in international journals between 1980 and March 2022 and dealing with wild and cultivated food plants known to have therapeutical properties were reviewed [6]. The results highlighted the presence of 13 wild and/or cultivated plants widespread all over the Italian peninsula and showing a high number of citations and of different medicinal uses. Among them, seven wild plant species (*Borago officinalis* L., *Cynodon dactylon* (L.) Pers., *Foeniculum vulgare* Mill., *Hypericum perforatum* L., *Malva sylvestris* L., *Sambucus nigra* L., *Urtica dioica* L.), were selected for the present comparative study given the fact that they were all spontaneously growing wild and present in the similar sampling areas all along the Italian peninsula. For all of them, both food and therapeutic characteristics have been previously reviewed [1, 6, 22].

B. officinalis is traditionally used as a food in salads, in soup (alone or with beans or with mixed vegetables), in pancakes and in pies [3, 23]. However, it was also widely reported in ethnobotanical research for its diuretic, emollient, expectorant, diaphoretic, anti-inflammatory and liver wellbeing properties, and its properties against several digestive system diseases, mainly via ingestion of raw and boiled leaves and cooking water, or by topical use [6, 23].

C. dactylon is used as substitute for coffee [24, 25] or tea [26], and its raw roots are eaten raw in salad [22]. Roots and rhizomes are also used in decoction form for a wide variety of diseases (e.g., cystitis, hepatitis, renal stones, cough, flu, fever, rheumatisms, dysmenorrhea, abscesses, prostatitis), and the whole plant extract is used both orally and topically against respiratory disorders and skin diseases [6].

F. vulgare is widely consumed in cooking and largely used in folk medicine. It is eaten raw or cooked, alone or in mixed vegetable salad or soups, and is commonly used as flavouring (e.g., seeds to aromatize salami giving them a recognizable taste), and in liqueur production [3, 23]. Fennel is also used for gastrointestinal disorders, respiratory issues, female ailments and as an anti-pyretic, antirheumatic and detoxifier [6, 23].

H. perforatum finds culinary applications mainly in liqueur production (i.e., flowers for grappa flavouring) or as herbal tea and food supplement [22, 27, 28]. Many more applications are found for *Hypericum* as a medicinal plant. Its flowers are widely used in phytotherapy all over the world for a wide range of diseases, as an infusion (e.g., to aid digestion, as an antidepressant, to treat constipation and sleep disorders), macerated in olive oil (e.g., applied to burns, sores, skin rash,

wounds, contusions, and erythema) or as decoction (e.g., against gastrointestinal and hepatic colic and as a digestive and blood depurative) [6]. Leaves and other aerial parts are used for menstrual pains, arthrosis, joint pain and rheumatisms [6].

M. sylvestris is used in several traditional pasta food recipes [22, 29], eaten with other boiled vegetables in a salad or in a soup [4], or utilized as a herbal tea [30]. Mallow is also reported to have many different therapeutical uses. Flower and leaf decoctions and infusions are useful for skin and mouth inflammations, gastrointestinal diseases, vaginal and urogenital system inflammations and respiratory ailments, while root decoction is a remedy for cough, sore throat, stomach ache, toothache, menstrual pain, hypertension, dermatitis and weakness [6].

S. nigra flowers and, to a lesser extent, fruits are diffused in omelette, pancake, jam and liqueur recipes [3, 23]. Sometimes, the same food preparations are used as folk medicine, such as jam reported as an antirheumatic remedy in the North of Italy [3]. Moreover, several different preparations (decoction, infusion, syrup, cataplasm) made with *S. nigra* flowers and bark are used against a wide range of respiratory, digestive, metabolic affections and skin diseases [6].

U. dioica is one of the most consumed wild species, both as food and medicine, being much more valued today than in the past. As food, it is eaten in mixed vegetable soups and salads, omelettes, is used to make green pasta or to fill and season hand-made pasta [3, 23]. As medicine, nettle is used both internally after ingestion (e.g., as expectorant, diuretic, depurative, digestive and to treat anaemia, cold and coughs) and externally topically (e.g., to treat dandruff and hair loss, dermatitis, painful joints, rheumatisms, chilblains, wounds) [6].

In the present research, the seven targeted wild plant species were collected in three different Italian regions (Liguria, Tuscany and Apulia) and selected organs were characterized for their primary and secondary metabolite levels and for antioxidant capacity. The main objectives of the present research were (i) to perform a preliminary biochemical screening by quantifying total polyphenols, reducing sugars and proteins and antioxidant activity, and to validate results by means of PCA analysis; (ii) to investigate the variability of the targeted compounds into the different plant organs traditionally consumed and/or used as medicinal remedies; (iii) to assess, within the same plant species and organs, possible differences linked to the gathering regions.

3.2. Materials and Methods

3.2.1. Study Areas

Three Italian regions were selected on the basis of their geographical North–South and East–West positioning in the Italian peninsula: Liguria (northeast), Tuscany (Eastcentral), Apulia (southwest). For each region, a search on Wikipantbase #Italia website (<http://bot.biologia.unipi.it/wpb>, accessed on 5 March 2021) was performed in order to choose a collection area as small as possible in which all the seven species could be located together. Specifically, the areas of Riomaggiore 44°06′01.6″ N, 9°44′05.7″ E (Liguria), Monte Ferrato 43°55′23.3″ N, 11°05′04.7″ E (Tuscany) and Gargano 41°51′39.2″ N, 15°21′03.4″00 E (Apulia) were selected (Figure 3.1).

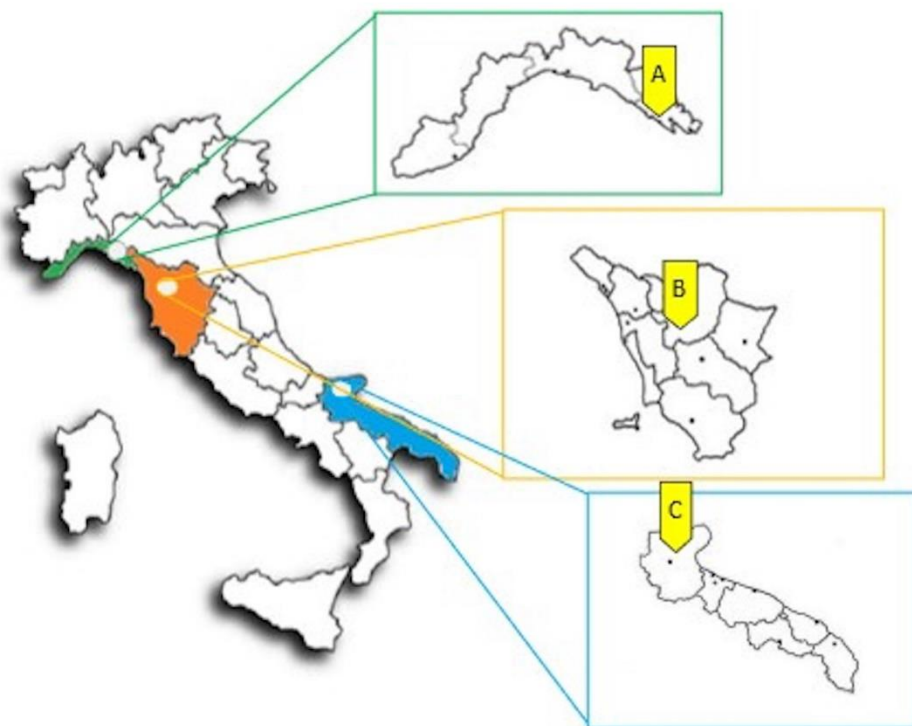


Figure 3.1. Map of the study areas. (A): Riomaggiore (Liguria region); (B): Monte Ferrato (Tuscany region); (C): Gargano (Apulia region).

3.2.2. Plant Material

In a previous paper, a complete database of Italian wild and/or cultivated edible plants also showing medicinal effects was created [6]. Results pointed out 13 wild and/or cultivated plant species being highly cited (citation index) and with a high number of reported medicinal uses

(use index). Based on the previous findings, in the present study, only the wild species were selected and specifically: *Borago officinalis* L. (fam. *Boraginaceae*), *Cynodon dactylon* (L.) Pers. (fam. *Poaceae*), *Foeniculum vulgare* Mill. (fam. *Apiaceae*), *Hypericum perforatum* L. (fam. *Hypericaceae*), *Malva sylvestris* L. (fam. *Malvaceae*), *Sambucus nigra* L. (fam. *Viburnaceae*), *Urtica dioica* L. (fam. *Urticaceae*).

Sampling was carried out, by following the geographic coordinates provided by Wikipantbase #Italia (<http://bot.biologia.unipi.it/wpb>, accessed on 5 March 2021), between April and May 2021. The plants were collected in all the regions at the following phenological stages: *B. officinalis*, *H. perforatum*, *S. nigra* and *M. sylvestris* at advanced flowering; *C. dactylon* at vegetative stage with blossom starting to appear; *F. vulgare* at vegetative phase (stem elongation 40–60 cm), *U. dioica* at vegetative phase (stem elongation 90–110 cm). Different individual plants (5–6) for each species in each sampling area were gathered and pooled together in order to have the same homogeneous starting sample for the following analysis. The collected plants were stored in water to preserve their conditions during travel back to laboratory, and immediately washed and separated into different organs (flowers, leaves, stems, roots, and when present bark, Figure 3.2). Each sample was frozen in liquid nitrogen, lyophilized and grinded with a blender, and the dry powder was stored at room temperature until further analysis.

3.2.3. Sample Extraction

For each organ, three biological replicates (0.2 g DW) were extracted with 100% (v/v) MilliQ water at 40°C, 1 h shaking, with a S/L of 1:40, and with 95% (v/v) methanol at room temperature, 5 min shaking, with a solid/liquid ratio (S/L) of 1:20 [31]. The resulting extracts were then centrifuged at 5000 rpm for 10 min at 20°C to separate the solid and liquid fractions. The latter were stored at – 20°C until spectrophotometric analyses.

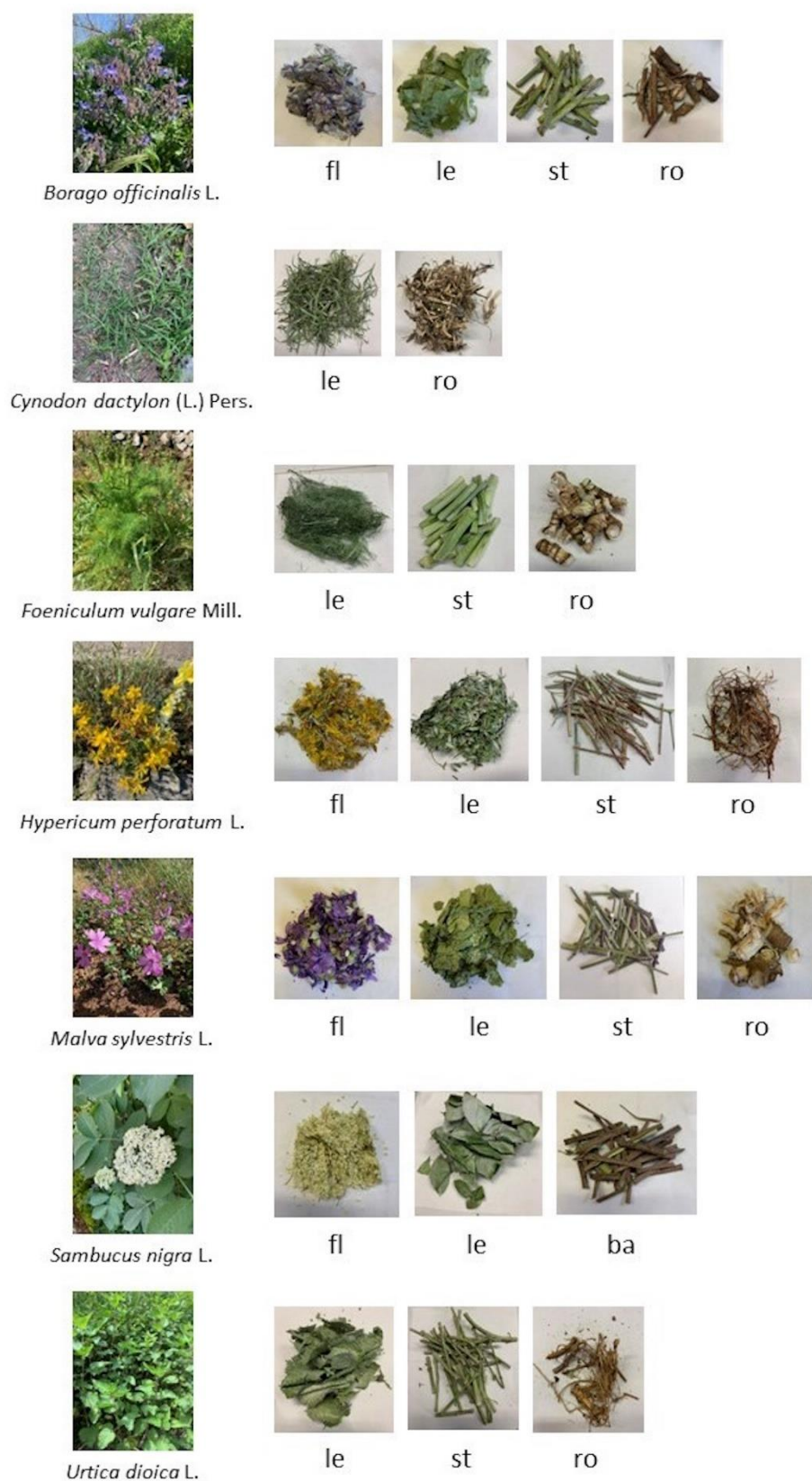


Figure 3.2. Selected species and different organs analysed. Fl: flowers; le: leaves; st: stems; ro: roots; ba: bark (Photos by S. Monari).

3.2.4. Spectrophotometric Analyses

Liquid aqueous and methanol extracts were analysed by means of different spectrophotometric assays to assess the total amount of polyphenols, reducing sugars and proteins and antioxidant activity. Total polyphenol content was determined using the Folin–Ciocalteu method [32]. Results were expressed as mg of gallic acid (GA) equivalent per g of dry weight (mg GA eq/gDW) by means of a dose–response calibration curve (between 0 and 15 µg of GA). Total reducing sugar content was measured with the 3,5-dinitrosalicylic acid (DNS) method [33]. Results were expressed as mg of D-glucose (GLU) equivalent per g of dry weight (mg GLU eq/gDW) by means of a dose–response calibration curve (between 50 and 500 µg of GLU). Total protein content was determined as described by Lowry et al. [34]. Results were expressed as mg of bovine serum albumin (BSA) equivalent per g of dry weight (mg BSA eq/gDW) by means of a dose–response calibration curve (between 0 and 200 µg of BSA). Antioxidant activity was assessed using the ABTS (2,20-azino-di-(3-ethylbenzthiazoline sulfonic acid)) method [5, 35]. Results were expressed as mg of ascorbic acid (AA) equivalent per g of dry weight (mg AA eq/gDW) by means of a dose–response calibration curve (between 0 and 2 µg of AA).

3.2.5. Statistical Analyses

All assay procedures were performed on three biological replicates, each of which were analysed in four technical replicates. The results were expressed as the mean ($n = 3$) $\pm$ SD per g DW (Figures 3.1 and S3.2). Data were tested for normality using the Shapiro–Wilk test, and for homogeneity of variance using the Levene’s test with default parameters from the package *car* (<https://CRAN.R-project.org/package=car>, accessed on 3 February 2023) [36]. Differences in polyphenol, reducing sugar and protein contents, and in antioxidant activity, were evaluated among plant organs and gathering regions (within the same species). When data were parametric, one-way ANOVA followed by a Tukey HSD test ($p < 0.05$) was used, while when data were not parametric, the Kruskal–Wallis test followed by the Dunn test was applied ($p < 0.05$). Principal Component Analysis (PCA) was performed to highlight the differences among species using original values for polyphenols, reducing sugars, proteins and antioxidant activity (Figures 3.5 and Appendix 2 at the end of the thesis). Normalized values (Appendix 2) with respect to the average of the species in each region were calculated as follows and used for PCA analysis (Figures 3.2 and S3.3) to highlight differences between plant organs:

$$\text{Poly_Norm}_{\text{sample } A, \text{ sp1, r1}} = \frac{0.5 \cdot \text{Poly}_{\text{sample } A, \text{ sp1, r1}}}{\text{average Poly}_{\text{sp1, r1}}}$$

$$\text{RedSug_Norm}_{\text{sample } A, \text{ sp1, r1}} = \frac{0.5 \cdot \text{RedSug}_{\text{sample } A, \text{ sp1, r1}}}{\text{average RedSug}_{\text{sp1, r1}}}$$

$$\text{Prot_Norm}_{\text{sample } A, \text{ sp1, r1}} = \frac{0.5 \cdot \text{Prot}_{\text{sample } A, \text{ sp1, r1}}}{\text{average Prot}_{\text{sp1, r1}}}$$

$$\text{Antiox_Norm}_{\text{sample } A, \text{ sp1, r1}} = \frac{0.5 \cdot \text{Antiox}_{\text{sample } A, \text{ sp1, r1}}}{\text{average Antiox}_{\text{sp1, r1}}}$$

Abbreviations: Poly_Norm: Normalized polyphenols; RedSug_Norm: Normalized reducing sugars; Prot_Norm: Normalized proteins; Antiox_Norm: Normalized antioxidant activity; sp: species; r: region.

All statistical analyses were performed using R software version 3.5.1 (R Core Team, Vienna, Austria).

3.3. Results and Discussion

The biochemical characterization of the different organs of the selected species was performed with specific spectrophotometric assays (Figures 3.3 and 3.4). Since these wild food plants are mainly used as herbal teas (like infusion or decoction) for medicinal purposes [6], the analysed aqueous extracts here represent the samples closer to the actual way of usage. Additionally, methanol extracts were obtained and analysed (Supplementary Materials, Figures S3.1–S3.3, Appendix 2). This paper reports for the first time the levels of several bioactive compound classes in different organs of seven wild edible/medicinal plants collected across three different Italian regions.

All data on water extracts after metabolite spectrophotometric analysis were elaborated with Principal Component Analysis (PCA) (Figure 3.3) demonstrating that *H. perforatum* and *S. nigra* were the most active species and had the best total polyphenols, proteins and antioxidant activity results, while *F. vulgare* and *B. officinalis* stems showed the highest reducing sugar values. The same PCA approach was adopted on methanolic sample data (Figure S3.1), and similar result clusters were highlighted. No differences among samples of the three regions were highlighted.

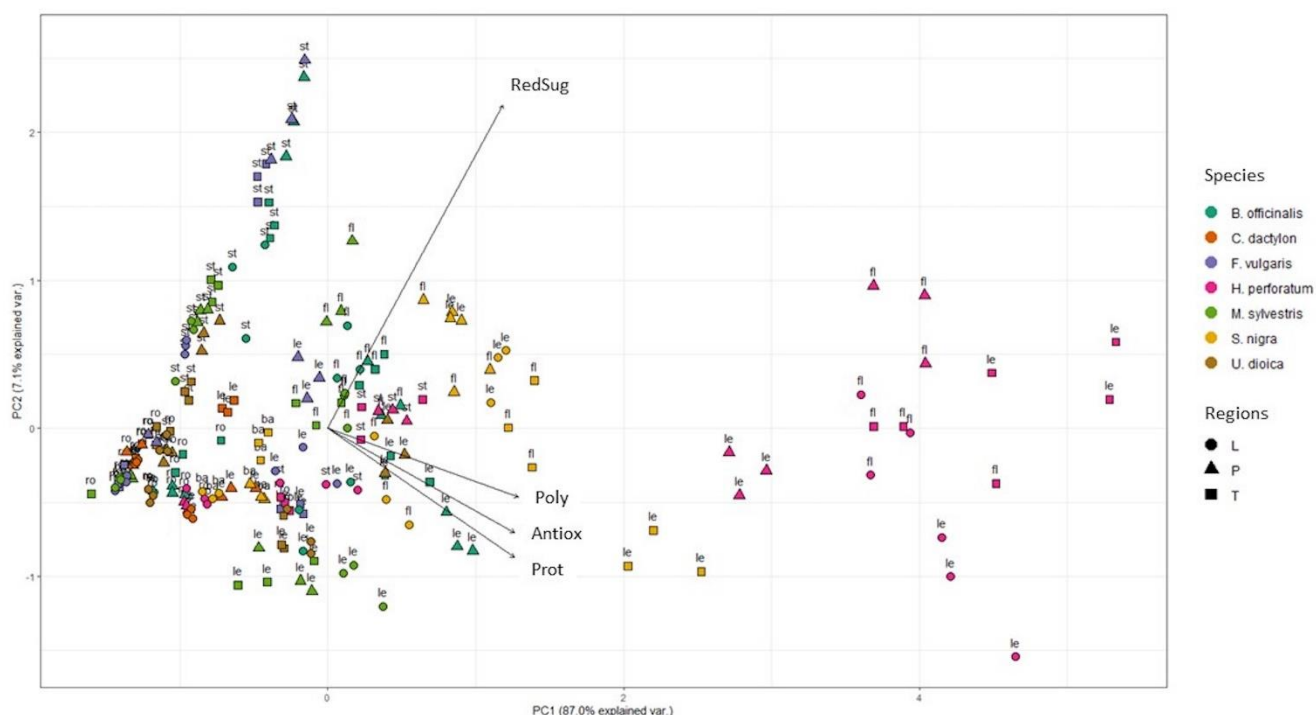


Figure 3.3. PCA analysis of spectrophotometric results on water extracts, showing the grouping of species according to total polyphenols, reducing sugars, proteins and antioxidant activity data (Figure 3.4). L: Liguria; P: Apulia; T: Tuscany; fl: flowers; le: leaves; st: stems; ro: roots; ba: bark; RedSug: reducing sugars; Poly: polyphenols; Antiox: antioxidant activity; Prot: proteins.

3.3.1. Total Amount of Polyphenols

The total amount of polyphenols was found to be significantly higher in aqueous extracts of flowers and leaves of all analysed plant species with respect to stems, roots and bark (Figure 3.4a). Similar data were observed in methanolic extracts (Figure S3.2a). This trend was clearly pointed out via PCA analysis on normalized values (Appendix 2), which were used to smooth differences among species and to highlight those among organs (Figures 3.5 and S3.3). After data normalization, in fact, flowers and leaves of all the plant species were clustered together at positive PC1 values.

Samples were similarly grouped when considering aqueous (Figure 3.3) and methanolic (Figure S3.3) extracts. No general differences could be clearly evidenced among similar samples of the three gathering regions. Samples were similarly grouped when considering aqueous (Figure 3.5) and methanolic (Figure S3.3) extracts. No general differences could be clearly evidenced among similar samples of the three gathering regions.

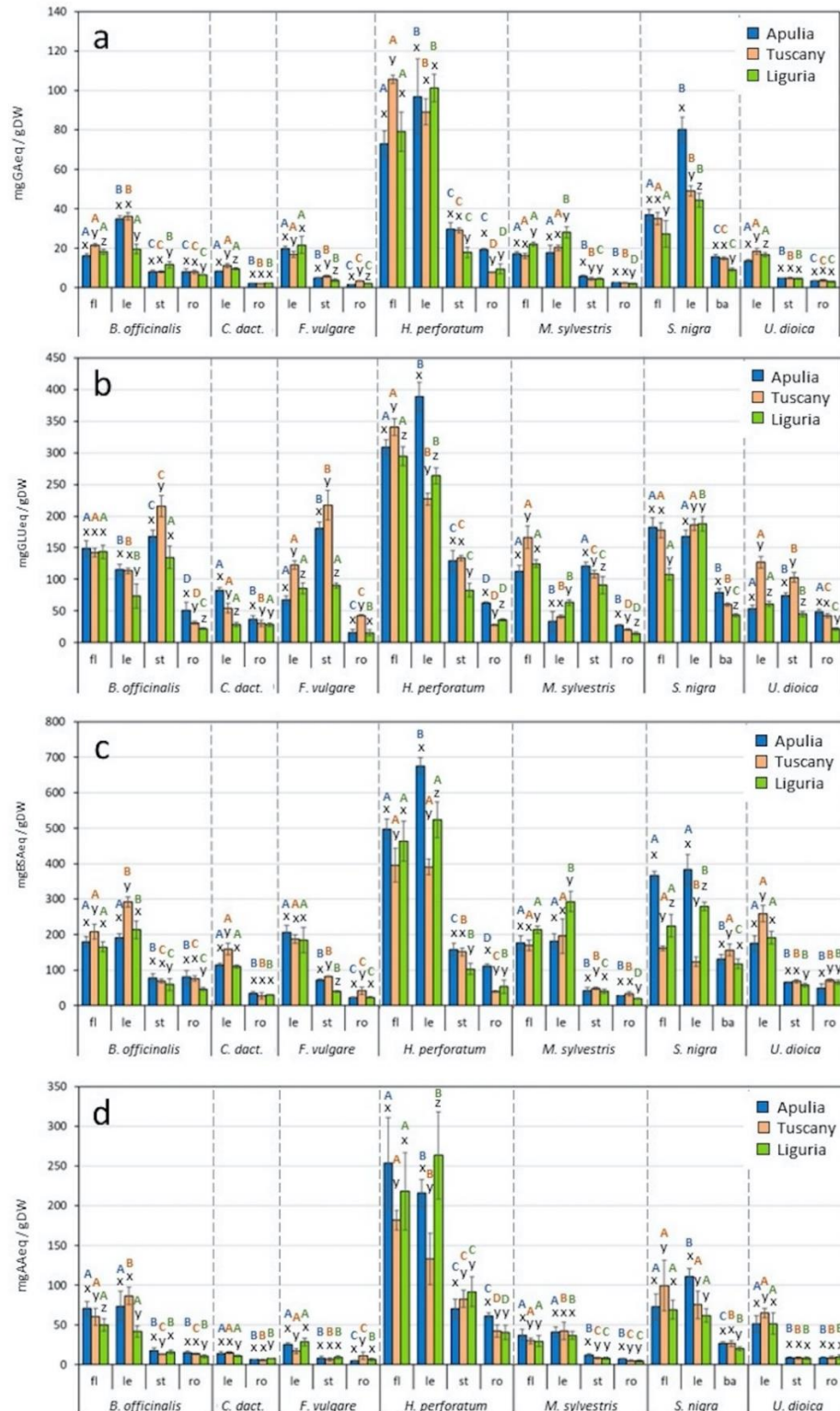


Figure 3.4. Total amounts of polyphenols (a), reducing sugars (b), proteins (c) and antioxidant activity (d) of aqueous extracts after spectrophotometric analysis. fl: flowers; le: leaves; st: stems; ro: roots; ba: bark; GA: gallic acid, GLU: D-glucose, BSA: bovine serum albumin, AA: ascorbic acid, eq: equivalent, DW: dry weight. Different letters indicate a statistically significant difference (one-way ANOVA followed by post hoc Tukey HSD test or Kruskal–Wallis followed by Dunn test, $p < 0.05$) among the regions (x, y, z) and among the organs of each species (A, B, C, D). Data are the mean $\pm$ SD ($n = 3$).

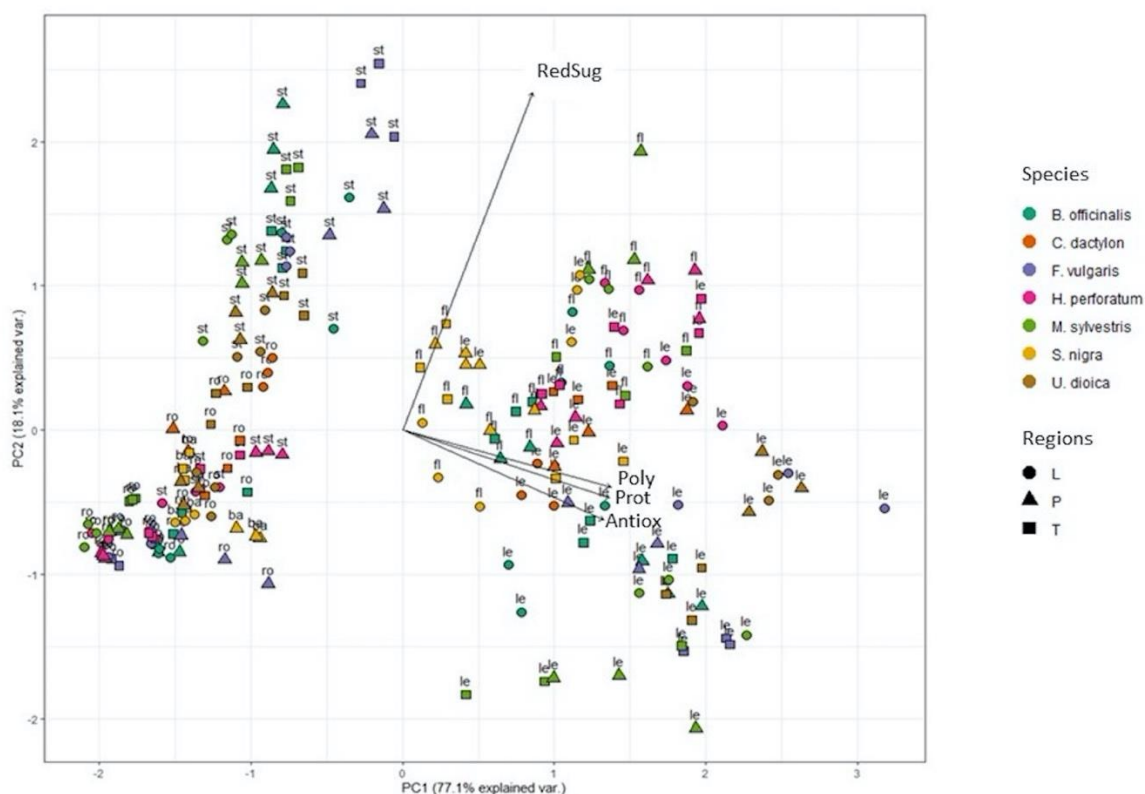


Figure 3.5. PCA analysis of aqueous extracts spectrophotometric results (Appendix 2) after normalization (see Section 3.2.5) showing the grouping of organs according to the concentrations of reducing sugars, polyphenols, proteins and antioxidant activity. L: Liguria; P: Apulia; T: Tuscany; fl: flowers; le: leaves; st: stems; ro: roots; ba: bark; RedSug: reducing sugars; Poly: polyphenols; Antiox: antioxidant activity; Prot: proteins.

Overall, aqueous extracts of *H. perforatum* L. showed the highest total polyphenol content with an average of 85.9 mg GA eq/gDW in flowers and of 95.9 mg GA eq/gDW in leaves. The data were not significantly different among regions (Figure 3.3) except for flowers from Tuscany (105.7 mg GA eq/gDW, $p < 0.01$) (Figure 3.4a). This species is extensively used as medicinal plant and is already known to have high polyphenol contents [37]. Reported results were in agreement with those of Sekeroglu et al. [35], who observed higher polyphenol concentrations in *H. perforatum* flowers and leaves with respect to stem aqueous extracts. According to the literature, in *H. perforatum* the most abundant phenolic compounds showing biological activity are flavonoids, such as quercetin, rutin, kaempferol and naringenin, and phenolic acids, like chlorogenic, gallic, caffeic, ferulic and vanillic acids [38, 39].

S. nigra L. leaves scored the second-best total polyphenol content, with Apulia samples (80.3 mg GA eq/gDW) showing almost double the level of Tuscany and Liguria (49.1 and 44.6 mg GA

eq/gDW, respectively), and with an average 1.7-fold significantly higher content with respect to flowers (on average 33.1 mg GA eq/gDW, $p < 0.01$) (Figure 3.4a). The present results seem to be totally comparable to those reported by Viapiana and Wesolowski [40] that detected a maximum 35.57 mg GA eq/gDW total amount of polyphenols in water infusions of *S. nigra* flowers.

Polyphenol levels of leaves and flower water extracts of *B. officinalis* L. (on average 30.1 and 18.7 mg GA eq/gDW, respectively) were seven-fold higher than those obtained by Karimi et al. [41] on leaves of the same species. Antioxidant and anti-inflammatory activities of *S. nigra* L. have been related to the presence of anthocyanins, phenolic acids and flavonoids (such as gallic acid, rutin and quercetin) [40, 42], while in *B. officinalis* L. to syringic and rosmarinic acids, myricetin, rutin and kaempferol [43, 44].

The results obtained from methanolic extracts (Figure S3.2a) showed the same trend detected in water extracts (Figure 3.4a) with slightly higher extraction efficiency given to the use of methanol organic solvent, as well documented in the literature [45]. In particular, after methanol extraction, polyphenol concentrations in flowers and leaves of *H. perforatum* and *S. nigra* were three to four-fold more concentrated respect to the aerial parts of methanolic extracts of some other wild plants (e.g., *Hypericum montbretii* Spach., *H. bupleuroides* Griseb., *Apium graveolens* L. and *Coriandrum sativum* L.) [46, 47].

3.3.2. Total Amount of Reducing Sugars

The content of reducing sugars in water and methanolic extracts of different plant organs was investigated (Figures 3.4b and S3.2b) and data were normalized and elaborated using PCA (Figures 3.5 and S3.3). After water extraction (Figure 3.5), samples of stems, flowers and leaves of all species were those more enriched in reducing sugars, while roots and bark had lower amounts. Analogous results were obtained in methanolic samples (Figure S3.3). In general, no statistically significant differences were detected among regions (Figure 3.5), with the exception of leaves of *H. perforatum* collected in Apulia, which showed reducing sugar levels of 389.2 mg GLU eq/gDW, respectively 1.7 and 1.5 times higher than in leaves from Tuscany and Liguria, respectively. No previous publication has reported the total amount of reducing sugars in water extracts of *H. perforatum*. Jaradat [48] quantified these compounds only in methanolic extracts of *Hypericum lanuginosum* Lam; Nahdi et al. [49] determined the reducing sugar contents in methanolic and aqueous extracts of *Hypericum humifusum* L. leaves showing a 3.7-fold higher content in the methanolic (1.9 g/L) with respect to the aqueous extract (0.51 g/L).

Among other species, *B. officinalis* and *F. vulgare* stems showed the highest reducing sugar levels (172.8 and 162.5 mg GLU eq/gDW, respectively), on average 1.7-, 1.2- and 5.7-fold higher than leaves, flowers and roots, respectively. The results from *F. vulgare* are in agreement with previous data from the literature [50], in which stems showed higher concentrations of reducing sugars (1.49 g/100 g) compared to leaves (0.72 g/100 g). Similar levels of reducing sugars were previously detected in other medicinal plants such as *Arisaema erubescens* (Wall.) Schott (134.47 mg/g DW) and *Lindera neesiana* (Wall. ex Nees) Kurz (176.72 mg/g DW) [51].

Data on methanolic extracts (Figure S3.2b) reported the highest concentrations of sugars in flowers and leaves of *H. perforatum* (on average 210.9 and 251.8 mg GLU eq/gDW, respectively) and in stems of *B. officinalis* and *F. vulgare* (on average 1.3 to 1.6-fold higher than flowers and leaves of the same species, respectively).

3.3.3. Total Amount of Proteins

The amount of total proteins was higher in flowers and leaves compared to other plant organs, both in aqueous and methanolic samples (Figures 3.4c, 3.5, S3.2c and S3.3). These results are in agreement with previous studies on different wild food and medicinal plants which detected higher levels of proteins in leaves and flowers with respect to stems and roots [52, 53].

In aqueous extracts, the highest protein level was detected in *H. perforatum* flowers (from 395.5 to 497.8 mg BSA eq/gDW, respectively in Tuscany and Apulia samples) and in leaves (from 390.2 to 675.7 mg BSA eq/gDW, respectively in Tuscany and Apulia samples). The few studies that have reported the total protein amount in *H. perforatum* [54–56] were carried out with extracts obtained with different methods to the present paper. In particular, Karppinen et al. [56], by means of an extraction with a sodium borate buffer, obtained higher protein yields in flowers and leaves with respect to stems and roots of *H. perforatum*, in accordance with data here reported in Figure 3.4c.

On average, flowers and leaves of *S. nigra* from Apulia showed 2.7-fold higher protein levels than samples from Tuscany, and 1.5-fold higher than samples from Liguria ($p < 0.01$). Interestingly, the present results are comparable to those obtained by Bosi et al. [57] on different leguminous, well-known important sources of vegetable proteins. Besides the previously mentioned results, no significant difference was detected among the three regions (Figure 3.3).

Methanol extracts showed the highest protein yield in all *H. perforatum* organs (with maximum levels in flowers, 800.6 mg BSA eq/gDW on average) with respect to all other samples (Figure S3.2c).

3.3.4. Antioxidant Activity

The antioxidant activity of aqueous and methanolic extracts was evaluated (Figures 3.4d and S3.2d). *H. perforatum* showed the highest activity, mainly in water samples of flowers from Apulia and Liguria (253.6 and 218.3 mg AA eq/gDW, respectively) and of leaves of the same regions (216.1 and 263.5 mg AA eq/gDW). Overall, the present results show a higher antioxidant activity in *H. perforatum* samples compared to those of well-known food plants such as *Brassica rapa* L. (0.12 mg AA eq/gDW) [58]. Apart from the previously reported data, no differences between organs of different regions were detected (Figure 3.3). In agreement with present results, previous reports showed higher antioxidant activity in aqueous extracts of *H. perforatum* aerial parts respect to those of other wild plant species [59]. Previous reports also showed a higher antioxidant concentration in leaves and flowers of *S. nigra* [60, 61], in line with data reported in Figure 3.4d, where flowers and leaves scored on average 80.5 and 82.8 mg AA eq/gDW, respectively, followed by flowers and leaves of *B. officinalis* (60.5 and 67.3 mg AA eq/gDW). Similarly to water samples, in methanol extracts (Figures S3.2d and S3.3), the highest antioxidant activity was detected in flowers, leaves and stems of *H. perforatum* (182.3, 176.4 and 90.9 mg AA eq/gDW, respectively), followed by flowers and leaves of *S. nigra* (83.9 and 72.1 mg AA eq/gDW, respectively). After PCA analysis of aqueous (Figure 3.5) and methanolic (Figure S3.3) normalized data, leaves and flowers of all species exhibited a stronger antioxidant activity respect to stems, roots and barks, in accordance with total polyphenol concentrations (Figures 3.4a and S3.2a).

3.4. Conclusions

Seven Italian wild edible plants (*Borago officinalis* L., *Cynodon dactylon* (L.) Pers., *Foeniculum vulgare* Mill., *Hypericum perforatum* L., *Malva sylvestris* L., *Sambucus nigra* L., *Urtica dioica* L.), also known for their multiple therapeutic uses, were selected and analysed to investigate the variability of health-related compounds in the different traditionally consumed plant organs, and at assessing possible differences linked to the different gathering regions. Results indicated that (i) the highest levels of polyphenols, reducing sugars and proteins, as well as of antioxidant

activity, were detected in the extracts of *H. perforatum* L. (on average up to 105.7 mg GA eq/gDW, 389.2 mg GLUC eq/gDW, 675.7 mg BSA eq/gDW and 263.5 mg AA eq/gDW, respectively), followed by *S. nigra* L. and *B. officinalis* L. extracts; (ii) flowers and leaves of all plant species exhibited a higher amount of bioactive compounds with respect to stems, roots and bark (e.g., total polyphenols of *H. perforatum* flowers and leaves from Tuscany were 13.6- and 11.5-fold higher than in roots, respectively), and these correlated with higher antioxidant potential; (iii) in general, very few differences were detected among samples collected in different regions along the Italian peninsula.

In the present paper, for the first time, seven different Italian wild edible species collected in three different regions were compared, enhancing the knowledge of some of the most widely and traditionally used food–medicine plants by providing additional evidence on the presence of bioactive compounds in their different organs. However, further research is needed to directly correlate the metabolite data with the traditionally reported health effects of these plants, and furthermore to validate them as possible substitutes of chemical drugs for therapeutic applications.

Author Contributions

Methodology: S.M.; material preparation: S.M. and M.S.; data collection and analysis: S.M.; writing—original draft preparation: S.M.; conceptualization: M.F. and A.T.; writing—review and editing: M.F. and A.T.; supervision. All authors have read and agreed to the published version of the manuscript.

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Supplementary Materials

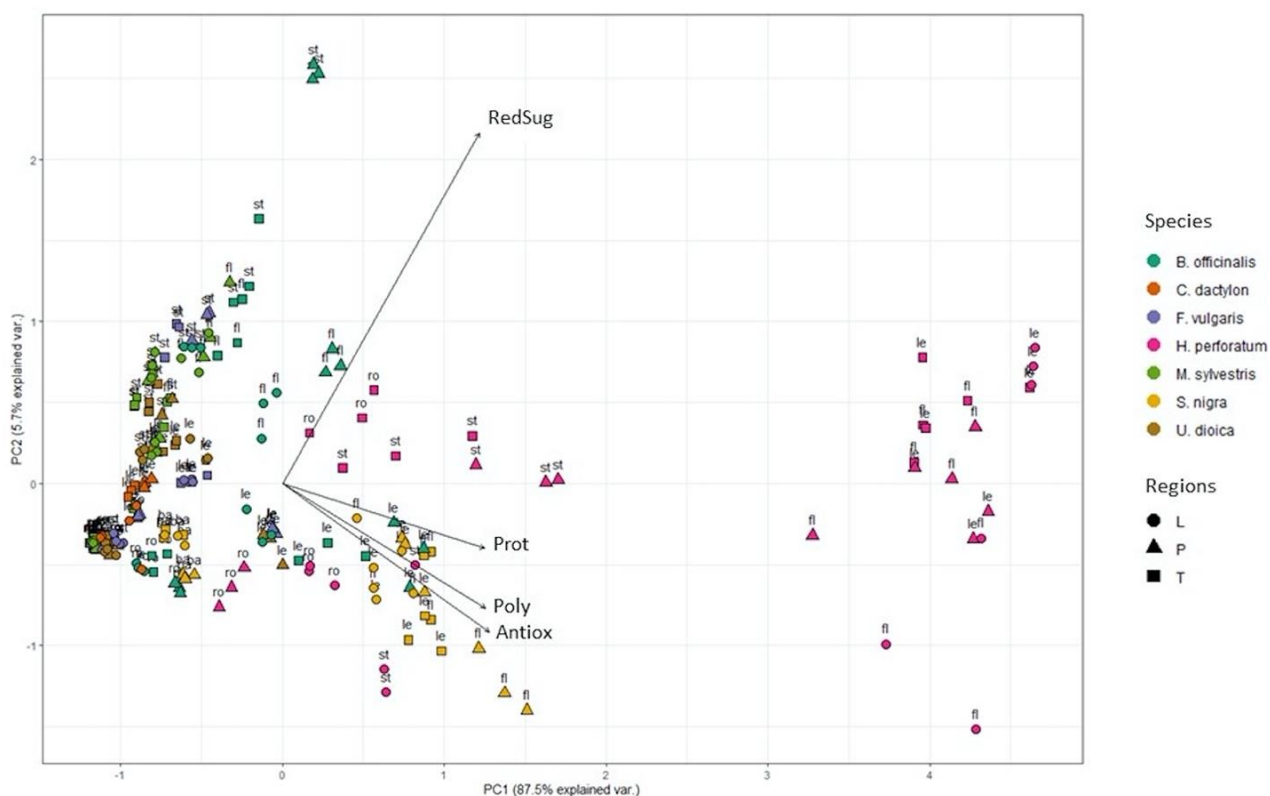


Figure S3.1. PCA analysis of spectrophotometric results on methanol extracts, showing the grouping of species according to total polyphenols, reducing sugars, proteins and antioxidant activity data (Fig. S3.2). L: Liguria; P: Apulia; T: Tuscany; fl: flowers; le: leaves; st: stems; ro: roots; ba: bark; RedSug: reducing sugars; Poly: polyphenols; Antiox: antioxidant activity; Prot: proteins.

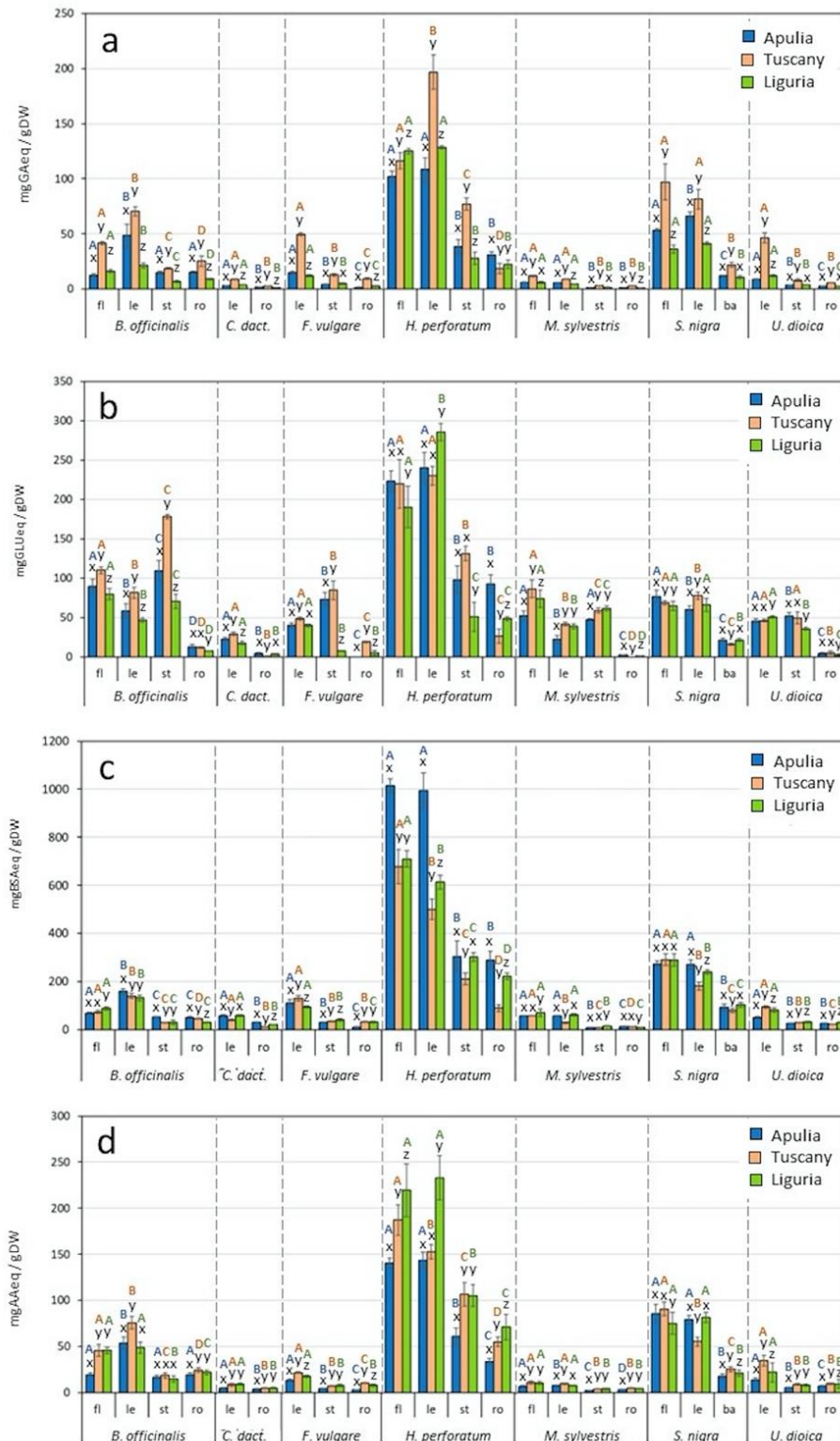


Figure S3.2. Total amounts of polyphenols (a), reducing sugars (b), proteins (c) and antioxidant activity (d) of methanol extracts after spectrophotometric analysis. fl: flowers; le: leaves; st: stems; ro: roots; ba: bark; GA: gallic acid, GLU: D-glucose, BSA: bovine serum albumin, AA: ascorbic acid, eq: equivalent, DW: dry weight. Different letters indicate a statistically significant difference (one-way ANOVA followed by post hoc Tukey HSD test or Kruskal-Wallis followed by Dunn test, $p < 0.05$) among the regions (x, y, z) and among the organs of each species (A, B, C, D). Data are the mean $\pm$ SD ($n = 3$).

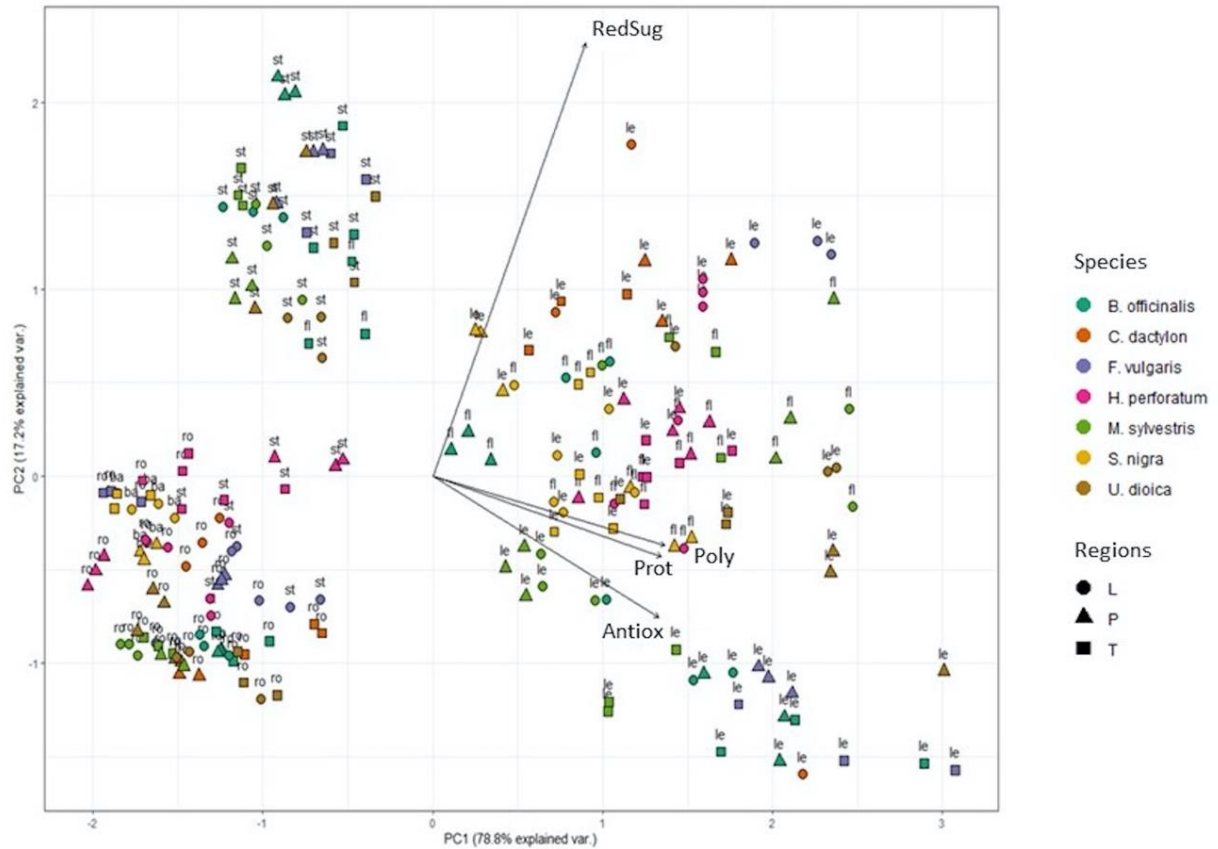


Figure S3.3. PCA analysis of methanol extracts spectrophotometric results (Appendix 2) after normalization (see Methods section) showing the grouping of organs according to the concentrations of reducing sugars, polyphenols, proteins and antioxidant activity. L: Liguria; P: Apulia; T: Tuscany; fl: flowers; le: leaves; st: stems; ro: roots; ba: bark; RedSug: reducing sugars; Poly: polyphenols; Antiox: antioxidant activity; Prot: proteins.

Chapter 4

Bioaccessibility of alimurgic plant preparations after simulated *in vitro* digestion, and biological activities for oral and topical applications

*Under preparation to be submitted for publication to Journal of Advanced Research as: Monari, S., *Escórcio, R., *Correia, V.G., *Cairrão, A., *Bento A., *Pereira C.S., Tassoni, A.*

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4.1. Introduction

The use of plants for therapeutic purposes dates back to ancient civilizations. Already during the Sumerian period, it was documented on clay tablets a list of treatments for various illnesses, while Egyptian and Greek medical knowledge was published in the Ebers Papyrus written around 1550 BC and the book *De Materia Medica* written by Pedanius Dioscorides between 50-70 AD, both reporting thousands of remedies involving medicinal plants [1]. Since then, the empirically obtained knowledge on natural medicines was generally handed down from generation to generation. Currently, approximately 30000 plant species are known to be used for therapeutic purposes [2] and the interest in unravelling nature-derived bioactive compounds from novel sources has been increasing in the last decades.

A recent survey on Italian ethnobotanical studies published in the last 40 years highlighted the presence of several wild and/or cultivated plants widespread all over the Italian peninsula and commonly used in Italian folk medicine purposes [3]. In this study, 13 plant species were identified as the most relevant Italian species used for medicinal purposes, by calculating the citation index and the use index, in order to find the species with the highest number of citations and therapeutic application. Among them, *Borago officinalis* L., *Foeniculum vulgare* Mill., *Hypericum perforatum* L. and *Sambucus nigra* L. were chosen as object of the present study. *B. officinalis* has been usually used as diuretic, laxative, menstrual analgesic and for the treatment

of swelling and inflammation, depression and respiratory and skin diseases [4]. *Foeniculum vulgare* Mill. is used for gastrointestinal disorders, respiratory issues, female ailments and as an anti-pyretic, antirheumatic and detoxifier [3, 5]. *H. perforatum* has been consumed against acute and chronic pain, toothache, burns, rheumatism, psychiatric and neurodegenerative disorders [6]. Several different *S. nigra* medicinal preparations (decoction, infusion, syrup, cataplasm) made with flowers and bark are used against a wide range of respiratory, digestive, metabolic affections and skin diseases [3].

Among the reported medicinal plant preparation techniques for therapeutic purposes [3], water extracts, namely infusion and decoction, were most commonly used in many different types of applications, such as oral intake and topical use. An infusion as well as a decoction, could be orally taken for digestion problems and stomachache, as laxative or diuretic, or as an expectorant for bronchial disorders. However, it is well-known that, due to pH variations and the presence of digestive enzymes, after oral intake, the bioaccessibility of metabolites following passage through the gut differs from the starting value in raw food. Bioaccessibility is defined as the quantity or fraction of the food matrix which is released in the gastrointestinal tract and becomes available for absorption [7], and bioavailability represents the portion of the digestates (phytochemicals in this case) that are absorbed by the intestinal cells and metabolized [8]. Additionally, the release of food nutrients and their delivery to target areas in the body is different along the digestion process [9]. It is therefore important to understand how the digestion affects the availability of the food components to better evaluate the therapeutic properties of medicinal plant preparations (e.g., infusion and decoction) on the human body.

There are two main ways to analyse the effects of digestion on food sources: 1) *in vitro* static or dynamic oro-gastrointestinal digestion assays that simulate the main phases of digestion process and allow to evaluate the bioaccessibility of food components [10, 11]; 2) *in vivo* models through administration of food to animals or humans under controlled conditions [12, 13]. *In vitro* methods do not include every step of the process *in vivo*, such as active transportation processes and presence and action of the gut microbiota [14]. On the other hand, compared to *in vivo* assays and clinical investigations, the *in vitro* protocols are cheaper, safer, faster, simpler and more reproducible. Static *in vitro* digestion simulation models take place in a beaker in continuous agitation, to simplify the intestinal mechanical forces and fluid dynamics, while dynamic models use single- or multi-chamber systems which mimic the digestion active mechanisms such as the continuous secretion of digestive juices, the variable enzyme

concentrations and pH changes [10, 15]. Brodkorb and colleagues [16] published a standardized static *in vitro* digestion protocol with the aim to optimise protocol conditions as close as possible to human digestion, and simple enough to be used with high sample numbers.

Plant bioactive compounds (both primary and secondary metabolites) are differently absorbed by gut cells depending on their chemical characteristics, as solubility, hydrophobicity, molecular weight, isomer configuration [8]. Numerous of these compounds have been isolated from traditional alimurgic plants, with proven human health beneficial properties.

Phenols represent the largest and most diversified group of secondary metabolites and are well known bioactive molecules with anti-inflammatory, antioxidant, antidiabetic, anticancer effects [17, 18]. Most of phenolic compounds are water soluble, and their bioaccessibility depends on their release facilitated by digestive enzymes from the vegetal matrix (where they interact with proteins and carbohydrates), their degree of complexation and possible co-precipitation with proteins or minerals during the digestion [18, 19]. Moreover, the absorption of different phenol classes depends on the chemical structure and in particular on their molecular weight (the higher the molecular weight, the lower the intestinal absorption), on the glycosylation degree (glycosylated polyphenols as such are usually not well absorbed by the intestine and are generally deglycosylated prior absorption), on the esterification degree (not-esterified polyphenols are well absorbed respect to esterified forms) [20]. Different studies demonstrated that chemical structure and biological properties of many polysaccharides (e.g. starch and soluble fibers) are significantly modified during the gastrointestinal digestion, while in other cases (such as for insoluble fibers) their structure may not be altered [14]. During the digestion process generally occurs the disruption of glycosidic bonds of polysaccharides, with the depolymerization of the molecules and the formation of reducing sugars (e.g., monosaccharides, disaccharides and oligosaccharides) [21]. Thanks to their reducing power and radical scavenging activity, reducing sugars may show a moderate antioxidant activity [22]. Plant proteins are essential macronutrients for human nutrition, and compared to animal proteins are valuable substitutes with lower cost and high environmental impact [23]. During gastro-intestinal digestion proteins are hydrolysed by intestinal enzymes (pepsin and pancreatic endo- and exopeptidases) resulting in the formation of highly bioavailable small molecular weight peptides (some of them also showing biological activities and defined bioactive peptides, see Chapter 1, [24]), and amino acids [20].

Alimurgic plant preparations were widely reported to have a topical medicinal application [3]. Infusions and decoctions were used to treat many skin and infectious diseases such as burns and wounds, as cicatrizing and lenitive, against acne and rheumatic pains, as anti-inflammatory and antimicrobial [3, 25, 26]. Antimicrobial activity of medicinal plants is a subject of interest in the search of new substances to fight the increasing occurrence of resistances to antibiotics and antivirals. In addition, antibiotic drugs are sometimes associated with adverse effects, and so there is a need for alternative natural antimicrobials for the treatment of infectious diseases.

In a previous paper [27] (Chapter 3), water and methanol extracts of raw plant organs of *B. officinalis*, *F. vulgare*, *H. perforatum*, *S. nigra* from Apulia, Tuscany and Liguria (Italy) were analysed, demonstrating that flowers and leaves had the highest total phenols, proteins contents and antioxidant activity results, while stems showed the highest reducing sugars values. Very few differences were detected among samples collected in different regions.

The aim of the present study is instead to evaluate the effect of the *in vitro* oro-gastrointestinal simulated static digestion on the bioaccessibility of phytochemical compounds and on biological activities of the medicinal preparations (infusion and decoction of selected organs of *B. officinalis*, *F. vulgare*, *H. perforatum* and *S. nigra*, in view of their oral ingestion or topical application. As far as we know no studies were previously published focusing on antimicrobial and antioxidant potential of infusion and decoction samples (before and after digestion process) of selected alimurgic plants and related organs.

4.2. Materials and Methods

4.2.1. Plant Materials and Sample Preparation

In Monari et al. (2023) (Chapter 3 [27]) seven wild food plant used for medicinal purposes and collected in three different Italian regions (Apulia, Tuscany and Liguria) were biochemically analysed. Following previous results, in the present study the most active organs were further analysed and specifically precisely flowers, leaves and stems of *Borago officinalis* L. (BO) and *Hypericum perforatum* L. (HP), stems of *Foeniculum vulgare* Mill. (FV), and flowers and leaves of *Sambucus nigra* L. (SN). After harvesting, each sample was frozen in liquid nitrogen, lyophilised and grinded by means of a blender.

Infusions and decoctions of each plant part were prepared: for infusions 20 ml of boiling water were added to 0.4 g of sample dry powder and left stand for 5 minutes at room temperature; for decoctions 20 ml of distilled water were added to 0.4 g of powder dry sample and boiled 5 minutes. Infusions and decoctions were centrifuged at 4500 *g* at 4°C for 10 minutes and supernatants recovered. Samples were stored at -20°C until further analyses.

4.2.2. *In Vitro* Oro-Gastrointestinal Digestion

The simulated *in vitro* oro-gastro-intestinal digestion was performed by applying the protocol of the static model system INFOGEST [16], which mimics the oral, gastric and small intestinal phases of human digestion process. Firstly, the number of activity units of each enzyme used in the protocol (i.e., α -amylase, pepsin and pancreatin) was determined by applying standard protocols [16] (Appendix 3). Then enzyme solutions and simulated digestion fluids were prepared as in Table 4.1.

Table 4.1. Volumes and concentrations of salt stock solutions and recipes for simulated digestion fluids for a volume of 400 ml (1.25x concentration). SSF: simulated salivary fluid; SGF: simulated gastric fluid; SIF: simulated intestinal fluid.

Salt solution added	Stock concentrations		SSF	SGF	SIF
	g / L	M	ml	ml	ml
KCl	37.3	0.5	15.1	6.9	6.8
KH ₂ PO ₄	68	0.5	3.7	0.9	0.8
NaHCO ₃	84	1	6.8	12.5	42.5
NaCl	117	2	-	11.8	9.6
MgCl ₂ (H ₂ O) ₆	30.5	0.15	0.5	0.4	1.1
(NH ₄) ₂ CO ₃	48	0.5	0.06	0.5	-
HCl	-	6	1.1	1.3	0.7
CaCl ₂ (H ₂ O) ₂	44.1	0.3	1.5	0.005	0.04

The digestion procedure was performed at 37°C by following the steps below:

- Oral phase (2 min – pH 7):
 - 3 ml of infusion/decoction were diluted 1:1 (v/v) with SSF (simulated salivary fluid, pre-warmed at 37°C) and the volume of the final digestion mixture was measured
 - 15 µl CaCl₂ were added, to achieve a final concentration of 1.5 mM of SSF
 - 0.3 mL of α-amylase (75 U/ml H₂O) were added
 - Volume brought to 6 ml with H₂O (to achieve a final concentration of 1x of SSF)
 - Incubation at 37°C, 2 min shaking (130 rpm)
- Gastric phase (2 h – pH 3):
 - The previous solution (oral bolus) was diluted 1:1 (v/v) with SGF (simulated gastric fluid, pre-warmed at 37°C)
 - pH was adjusted to 3.0 with HCl 1M
 - 3 µl CaCl₂ were added, to achieve a final concentration of 0.15 mM of SGF
 - 0.3 mL of pepsin (2,000 U/ml H₂O) were added
 - pH was verified and adjusted to 3.0 if necessary
 - volume brought to 12 ml with H₂O (to achieve a final concentration of 1x of SGF)
 - Incubation at 37°C, 2 h shaking (130 rpm)
- Intestinal phase (2 h – pH 7):
 - The previous solution (gastric chyme) was diluted 1:1 (v/v) with SIF (simulated intestinal fluid, pre-warmed at 37°C)
 - pH was adjusted to 7.0 with NaOH 1M
 - 40 µl CaCl₂ were added, to achieve a final concentration of 0.6 mM of SIF
 - 3 mL of pancreatin (100 U/ml H₂O) were added
 - pH was verified and adjusted to 7.0 if necessary
 - volume brought to 24 ml with H₂O (to achieve a final concentration of 1x of SIF)
 - Incubation at 37°C, 2 h shaking (130 rpm)

Samples were immediately stored in ice to stop enzymatic reactions and frozen at -20°C until analysis.

4.2.3. Spectrophotometric Analyses

Infusion and decoction samples digested and not digested were analysed to assess total amount of phenols, reducing sugars and proteins, and the antioxidant activity. Total phenols content was determined using the Folin-Ciocalteu method [28]. Results were expressed as mg of gallic acid (GA) equivalent per g of dry weight (mg GAeq/gDW) with a dose-response calibration curve (0 - 15 µg of GA). Total reducing sugars content was measured with the 3,5-dinitrosalicylic acid (DNS) method [29]. Results were expressed as mg of D-glucose (GLU) equivalent per g of dry weight (mg GLUeq/gDW) with a dose-response calibration curve (50 - 500 µg of GLU). Total proteins content was determined as described by Lowry et al. [30]. Results were expressed as mg of bovine serum albumin (BSA) equivalent per g of dry weight (mg BSAAeq/gDW) with a dose-response calibration curve (0 – 200 µg of BSA). Antioxidant activity was assessed using the ABTS (2,20-azino-di-(3-ethylbenzthiazoline sulfonic acid)) method [31]. Results were expressed as mg of ascorbic acid (AA) equivalent per g of dry weight (mg AAeq/gDW) with a dose-response calibration curve (0 - 2 µg of AA).

4.2.4. NMR Baseline Characterisation

Nuclear magnetic resonance (NMR) spectroscopy is an analytical technique to observe local magnetic fields around atomic nuclei, which are characteristic to individual compounds and allows the identification of molecules and classes of molecules. This technique will provide a snapshot of the compositional functional groups in the extracts and insights of structural related features such as bonds between molecules (macromolecular view).

Infusions and decoctions before and after digestion were previously lyophilized, and NMR spectra of plant extracts were recorded using an Avance III 800 MHz CRYO (Bruker Biospin, Rheinstetten, Germany). All NMR spectra (^1H , ^1H - ^{13}C HSQC, ^1H - ^{13}C HSQC-TOCSY) were acquired in deuterium oxide (D_2O) using 5 mm diameter NMR tubes, at 25 °C as follows: 5 mg of each sample powder in 500 µL of D_2O . MestReNova, Version 11.04-18998 software (Mestrelab Research, S.L.) was used to process the acquired raw data. All samples were analysed in biological triplicates.

4.2.5. Phenols Characterization by HPLC-DAD

Phenols were extracted from 1 ml of plant infusion/decoction aqueous extracts. The samples were loaded onto a Strata-X column (33 µm polymeric sorbent, 60 mg / 3 mL, Phenomenex,

Torrence, CA, USA) and phenols were eluted with 100% (v/v) methanol, completely dried and resuspended in 200 µl of 1:9 acetonitrile 100%/water 99.8%:0.2% acetic acid (v/v) before being injected into the HPLC-DAD (column Gemini C18, 5 µm particles 250 x 4.6 mm, pre-column Security- Guard Ea, Phenomenex, Torrence CA, USA) equipped with an on-line diode array detector (MD-2010, Plus, Jasco Instruments, Großumstad, Germany) [32]. The adopted HPLC-DAD separation procedure allowed the simultaneous analysis of 25 compounds by means of a 45 min dynamic gradient in which eluents were acetonitrile 100% (v/v) and water 99.8%:0.2% acetic acid (v/v). The HPLC standards were purchased from Sigma-Aldrich (Milano, Italy). Standard compounds were injected at several concentrations (between 2 and 50 µM) to verify retention time and spectrum for peak identification and to assess the linearity range for quantification. For each plant samples, five chromatograms obtained at different wavelengths (270, 285, 305, 323 and 365 nm) were analysed to determine the concentration of single compounds, depending on their maximum absorbance. In detail: gallic (GA), protocatechuic (PROTA), syringic (SIRA), vanillic (VANA) and trans-cinnamic (CINA) acids, epigallocatechin (EGC), catechin (CAT), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin-gallate (EGCG), and vanillin (VAN) were quantified at 270 nm; naringenin (NAR) at 285 nm; p-coumaric acid (COUMA) at 305 nm; chlorogenic (CHLORA), caffeic (CAFA), trans-ferulic (FERA), sinapic (SINA) acids, apigenin (API) and piceatannol (PICEAT) at 323 nm; rutin (RUT), myricetin (MYR), quercetin (QUERC), kaempferol (KAE), luteolin (LUT) and luteolin-7-glucoside (LUT-7-glu) at 365 nm.

4.2.6. Antimicrobial Activity Assays

4.2.6.1. Minimal Inhibitory Concentration (MIC)

Stock solutions of each plant organ extract powder were prepared (30 mg/ml in water). *S. aureus* NCTC8325 and *E. coli* TOP 10 bacterial strains were grown in Mueller-Hinton broth (MHB) (Merck KGaA, Darmstadt, Germany) overnight at 37°C. Aliquots of 100 µl of bacteria suspension were added to 96-well microplates containing two-fold serial dilutions in water (100 µl) of plant extracts to obtain a cell density of 10⁵ cells/ml in each well. Tested concentrations of plant extracts ranged from 15 to 0.03 mg/ml. The microplates were incubated for 24 h at 37 °C, and the optical density at 600 nm (OD₆₀₀) was measured to evaluate bacterial growth (Infinite M nano+ Tecan microplate reader, city, state). Wells with only media (abiotic), bacteria (biotic), and extracts with medium (negative) were utilized as controls. Minimal Inhibitory Concentration

(MIC) was defined as the lowest concentration of a compound at which bacterial growth was inhibited.

4.2.6.2. Thiazolyl Blue Tetrazolium Bromide (MTT) Assay

After 24h of incubation with the plant extracts, 100 µl of each well solution were transferred to a new plate and 10 µl of MTT (thiazolyl blue tetrazolium bromide) were added and incubated at 37°C in the dark. After 30 min, 100 µl of sodium dodecyl sulphate (SDS) was added to each well to solubilize formazan crystal produced in the solution, and the plate incubated 2h at room temperature in the dark. The sample absorbance was measured at 560 nm and 700 nm and the values were subtracted to obtain the final results (560nm – 700nm). Microbial growth inhibition (GI) percentage was calculated relatively to the biotic control.

4.2.6.3. Colony Forming Unit (CFU) Assay

Samples with the lowest MIC values were selected to assess bacterial cells' viability and were used at the concentrations previously inducing at least 70% of bacteria growth inhibition. After 24h incubation at 37°C, bacterial cells were plated on Petri dishes containing Mueller Hinton Agar. As controls, samples with only media (abiotic), bacteria (biotic), and extracts with medium (negative) were utilized. After 24 h at 37°C, the number of distinct growing colony forming units (CFUs) was counted. The percentage of growth was calculated as follows:

$$\% \text{ growth} = \left(\frac{(CFU_{sample}) - (CFU_{control})}{CFU_{control}} \right) * 100$$

4.2.7. Statistical Analyses

All spectrophotometric analyses were performed using 3 biological replicates and analysed in 4 technical replicates. The results were expressed as the mean (n=3) ± standard deviation (SD) per g of dry weight (DW). The statistically significant differences between digested and not digested infusion/decoction obtained by plant organs were evaluated by *t*-test at *p*<0.05 significance level. All statistical analyses were performed using R software version 3.5.1 (R Core Team, Vienna, Austria). The correlation indexes between total polyphenols content and antioxidant activity were calculated using Microsoft Excel (Microsoft 365 MSO; 2307 version, Build

16.0.16626.20170). For the principal component analysis (PCA) of the ^1H NMR spectra, RStudio version 1.4.1106 with Rnmr1D package (Jacob, n.d.) was used. All spectra were first aligned, baseline correction was applied and the segments [8.0;7.46]; [7.43;4.90]; [4.70;.2.71]; [2.68;0.5] ppm analysed. The segments associated to solvents were deleted.

4.3. Results and Discussion

4.3.1. Spectrophotometric Analysis

4.3.1.1. Total Phenols Content

The spectrophotometric assessment of the total amount of phenols in plant extracts before and after digestion is shown in Fig. 4.1. In not digested extracts, phenols content was in a wide range of 6.5-144.1 mg GAeq/gDW, and of 12.0-41.2 mg GAeq/gDW in digested samples. Not digested samples of leaves and flowers of *Hypericum perforatum* (HP) showed overall the highest values both in infusions and decoctions, while stems of *Foeniculum vulgare* (FV) showed the lowest (Figs. 4.1 a,b). No specific difference was detected among samples of the three different collection regions.

In general, a statistically significant decrement ($p<0.05$) of phenols concentration after digestion was recorded both in infusions and decoctions of leaves and flowers (Fig. 4.1). Specifically, the highest decrease was detected in leaves followed by flowers (for example 56.5 to 80.4% in flowers and leaves infusions and decoctions of both BO and HP compared to not digested samples). The reduction of the total amount of phenols after the intestinal digestion phase was widely observed in other bioaccessibility studies on different food matrixes such as guaraná aqueous extracts (-57% compared to not digested samples) [33], coffee grounds (-10-20%) [34], blackberries (- 70%) [35], tea infusions (-50-85%) [36–38].

Conversely, after digestion stem samples showed a very minor decrease such as stem infusions of *Borago officinalis* (BO) (- 11.1%) (Fig. 4.1a). Moreover, stems were the only plant organ in which a statistically significant ($p<0.05$) higher content of phenols was detected compared to undigested ones. In particular all FV stems (+ 50.8%), HP stems from Liguria (+48.4%), and in BO stems decoctions from Tuscany and Liguria (+63.7% on average) (Fig. 4.1 a,b).

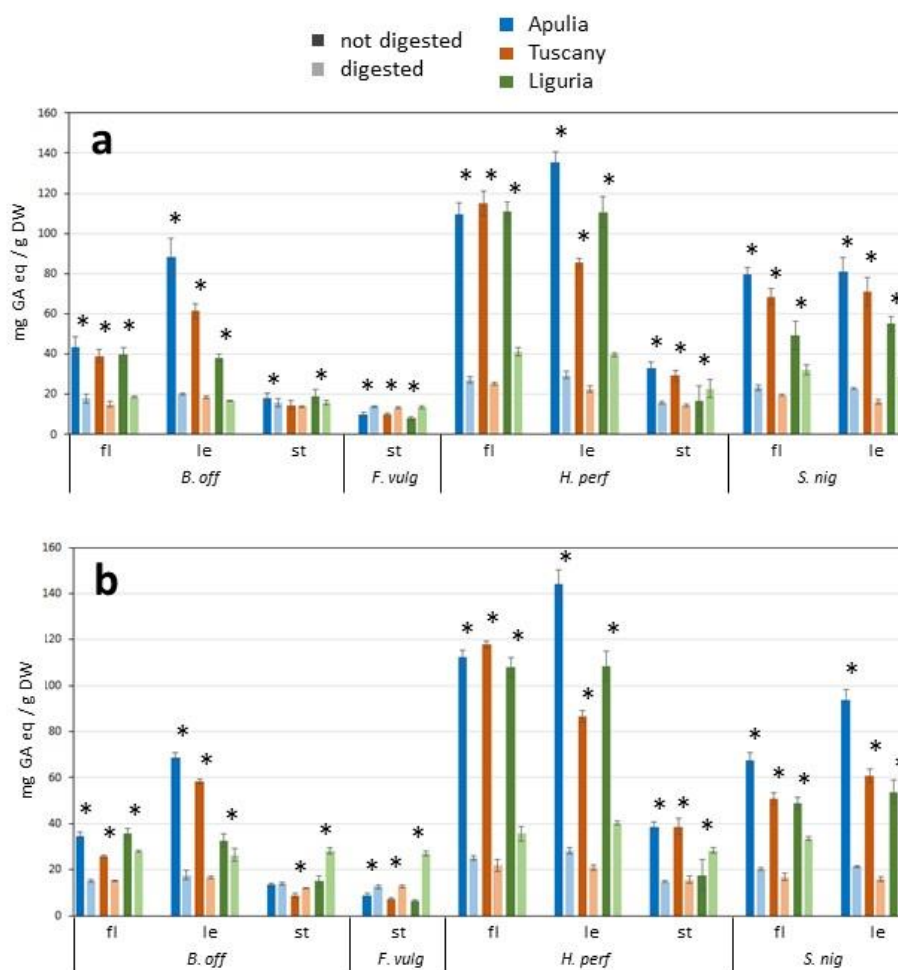


Figure 4.1. Total phenol amounts in infusions (a) and decoctions (b) of *Borago officinalis* (B.off), *Foeniculum vulgare* (F.vulg), *Hypericum perforatum* (H.perf) and *Sambucus nigra* (S.nig) determined by spectrophotometric analysis. Fl: flowers; le: leaves; st: stems; GA: gallic acid, DW: dry weight. Star symbol (*) indicates statistically significant differences ($p < 0.05$, t test) among not digested and digested samples. Data are the mean $\pm$ SD ($n = 3$).

These results match those reported on which found a lower phenols concentration after the simulated digestion process. Phenols bioaccessibility and concentration variation after digestion were previously documented [19] and can be caused by several factors: the molecule sensibility to the gastrointestinal pH changes [39], the possible degradation of tetramer and trimer into monomer and dimer compounds [40], the interaction with simulated digestive juices and proteolytic enzymes [41].

Our results also showed different effects of the digestion process according to treated matrixes, and this could be explained by the presence of different phenol profiles in the plant organ extracts [42, 43], depending on developmental stages, cell types and environmental cues [44]. Phenols increase, constancy or loss after digestion can be influenced by several factors such as

their interaction with other dietary components (e.g., proteins, carbohydrates), generation of complex phenolic metabolites (e.g., quinones), enzymatic actions, different pH conditions, physicochemical properties of phenols themselves like hydrophilicity/lipophilicity [10].

Plant cell walls represent a barrier to digestion, and when this barrier is broken by mastication or cooking, phytochemicals can associate with fibres, proteins and other food components changing their relative bioaccessibility [45, 46].

4.3.1.2. Total Reducing Sugars Content

Results on total reducing sugar contents before and after digestion are shown in Fig. 4.2. The reducing sugars content varied considerably in not digested extracts, ranging from 34.3 mg GLUeq/gDW in stems infusion of HP from Liguria up to 451.2 mg GLUeq/gDW in leaves decoction of HP from Apulia, while in digestates the variation was lower, ranging from 239.6 mg GLUeq/gDW, in stem decoction of HP, to 345.7 mg GLUeq/gDW in leaves decoction of HP, both from Apulia.

A significative increment ($p < 0.05$) of total reducing sugars amount was observed after the digestion process. Among organs, leaves showed on average the highest increase with +197.4 % in infusions and +192.3% in decoctions, followed by flowers (averages of +89.2% in infusions and +100.6% in decoctions) (Fig. 4.2). This increase can be explained by the enzymatic hydrolysis action (α -amylase in particular) causing the breakdown of glycosidic bonds of polysaccharides and resulting in the accumulation of monosaccharides (as reducing sugars) with more reducing residues detected by the DNS reagent of the assay [47].

These results match those observed in other studies that performed simulated *in vitro* digestion on different vegetable matrices: a significant increase in reducing sugars content was obtained in Jackfruit pulp (+10.6%) [48] in Okra fruits [49] and in aloe (*Aloe vera* (L.) Burm.f.) leaves [14]. Among the analysed plants, only HP flowers from all the regions analysed showed a decrement in total amount of reducing sugars both infusions and decoctions (-17.9% on average) as well as leaves from Apulia (-23.8 %). Flower infusions from Apulia, and leaf infusions and decoctions from Tuscany and Liguria showed no statistical differences before and after digestion ($p > 0.05$) (Fig. 4.2). When a decrease of sugars amount occurs, may mean that some polysaccharides showed resistance to be broken down in the simulated digestive tract [50]. Previous published results in fresh squash (*Cucurbita moschata* Duchesne) showed no significative change in

reducing sugar content, while in *Opilia amentacea* Roxb. fruits a decrease of sugars content was measured after *in vitro* digestion [14].

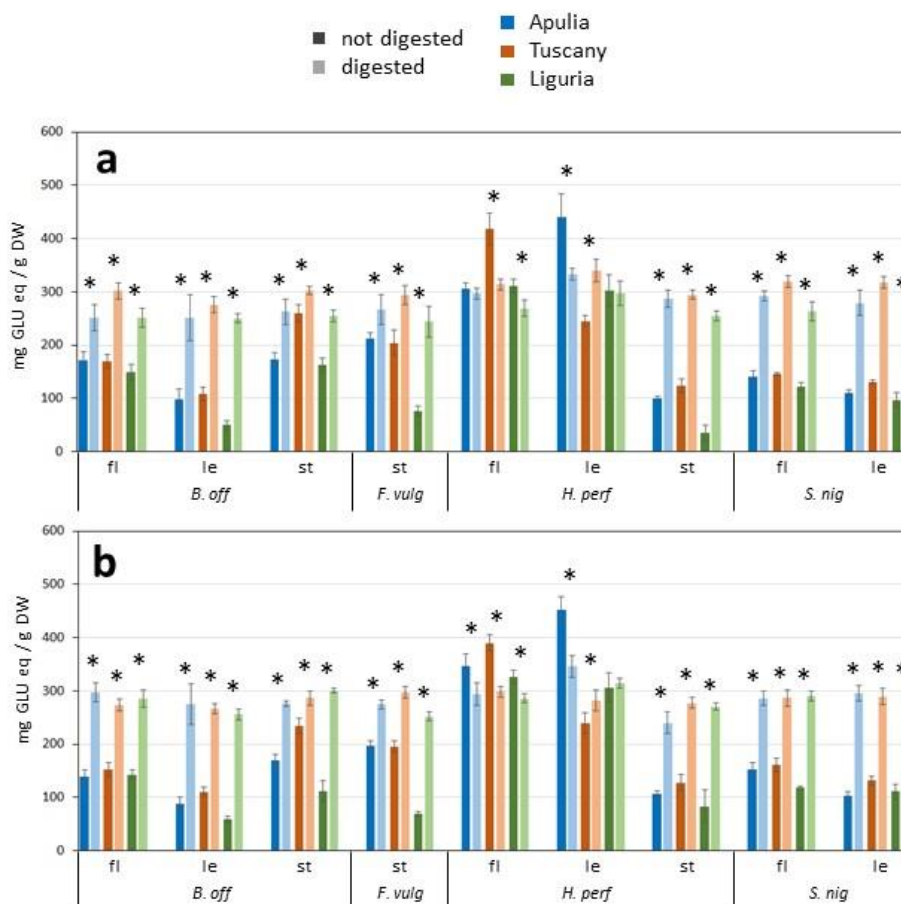


Figure 4.2. Total amount of reducing sugars in infusions (a) and decoctions (b) of *Borago officinalis* (B.off), *Foeniculum vulgare* (F.vulg), *Hypericum perforatum* (H.perf) and *Sambucus nigra* (S.nig) determined by spectrophotometric analysis. Fl: flowers; le: leaves; st: stems; GLU: D-glucose, DW: dry weight. Star symbol (*) indicates statistically significant differences ($p < 0.05$, t test) among not digested and digested samples. Data are the mean $\pm$ SD ($n = 3$).

The different results obtained among the sampled species and organs may therefore depend on the heterogeneity in the polysaccharidic constituents of plant cells (either components of the cell walls or reserve sugars), which in turn are dependent on the type, function, and growth stage of the plant organ [51]. Therefore, different polysaccharides undergo the process of breakdown of glycosidic bonds and release monosaccharides, disaccharides or short oligosaccharides reducing sugars in different types and amounts.

4.3.1.3. Total Proteins Content

The total yield of proteins before and after digestion was detected by means of Lowry assay, and results are showed in Fig. 4.3. In not digested samples, protein contents varied from 57.6 mg BSAeq/gDW (stems decoction of FV from Liguria, Fig. 4.3b) to 749.1 mg BSAeq/gDW (leaves infusion of HP from Apulia, Fig. 4.3a), while in digestates between 378.4 mg BSAeq/gDW (stems decoction of BO from Toscana, Fig. 4.3b) and 1231.4 mg BSAeq/gDW (flowers decoction of HP from Liguria, Fig. 4.3b).

A general increase of total amount of proteins was observed after digestion. Stems showed the highest percentage of increase, with a maximum of 1518.5 % increase in decoctions (Fig. 4.3b) and 901.9 % increase in infusions respect to not digested samples in HP stems from Liguria (Fig. 4.3a). This increment is mainly due to the hydrolyzation of big folded proteins during digestion, by means of the enzyme pancreatin [52], which releases a large amount of short peptides (to be notice that Lowry reagent detect the reaction of copper ions with the peptide bonds under alkaline conditions [30]). These short peptides can be bioactive peptides derived from plant proteins, which exhibit benefits to human health as anti-inflammatory, antimicrobial and antioxidant effects) [20].

The reported digested protein increases are consistent with those observed in other studies in which the effects of digestion on different foods, for example chickpea seeds [53], corn gluten meal [54], amaranth flour [55].

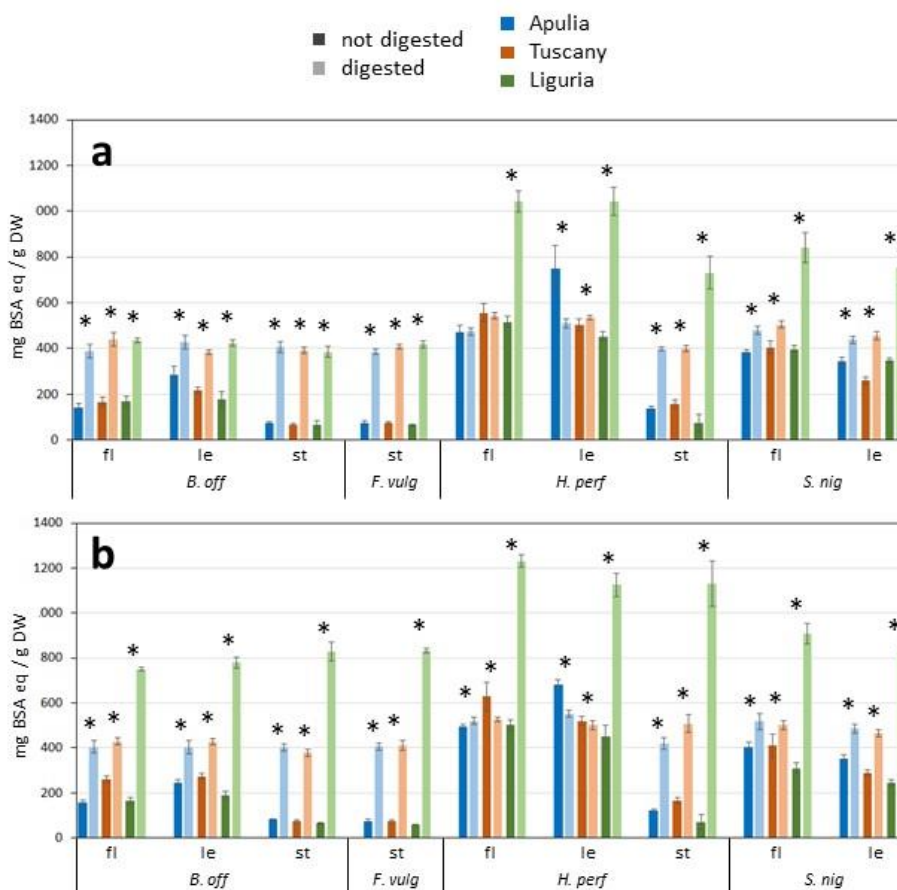


Figure 4.3. Total amounts of proteins of infusions (a) and decoctions (b) of *Borago officinalis* (B.off), *Foeniculum vulgare* (F.vulg), *Hypericum perforatum* (H.perf) and *Sambucus nigra* (S.nig) determined by spectrophotometric analysis. Fl: flowers; le: leaves; st: stems; BSA: bovine serum albumin, DW: dry weight. Star symbol (*) indicates statistically significant differences ($p < 0.05$, t test) among not digested and digested samples. Data are the mean $\pm$ SD ($n = 3$).

4.3.1.4. Antioxidant Activity

The antioxidant capacity of plant organ water extracts was detected in samples before and after digestion by ABTS assay (Fig. 4.4).

The activity of the not digested samples varied from 9.5 mg AAeq/gDW (on average in stems of FV of the three regions) to 178.2 mg AAeq/gDW (on average in flowers of HP), while digestates showed an activity between 24.2 mg AAeq/gDW (stems infusion of FV) and 86.0 mg AAeq/gDW (leaves decoction of HP from Liguria) (Fig. 4.4).

After the *in vitro* oro-gastro-intestinal digestion, a general significantly decrease ($p < 0.05$) of antioxidant capacity was observed. Specifically, an average 52.7% loss of activity was detected in flowers and leaves of the species analysed from the three regions (Fig. 4.4). This result is consistent with other researches which reported a decrease of the antioxidant potential after

simulated *in vitro* digestion process of the samples analysed, for instance a 47% loss of activity in wine samples [56], a 38.09-52.62% reduction in different Madeira sorrel morphological parts [39], herbal teas (-25-51%) [38, 57], leaves soup (-62-67%) [58], and fruits (-20-67%) [59, 60]. Conversely, in agreement with previously detected phenol levels (Figs. 4.1 a,b), stems of BO and FV showed an increment in the antioxidant activity (+111.6% and +327.3% on average among the three regions respectively in infusions and decoctions, Figs. 4.4 a,b). These differences among plant organs after digestion may also be explained by the fact that the antioxidant activities of plant extracts are closely related to the changes of individual phenolic structures. It is in fact a well-known fact that types of phenols, more than their concentration, are strictly correlated with antioxidant activity [18] such as for examples catechins and phenolic acids (i.e. caffeic acid).

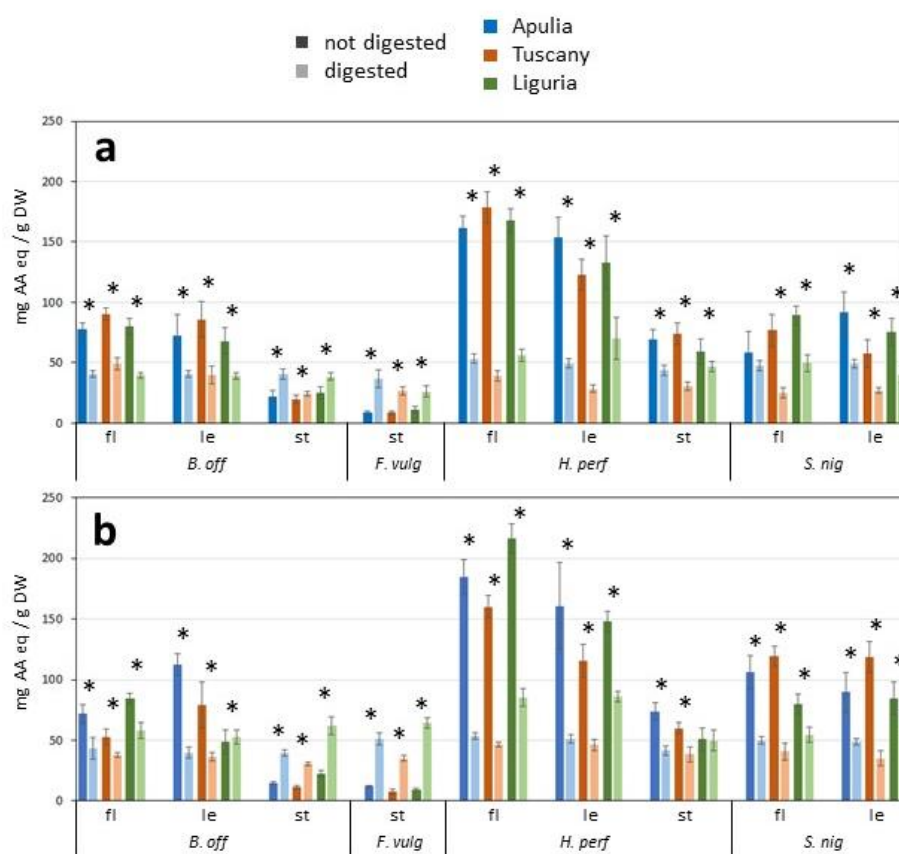


Figure 4.4. Antioxidant activity of infusions (a) and decoctions (b) of *Borago officinalis* (B.off), *Foeniculum vulgare* (F.vulg), *Hypericum perforatum* (H.perf) and *Sambucus nigra* (S.nig) determined by spectrophotometric analysis. Fl: flowers; le: leaves; st: stems; AA: ascorbic acid, DW: dry weight. Star symbol (*) indicates statistically significant differences ($p < 0.05$, t test) among not digested and digested samples. Data are the mean $\pm$ SD ($n = 3$).

Thus, the higher or lower antioxidant capacity after simulated *in vitro* digestion depends on the partial phenolic compound hydrolysis and/or modification that generates new compounds exposing a higher or lower number of hydroxyl groups responsible for the antioxidant activity. To demonstrate the close correlation between total amount of phenols and antioxidant activity, the correlation index was calculated by using Microsoft Excel (Microsoft 365 MSO; 2307 version, Build 16.0.16626.20170), indicating a high correlation, exactly $R^2 = 0.896$ for not digested and $R^2 = 0.787$ for digested samples.

4.3.2. *H. perforatum* Phenols Characterization by HPLC-DAD

HP digested and not digested samples were selected for the evaluation of the detailed phenolic composition by means of HPLC-DAD characterization (Fig. 4.5). Similar analysis for *Borago officinalis* samples is actually still in progress and due to lack of time could not yet be here reported.

A total of three flavanols (catechin, epicatechin and epigallocatechin), one hydroxycinnamic acid (caffeic acid) and one flavonol (rutin) were identified in infusions and decoctions of HP from the three regions (Fig. 4.5). Very close to the rutin peak (RUT), a second peak with the same spectrum and maximum absorbance wavelength (365 nm) of rutin, was detected in all the extracts, and on the basis of these characteristics it was identified as a rutin structural isomer (RUT2) (Fig. S4.1).

Epicatechin, caffeic acid and rutin were detected in all plant organs, both in infusions and decoctions, whereas catechin was detected only in flowers decoction and epigallocatechin in infusions and decoctions of flowers and stems. Flowers and leaves resulted to have higher contents of phenolic compounds respect to stems, and these results match with those observed in another study on *Rumex maderensis* Lowe, a wild food plant traditionally used in the treatment of several diseases [39].

In general, rutin resulted to be the phenolic compound with the highest concentration in the analysed extracts (51.9% on average in not digested samples and 58.0% on average in digested, Fig. 4.5). Rutin is a flavonoid with a wide range of biological activities [61], well studied for its neuroprotective properties (for instance on cerebral ischemia, Parkinson and Alzheimer's diseases) [62], therefore the presence of a rutin high concentration could be correlated with the reported protective action of HP extracts on the nervous system [62].

Catechin and epicatechin were already previously detected in aerial parts of HP [63], and studies on phenolic contents of *H. perforatum* showed that caffeic acid [64], rutin [65], catechin and epicatechin [66] are most abundant compounds present in the aerial parts of this plant.

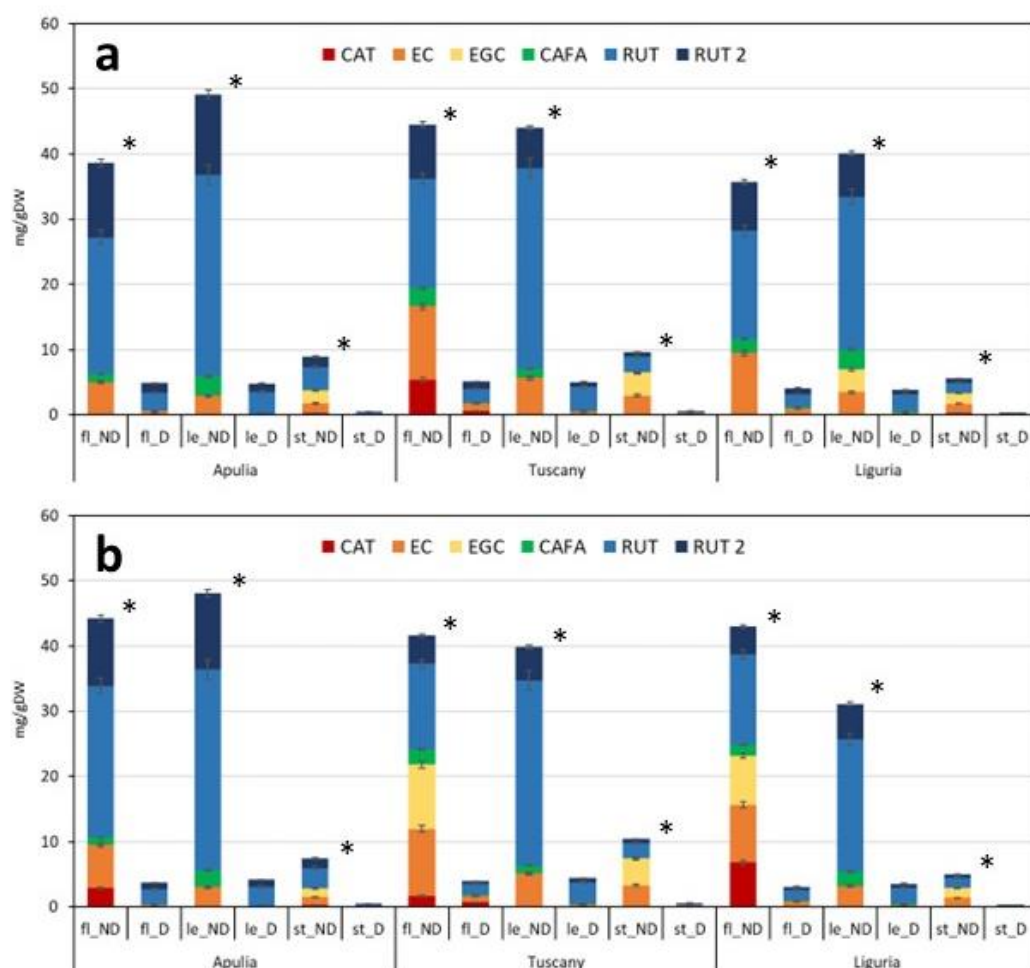


Figure 4.5. Phenols characterisation of *H. perforatum* infusions (a) and decoctions (b) by HPLC-DAD. Fl: flowers; le: leaves; st: stems; ND: not digested; D: digested; CAT: catechin; EC: epicatechin; EGC: epigallocatechin; CAFA: caffeic acid; RUT: rutin; RUT2: rutin2; DW: dry weight. Star symbol (*) indicates statistically significant differences ($p < 0.05$, t test) among not digested and digested samples. Data are the mean $\pm$ SD ($n = 3$).

The concentration of all phenolic compounds significantly decreased ($p < 0.05$) after exposure to simulated oro-gastro-intestinal digestion (Fig. 4.5), in accordance with results obtained by spectrophotometric total phenols quantification assay (Fig. 4.1). In the extracts after digestion, rutin concentration resulted to be on average 11.1% lower than in not digested samples, and caffeic acid was present only in traces (from 0 to 0.26 mg/gDW in infusions and between 0 and 0.22 mg/gDW in decoctions, (Figs. 4.5 a,b). This loss of phenolic compounds after oro-gastro-

intestinal digestion is supported by several studies [19, 67], and, as previously stated, is caused by their degradation or transformation during the process [68].

4.3.3. Chemical Characterisation by Nuclear Magnetic Resonance (NMR)

During a 4-months work period at the laboratory of ITQB NOVA (Instituto de Tecnologia Quimica e Biologica Antonio Xavier), Oeiras, Portugal, given the short time available only samples of *Borago officinalis* and *Hypericum perforatum* were selected and chemically characterized using NMR (Fig. 4.6), in order to obtain a snapshot of the compositional functional groups in the extracts of the two species and insights of structural related features such as bonds between molecules, with the aim of verifying any differences between species and not digested and digestates.

The acquired ^1H NMR spectra were processed using the statistical tool Rnmr 1D package to categorize the extracts based on the degree of similarity (RStudio version 1.4.1106 with Rnmr1D package). In Fig. 4.6a as example ^1H NMR spectra of HP flower infusions and decoctions before and after digestion are shown: the blue square part represents aromatic compounds region, the green square part proteins region and the red square part aliphatic compounds region. In the case of *Hypericum* species, aromatic compounds can be hypericin, phenolic acids and flavonoids [69], and aliphatics can be hyperforin, fatty acids, terpenes and terpenoids [70]. As regards *Borago* species, aromatics can be phenolic and flavonoid compounds [71], and aliphatic compounds can be fatty acids and volatile compounds as aliphatic alcohol and terpenes [72, 73]. The dispersity displayed in the PCA graph (Fig. 4.6b) comparing both HP and BO extracts shows that the main difference between the samples is due to the digestion process that increases the similarity between the samples. The samples are grouped according to plant organ rather than according to extraction method within each species with no clear differences respect to how infusion or decoction impacts on the chemical profile. These results differ from a previous study, which findings showed that infusion and decoction of ten analysed herbs were discriminated according to the different preparation methods, infusion and decoction [74].

Undigested HP samples are arranged in 2 groups, one containing the flowers and leaves (HP_fl and HP_le) and the other the stems (HP_st), leading to conclude that in this species the extracts from flowers and leaves are more similar to each other respect to stem extracts. When analysing the undigested BO extracts, the flowers (BO_fl), leaves (BO_le) and stems (BO_st) samples are scattered in the graph (Fig. 4.6b). These findings match those observed in earlier studies on

traditional medicinal plants in which different plant organs of *Lycium barbarum* L. [75] and *Peganum harmala* L. [76] showed different spectra.

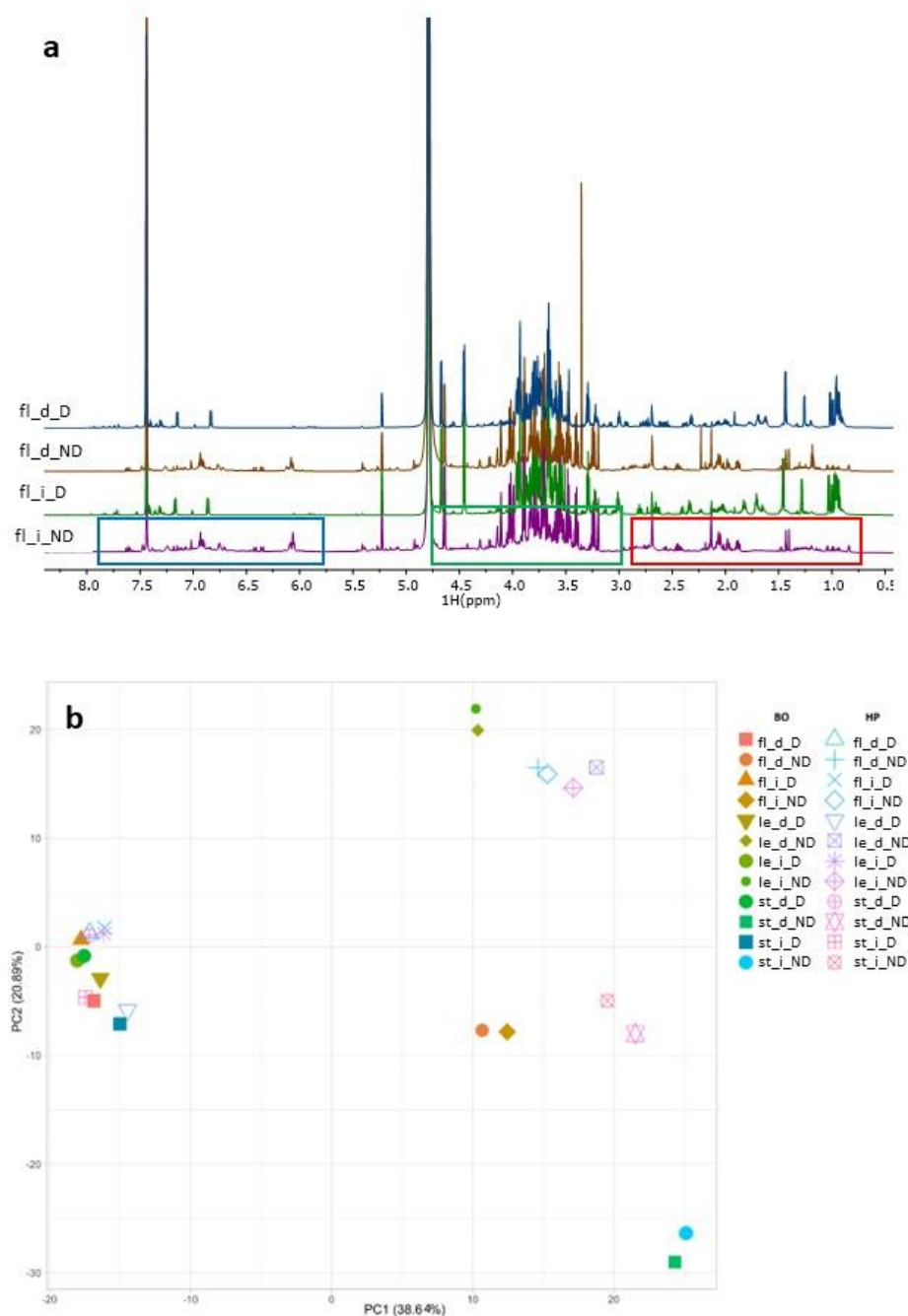


Figure 4.6. (a) Examples of ^1H NMR spectra obtained from *H. perforatum* flowers (fl). d: decoction; i: infusion; ND: not digested; D: digested. (b) Principal component analysis (PCA) of all *H. perforatum* and *B. officinalis* NMR spectra (Rnmr1D package). d: decoction; i: infusion; ND: not digested; D: digested; fl: flowers; le: leaves; st: stems.

4.3.4. Antimicrobial Activities

Given their traditional use as topical application against skin infection and wounds, the not digested infusions and decoctions of HP and BO were also tested for their broad antimicrobial activity.

An initial extract stock concentration of each plant extract of 15 mg/ml was initially tested. Two model bacteria were selected: *S. aureus*, a gram-positive, and *E. coli*, a gram-negative. *E. coli* is a harmless microorganism inhabitant of the gastrointestinal tract, but outside the intestine is a pathogenic bacteria that can cause urinary tract and skin infections, meningitis, bloodstream infections, etc. [77, 78]. *S. aureus* is one of the major causes of skin infections, both mild infections as furuncles, abscesses and wound infections, and invasive infections as respiratory tract and cardiovascular infections [79, 80].

By definition, the minimum inhibitory concentration (MIC) is the lowest concentration of a compound inhibiting the growth of a given strain of bacteria [81]. In this work, the MIC was also defined (Table 4.2).

Table 4.2. Minimum inhibitory concentration (MIC) values (mg/ml) of *H. perforatum* and *B. officinalis* plant not digested extracts against *E. coli* and *S. aureus* cells. MIC values identified as >15 indicate plants extracts without observed bacterial activity in the tested concentration range. Concentration at which >70% of bacterial growth was inhibited. fl: flowers; le: leaves; st: stems; i: infusion; d: decoction.

Sample	<i>H. perforatum</i> MIC (mg/ml)		<i>B. officinalis</i> MIC (mg/ml)	
	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>
fl_i	7.5	1.88	0.94	15
fl_d	0.94	0.94	0.47	15
le_i	0.94	0.94	0.94	15
le_d	>15	0.94	3.75	>15
st_i	-	-	7.5	>15
st_d	-	-	1.88	>15

Overall HP extracts exhibited activity against both gram-positive and gram-negative bacteria, while BO samples were more active against the gram-negative *E. coli* at tested concentrations. Decoctions of HP flowers (fl_d, 0.94 mg/ml) and decoctions and infusions of BO flowers and leaves (fl_d, 0.47 mg/ml; le_i, 0.94 mg/ml) were the most effective against *E. coli*. HP le_i (0.94

mg/ml) and fl_d (0.94 mg/ml) were the most effective against *S. aureus* (Table 4.2). No clear differences were detected between antimicrobial activity of infusion or decoction samples in agreement with the spectrophotometric data that showed no difference of these extraction processes on the content and quality of bioactive compounds (Fig. 4.1).

Additionally, after 24h all samples were subjected to the MTT assay, to evaluate the metabolic activity of the bacterial cells exposed to the plant extracts (Table 4.3). MTT is a reagent, yellow tetrazole, which when reduced is transformed to purple formazan in living cells: cells with a low metabolism reduce low quantity of MTT, while rapidly dividing cells exhibit high rates of MTT reduction. Low metabolism means no cells viability or high level of stressed cells.

Table 4.3. Percentage of growth inhibition after the optical density (OD) measurement and after the thiazolyl blue tetrazolium bromide reagent addition (MTT). *indicates data from only one replicate; n.c.: not correlated. fl: flowers; le: leaves; st: stems; i: infusion; d: decoction.

	<i>H. perforatum</i> MIC (mg/ml)		<i>B. officinalis</i> MIC (mg/ml)	
Sample	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>
fl_i	OD: 75.6 ± 7.7; MTT: 88.4 ± 17.2	OD: 118.7 ± *; MTT: 102.3 ± *	OD: 71.2 ± 1.1; MTT: n.c.	OD: 117.6 ± 3.9; MTT: 97.1 ± 13.1
fl_d	OD: 80.3 ± 7.8; MTT: 66.3 ± 5.2	OD: 122.0 ± *; MTT: 94.3 ± *	OD: 82.2 ± 13.1; MTT: 72.6 ± 52.0	OD: n.c.; MTT: 91.3 ± 1.1
le_i	OD: 71.1 ± 3.9; MTT: 79.7 ± 9.5	OD: 120.4 ± *; MTT: 107.2 ± *	OD: 67.4 ± 0.5; MTT: n.c.	OD: 88.7 ± 6.2; MTT: 92.7 ± 8.1
le_d	-	OD: 89.3 ± 47.9; MTT: 61.4 ± 48.0	OD: 74.6 ± 4.2; MTT: 81.8 ± 3.7	-
st_i	-	-	OD: 64.5 ± 6.0; MTT: 83.0 ± 3.0	-
st_d	-	-	OD: 72.3 ± 5.0; MTT: 51.0 ± 4.8	-

MTT data correlate with the OD measurements (Table 4.3), confirming the MIC previously analysed of all the extracts.

The samples giving the lowest MIC values were then selected to perform a viability assay (Fig. 4.7) by plating and counting the surviving cells capable of forming colonies (CFU, colony forming units) in solid media at that specific concentration previously detected (Table 4.2).

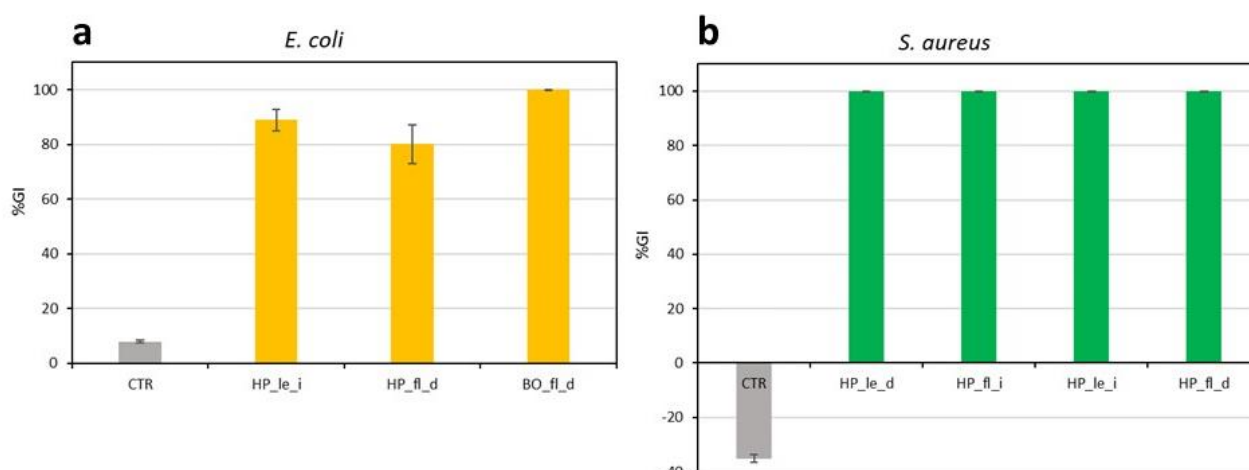


Figure 4.7. *H. perforatum* (HP) and *B. officinalis* (BO) not digested infusion and decoction extracts bactericidal activity against *E. coli* (a) and *S. aureus* (b) presented as the growth inhibition (GI %) relative to the corresponding control. The error bar represents the standard deviation between three replicates. CTR: control; le: leaves; fl: flowers; i: infusion; d: decoction.

Not digested infusion of HP leaves and decoction of HP flowers (HP_le_i; HP_fl_d) and not digested decoction of BO flowers (BO_fl_d) resulted to be the most active against *E. coli* (from 80% to 100% inhibition of bacteria growth compared to the control) (Fig. 4.7a). Not digested infusion and decoction of HP flowers and leaves (HP_le_d, HP_fl_i, HP_le_i, HP_fl_d) gave 100% inhibition of *S. aureus* bacteria growth compared to the control (Fig. 4.7b). These results are in accordance with the MIC and MTT assays (Tables 4.2 and 4.3). The controls tested presented low inhibition activity (7.9% GI, Fig.4.7a) in *E. coli*, and no inhibition activity in *S. aureus* (-35.12% GI, Fig. 4.7b), confirming the antimicrobial activity of the extracts.

Even though these results differ from previous studies in which HP extracts showed activity against *S. aureus* but not against *E. coli* [82, 83], they are consistent with others which found HP extracts having activity against both bacteria [84]. The high antimicrobial activity of HP could be due to the presence of hyperforin and hypericin, together with other phenolic compounds as flavonoids and xanthones [85]. Regarding BO, in accordance with the present results, previous studies have demonstrated that water extracts showed higher antimicrobial activity against gram-negative respect to gram-positive [71]. The antibacterial potential of BO extracts could be related to the presence of fatty acids, essential oil, flavonoids and other polyphenols [86].

4.4. Conclusions

Food and medicine of plant origin are valuable sources of many bioactive components, but the determination of the bioactivities of these phytochemicals in raw plant extracts is not sufficient for the prediction of their potential *in vivo* effects once ingested, because of the intensive metabolic modifications that they face in the oro-gastrointestinal tract. An evaluation of the influence of the digestion process on the phytochemical bioaccessibility is essential to better understand the real effects of herbal preparations on human health. In this study, the impact of the digestion process on total amount of phenols, reducing sugars and proteins, as well as their antioxidant and antimicrobial activities, was evaluated before and after digestion by means of an *in vitro* simulated human digestion protocol. In summary, results showed that phenols bioaccessibility and antioxidant activity in general decrease, while reducing sugars and proteins generally increase after digestion. Despite the decrease of antioxidant activity after digestion, these herbal preparations nonetheless showed a remarkable activity and, after having reached the colon, may contribute to protect the gastro-intestinal tract from oxidative damage.

In addition, *H. perforatum* and *B. officinalis* not digested infusion and decoction extracts showed high antimicrobial activity against the tested gram positive and negative bacteria, confirming the potential of these plants as natural antimicrobials that could be effective for topical use against skin infection and wounds but also as alternatives against multi-drug resistant bacteria. Further identification and quantification of phytochemicals is needed to narrow down likely candidates for the bioactivity against both bacteria.

To the best of our knowledge, this is the first study so far analysing the phytochemical composition of different medicinal preparations (infusions and decoctions) obtained from several plant organs of seven wild alimurgic plant species collected in three different Italian regions. In conclusion studied herbal preparations, provide important amounts of bioactive and nutritive compounds which may be part of a healthy diet and may contribute, among other actions, to prevent or decrease oxidative stress-related disorders, and/or be used to treat several general or topical diseases. Further studies are needed to fully understand and characterize the activity of these plant extracts, their biochemical profiles and possible therapeutic activities.

Author Contributions

Methodology: M.S., E.R., C.V.G., C.A.; material preparation: M.S., E.R., C.V.G., C.A.; data collection and analysis: M.S., E.R., B.A.; writing—original draft preparation: S.M.; conceptualization: M.S., E.R., C.V.G., C.A., P.C.S., A.T.; writing—review and editing: M.S., E.R., A.T.; supervision: A.T. All authors have read and agreed to the published version of the manuscript.

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Supplementary Materials

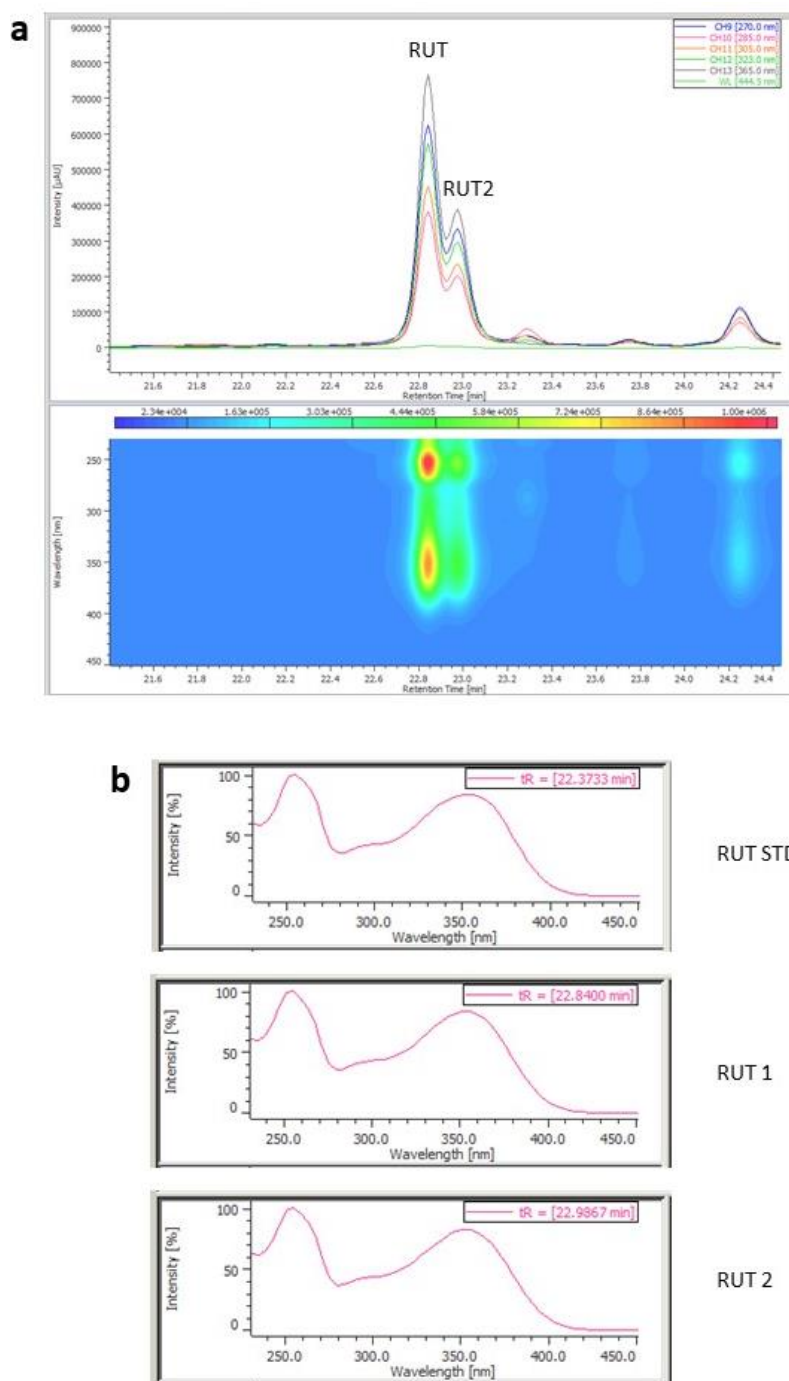


Figure S4.1. Example of chromatogram obtained from *H. perforatum* flowers infusion. A) Focus on rutin peaks (RUT and RUT2); B) spectra of rutin standard (RUT STD), rutin (RUT1) and rutin isomer (RUT2).

Chapter 5

Anti-tyrosinase, anti-inflammatory activities and hormesis effect of infusions and decoctions of Italian alimurgic plant species

*Preliminary biological activity results on extracts of selected plant species organs. Analysis on a larger number of plant extracts than those here presented (different selected organs and species) are still on-going to demonstrate the potential hormesis effect of extracts. Data will be organised in a manuscript having following authors: Monari S., *Calabretta M.M., *Michelini E., Tassoni A.*

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5.1. Introduction

Alimurgic plants are known to be used for centuries for several purposes, as food, feed and medicine, mainly in period of poverty and wars [1, 2]. Nowadays the renewed interest in alimurgic species led to a broad knowledge of their phytochemical compounds, which are responsible for their numerous biological activities [3]. In the present research few alimurgic species (*Borago officinalis* L., *Foeniculum vulgare* Mill., *Hypericum perforatum* L. and *Sambucus nigra* L.) were selected, collected and their infusions and decoctions (not digested and digested by *in vitro* oro-gastro-intestinal digestion process) were analysed for their biochemical profile (Chapters 3 and 4). In Italian folk medicine, infusions and decoctions represent the most used plant medicinal preparations [4], with both external and internal medical applications. During infusion the plant constituents are extracted by letting the material float in the solvent, mostly water over time; during decoction extraction process the plant material is boiled with the solvent [5].

Borago officinalis L., commonly known as borage, is a perennial herb widely use in traditional medicine for respiratory ailments, as antihypertensive, nerve and cardiac tonic, depurative, diuretic, antistress, blood cleanser, with antioxidant, anti-inflammatory, anti-diabetic, antibacterial properties, etc. [6]. *Foeniculum vulgare* Mill., fennel, is a wild edible plant highly

used as flavouring and known in folk medicine for its diuretic and digestive properties, and its extracts had proven antioxidant, anti-inflammatory, anti-diabetic and hepatoprotective properties [7]. *Hypericum perforatum* L., popularly called St. John's wort, is a spontaneous perennial herb common all over the world, known for its antidepressant activity but also widely used for external (against wounds, sunburns, haemorrhoids, rheumatism, cramps, etc.) and internal (as astringent and diuretic) applications, with proven anti-inflammatory, antimicrobial and anticancer activities [8]. *Sambucus nigra* L., also called elder, is a deciduous shrub widely used in food and beverage flavouring and in folk medicine, with known biological activities as antioxidant, anti-inflammatory, anticancer, antimicrobial, antidiabetic, cardiovascular protective, neuroprotective activities [9].

Among the most considered biological activities of wild food plants there are anti-tyrosinase and anti-inflammatory activity.

Tyrosinase is the key enzyme in melanin biosynthesis, widely distributed in both plant and animal cells. Melanin formation, involved in determining the colour of skin and hair in mammals, can be a dangerous process which can lead to dermatological diseases (freckles, melisma, age spots, post-inflammatory hyperpigmentation and melanoma) [10] and is also responsible for fruits and vegetables browning [11]. For these reasons, preparations with tyrosinase inhibition action became very important to contrast collateral effects of the overproduction of melanin, both in cosmetic and nutraceutical fields.

Inflammation is a tissue response to injury which might be caused by a physical trauma, by a microorganism or by a disease [12]. During inflammation oxidative reaction damage is generated and a prolonged inflammatory process can lead to chronic inflammation which causes several diseases as arthritis, bronchitis, pancreatitis, cardiovascular diseases, neurodegenerative disorders and cancer [13]. A wide range of plant molecules show anti-inflammatory activity [14] and thus it is important to investigate the presence of this activity in view of the discovery of new plant nutraceutical products and medicinal preparations.

Another important effect of plant metabolites on humans and other heterotrophic organisms is the hormesis effect. Plants, as sessile organisms, cannot physically escape from environmental biotic and abiotic stressors, therefore have evolved a complex stress response system to face environmental challenges which also includes the production of several secondary metabolites. Since animals (including humans) are highly dependent on plants for their survival, they have co-evolved with plants and are able to somehow react and adapt to the plant phytochemicals, so

that most of the known health beneficial effects of wild edible plants derive from pharmacologically active substances that plants produce under stress conditions [15].

To describe this process the term *hormesis* was coined, referring to a non-linear, biphasic biological response where the exposure to a low dose of the stressor (environmental factor or chemical compound) induces an adaptative beneficial effect, while the same stressor exerts a toxic and damaging effect at higher doses [16] (Fig. 5.1).

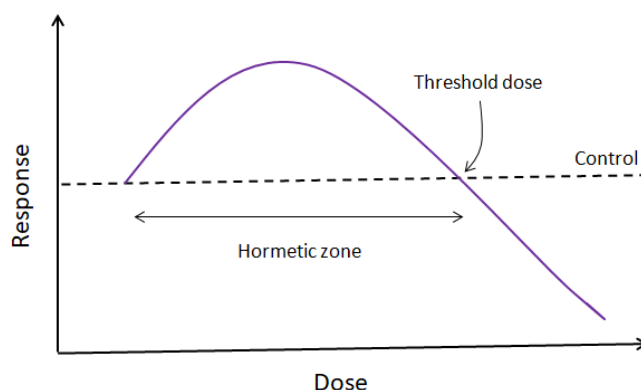


Figure 5.1. Hormetic dose-response curve (from [17]).

When the stress occurs in a species (e.g., autotrophic plant), with consequent production of secondary metabolites, and the beneficiaries include organisms of other species that eat the stressed plant (e.g., heterotrophic), this interspecies hormesis is called *xenohormesis* [18].

Resveratrol, a phenol synthesized by plants against UV and pathogen stress, is one of the phytochemicals found to stimulate *xenohormetic* effects, by activating sirtuine enzymes (highly conserved signalling proteins involved in cellular processes as aging, transcription and apoptosis) in yeast and mammals [19]. Nuclear factor erythroid 2 (Nrf2) is a transcription factor representing the primary cellular defence against the cytotoxic effects of oxidative stress [20]. It regulates genes with cytoprotective functions, and has hormetic effects given that its normal activation provokes a moderate stress response with health benefits and prolonged lifespan in animals, but excessive long-term stimulation can be pathological for the organism [21]. Many dietary phytochemicals can stimulate the Nrf2 signalling pathway [22] and have proven hormetic capacities, for example flavonoids, phytoalexins [23], phenols [16], the extravergin olive oil polyphenols namely secoiridoids (e.g. oleuropein) [24] and ferulic acid [25].

Another example is the NF- κ B (nuclear factor κ B) inflammation pathway, closely link to Nrf2 pathway [22], where NF- κ B is a transcription factor that regulates genes involved in several

cellular processes as inflammation, proliferation, immune response and apoptosis [26]. The activation of NF- κ B is regulated by the binding protein I κ B α , and phytochemicals (such as curcumin and resveratrol [27]) that inhibit the phosphorylation of I κ B α leading to a physiological anti-inflammatory action [28, 29].

These and many other examples of xenohormesis effects on mammals of plant phytochemicals demonstrate that herbs, spices and wild food plants can be exploited also in the field of nutrition and medicine. Martucci and colleagues [30] proposed that the Mediterranean diet, rich in natural compounds as vitamins, minerals and phytochemicals (called in this contest *hormetins*), could be ascribed to a moderate activation of stress-response mechanisms stimulated by low-dose constant exposure to these hormetins.

Together with nutraceutical uses, hormetic compounds can be used to contrast many chronic diseases as antiaging by acting in prenatal and postnatal life [31], for anticancer drug discovery in combined therapies [32], and also against endocrine, nervous and immune systems diseases [33].

In the present Chapter, anti-tyrosinase and anti-inflammatory activities and possible implications on xenohormesis effects of infusions and decoctions of selected alimurgic species were assessed in order to demonstrate their efficacy in topical application against skin problems, or, to a lesser extent, after ingestion as food components.

5.2. Materials and Methods

5.2.1. Plant Materials

The same samples selected and analysed in Chapter 4 (flowers, leaves and stems of *B. officinalis* and *H. perforatum*, stems of *F. vulgare*, and flowers and leaves of *S. nigra*) (Figs. 4.1, 4.2, 4.3) were selected and their water extraction as infusions and decoctions were analysed before and after *in vitro* oro-gastrointestinal digestion for their anti-tyrosinase activity.

Not digested and digested infusions and decoctions of *Sambucus nigra* L. samples from Apulia region previously analysed in Chapter 4 for their biochemical profile (Figs. 4.1, 4.2, 4.3) were selected to assess anti-inflammatory activity.

5.2.2. Anti-tyrosinase Activity

Anti-tyrosinase activity was assessed by an optimised tyrosinase inhibition assay [34]. Tyrosinase from mushroom (10U) as enzyme, and L-DOPA (3,4-dihydroxy-L-phenylalanine, 2mM) as substrate were purchased from Sigma Aldrich (St. Louis, MO, USA) and used in this experiment. The kinetic of brown colour formation was evaluated in a 96-well plate (490 nm absorbance measurement, VersaMax Microplate reader) in a reaction containing 10 U of tyrosinase and 2 mM L-DOPA in the presence of 40µl of the sample. The enzyme activity was monitored every 30 seconds in 10 minutes, at 30°C. The results were expressed as mg of kojic acid (KA, a well-known tyrosinase inhibitor) equivalents/ gDW by means of a dose-response calibration curve (between 0 and 400 µM of KA).

5.2.3. Anti-inflammatory Activity

A bioluminescent cell-based assay for anti-inflammatory activity was performed using human embryonic kidney HEK293 cells ATCC (American Type Culture Collection, Manassas, VA, USA) routinely grown in Dulbecco modified essential medium (DMEM high glucose 4.5 g/L, GE Healthcare) supplemented with 10% (v/v) foetal bovine serum, 2 mM L-glutamine, 50 U/µL penicillin, and 50 µg/mL streptomycin. HEK293 cells were plated in 96-well plates the day before transfection at a density of 2×10^4 cells/100 µL of growth medium per well.

Transient transfections were performed with plasmid pGL4.32 [luc2P/NFκB-RE/Hygro] containing five copies of the NFκB response element (NFκB-RE) driving transcription of the luc2P reporter protein (Promega, Madison, WI) by using FuGENE®HD (Promega) according to the manufacturer's instructions (Fig. 5.2a). The luciferase (Luc) is placed under the control of NFκB response elements in the plasmid pGL4.32: in such configuration, the binding of TNF-α (tumour necrosis factor) to its specific receptor (TNFR) activates the intracellular inflammatory pathway which leads to the expression of luciferase.

During transfection, cells were incubated for 24 h at 37 °C with 5% CO₂. Then, the medium was changed, and cells were treated in triplicate with 30 µL of TNFα (tumour necrosis factor) solutions in culture medium (concentration range 0.1–20 ng/mL) or with 30 µL of culture medium as a control, to obtain the calibration curve. In addition, co-incubation for 5 h with fresh medium containing real samples and 1 ng/mL TNFα (Sigma Aldrich, Saint Louis, Missouri, USA) were performed to evaluate (anti)-inflammatory activity of vegetable extracts. Bioluminescent

(BL) measurements were performed with a conventional luminometer (Varioskan™ Flash Multimode Reader) acquiring BL signals after injection of 100 µL D-Luciferin substrate (Fig. 5.2b).

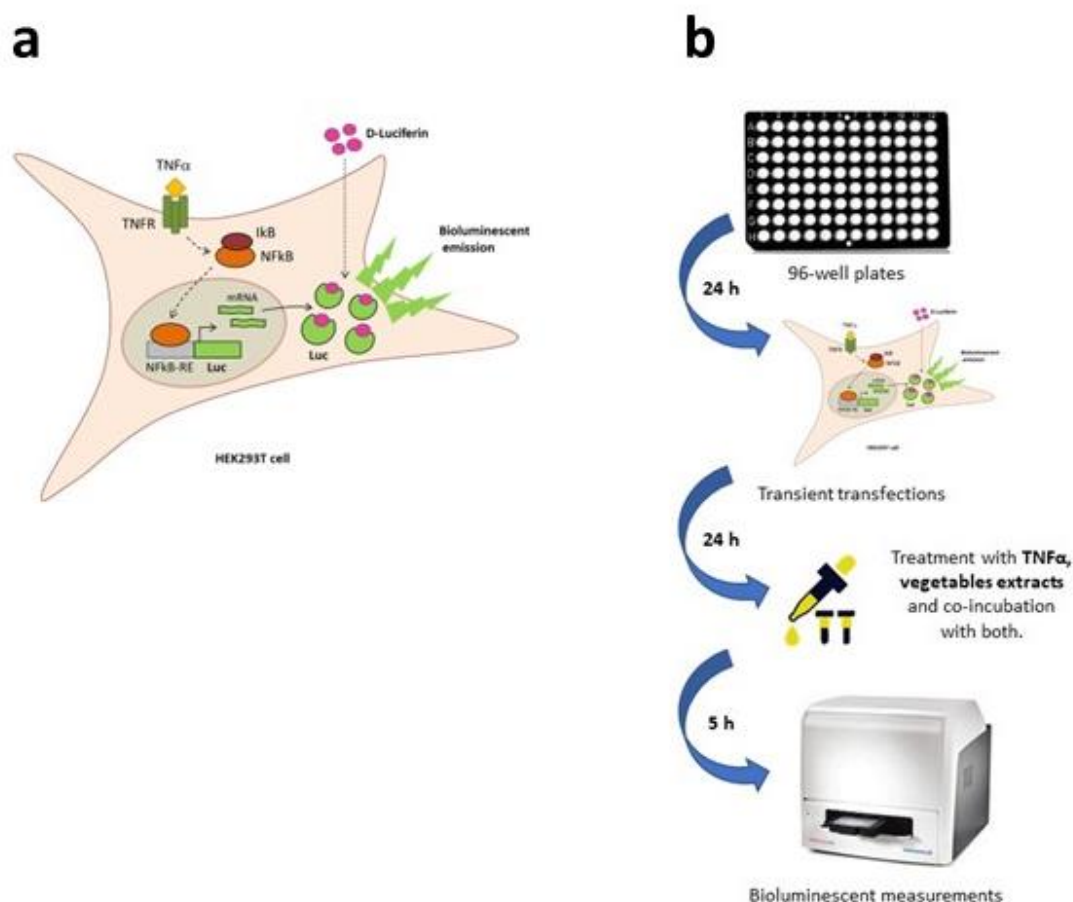


Figure 5.2. a) Schematic representation of the cell biosensor developed for the evaluation of anti-inflammatory activity. Hek293T cells were engineered with a reporter construct (pGL4.32) containing five copies of NFκB-RE and the Luc luciferase as reporter. The binding of TNF-α to the TNFR activates the intracellular signalling pathway which leads to the expression of Luc luciferase; b) Scheme of transient transfection process. HEK: human embryonic kidney; Luc: luciferase; NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells; NFκB-RE: nuclear factor kappa-light-chain-enhancer of activated B cells response elements; TNFα: tumour necrosis factor; TNFR: tumour necrosis factor receptor; IκB: inhibitor of nuclear factor kappa B.

5.2.4. Toxicity Assay

Toxicity assay was performed using a Microtox® assay [35]. *Aliivibrio fischeri* strain, a naturally bioluminescent non-pathogenic bacteria, was used to quantitatively assess sample toxicity. The light emitted reduction is directly related to the relative toxicity of the sample.

In particular, *A. fischeri* strain was cultured at 19 °C, with orbital shaking at 200 rpm, in LB Broth Miller (Luria-Bertani broth, Miller) medium with high salinity (10 g/L peptone, 30 g/L NaCl, 5 g/L

yeast extract), without antibiotic selection. To perform the toxicity assay a volume of 100 μ L of *A. fischeri* (OD600 = 2.0) was seeded in a black 96 well-plate. Then a volume of 10 μ L of sample solutions were dispensed in bacterial suspension while 10 μ L of bidistilled water or black of digestion (only enzymes) were used as control. Bioluminescence signals were acquired after 15 min and 4 h incubation at room temperature with the Varioskan™ LUX multimode microplate reader. All measurements were performed in triplicate.

5.2.5. Statistical Analyses

All spectrophotometric analyses were performed using 3 biological replicates and analysed in 4 technical replicates. The results were expressed as the mean ($n=3$) $\pm$ standard deviation (SD) per g of dry weight (DW). The differences of anti-inflammatory activity and toxicity between digested and not digested infusions/decoctions obtained from different plant organs were evaluated by *t*-test at $p<0.05$ significance level. All statistical analyses were performed using R software version 3.5.1 (R Core Team, Vienna, Austria) (<https://cran.r-project.org>).

5.3. Results and Discussion

5.3.1. Anti-tyrosinase Activity

The anti-tyrosinase activity results on infusions and decoctions before and after *in vitro* orogastrointestinal digestion are showed in Fig. 5.3.

All the plant extracts, both not digested and digested, showed anti-tyrosinase activity. For infusion samples, the activity ranged between 0.35 mg KAeq/gDW (not digested flowers of *S. nigra* from Liguria) and 0.58 mg KAeq/gDW (not digested flowers of *S. nigra* from Apulia) (Fig. 5.3a), and for decoctions between 0.31 mg KAeq/gDW (digestates of *S. nigra* flowers from Liguria) and 0.87 mg KAeq/gDW (not digested flowers of *S. nigra* from Apulia) (Fig. 5.3b).

There is not a specific trend detectable in results obtained on digested samples, nevertheless most of the extracts showed a statistically difference ($p<0.05$) between samples before and after digestion, with some slightly increasing and others slightly decreasing after digestion in comparison to samples not digested.

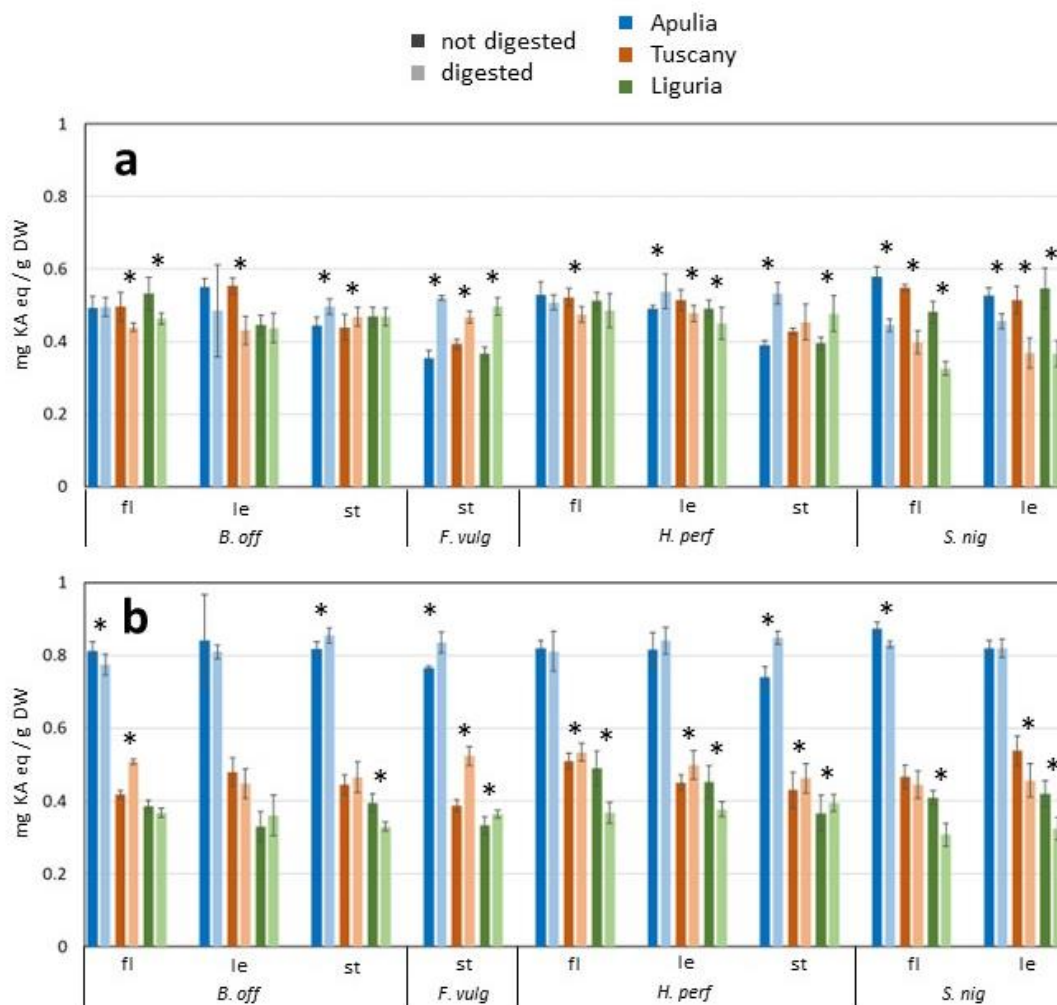


Figure 5.3. Anti-tyrosinase activity of infusions (a) and decoctions (b) samples of *Borago officinalis* (B.off), *Foeniculum vulgare* (F.vulg), *Hypericum perforatum* (H.perf) and *Sambucus nigra* (S.nig) detected by spectrophotometric analysis. Fl: flowers; le: leaves; st: stems; KA: kojic acid, DW: dry weight. Star symbol (*) indicates statistically significant differences ($p < 0.05$, t-test) among not digested and digested samples. Data are the mean $\pm$ SD ($n = 3$).

Given the results obtained in Chapter 4, it could be hypothesised that the anti-tyrosinase activity showed by not digested and digested can be ascribed to different types of active compounds. For not digested samples activity could be due to the high concentrations of polyphenols in the former case (see chapter 4, Fig. 4.1), while for digested samples anti-tyrosinase capacity could be related to high concentrations of bioactive peptides produced from integral proteins during digestion (see Chapter 4, Fig. 4.3). It is in fact well known that natural phenolic compounds have tyrosinase inhibition properties [36, 37], and constituents of these plant extracts have depigmenting properties resulting from the inhibition of tyrosinase activity, such as rutin and caffeic acid [38] which were also here detected in *Hypericum* samples (Chapter 4, Fig. 4.5). Moreover, it is well known that plant proteins are a common source of tyrosinase inhibitory

peptides [39], as reported by Ferri et al. [40] that demonstrated that small peptides, released after enzymatic hydrolysis, show higher anti-tyrosinase activity respect to their native proteins.

5.3.2. Anti-inflammatory Activity

Anti-inflammatory activity of both infusions and decoctions, before and after *in vitro* digestion, of *Sambucus nigra* L. from Apulia was investigated using a cell-based bioluminescent assay (Fig. 5.2) and preliminary results are shown in Fig. 5.4. Due to time constraints, *S. nigra* was preliminarily selected among all analysed species target of this thesis, as it previously resulted the species mostly cited in ethnobotanical studies to be used for anti-inflammatory treatments (Chapter 2, [4]).

The cell-based bioluminescent system employed evaluates the inflammatory response via the NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells) signal transduction pathway, which is induced by several types of stressors and is involved in the regulation of cell-cycle/growth, inflammation, apoptosis, and immunity [41].

Data related to the water control (CTR H₂O Fig. 5.4 a,b), corresponding to the maximum level of cell bioluminescence, report the maximum level of induced active inflammation as only the stressor substance (TNF α) was added to these samples. When stressed cells were co-incubated with aliquots of not digested (ND) or digested (D) infusions and decoctions, the most active extracts (showing the lowest bioluminescent signal corresponding to the highest detected anti-inflammatory activity), were *S. nigra* infusions or decoctions before the digestion (-51.4 and 74.6% of signal decrement in flowers and leaves infusion, and -36.9 and 65.7% of signal decrement in flowers and leaves infusions, respect to the control), followed by the digestates (-44.1 and 41.6% of signal decrement in flowers and leaves infusion, and -41.5 and 39.5% of signal decrement in flowers and leaves infusions, respect to the control) and the digestion control CTR Enz (sample only with enzymes, without plant extracts) (-42.9% of signal decrement respect to the water control). When samples were diluted 1:10 all data resulted to be very similar to each other ($p>0.05$) with much less anti-inflammatory activity and slightly lower than the CTR H₂O (Fig. 5.4), and so showed no activity. No difference was detected between infusion (Fig. 5.4a) and decoction samples (Fig. 5.4b). Among different organs, only between flowers and leaves not digested were detected significantly differences ($p<0.05$) (Fig. 5.4 a,b).

The anti-inflammatory activity of *S. nigra* extracts was well documented. Ho et al. [42] demonstrated the high anti-inflammatory effects of polyphenols present in flowers and fruits

extracts, and Mota and colleagues [43] studied the *in vitro* and *in vivo* anti-inflammatory activity of elder flowers extracted with different methodologies.

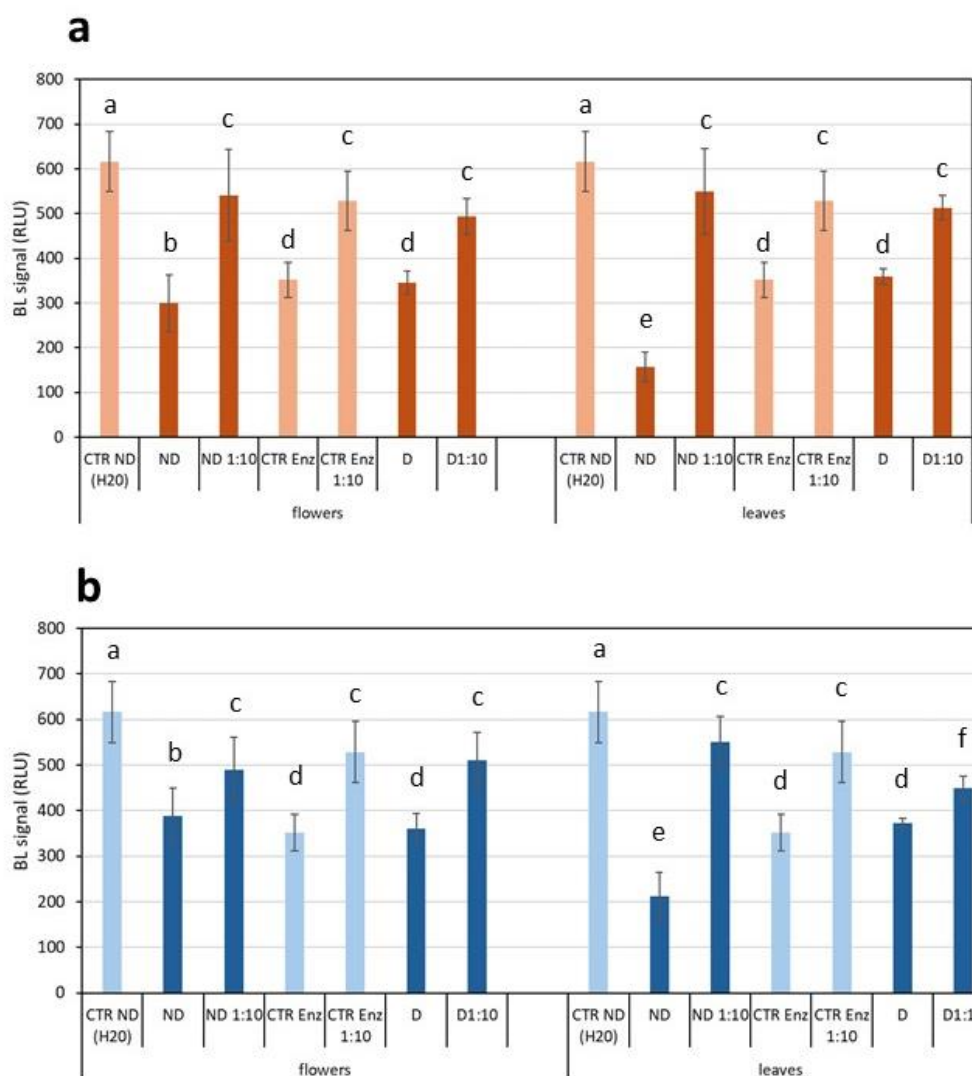


Figure 5.4. Anti-inflammatory activity obtained with HEK293 cells co-transfected with pGL4.32[luc2P/NF-kB-RE/Hygro] and co-incubated for 5 h at 37 °C with *S. nigra* infusions (a) and decoctions (b) in the presence of 1 ng/mL TNF α . BL: bioluminescent signal; RLU: relative light units; CTR H₂O: water control; ND: not digested sample; ND1:10: not digested sample 1:10; CTR Enz: control of digestion (only enzymes); CTR Enz 1:10: control of digestion (only enzymes 1:10); D: digested samples; D1:10: digested samples 1:10. Different letters indicate statistically significant difference (one way ANOVA followed by post hoc two tail Student's t-test, $p < 0.05$) between extracts data (flowers or leaves) and related controls. Data are the mean ($n = 3$) $\pm$ SD.

Two possible explanations are possible for the reported results: i) infusions and decoctions decreased the activation of the expression of the transcription factor causing the inflammation

and so showed an anti-inflammatory activity, or ii) the used human embryonic kidney cells died, with a consequent decrease of BL, due to the toxicity of infusions and decoctions.

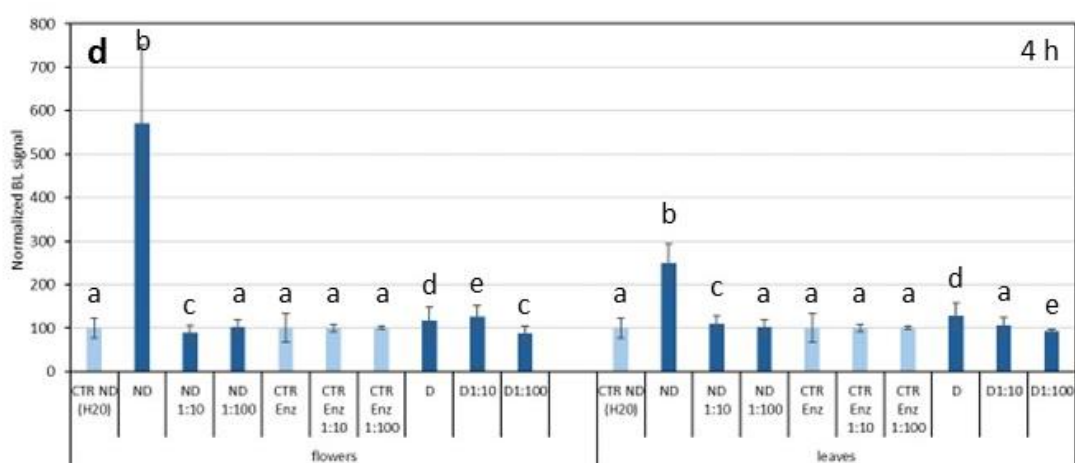
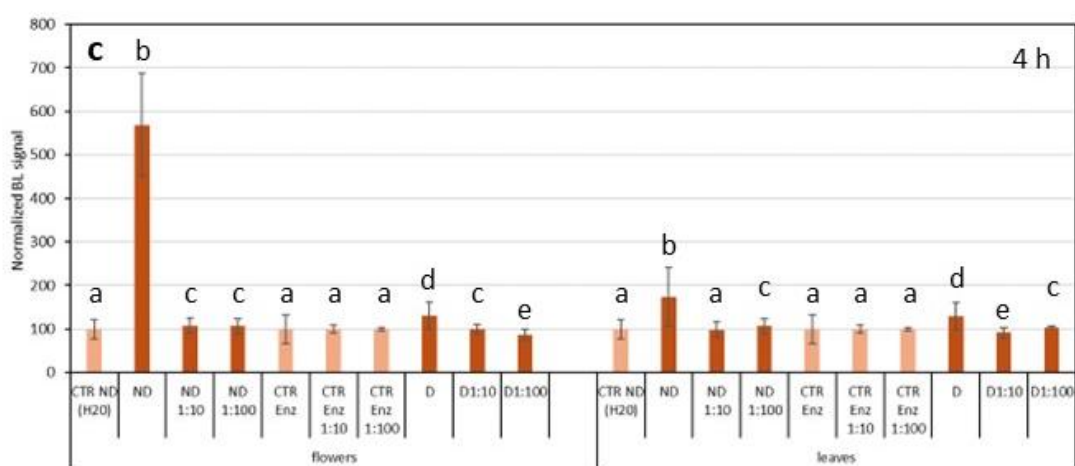
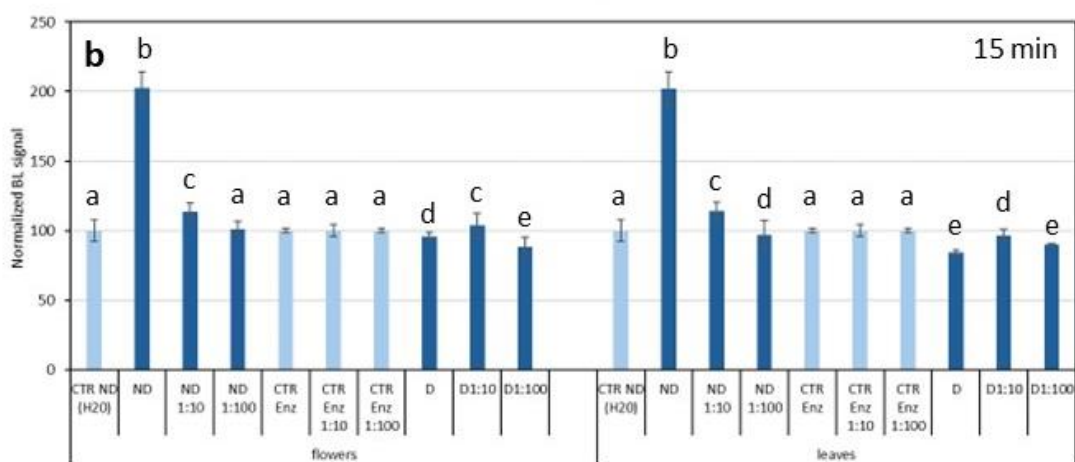
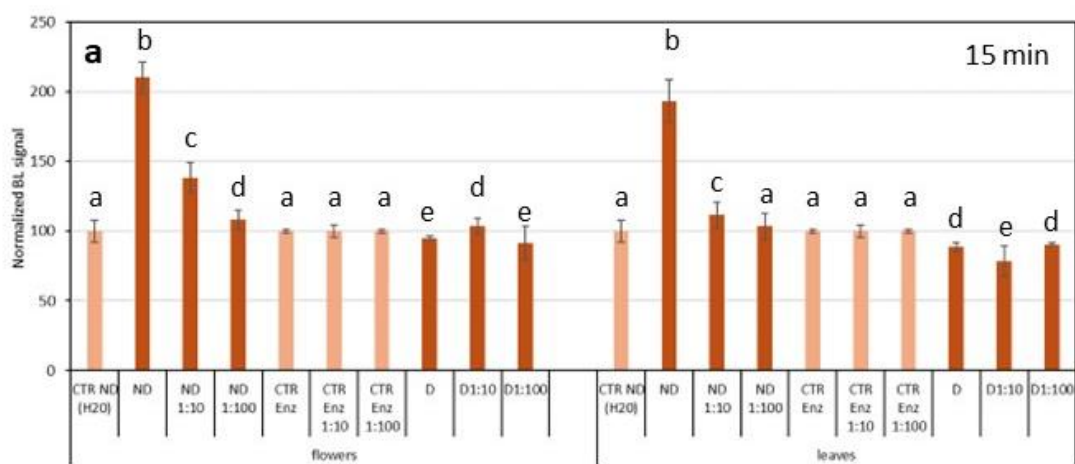


Figure 5.5. Toxicity test after 15 minutes (a,b) and 4 h (c,d) of infusions/decoctions of *S. nigra* before and after digestion. Bioluminescent signal of controls wells was set at 100 to normalize results. Different letters indicate statistically significant difference (one way ANOVA followed by post hoc two tail Student's t-test, $p < 0.05$) between flowers or leaves data and control. Data are the mean ($n=3$) $\pm$ SD.

To better understand which one of the two hypothesis is the right one, the plant extracts' toxicity was evaluated by Microtox® test with *Aliivibrio fischeri*, a naturally bioluminescent non-pathogenic bacterium [44]. This test is based on the intensity of the bacteria bioluminescence signal: strong BL signal indicates healthy bacteria, light BL signal indicates diseased bacteria. The more toxic the sample, the weaker the light emitted.

The results of the toxicity assay are shown in Fig. 5.5. After 15 minutes of incubation with plant extracts it is already noticeable that the bioluminescence signal of not digested samples, both infusion and decoction of flowers and leaves, resulted to be 2-fold higher than water control (CTR ND (H₂O), Fig. 5.5 a,b), and after 4 hours flower infusions and decoctions further increased BL up to 6-fold higher respect to water control (Fig. 5.5 c,d). This increase is probably due to the presence of beneficial substances which are absent in the water control. Infusion and decoction after digestion led to an increase of the signal only after 4 hours, while all the diluted samples showed a signal comparable to the control (Fig. 5.5 c,d). These results demonstrated that the analysed samples have no toxic activities in bacteria cells, thus confirming their anti-inflammatory activity (Fig. 5.4).

The present data could also lead to hypothesise a possible xenohormetic effect of the selected plant extracts. In recent years, the effects of hormetic phenomena on the bioluminescence of *A. fischeri* have been widely studied for a broad range of chemicals [45], and it has been established that the quorum sensing (the communication process by which bacteria can share information about changes in their cell numbers and other environmental factors, leading to the increase of bacteria population) in *A. fischeri* has a close relationship with the hormetic phenomena [46]. So, the consistent increase of the BL signal respect to control due to the addition of not diluted samples, the slight increase of BL due to digested samples, and the values of diluted samples close to those of the control, could indicate a possible induced hormetic dose-response that is time and concentration dependent. A similar effect was already demonstrated on *A. fischeri* by using several different chemicals such as sulfadoxine [47], sulfapyridine [46], sulfonamides [48]. Further analyses on several dilutions of the extracts and on other organs and plant species are ongoing to confirm this hypothesis.

5.4. Conclusions

Alimurgic plants in traditional medicine are widely used in external application to treat skin infections, wounds, burns, and also as cosmetic treatment for acne, skin spots and anti-aging. *B. officinalis*, *F. vulgare*, *H. perforatum* and *S. nigra* infusions and decoctions were selected and analysed to detect their anti-tyrosinase activity. Plant extracts of the selected alimurgic plants showed anti-tyrosinase activities, both before and after digestion, probably due to the presence of phenolic compounds in the former and of bioactive peptides in the latter.

S. nigra extracts were also selected to detect their anti-inflammatory activity and possible hormetic effect. Infusions and decoctions showed activity in their not diluted concentrations and moreover exhibit a possible correlation with hormesis effect on model bacteria metabolism.

Although more investigations are required to evaluate if the reported biological activities are dose-dependent, and if a true hormetic effects was obtained, the present results seem promising and lead us to demonstrate with scientific data the usefulness of using plant preparations against skin infections and injuries.

Author Contributions

Methodology: M.S. and C.M.M.; material preparation: M.S. and C.M.M.; data collection and analysis: M.S. and C.M.M.; writing—original draft preparation: S.M.; conceptualization: M.S., C.M.M., M.E., A.T.; writing—review and editing: M.S., A.T.; supervision: A.T. All authors have read and agreed to the published version of the manuscript.

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Chapter 6 - Discussion and final remarks

Traditional medicine represents an important part of human life: it is the oldest form of health care, used to prevent or treat physical and mental illness [1], and arises from the knowledge, practices and experiences of different cultures in the past, mostly orally transmitted until the present days. In the last decades a large amount of ethnobotanical research was carried out all over the world in order to valorise and to preserve this important cultural heritage, also aiming at exploiting it to find new natural drugs [2].

Wild alimurgic plants have a key role in nourishment and traditional medicine due to the presence of several classes of phytochemical constituents as terpenes, flavonoids, alkaloids having several biological activities [3–5]. A considerable amount of literature was published on biochemical analysis and bioactivities studies on wild food species. These studies mainly dealt with the assessment of nutritional and phytochemical compositions and of biological activities (e.g., antioxidant, antibacterial, anti-inflammatory, anti-cancer) of plants and their extracts obtained with water or several other organic polar and non-polar solvents [6–9].

In general phytochemical studies about wild plants are based on previous ethnobotanical and ethnopharmacological knowledge, and are follow several steps, well described by Gonzalez Mera and colleagues [10], that are: i) extraction of the plant, ii) qualitative phytochemical screening (e.g., by spectrophotometric colorimetric or fluorometric assays) to determine the main molecule chemical classes, iii) quantification (e.g., by TLC, HPLC, NMR) and in some cases purification of the individual components, iv) determination of biological activities of the extracts through *in vitro*, cell-based and *in vivo* assays.

The aim of this PhD project was to characterize and compare the phytochemical profile and biological activities of organs from several alimurgic plants widely used in Italian folk medicine, and their medicinal preparations (i.e., infusion and decoction), in order to acquire preliminary scientific data which can possibly confirm the traditionally reported effectiveness of these remedies in treating human diseases and detect their phytochemical compositions. Extensive biochemical and bioactivity studies *in vitro* represent in fact the first steps are required to validate the traditional uses of medicinal plants.

The first step of the work was a detailed bibliography analyses of the most cited and used Italian wild food plants in traditional medicine, by which seven species were selected: *Borago officinalis*

L., *Cynodon dactylon* (L.) Pers., *Foeniculum vulgare* Mill., *Hypericum perforatum* L., *Malva sylvestris* L., *Sambucus nigra* L., *Urtica dioica* L. (Chapter 2, [11]). Plants were collected in three different Italian regions (Apulia, Liguria and Tuscany) to assess possible differences linked to the area of gathering. A preliminary biochemical screening by means of spectrophotometric assays of the selected species was performed to characterize raw plant extracts obtained with water and methanol extraction of the different plant organs (Chapter 3, [12]). Three important plant metabolite classes, polyphenols, reducing sugars and proteins, were quantified, together with the antioxidant activity of the extracts (Figs. 3.4 and S3.2). These initial results revealed that targeted metabolites, as well as antioxidant activity, were generally higher in flowers and leaves respect to stems, roots and bark (Figs. 3.5 and S3.3), in agreement with previous publications that reported flowers and leaves as the most cited organs in folk medicine (alone or included in “aerial parts”), and having high number of medicinal uses [13–15].

Extracts of the same species showed very minor differences among the three regions demonstrating a very limited effect of collection sites on the phytochemical levels (Figs. 3.5 and S3.3).

The effectiveness of phytochemicals in the human body is largely dependent on the bioaccessibility of the plant metabolites in the passage through the gastrointestinal human tract. To further understand the potential effects of a medicinal plant preparation is essential to analyse how human digestion affects its composition and bioactivity [16]. Over the past decades there has been an increasing interest in substituting *in vivo* animal/human-based food digestion experiments (administration of food to animals or humans under controlled conditions) generally costly and time consuming, with *in vitro* analyses (static or dynamic oro-gastrointestinal digestion assays), largely reproducible and with no ethic limits. Most studies on bioavailability and bioaccessibility have analysed the effects of the *in vitro* digestion process on food stuff and beverages, both plant-based (vegetables, fruits, rice, legumes, spices, juices, tea, spices) [17, 18] and animal-based (dairy products as milk and its derivatives, eggs, meat and seafood products) [19, 20]. Although a wide range of data are available on bioaccessibility of metabolites from various plant sources, studies considering the effects of digestion on medicinal alimurgic plant preparations of the species selected in this thesis are absent. As well as food, also medicinal preparations orally taken undergo changes after ingestion, and therefore the second part of the project aimed at verifying how plant metabolites and their biological activities are affected by human digestion process (Chapter 4).

The aqueous extracts corresponding to infusion and decoction of plant organs were selected as they resulted the most used preparations in Italian folk medicine [11]. On the basis of the previous analyses (Chapter 3, Figs. 3.4 and 3.5), infusions and decoctions of the organs showing high concentrations of metabolites and high antioxidant activity, and obtained from the most promising species (flowers, leaves and stems of *B. officinalis* and *H. perforatum*, stems of *F. vulgare* and flowers and leaves of *S. nigra*), were prepared and subjected to an *in vitro* simulated oro-gastrointestinal digestion protocol. The total phenols, reducing sugars and proteins level were again detected in not digested and digested samples (Chapter 4). In general, total amount of phenols in infusions and decoctions decreased after digestion (Fig. 4.1), leading to an overall loss in antioxidant activity (Fig. 4.4), thus suggesting that part of these compounds were degraded or converted into other undetected and not active metabolites [18, 21, 22]. In fact, antioxidant properties of a plant are mainly attributed to the chemical structure of its phenolic compounds, and thus followed the same trend of phenolics concentration [23, 24]. Conversely, total reducing sugar (Fig. 4.2) and protein concentrations (Fig. 4.3) increased after digestion due to the action of pepsin and pancreatin digestion enzymes which caused, in the former case the breakdown of carbohydrates glycosidic bonds, leading to an increase in the concentration of mono- and disaccharides [25–27], and in the latter case the hydrolysis of proteins into amino acids and small peptides [28–30].

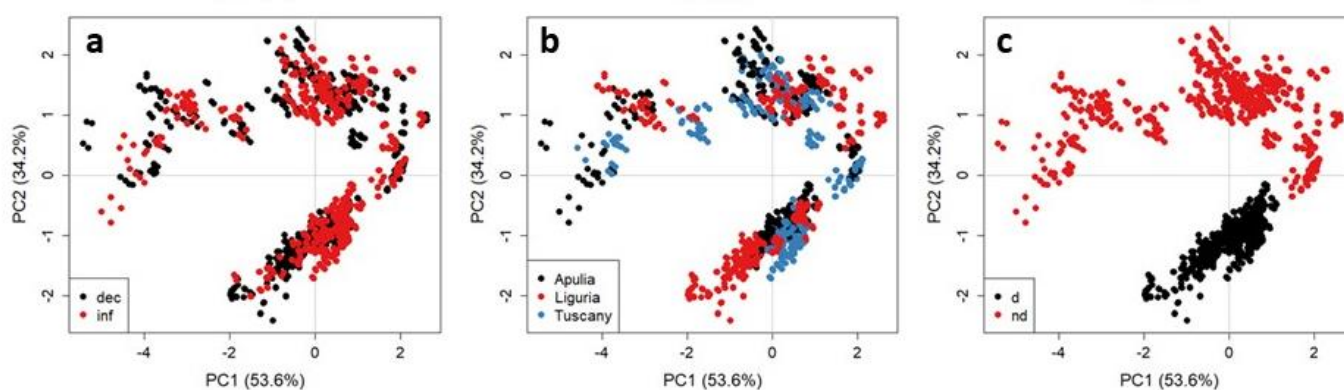


Figure 6.1. Principal component analysis (PCA) of spectrophotometric results on infusions and decoctions before and after digestion, showing the grouping of the samples according to total polyphenols, reducing sugars, protein and antioxidant activity data. Dec: decoction; inf: infusion; d: digestates; nd: not digested.

All data on infusion and decoction extracts after metabolite spectrophotometric analysis were elaborated with Principal Component Analysis (PCA) (Fig. 6.1), demonstrating that no differences

among the two preparations (Fig. 6.1a) and among regions (Fig. 6.1b) were highlighted, while the predominant discrimination variant is the digestion process (Fig. 6.1c).

H. perforatum species preparations, showing the highest phenols content (Fig. 4.1), was then analysed by means of HPLC-DAD to characterize its detailed phenolic profile (Fig. 4.5). The detected phenolic compounds were, in order of concentration from the highest to the lowest: rutin, epicatechin, epigallocatechin, catechin, caffeic acid. Their concentrations decreased after digestion, confirming results obtained by spectrophotometric total phenols quantification assay (Fig. 4.1). *H. perforatum* and *B. officinalis* digested and not digested extracts were also chemically characterized by means of NMR (Fig. 5.2), in order to detect the compositional functional groups in the extracts of which the analysed species are composed, and so to characterize the structure of the samples. Results showed three main classes of molecules, aliphatic, proteins and aromatic compounds (Fig. 4.6), and the obtained sample spectra also highlighted differences in the chemical structures between digested and not digested preparations.

Given the extensive traditional use of infusions and decoctions in topical application against burns, wounds and other skin diseases [11], the last part of the present thesis concerned the analyses of antimicrobial, anti-tyrosinase and anti-inflammatory activities of *B. officinalis* and *H. perforatum* (flowers, leaves, stems), *F. vulgare* (stems) and *S. nigra* (flowers, leaves) extracts to verify their efficacy in external treatments (Chapter 4 and 5, Tables 4.2 and 4.3, Figs. 4.7 and 5.3). Antimicrobial activity of not digested infusions and decoctions was tested against two, gram negative and gram positive, model bacteria which can cause wounds infections and other skin problems, *E. coli* and *S. aureus* [31–34]. The extracts showed activity against the two bacteria, demonstrating that infusions and decoctions made with *H. perforatum* and *B. officinalis* could be effective for topical applications against skin diseases as reported by ethnobotanical studies [11, 35, 36]. In general, *B. officinalis* resulted to be more active against *E. coli*, while *H. perforatum* showed higher activity against *S. aureus* (Tab. 5.1).

B. officinalis and *H. perforatum* (flowers, leaves, stems), *F. vulgare* (stems) and *S. nigra* (flowers, leaves) preparations were analysed for their anti-tyrosinase activity (activity that counteracts the overproduction of melanin in order to avoid browning of fruits and vegetables and dermatological diseases) (Chapter 5), to demonstrate their wide use for skin treatments. All plant infusions/decoctions showed activity both before and after digestion (Fig. 5.3), probably due to the presence of high concentrations of phenolic compounds in not digested [37], and the presence of high concentrations of bioactive peptides in digested samples [38].

In infusions and decoctions of flowers and leaves of *S. nigra*, before and after digestion, anti-inflammatory activity was also detected. The most active extracts resulted to be infusions or decoctions before the digestion (Fig. 5.4), but to demonstrate that data were the result of plant extracts activity and not of their toxicity, a toxicity test with natural bioluminescent bacteria (*A. fischeri*) was done demonstrating that plant preparations did not exert any toxicity (Fig. 5.5). Furthermore, it was possible to hypothesize a potential hormetic effect on the model bacteria cells because, while the addition of not diluted samples showed a consistent increase in the BL signal (wellness of bacteria), the values of diluted samples resulted to be close to those of the control, indicating a possible induced hormetic dose-response that is time and concentration dependent.

In conclusion, starting from traditional knowledge on wild alimurgic plants and folk medicine originated from popular therapeutic remedies handed down from generation to generation by local populations, the most cited and used Italian wild species and their most used medicinal preparations were selected, collected and analysed. To the best of our knowledge, this is the largest study so far analysing several wild food plants from different Italian regions, their organs and their traditional preparations (infusion and decoction) used for medicinal purposes. Plant preparations (not digested and digested *in vitro*) of the selected alimurgic species showed high concentrations of phytochemicals with ascertained healthy properties, and furthermore demonstrated to have significant biological activities. In particular, results on not digested samples showed evidences linked to their traditional topical application as remedies against burns, wounds and skin infection in general, together with cosmetic uses such as against pimples, wrinkles and skin blemishes. On the other hand, digested samples, mimicking the traditional use (e.g., against intestinal and urinary problems) of infusions or decoctions through oral ingestion followed by gut digestion, demonstrated also to be active.

Although *in vitro* digestion simulations were very close to *in vivo* conditions, many other variables acting in the human organism could not be replicated in laboratory during the present thesis work (such as gut microbiome effect, digested molecules gut uptake, etc), so further analyses are needed to link the detected phytochemical profile with the traditionally reported effects and to confirm and justify the folk medicinal uses of these plant preparations.

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Appendix 1. Dataset of Italian wild and cultivated plant species used for medicinal purpose. w: wild, c: cultivated, w/c: wild and cultivated; CI: citation index, UI: use index; cit.n., number of citations; nd: not determined. Pathological groups: 1) oro-gastro-intestinal tract diseases, 2) skin-ears-eyes-hair diseases and wounds, 3) systemic disorders, 4) genito-urinary tract diseases, 5) respiratory tract diseases, 6) nervous system diseases, 7) cardio-circulatory system diseases, 8) muscular-skeletal diseases, 9) metabolic diseases.

Species	Family	Vernacular name	w/c	CI	UI	Papers	cit. n.	Pathological groups								
								1	2	3	4	5	6	7	8	9
<i>Abies alba</i> Mill.	Pinaceae	Abete bianco	w	0.12	0.89	Lokar and Poldrini, 1988; Pieroni, 2000; Camangi et al, 2003; Passalacqua et al, 2007; Vitalini et al, 2009; Di Sanzo et al, 2013; Cornara et al, 2014; Bellia and Pieroni, 2015; Petalka et al, 2020	9	X	X	X		X	X	X	X	X
<i>Abies</i> sp.pl.	Pinaceae	-	nd	0.01	0.11	Mattalia et al, 2019	1		X							
<i>Acacia karroo</i> Hayne	Fabaceae	-	c	0.01	0.22	Palmese et al, 2001	1			X	X					
<i>Acanthus mollis</i> L.	Acanthaceae	Acanto	c	0.05	0.44	Bruni et al, 1997; Leto et al, 2012; Tuttolomondo et al, 2014; Gargano et al, 2018	4	X	X					X	X	
<i>Acer campestre</i> L.	Sapindaceae	Acerio campestre	w	0.01	0.11	Mautone et al, 2019	1				X					
<i>Acer neapolitanum</i> (Ten.) Gmas.	Sapindaceae	Acerio napoletano	w	0.01	0.11	Guarrera and Lucia, 2007	1		X							
<i>Acer opalus</i> subsp. <i>obtusatum</i> (Waldst. & Kit. ex Willd.) Gams	Sapindaceae	Opalo	w	0.01	0.11	Leporatti et al, 1985b	1	X								
<i>Acer pseudoplatanus</i> L.	Sapindaceae	Acerio di monte	w	0.01	0.11	Bellia and Pieroni, 2015	1					X				
<i>Achillea ageratum</i> L.	Compositae	Millefoglio	w	0.01	0.67	Guarino et al, 2008	1	X	X	X	X	X		X		
<i>Achillea atrata</i> L.	Compositae	Millefoglio del calcare	w	0.01	0.56	Petalka et al, 2020	1		X			X	X		X	
<i>Achillea clavinae</i> L.	Compositae	Millefolio	w	0.03	0.67	Lokar and Poldrini, 1988; Petalka et al, 2020	2	X	X		X	X	X		X	
<i>Achillea collina</i> (Becker ex Rchb.f.) Heimerl	Compositae	Millefoglio	w	0.05	0.67	Guarrera et al, 2005; Guarino et al, 2008; Cornara et al, 2014; Lucchetti et al, 2019	4	X	X		X	X		X	X	
<i>Achillea erba-rotta</i> All.	Compositae	Millefoglio erba rotta	w	0.08	0.78	Pieroni and Giusti, 2009; Mattalia et al, 2012; Cornara et al, 2014; Bellia and Pieroni, 2015; Fontefrancesco and Pieroni, 2020; Danna et al, 2022	6	X	X	X		X	X			
<i>Achillea erba-rotta</i> subsp. <i>moschata</i> (Wulfen) I.Richardson	Compositae	Millefoglio erba rotta	w	0.01	0.11	Bruschi et al, 2019	1	X								
<i>Achillea ligustica</i> All.	Compositae		w	0.09	0.67	Bruni et al, 1997; Palmese et al, 2001; Signorini et al, 2009; Leto et al, 2012; Cornara et al, 2014; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b	7	X	X	X			X		X	
<i>Achillea millefolium</i> L.	Compositae	Millefoglio	w	0.41	1.00	Lokar and Poldrini, 1988; Bruni et al, 1997; Pieroni, 2000; Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Pieroni et al, 2004b; Pieroni and Quave, 2005; Menale et al, 2006; Passalacqua et al, 2007;	30	X	X	X	X	X	X	X	X	X

<i>Anagallis arvensis</i> L.	Primulaceae	-	w	0.07	0.67	Leporatti and Ivancheva, 2003; Loi et al, 2005; Guarino et al, 2008; Leto et al, 2012; Lucchetti et al, 2019	5	X	X	X	X	X	X	X	X
<i>Anagallis arvensis</i> subsp. <i>arvensis</i>	Primulaceae	-	w	0.01	0.11	De Natale and Pollio, 2007	1					X			
<i>Anchusa officinalis</i> L.	Boraginaceae	Buglossa	w	0.01	0.22	Vitalini et al, 2015	1	X	X						
<i>Anchusa undulata</i> L. subsp. <i>hybrida</i> (Ten.) Bég.	Boraginaceae	Buglossa undulata	w	0.03	0.22	Fortini et al, 2016; Motti and Motti, 2017	2	X						X	
<i>Anemone hortensis</i> L.	Ranunculaceae	Anemone viola	w	0.01	0.33	Guarino et al, 2008	1		X	X					X
<i>Anemone nemorosa</i> L.	Ranunculaceae	Anemone dei boschi	w	0.01	0.33	Guarino et al, 2008	1		X	X					X
<i>Anemone pulsatilla</i> L.	Ranunculaceae	-	w	0.01	0.22	Leporatti and Ivancheva, 2003	1						X	X	
<i>Anemone vernalis</i> L.	Ranunculaceae	-	w	0.01	0.44	Petalka et al, 2020	1	X	X			X			X
<i>Anethum graveolens</i> L.	Apiaceae	Aneto	c	0.08	0.44	Leporatti and Ivancheva, 2003; Menale et al, 2006; Leporatti and Ghedira, 2009; Vitalini et al, 2009; Fortini et al, 2016; Galuzzo et al, 2021	6	X		X					
<i>Angelica archangelica</i> L.	Apiaceae	-	w	0.04	0.33	Leporatti and Ivancheva, 2003; Guarino et al, 2008; Fontefrancesco and Pieroni, 2020	3	X	X	X					
<i>Angelica sylvestris</i> L.	Apiaceae	Angelica selvatica	w	0.12	0.67	Lokar and Poldrini, 1988; Guarino et al, 2008; Idolo et al, 2010; Vitalini et al, 2012; Cornara et al, 2014; Vitalini et al, 2015; Mautone et al, 2019; Fontefrancesco and Pieroni, 2020; Petalka et al, 2020	9	X	X				X	X	X
<i>Antennaria dioica</i> L.	Compositae	Sempiterni di monte	w	0.04	0.67	Lokar and Poldrini, 1988; Pieroni and Giusti, 2009; Petalka et al, 2020	3	X	X	X	X	X			
<i>Anthemis arvensis</i> L.	Compositae	Falsa camomilla	w	0.05	0.44	Uncini Manganeli and Tomei, 1999; Loi et al, 2005; Tuttolomondo et al, 2014; Galuzzo et al, 2021	4	X	X	X				X	
<i>Anthemis arvensis</i> L. subsp. <i>incrassata</i> (Loisel.) Nyman	Compositae	Falsa camomilla	w	0.01	0.56	Menale et al, 2016	1	X	X			X	X		
<i>Anthemis cotula</i> L.	Compositae	Camomilla fetida	w	0.01	0.11	Guarino et al, 2008	1			X					
<i>Anthoxanthum odoratum</i> L.	Poaceae	Paleo odoroso	w	0.01	0.11	Guarino et al, 2008	1			X					
<i>Anthriscus cerefolium</i> (L.) Hoffm.	Apiaceae	Cerfoglio	w/c	0.03	0.44	Guarino et al, 2008; Galuzzo et al, 2021	2	X	X	X	X				
<i>Anthriscus sylvestris</i> (L.) Hoffm.	Apiaceae	Cerfoglio	w	0.04	0.67	Guarino et al, 2008; Leporatti and Ghedira, 2009; Petalka et al, 2020	3		X	X	X			X	X
<i>Anthyllis vulneraria</i> L.	Fabaceae	Vulneraria	w	0.05	0.22	Lokar and Poldrini, 1988; Guarino et al, 2008; Vitalini et al, 2015; Petalka et al, 2020	4		X	X					
<i>Antirrhinum majus</i> L.	Plantaginaceae	Bocca di leone	w	0.01	0.44	Guarino et al, 2008	1	X	X	X			X		
<i>Apium graveolens</i> L.	Apiaceae	Sedano	c	0.22	0.78	Leporatti et al, 1985a; Leporatti et al, 1985b; Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003;	16	X	X	X	X	X	X		X

<i>Aristolochia clematitis</i> L.	Aristolochiaceae	Aristolochia	w	0.04	0.44	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003; Danna et al, 2022	3	X	X	X	X	X	X
<i>Aristolochia fontanesii</i> Boiss. & Reut.	Aristolochiaceae	Aristolochia	w	0.01	0.11	Leto et al, 2012	1						X
<i>Armoracia rusticana</i> P. Gaertn, B.Mey & Scherb.	Brassicaceae	-	w	0.08	0.44	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Pieroni and Quave, 2005; Vitalini et al, 2015	6	X	X				X
<i>Arnica montana</i> L.	Compositae	Arnica	w	0.19	1.00	Lokar and Poldrini, 1988; Pieroni and Giusti, 2009; Vitalini et al, 2009; Mattalia et al, 2012; Vitalini et al, 2012; Cornara et al, 2014; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Bruschi et al, 2019; Fontefrancesco and Pieroni, 2020; Mattalia et al, 2020a; Petalka et al, 2020; Danna et al, 2022	14	X	X	X	X	X	X
<i>Artemisia abrotanum</i> L.	Compositae	-	w	0.03	0.33	Di Novella et al, 2013; Tuttolomondo et al, 2014a	2	X	X	X			
<i>Artemisia absinthium</i> L.	Compositae	Assenzio	w	0.32	1.00	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Pieroni, 2000; Leporatti and Corradi, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Maccioni et al, 2004; Menale et al, 2006; Guarino et al, 2008; Leporatti and Ghedira, 2009; Pieroni and Giusti, 2009; Vitalini et al, 2009; Mattalia et al, 2012; Vitalini et al, 2012; Cornara et al, 2014; Menale and Muoio, 2014; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Mautone et al, 2019; Mattalia et al, 2020a; Petalka et al, 2020; Galuzzo et al, 2021; Danna et al, 2022	24	X	X	X	X	X	X
<i>Artemisia alba</i> Turra	Compositae	Assenzio maschio	w	0.04	0.22	Leto et al, 2012; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b	3	X	X				
<i>Artemisia arborescens</i> (Vaill.) L.	Compositae	-	w	0.20	1.00	Bruni et al, 1997; Ballero et al, 2001; Palmese et al, 2001; Loi et al, 2004; Loi et al, 2005; De Natale and Pollio, 2007; Maxia et al, 2008; Leonti et al, 2009; Signorini et al, 2009; Savo et al, 2011; Leto et al, 2012; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Gargano et al, 2018	15	X	X	X	X	X	X
<i>Artemisia campestris</i> L.	Compositae	Assenzio selvatico	w	0.01	0.33	Leporatti and Ghedira, 2009	1	X	X				X
<i>Artemisia campestris</i> L. subsp. <i>glutinosa</i> (Besser) Batt.	Compositae	Assenzio selvatico	w	0.01	0.33	Guarino et al, 2008	1	X	X		X		
<i>Artemisia campestris</i> L. subsp. <i>variabilis</i> (Ten.) Greuter	Compositae	Assenzio selvatico	w	0.03	0.22	Menale et al, 2016; Menale et al, 2021	2	X				X	
<i>Artemisia coerulescens</i> L.	Compositae	-	w	0.01	0.22	Lokar and Poldrini, 1988	1	X	X				

<i>Artemisia genipi</i> Weber ex Stechm.	Compositae	Assenzio genipi	w	0.09	0.44	Pieroni and Giusti, 2009; Vitalini et al, 2012; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Fontefrancesco and Pieroni, 2020; Danna et al, 2022	7	X	X	X	X					
<i>Artemisia glacialis</i> L.	Compositae	Assenzio alpino	w	0.03	0.33	Mattalia et al, 2012; Bellia and Pieroni, 2015	2	X	X	X	X					
<i>Artemisia maritima</i> L.	Compositae	-	w	0.01	0.11	Mattalia et al, 2020a	1	X								
<i>Artemisia umbelliformis</i> Lam.	Compositae	-	w	0.11	0.67	Pieroni and Giusti, 2009; Mattalia et al, 2012; Vitalini et al, 2012; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Petalka et al, 2020; Danna et al, 2022	8	X	X	X	X	X	X			
<i>Artemisia verlotiorum</i> Lamotte	Compositae	-	w	0.01	0.11	Menale et al, 2016	1							X		
<i>Artemisia vulgaris</i> L.	Compositae	Assenzio selvatico	w	0.15	0.78	Leporatti and Ivancheva, 2003; Guarino et al, 2008; Pieroni and Giusti, 2009; Vitalini et al, 2009; Menale and Muoio, 2014; Bellia and Pieroni, 2015; Vitalini et al, 2015; Bruschi et al, 2019; Lucchetti et al, 2019; Petalka et al, 2020; Danna et al, 2022	11	X	X	X	X	X	X			
<i>Arum cylindraceum</i> Gasp.	Araceae	-	w	0.01	0.56	Guarino et al, 2008	1	X		X	X				X	X
<i>Arum italicum</i> Mill.	Araceae	Gigaro chiaro	w	0.22	0.56	Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Camangi et al, 2003; Pieroni et al, 2004a; Pieroni and Quave, 2005; De Natale and Pollio, 2007; Gonzalez-Tejero et al, 2008; Leporatti and Ghedira, 2009; Leto et al, 2012; Montesano et al, 2012; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b; Fortini et al, 2016; Lucchetti et al, 2019; Maruca et al, 2019; Mautone et al, 2019	16	X	X	X	X					
<i>Arum maculatum</i> L.	Araceae	Gigaro scuro	w	0.01	0.44	Leporatti and Ivancheva, 2003	1	X			X				X	X
<i>Arum pictum</i> L.f.	Araceae	-	w	0.03	0.33	Bruni et al, 1997; Loi et al, 2004	2		X	X					X	X
<i>Aruncus dioicus</i> (Walter) Fernald	Rosaceae	-	w	0.01	0.44	Lokar and Poldrini, 1988	1		X	X	X				X	
<i>Arundo donax</i> L.	Poaceae	Canna domestica	w	0.31	0.67	Bruni et al, 1997; Ballero et al, 2001; Leporatti and Corradi, 2001; Palmese et al, 2001; Pieroni et al, 2004a; Loi et al, 2005; Pieroni and Quave, 2005; Menale et al, 2006; Passalacqua et al, 2007; Guarino et al, 2008; Maxia et al, 2008; Leporatti and Ghedira, 2009; Leto et al, 2012; Montesano et al, 2012; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Menale et al, 2016; Lucchetti et al, 2019; Maruca et al, 2019; Mattalia et al, 2019; Mautone et al, 2019; Menale et al, 2021	23	X	X	X	X	X	X			
<i>Asarum europaeum</i> L.	Aristolochiaceae	Baccaro	w	0.01	0.22	Leporatti and Ivancheva, 2003	1	X				X				
<i>Asclepias curassavica</i> L.	Apocynaceae	-	w	0.01	0.11	Tuttolomondo et al, 2014a	1		X							

<i>Aster alpinus</i> L.	Compositae	Astro alpino	w	0.01	0.11	Vitalini et al, 2015	1	X											
<i>Astracantha sicula</i> (Biv.) Greuter	Fabaceae	-	w	0.01	0.22	Tuttolomondo et al, 2014a	1	X	X										
<i>Astragalus glycyphyllos</i> L.	Fabaceae	Astragalo	w	0.04	0.67	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003; Guarino et al, 2008	3	X		X	X	X	X	X	X	X	X	X	X
<i>Astragalus monspessulanus</i> L.	Fabaceae	Astragalo	w	0.01	0.11	Guarino et al, 2008	1			X									
<i>Athamanta cretensis</i> L.	Apiaceae	Atamanta comune	w	0.01	0.22	Petalka et al, 2020	1				X	X							
<i>Athamanta sicula</i> L.	Apiaceae	-	w	0.01	0.11	Tuttolomondo et al, 2014	1				X								
<i>Athyrium filix-femina</i> (L.) Roth	Athyriaceae	Felce femmina	w	0.01	0.11	Di Novella et al, 2013	1						X						
<i>Atropa bella-donna</i> L.	Solanaceae	Belladonna	w	0.18	0.89	Leporatti et al, 1985b; Leporatti and Ivancheva, 2003; De Natale and Pollio, 2007; Guarino et al, 2008; Idolo et al, 2010; Leto et al, 2012; Mattalia et al, 2012; Fortini et al, 2016; Bruschi et al, 2019; Mattalia et al, 2019; Mattalia et al, 2020b; Petalka et al, 2020; Mattalia et al, 2021	13	X	X	X	X	X	X	X	X	X	X	X	X
<i>Avena barbata</i> Pott ex Link	Poaceae	Avena selvatica	w	0.01	0.44	Guarino et al, 2008	1					X	X	X	X	X	X	X	X
<i>Avena fatua</i> L.	Poaceae	Avena selvatica	w	0.07	0.67	Guarino et al, 2008; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Gargano et al, 2018	5	X	X			X	X	X	X	X	X	X	X
<i>Avena sativa</i> L.	Poaceae	Avena	c	0.22	0.89	Bruni et al, 1997; Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Pieroni et al, 2004b; Pieroni and Quave, 2005; Guarino et al, 2008; Maxia et al, 2008; Leporatti and Ghedira, 2009; Montesano et al, 2012; Di Novella et al, 2013; Cornara et al, 2014; Menale and Muoio, 2014; Menale et al, 2016; Motti and Motti, 2017; Geraci et al, 2018; Lucchetti et al, 2019	16	X	X	X	X	X	X	X	X	X	X	X	X
<i>Ballota nigra</i> L.	Lamiaceae	Cimiciotta comune	w	0.07	0.89	Pieroni, 2000; Pieroni et al, 2004a; Pieroni and Quave, 2005; Guarino et al, 2008; Galuzzo et al, 2021	5	X	X	X	X	X	X	X	X	X	X	X	X
<i>Ballota nigra</i> subsp. <i>ruderalis</i> (Sw.) Briq.	Lamiaceae	Cimiciotta comune	w	0.01	0.11	Signorini et al, 2009	1	X											
<i>Barbarea vulgaris</i> R.Br.	Brassicaceae	Erba di santa Barbara	w	0.01	0.22	Guarino et al, 2008	1			X									X
<i>Bartsia alpina</i> L.	Orobanchaceae	Bartsia	w	0.04	0.33	Bellia and Pieroni, 2015; Dei Cas et al, 2015; Petalka et al, 2020	3		X				X				X		X
<i>Beckwithia glacialis</i> (L.) A.Löve & D.Löve	Ranunculaceae	-	w	0.01	0.22	Petalka et al, 2020	1								X				X
<i>Bellis perennis</i> L.	Compositae	Pratolina	w	0.15	0.89	Lokar and Poldrini, 1988; Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; Passalacqua	11	X	X	X	X	X	X	X	X	X	X	X	X

<i>Brassica cretica</i> Lam.	Brassicaceae	-	w	0.01	0.22	Motti and Motti, 2017	1	X	X									
<i>Brassica montana</i> Pourr.	Brassicaceae	-	w	0.01	0.00	Guarino et al, 2008	1				X							
<i>Brassica napus</i> L.	Brassicaceae	Senape bianca	c	0.04	0.22	Ballero et al, 2001; Guarino et al, 2008; Leporatti and Ghedira, 2009	3		X	X								
<i>Brassica nigra</i> (L.) K.Koch	Brassicaceae	-	c	0.05	0.56	Camangi et al, 2003; Leporatti and Ivancheva, 2003; Guarino et al, 2008; Idolo et al, 2010	4		X	X	X	X	X	X	X	X	X	
<i>Brassica oleracea</i> L.	Brassicaceae	Cavolo nero	c	0.43	0.89	Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Maccioni et al, 2004; Pieroni et al, 2004a; Pieroni et al, 2004b; Guarrera et al, 2005; Pieroni and Quave, 2005; Passalacqua et al, 2007; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Motti et al, 2009; Idolo et al, 2010; Savo et al, 2011; Montesano et al, 2012; Cornara et al, 2014; Menale and Muoio, 2014; Tuttolomondo et al, 2014b; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Guarrera et al, 2015; Fortini et al, 2016; Menale et al, 2016; Motti and Motti, 2017; Bruschi et al, 2019; Lucchetti et al, 2019; Maruca et al, 2019; Mautone et al, 2019; Fontefrancesco and Pieroni, 2020; Menale et al, 2021; Danna et al, 2022	32	X	X	X	X	X	X	X	X	X	X	X
<i>Brassica rapa</i> L.	Brassicaceae	Rapa	c	0.09	0.67	Guarrera et al, 2005; Guarino et al, 2008; Bellia and Pieroni, 2015; Menale et al, 2016; Motti and Motti, 2017; Petalka et al, 2020; Danna et al, 2022	7	X	X	X	X	X	X	X	X			
<i>Brassica rupestris</i> Raf.	Brassicaceae	-	w	0.03	0.22	Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b	2	X								X		
<i>Brassica</i> sp.pl.	Brassicaceae	-	nd	0.01	0.33	Cornara et al, 2009	1	X	X		X					X		
<i>Bryonia alba</i> L.	Cucurbitaceae	-	w	0.01	0.44	Leporatti and Ivancheva, 2003;	1	X	X	X		X				X		
<i>Bryonia dioica</i> Jacq.	Cucurbitaceae	Brionia comune	w	0.11	0.56	Lokar and Poldrini, 1988; Pieroni, 2000; Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; Pieroni et al, 2004b; Guarino et al, 2008; Leporatti and Ghedira, 2009; Menale and Muoio, 2014	8	X	X	X	X	X	X	X		X		
<i>Buglossoides purpureoerulea</i> (L.) I. M. Johnston.	Boraginaceae	Erba perla	w	0.01	0.11	Guarino et al, 2008	1	X										
<i>Bunias erucago</i> L.	Brassicaceae	Cascellone	w	0.05	0.22	Pieroni, 2000; Guarino et al, 2008; Tuttolomondo et al, 2014a; Ranfa and Bodesmo, 2017	4	X					X					
<i>Bupthalmum salicifolium</i> L.	Compositae	-	w	0.01	0.11	Lokar and Poldrini, 1988	1		X									
<i>Bupleurum rotundifolium</i> L.	Apiaceae	Buplero	w	0.01	0.22	Guarino et al, 2008	1	X	X									
<i>Buxus sempervirens</i> L.	Buxaceae	Bosso	w/c	0.05	0.33	Leporatti et al, 1985b; Guarino et al, 2008; Leporatti and Ghedira, 2009; Di Novella et al, 2013	4	X	X	X	X					X		

<i>Calendula arvensis</i> M. Bieb.	Compositae	Calendula	w	0.11	0.56	Loi et al, 2005; Passalacqua et al, 2007; Leporatti and Ghedira, 2009; Signorini et al, 2009; Tuttolomondo et al, 2014b; Fortini et al, 2016; Motti and Motti, 2017; Ranfa and Bodesmo, 2017	8	X	X	X	X	X	X	X
<i>Calendula officinalis</i> L.	Compositae	Calendula	w/c	0.26	0.89	Lokar and Poldrini, 1988; Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; Pieroni et al, 2004b; Guarrera et al, 2005; Menale et al, 2006; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Vitalini et al, 2012; Cornara et al, 2014; Tuttolomondo et al, 2014a; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Fortini et al, 2016; Menale et al, 2016; Bruschi et al, 2019; Lucchetti et al, 2019; Mattalia et al, 2020a	19	X	X	X	X	X	X	X
<i>Calendula</i> sp.pl.	Compositae	-	nd	0.03	0.56	Idolo et al, 2010; Galuzzo et al, 2021	2	X	X	X	X	X	X	X
<i>Calicotome villosa</i> (Poir.) Link	Fabaceae	Ginestra villosa	w	0.01	0.11	Loi et al, 2004	1		X					
<i>Calluna vulgaris</i> (L.) Hull	Ericaceae	Brugo	w	0.03	0.67	Fontefrancesco and Pieroni, 2020; Petalka et al, 2020	2	X		X	X	X	X	X
<i>Calystegia sepium</i> (L.) R. Br.	Convolvulaceae	Campanella bianca	w	0.07	0.44	Passalacqua et al, 2007; Di Sanzo et al, 2013; Menale and Muio, 2014; Lucchetti et al, 2019; Mautone et al, 2019	5	X	X				X	X
<i>Calystegia silvatica</i> (Kit.) Griseb.	Convolvulaceae	Campanella bianca	w	0.03	0.22	Passalacqua et al, 2007; Savo et al, 2011	2	X	X					
<i>Camelina sativa</i> (L.) Crantz	Brassicaceae	-	w	0.01	0.22	Leporatti and Ghedira, 2009	1	X	X					
<i>Camellia japonica</i> L.	Theaceae	-	w	0.01	0.11	Menale et al, 2021	1	X						
<i>Camellia sinensis</i> (L.) Kuntze	Theaceae	Te'	w	0.01	0.44	Bruschi et al, 2019	1	X	X		X	X		
<i>Campanula dichotoma</i> L.	Campanulaceae	-	w	0.01	0.11	Scherrer et al, 2005	1				X			
<i>Campanula fragilis</i> subsp. <i>fragilis</i>	Campanulaceae	Campanula delicata	w	0.01	0.11	Savo et al, 2011	1		X					
<i>Campanula patula</i> L.	Campanulaceae	-	w	0.01	0.33	Petalka et al, 2020	1					X	X	X
<i>Campanula rapunculus</i> L.	Campanulaceae	-	w	0.03	0.33	Pieroni, 2000; Ranfa and Bodesmo, 2017	2	X	X		X			
<i>Campanula rotundifolia</i> L.	Campanulaceae	Campanula	w	0.01	0.33	Petalka et al, 2020	1					X	X	X
<i>Campanula scheuchzeri</i> Vill.	Campanulaceae	Campanula di Scheurzer	w	0.01	0.11	Vitalini et al, 2015	1		X					
<i>Cannabis sativa</i> L.	Cannabaceae	Canapa	c	0.01	0.33	Guarrera et al, 2005	1	X	X			X		
<i>Capparis orientalis</i> Veill.	Capparaceae	Cappero	w	0.01	0.11	Menale et al, 2021	1		X					
<i>Capparis spinosa</i> L.	Capparaceae	Cappero	w/c	0.04	0.67	Cornara et al, 2009; Leporatti and Ghedira, 2009; Tuttolomondo et al, 2014a	3	X	X		X	X	X	X
<i>Capsella bursa-pastoris</i> (L.) Medik.	Brassicaceae	Borsa del pastore	w	0.27	0.89	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003; Loi et al, 2005; De Natale and Pollio, 2007; Guarino et al, 2008; Leporatti and Ghedira, 2009;	20	X	X	X	X	X	X	X

<i>Carthamus lanatus</i> L.	Compositae	-	w	0.01	0.44	Guarino et al, 2008	1	X	X	X	X	X	X	X	X				
<i>Carthamus tinctorius</i> L.	Compositae	Cartamo	w/c	0.03	0.22	Galuzzo et al, 2021; Leporatti and Ghedira, 2009	2	X										X	
<i>Carum carvi</i> L.	Apiaceae		w	0.19	0.78	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003; Leporatti and Ghedira, 2009; Pieroni and Giusti, 2009; Vitalini et al, 2009; Vitalini et al, 2012; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Fontefrancesco and Pieroni, 2020; Mattalia et al, 2020a; Petalka et al, 2020; Galuzzo et al, 2021; Danna et al, 2022	14	X	X	X	X	X	X	X	X	X	X	X	X
<i>Castanea sativa</i> Mill.	Fagaceae	Castagno	w/c	0.19	0.67	Pieroni, 2000; Ballero et al, 2001; Camangi et al, 2003; Pieroni et al, 2004b; Guarrera et al, 2005; Scherrer et al, 2005; Passalacqua et al, 2007; Cornara et al, 2009; Idolo et al, 2010; Vitalini et al, 2015; Bruschi et al, 2019; Maruca et al, 2019; Petalka et al, 2020; Menale et al, 2021	14	X	X	X	X	X	X	X	X	X	X	X	
<i>Caucalis platycarpus</i> L.	Apiaceae		w	0.01	0.22	Guarino et al, 2008	1	X			X								
<i>Celtis australis</i> L.	Cannabaceae	Spacca sassi	w	0.04	0.22	Guarino et al, 2008; Di Novella et al, 2013; Lucchetti et al, 2019	3	X	X										
<i>Centaurea benedicta</i> (L.) L.	Compositae	Fiordaliso benedetto	w	0.01	0.33	Leporatti and Ivancheva, 2003	1	X	X	X	X	X	X	X	X	X	X		
<i>Centaurea calcitrapa</i> L.	Compositae	-	w	0.11	0.67	Guarino et al, 2008; Maxia et al, 2008; Leporatti and Ghedira, 2009; Leto et al, 2012; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b; Geraci et al, 2018; Galuzzo et al, 2021	8	X	X	X	X	X	X	X	X	X	X	X	X
<i>Centaurea cyanus</i> L.	Compositae	Fiordaliso	w	0.01	0.44	Leporatti and Ivancheva, 2003	1	X	X	X	X	X	X	X	X	X	X		
<i>Centaurea jacea</i> subsp. <i>gaudinii</i> (Boiss. & Reut.) Grelli	Compositae	Fordaliso stoppione	w	0.01	0.22	Guarrera and Lucia, 2007	1						X	X	X	X			
<i>Centaurea nigrescens</i> Willd.	Compositae	Fiordaliso nerastro	w	0.01	0.11	Guarino et al, 2008	1	X	X	X	X	X	X	X	X	X	X		
<i>Centaurea pratensis</i> Thuill.	Compositae	fiordaliso	w	0.03	0.11	Leporatti et al, 1985b; Guarrera and Lucia, 2007	2	X	X	X	X	X	X	X	X	X	X		
<i>Centaurea solstitialis</i> subsp. <i>schaauwii</i> (DC.) Gugler	Compositae	-	w	0.01	0.11	Leto et al, 2012	1						X	X	X	X	X		
<i>Centaurea</i> sp.pl.	Compositae	-	nd	0.01	0.11	Mattalia et al, 2020a	1	X	X	X	X	X	X	X	X	X	X		
<i>Centaureum erythraea</i> Rafn	Gentianaceae	Centauro eritreo	w	0.26	0.89	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Ballero et al, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Maccioni et al, 2004; Pieroni et al, 2004a; Pieroni and Quave, 2005; Guarrera and Lucia, 2007; Guarino et al, 2008; Leporatti and Ghedira, 2009; Idolo et al, 2010; Di Novella et al, 2013; Di Sanzo et al, 2013; Cornara et al, 2014;	19	X	X	X	X	X	X	X	X	X	X	X	X

<i>Cistus monspeliensis</i> L.	Cistaceae	Gisto di Montpellier	w	0.01	0.22	Uncini Manganelli and Tomei, 1999	1	X	X	X							
<i>Cistus salvifolius</i> L.	Cistaceae	Cisto salvia	w	0.03	0.11	Leto et al, 2012; Tuttolomondo et al, 2014b	2	X									
<i>Cistus</i> sp.pl.	Cistaceae	-	nd	0.03	0.33	Ballerio et al, 2001; Loi et al, 2005	2	X	X								X
<i>Citrullus colocynthis</i> (L.) Schrad.	Cucurbitaceae	-	w	0.01	0.22	Leporatti and Ghedira, 2009	1	X		X							
<i>Citrus aurantium</i> L.	Rutaceae	-	c	0.08	0.56	Palmease et al, 2001; Pieroni et al, 2004b; Passalacqua et al, 2007; Gonzalez-Tejero et al, 2008; Menale et al, 2016; Menale et al, 2021	6	X	X	X	X			X			
<i>Citrus limon</i> (L.) Osbeck	Rutaceae	limone	c	0.38	1.00	Leporatti et al, 1985a; Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Ballero et al, 2001; Palmease et al, 2001; Camangi et al, 2003; Pieroni et al, 2004b; Guarrera et al, 2005; Guarrera et al, 2005a; Loi et al, 2005; Pieroni and Quave, 2005; Scherrer et al, 2005; Passalacqua et al, 2007; Maxia et al, 2008; Motti et al, 2009; Idolo et al, 2010; Savo et al, 2011; Montesano et al, 2012; Cornara et al, 2014; Menale and Muoio, 2014; Guarrera et al, 2015; Menale et al, 2016; Motti and Motti, 2017; Gargano et al, 2018; Bruschi et al, 2019; Lucchetti et al, 2019; Mautone et al, 2019; Menale et al, 2021	28	X	X	X	X	X	X	X	X	X	X
<i>Citrus nobilis</i> Lour.	Rutaceae	Arancio	c	0.01	0.22	Savo et al, 2011	1	X			X						
<i>Citrus paradisi</i> Macfad.	Rutaceae	Pompelmo	c	0.01	0.11	Menale et al, 2016	1						X				
<i>Citrus reticulata</i> Blanco	Rutaceae	Madarino	c	0.05	0.56	Uncini Manganelli and Tomei, 1999; Palmease et al, 2001; Menale et al, 2016; Motti and Motti, 2017	4	X	X	X	X			X			
<i>Citrus sinensis</i> (L.) Osbeck	Rutaceae	Limone	c	0.14	0.67	Palmease et al, 2001; Pieroni et al, 2004a; Pieroni et al, 2004b; Pieroni and Quave, 2005; Motti et al, 2009; Savo et al, 2011; Montesano et al, 2012; Fortini et al, 2016; Menale et al, 2016; Bruschi et al, 2019	10	X	X	X	X	X	X				
<i>Clematis cirrhosa</i> L.	Ranunculaceae	-	w	0.01	0.44	Palmease et al, 2001	1	X	X	X						X	
<i>Clematis flammula</i> L.	Ranunculaceae	Clematide fiammola	w	0.04	0.67	Leporatti and Corradi, 2001; Guarino et al, 2008; Leporatti and Ghedira, 2009	3	X	X	X			X	X	X	X	X
<i>Clematis</i> sp.pl.	Ranunculaceae	-	w	0.01	0.44	Ballerio et al, 2001	1	X	X	X	X					X	
<i>Clematis vitalba</i> L.	Ranunculaceae	Vitalba	w	0.24	0.89	Lokar and Poldrini, 1988; Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Leporatti and Corradi, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Loi et al, 2004; Maccioni et al, 2004; Pieroni et al, 2004a; Pieroni et al, 2004b; Guarrera et al, 2005; Pieroni and Quave, 2005; Guarino et al, 2008; Leporatti and Ghedira, 2009; Montesano et al, 2012; Di Sanzo et al, 2013; Lucchetti et al, 2019; Mattalia et al, 2019; Mattalia et al, 2020b	18	X	X	X	X	X	X	X	X	X	X

<i>Clinopodium alpinum</i> (L.) Kuntze	Lamiaceae	Clinopodio alpino	w	0.01	0.22	Petalka et al, 2020	1							X		
<i>Clinopodium menthifolium</i> (Host) Stace	Lamiaceae	-	w	0.01	0.11	Guarino et al, 2008	1	X								
<i>Clinopodium nepeta</i> (L.) Kuntze	Lamiaceae	Erba nepetella	w	0.32	0.89	Pieroni, 2000; Leporatti and Corradi, 2001; Camangi et al, 2003; Guarrera et al, 2005; Guarrera et al, 2005a; Scherrer et al, 2005; Passalacqua et al, 2007; Guarino et al, 2008; Cornara et al, 2009; Leonti et al, 2009; Savo et al, 2011; Leto et al, 2012; Di Sanzo et al, 2013; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Fortini et al, 2016; Menale et al, 2016; Motti and Motti, 2017; Ranfa and Bodesmo, 2017; Maruca et al, 2019; Lucchetti et al, 2019; Mattalia et al, 2020b; Motti et al, 2020	24	X	X	X	X	X	X	X	X	X
<i>Clinopodium vulgare</i> L.	Lamiaceae	Clinopodio comune	w	0.01	0.22	Guarino et al, 2008	1	X			X					
<i>Coffea arabica</i> L.	Rubiaceae	Caffè	c	0.04	0.44	Palmease et al, 2001; Maxia et al, 2008; Savo et al, 2011	3			X	X	X	X	X		
<i>Coffea</i> sp.pl.	Rubiaceae	-	c	0.01	0.22	Danna et al, 2022	1				X		X			
<i>Colchicum autumnale</i> L.	Colchicaceae	Colchico	w	0.03	0.44	Lokar and Poldrini, 1988; De Natale and Pollio, 2007	2	X		X	X					X
<i>Colchicum lusitanicum</i> Brot.	Colchicaceae	Colchico	w	0.01	0.44	Leporatti et al, 1985b	1	X			X			X		
<i>Colchicum neapolitanum</i> (Ten.) Ten.	Colchicaceae	Colchico meridionale	w	0.01	0.44	Guarino et al, 2008	1	X			X				X	X
<i>Colutea arborescens</i> L.	Fabaceae	Vesicaria	w	0.03	0.33	Leporatti et al, 1985b; Guarino et al, 2008	2	X			X				X	
<i>Conium maculatum</i> L.	Apiaceae	Cicuta maggiore	w	0.16	0.78	Lokar and Poldrini, 1988; Ballero et al, 2001; Pieroni et al, 2004a; Pieroni and Quave, 2005; Guarino et al, 2008; Leto et al, 2012; Cornara et al, 2014; Menale and Muoio, 2014; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b; Bellia and Pieroni, 2015; Mattalia et al, 2021	12	X	X	X	X	X	X	X	X	
<i>Consolida regalis</i> Gray	Ranunculaceae	Consolida	w	0.04	0.33	Leporatti et al, 1985b; Leporatti and Ivancheva, 2003; Guarino et al, 2008	3	X		X	X					
<i>Convallaria majalis</i> L.	Asparagaceae	Mughetto	w	0.03	0.22	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003	2							X		
<i>Convolvulus althaeoides</i> L.	Convolvulaceae	Vilucchio	w	0.01	0.11	Loi et al, 2005	1	X								
<i>Convolvulus arvensis</i> L.	Convolvulaceae	Vilucchio	w	0.11	0.44	Lokar and Poldrini, 1988; Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; De Natale and Pollio, 2007; Leporatti and Ghedira, 2009; Leto et al, 2012; Di Sanzo et al, 2013; Lucchetti et al, 2019	8	X	X	X	X					X
<i>Convolvulus cantabrica</i> L.	Convolvulaceae	Vilucchio a bicchiere	w	0.01	0.11	Leporatti and Corradi, 2001	1	X								

<i>Coriandrum sativum</i> L.	Apiaceae	Coriandolo	w/c	0.07	0.33	Leporatti and Ivancheva, 2003; Guarino et al, 2008; Leporatti and Ghedira, 2009; Bruschi et al, 2019; Galuzzo et al, 2021	5	X	X	X									
<i>Cornus mas</i> L.	Cornaceae	Corniolo	w	0.08	0.44	Leporatti et al, 1985b; Pieroni, 2000; Leporatti and Ivancheva, 2003; Di Sanzo et al, 2013; Fortini et al, 2016; Lucchetti et al, 2019	6	X	X	X						X			
<i>Cornus sanguinea</i> L.	Cornaceae	Sanguinello	w	0.03	0.22	Leporatti et al, 1985b; Di Novella et al, 2013	2	X	X										
<i>Corydalis cava</i> (L.) Schweigg. & Körte	Papaveraceae	Colombina	w	0.01	0.11	Guarino et al, 2008	1								X				
<i>Corylus avellana</i> L.	Betulaceae	Nocciolo	w/c	0.07	0.67	Leporatti et al, 1985b; Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; Vitalini et al, 2015; Petalka et al, 2020	5	X	X	X			X	X					
<i>Cota altissima</i> (L.) J.Gay	Compositae	Camomilla orticante	w	0.03	0.11	Pieroni et al, 2004a; Pieroni and Quave, 2005;	2	X											
<i>Cota tinctoria</i> (L.) J.Gay	Compositae	Camomilla tintoria	w	0.01	0.11	Guarino et al, 2008	1				X								
<i>Cotinus coggygria</i> Scop.	Anacardiaceae	Sommacco	w	0.03	0.22	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003	2	X				X							
<i>Cotyledon macrantha</i> A.Berger	Crassulaceae	-	w	0.01	0.22	Menale et al, 2016	1	X	X										
<i>Cotyledon orbiculata</i> L.	Crassulaceae		w	0.01	0.22	Palmese et al, 2001	1		X	X									
<i>Crataegus laevigata</i> (Poir.) DC.	Rosaceae	Biancospino	w	0.08	0.67	Leporatti and Ivancheva, 2003; Guarino et al, 2008; Vitalini et al, 2009; Guarrera et al, 2015; Vitalini et al, 2015; Mattalia et al, 2021	6	X	X	X			X	X					
<i>Crataegus monogyna</i> Jacq.	Rosaceae	Biancospino	w	0.49	0.89	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Uncini Manganelli and Tomei, 1999; Leporatti and Corradi, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Loi et al, 2004; Maccioni et al, 2004; Pieroni et al, 2004a; Pieroni et al, 2004b; Loi et al, 2005; Pieroni and Quave, 2005; Passalacqua et al, 2007; Cornara et al, 2009; Signorini et al, 2009; Idolo et al, 2010; Leto et al, 2012; Mattalia et al, 2012; Vitalini et al, 2012; Di Novella et al, 2013; Cornara et al, 2014; Menale and Muoio, 2014; Sansanelli and Tassoni, 2014; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b; Bellia and Pieroni, 2015; Guarrera et al, 2015; Vitalini et al, 2015; Bruschi et al, 2019; Lucchetti et al, 2019; Maruca et al, 2019; Mattalia et al, 2019; Mautone et al, 2019; Mattalia et al, 2020b; Fontefrancesco and Pieroni, 2020; Mattalia et al, 2020a; Petalka et al, 2020	37	X		X	X			X	X				X
<i>Crataegus rhipidophylla</i> Gand.	Rosaceae	-	w	0.05	0.33	Bruni et al, 1997; Menale et al, 2006; Passalacqua et al, 2007; Menale and Muoio, 2014	4	X								X		X	
<i>Crepis biennis</i> Lapeyr.	Compositae	Radicchiella	w	0.01	0.11	Mattalia et al, 2020b	1			X									

<i>Crepis capillaris</i> (L.) Wallr.	Compositae	Radicchiella	w	0.03	0.22	Uncini Manganelli and Tomei, 1999; Pieroni, 2000	2	X					X	
<i>Crepis conyzifolia</i> (Gouan) A.Kern.	Compositae	Radicchiella	w	0.01	0.22	Petalka et al, 2020	1		X					
<i>Crepis vesicaria</i> L.	Compositae	Radicchiella vesciosa	w	0.05	0.56	Bruni et al, 1997; Loi et al, 2004; Sansanelli and Tassoni, 2014; Tuttolomondo et al, 2014; Geraci et al, 2018	5	X	X	X			X	X
<i>Crithmum maritimum</i> L.	Apiaceae	Finocchio di mare	w	0.03	0.44	Cornara et al, 2009; Savo et al, 2011	2	X		X	X			
<i>Crocus imperati</i> Ten.	Iridaceae	Zafferano selvatico	w	0.01	0.11	Guarino et al, 2008	1	X						
<i>Crocus ligusticus</i> Mariotti	Iridaceae	-	w	0.01	0.22	Cornara et al, 2014	1			X			X	
<i>Crocus sativus</i> L.	Iridaceae	Zafferano	c	0.03	0.33	Idolo et al, 2010; Bruschi et al, 2019	2	X			X			
<i>Crocus vernus</i> (L.) Hill	Iridaceae	Zafferano alpino	w	0.03	0.22	Pieroni, 2000; Vitalini et al, 2015	2	X	X					
<i>Cruciata laevipes</i> Opiz	Rubiaceae	Crocettona	w	0.01	0.11	Lucchetti et al, 2019	1	X						
<i>Cucumis melo</i> L.	Cucurbitaceae	Melone	w	0.01	0.11	Passalacqua et al, 2007	1	X						
<i>Cucumis sativus</i> L.	Cucurbitaceae	Cetriolo	c	0.09	0.56	Pieroni et al, 2004b; Guarrera and Lucia, 2007; Passalacqua et al, 2007; Guarino et al, 2008; Fortini et al, 2016; Menale et al, 2016; Motti and Motti, 2017	7	X	X	X	X			
<i>Cucurbita maxima</i> Duchesne	Cucurbitaceae	Zucca	c	0.09	0.33	Cornara et al, 2014; Dei Cas et al, 2015; Menale et al, 2016; Motti and Motti, 2017; Bruschi et al, 2019; Menale et al, 2021; Danna et al, 2022	7	X		X				
<i>Cucurbita pepo</i> L.	Cucurbitaceae	Zucchini	c	0.09	0.44	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Passalacqua et al, 2007; Guarino et al, 2008; Leporatti and Ghedira, 2009; Menale et al, 2016; Mautone et al, 2019	7	X	X		X		X	
<i>Cuminum cyminum</i> L.	Apiaceae	Cumino	c	0.04	0.33	Leporatti and Ghedira, 2009; Tuttolomondo et al, 2014b; Galuzzo et al, 2021	3	X			X			
<i>Cupressus macrocarpa</i> Hartw.	Cupressaceae	Cipresso	c	0.01	0.22	Leporatti et al, 1985a	1	X	X					
<i>Cupressus sempervirens</i> L.	Cupressaceae	Cipresso	w/c	0.11	0.78	Bruni et al, 1997; Camangi et al, 2003; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Leporatti and Ghedira, 2009; Bruschi et al, 2019; Maruca et al, 2019; Mattalia et al, 2019	8	X	X	X	X		X	X
<i>Curcuma longa</i> L.	Zingiberaceae	Curcuma	c	0.01	0.33	Bruschi et al, 2019	1			X	X		X	
<i>Cuscuta europaea</i> L.	Convolvulaceae	Cuscuta	w	0.01	0.22	Leporatti and Ivancheva, 2003	1	X				X		
<i>Cyanus segetum</i> Hill	Compositae	-	w	0.08	0.56	Pieroni et al, 2004b; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Menale et al, 2016; Petalka et al, 2020; Danna et al, 2022	6		X	X	X			
<i>Cyclamen hederifolium</i> Aiton	Primulaceae	Ciclaminio	w	0.09	0.44	Leporatti et al, 1985b; Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Guarrera et al, 2005; Pieroni and Quave, 2005; Guarino et al, 2008; Leto et al, 2012	7	X	X	X	X			

<i>Digitalis ferruginea</i> L.	Plantaginaceae	-	w	0.01	0.11	Guarino et al, 2008	1											X	
<i>Digitalis grandiflora</i> Mill.	Plantaginaceae	Digitale grande	w	0.01	0.11	Lokar and Poldrini, 1988	1											X	
<i>Digitalis lutea</i> subsp. <i>australis</i> (Ten.) Arcang.	Plantaginaceae	Digitale piccola	w	0.01	0.11	De Natale and Pollio, 2007	1											X	
<i>Digitalis purpurea</i> L.	Plantaginaceae	Digitale rossa	w	0.04	0.44	Bruni et al, 1997; Loi et al, 2004; Menale et al, 2006	3											X	
<i>Dioscorea communis</i> (L.) Caddick & Wilkin	Dioscoreaceae	Tamaro	w	0.23	0.78	Lokar and Poldrini, 1988; Bruni et al, 1997; Ballero et al, 2001; Palmese et al, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Guarrera et al, 2005; Loi et al, 2005; Guarrera and Lucia, 2007; Guarino et al, 2008; Leporatti and Ghedira, 2009; Leto et al, 2012; Di Sanzo et al, 2013; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b; Guarrera et al, 2015; Mattalia et al, 2019	17	X	X	X	X	X	X	X	X	X	X	X	X
<i>Diplotaxis eruroides</i> (L.) DC.	Brassicaceae	Rucola	w	0.03	0.33	Leporatti et al, 1985b; Lucchetti et al, 2019	2	X											
<i>Diplotaxis tenuifolia</i> (L.) DC.	Brassicaceae	Rucola	w/c	0.15	0.56	Leporatti and Corradi, 2001; Pieroni et al, 2004a; Pieroni and Quave, 2005; Gonzalez-Tejero et al, 2008; Savo et al, 2011; Montesano et al, 2012; Motti and Motti, 2017; Lucchetti et al, 2019; Mautone et al, 2019; Motti et al, 2020; Petalka et al, 2020	11	X	X	X	X	X	X	X	X	X	X	X	
<i>Dipsacus fullonum</i> L.	Caprifoliaceae	Scardaccione	w	0.08	0.78	Bruni et al, 1997; Guarino et al, 2008; Di Sanzo et al, 2013; Cornara et al, 2014; Motti and Motti, 2017; Petalka et al, 2020	6	X	X	X	X	X	X	X	X	X	X	X	X
<i>Dittrichia graveolens</i> (L.) Greuter	Compositae	Enula	w	0.03	0.44	Maxia et al, 2008; Galuzzo et al, 2021	2	X	X	X	X	X	X	X	X	X	X		
<i>Dittrichia viscosa</i> (L.) Greuter	Compositae	Enula appiccicosa	w	0.18	0.56	Uncini Manganelli and Tomei, 1999; Loi et al, 2004; Maccioni et al, 2004; Passalacqua et al, 2007; Guarino et al, 2008; Cornara et al, 2009; Leonti et al, 2009; Motti et al, 2009; Leto et al, 2012; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Gargano et al, 2018	13	X	X	X	X	X	X	X	X	X	X		
<i>Drimys maritima</i> (L.) Stearn	Asparagaceae	-	w	0.08	0.44	Bruni et al, 1997; Ballero et al, 2001; Guarrera et al, 2005; Leporatti and Ghedira, 2009; Leto et al, 2012; Tuttolomondo et al, 2014	6	X	X	X	X	X	X	X	X	X	X	X	
<i>Drosera rotundifolia</i> L.	Droseraceae	Acchiappamosche	w	0.03	0.33	Leporatti and Ivancheva, 2003; Petalka et al, 2020	2	X	X	X	X	X	X	X	X	X	X		
<i>Dryas octopetala</i> L.	Rosaceae	Camedrio alpino	w	0.03	0.33	Petalka et al, 2021	2											X	
<i>Dryopteris filix-mas</i> (L.) Schott.	Dryopteridaceae	Felce maschio	w	0.15	0.67	Lokar and Poldrini, 1988; Bruni et al, 1997; Leporatti and Ivancheva, 2003; Loi et al, 2004; Guarino et al, 2008; Leporatti and Ghedira, 2009; Di Sanzo et al, 2013; Dei Cas et al, 2015; Vitalini et al, 2015; Petalka et al, 2020; Danna et al, 2022	11	X	X	X	X	X	X	X	X	X	X	X	X

<i>Dryopteris mindshelkensis</i> Pavlov	Dryopteridaceae	-	w	0.01	0.11	Guarino et al, 2008	1			X							
<i>Dysphania ambrosioides</i> (L.) Mosyakin & Clemants	Amaranthaceae	Dripide	w	0.01	0.22	Leporatti and Ghedira, 2009	1	X			X						
<i>Ecballium elaterium</i> (L.) A.Rich.	Cucurbitaceae	Cocomero asinino	w	0.15	0.44	Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Loi et al, 2005; Pieroni and Quave, 2005; Guarino et al, 2008; Leporatti and Ghedira, 2009; Leto et al, 2012; Montesano et al, 2012; Di Novella et al, 2013; Tuttolomondo et al, 2014	11	X	X		X		X				
<i>Echinacea purpurea</i> (L.) Moench	Compositae	Echinacea	w	0.01	0.11	Bruschi et al, 2019	1					X					
<i>Echium italicum</i> L.	Boraginaceae	Viperina maggiore	w	0.05	0.56	Pieroni, 2000; De Natale and Pollio, 2007; Leto et al, 2012; Tuttolomondo et al, 2014	4	X	X	X	X		X				
<i>Echium plantagineum</i> L.	Boraginaceae	-	w	0.01	0.11	Motti et al, 2009	1				X						
<i>Echium vulgare</i> L.	Boraginaceae	Viperina azzurra	w	0.03	0.44	Guarino et al, 2008; Ranfa and Bodesmo, 2017	2	X	X	X	X						
<i>Elaeagnus rhamnoides</i> (L.) A.Nelson	Elaeagnaceae	Olivagno	w	0.04	0.67	Vitalini et al, 2015; Petalka et al, 2020; Danna et al, 2022	3		X	X	X	X	X	X			X
<i>Elymus hispidus</i> (Opiz) Melderis	Poaceae	-	w	0.01	0.11	Tuttolomondo et al, 2014a	1					X					
<i>Elymus repens</i> (L.) Gould	Poaceae	-	w	0.11	0.78	Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; Pieroni and Quave, 2005; Maxia et al, 2008; Tuttolomondo et al, 2014a; Menale et al, 2016; Lucchetti et al, 2019; Petalka et al, 2020	8	X	X	X	X	X	X	X			
<i>Ephedra aphylla</i> Forssk.	Ephedraceae	Efedra	w	0.01	0.11	Gonzalez-Tejero et al, 2008	1				X						
<i>Epilobium angustifolium</i> L.	Onagraceae	Garofanino a foglie strette	w	0.07	0.33	Menale et al, 2006; Fontefrancesco and Pieroni, 2020; Petalka et al, 2020; Mattalia et al, 2021; Danna et al, 2022	5	X			X						X
<i>Epilobium montanum</i> L.	Onagraceae	Garofanino di montagna	w	0.03	0.22	Fortini et al, 2016; Petalka et al, 2020	2					X					X
<i>Epilobium palustre</i> L.	Onagraceae	Garofanino acquatico	w	0.01	0.11	Petalka et al, 2020	1					X					
<i>Epilobium parviflorum</i> Schreb.	Onagraceae	-	w	0.03	0.22	Fortini et al, 2016; Petalka et al, 2020	2					X					X
<i>Epilobium</i> sp.pl.	Onagraceae	-	nd	0.01	0.11	Bottoni et al, 2020	1					X					
<i>Equisetum arvense</i> L.	Equisetaceae	Equiseto dei campi	w	0.38	1.00	Lokar and Poldrini, 1988; Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Loi et al, 2004; Maccioni et al, 2004; Pieroni et al, 2004b; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Cornara et al, 2009; Leto et al, 2012; Mattalia et al, 2014; Vitalini et al, 2012; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Fortini et al,	28	X	X	X	X	X	X	X			X

<i>Eryngium maritimum</i> L.	Apiaceae	Calcatreppola marina	w	0.01	0.11	Palmese et al, 2001	1	X											
<i>Erysimum cheiri</i> (L.) Crantz	Brassicaceae	-	w	0.03	0.22	Passalacqua et al, 2007; Guarino et al, 2008	2			X								X	
<i>Erysimum</i> sp.pl.	Brassicaceae	-	nd	0.01	0.11	Di Sanzo et al, 2013	1	X											
<i>Eucalyptus camaldulensis</i> Dehnh.	Myrtaceae	Eucalipto	w/c	0.08	0.44	Scherrer et al, 2005; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Cornara et al, 2009; Motti and Motti, 2017; Lucchetti et al, 2019	6	X		X									X
<i>Eucalyptus globulus</i> Labill.	Myrtaceae	Eucalipto	w/c	0.22	0.89	Uncini Manganeli and Tomei, 1999; Leporatti and Ivancheva, 2003; Maccioni et al, 2004; Loi et al, 2005; Guarino et al, 2008; Maxia et al, 2008; Cornara et al, 2009; Savo et al, 2011; Montesano et al, 2012; Cornara et al, 2014; Menale and Muoio, 2014; Menale et al, 2016; Motti and Motti, 2017; Bruschi et al, 2019; Mautone et al, 2019; Menale et al, 2021	16	X		X								X	X
<i>Eucalyptus</i> sp.pl.	Myrtaceae	-	nd	0.08	0.67	Ballero et al, 2001; Palmese et al, 2001; Leporatti and Ghedira, 2009; Motti et al, 2009; Mattalia et al, 2019; Mattalia et al, 2020b	6	X		X								X	X
<i>Euonymus europaeus</i> L.	Celastraceae	Evonimo	w	0.05	0.22	Leporatti et al, 1985b; Bruni et al, 1997; Guarino et al, 2008; Di Novella et al, 2013	4	X		X									
<i>Eupatorium cannabinum</i> L.	Compositae	Canapa acquatica	w	0.07	0.67	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Pieroni et al, 2004b; Guarino et al, 2008; Galuzzo et al, 2021	5	X		X								X	
<i>Euphorbia amygdaloides</i> L.	Euphorbiaceae	-	w	0.04	0.22	De Natale and Pollio, 2007; Guarino et al, 2008; Menale et al, 2016	3			X								X	
<i>Euphorbia characias</i> L.	Euphorbiaceae	Euforbia cespugliosa	w	0.04	0.22	Di Novella et al, 2013; Tuttolomondo et al, 2014; Guarrera et al, 2015	3	X		X									
<i>Euphorbia characias</i> subsp. <i>wulfenii</i> (Hoppe ex W.D.J.Koch) Radcl.-Sm.	Euphorbiaceae	Euforbia cespugliosa	w	0.01	0.22	Lokar and Poldrini, 1988	1	X		X									
<i>Euphorbia cyparissias</i> L.	Euphorbiaceae	Euforbia cyparissias	w	0.08	0.22	Pieroni, 2000; Pieroni et al, 2004a; Pieroni and Quave, 2005; Dei Cas et al, 2015; Vitalini et al, 2015; Fortini et al, 2016	6	X		X									
<i>Euphorbia dendroidea</i> L.	Euphorbiaceae	Euforbia arborea	w	0.07	0.11	Savo et al, 2011; Di Sanzo et al, 2013; Gargano et al, 2018; Mautone et al, 2019; Menale et al, 2021	5			X									
<i>Euphorbia falcata</i> L.	Euphorbiaceae	-	w	0.01	0.22	Leporatti et al, 1985b	1	X		X									
<i>Euphorbia helioscopia</i> L.	Euphorbiaceae	Euforbia calenzuola	w	0.20	0.56	Pieroni et al, 2004a; Pieroni and Quave, 2005; De Natale and Pollio, 2007; Guarino et al, 2008; Cornara et al, 2009; Signorini et al, 2009; Idolo et al, 2010; Di Novella et al, 2013; Tuttolomondo et al, 2014; Vitalini et al, 2015; Fortini et al, 2016; Menale et al, 2016; Motti and Motti, 2017; Mattalia et al, 2021; Danna et al, 2022	15	X		X							X	X	

<i>Euphorbia lathyris</i> L.	Euphorbiaceae	Euforbia catapunza	w	0.05	0.22	Pieroni, 2000; Guarino et al, 2008; Cornara et al, 2009; Tuttolomondo et al, 2014b	4	X	X										
<i>Euphorbia paralias</i> L.	Euphorbiaceae	-	w	0.01	0.11	Guarrera and Lucia, 2007	1												
<i>Euphorbia peplus</i> L.	Euphorbiaceae	Euforbia minore	w	0.03	0.33	Guarino et al, 2008; Lucchetti et al, 2019	2	X	X										
<i>Euphorbia rigida</i> M.Bieb.	Euphorbiaceae	-	w	0.03	0.11	Leto et al, 2012; Gargano et al, 2018	2												X
<i>Euphorbia seguieriana</i> Neck.	Euphorbiaceae	-	w	0.01	0.11	Danna et al, 2022	1		X										
<i>Euphorbia</i> sp.pl.	Euphorbiaceae	-	nd	0.03	0.11	Pieroni et al, 2004b; Loi et al, 2005	2		X										
<i>Euphrasia minima</i> Jacq. ex DC.	Orobanchaceae	Eufrasia	w	0.01	0.22	Petalka et al, 2020	1						X					X	
<i>Euphrasia officinalis</i> L.	Orobanchaceae	Eufrasia	w	0.05	0.44	Leporatti and Ivancheva, 2003; Vitalini et al, 2009; Petalka et al, 2020; Danna et al, 2022	4		X	X			X					X	
<i>Euphrasia officinalis</i> subsp. <i>versicolor</i> (A.Kern.) Vitek	Orobanchaceae	Eufrasia	w	0.01	0.22	Petalka et al, 2020	1						X					X	
<i>Euphrasia rostkoviana</i> Hayne	Orobanchaceae	-	w	0.04	0.67	Vitalini et al, 2012; Vitalini et al, 2015; Petalka et al, 2020	3	X	X				X	X				X	
<i>Euphrasia</i> sp.pl.	Orobanchaceae	-	nd	0.01	0.22	Lokar and Poldrini, 1988	1		X	X									
<i>Euphrasia stricta</i> D. Wolff	Orobanchaceae	-	w	0.01	0.22	Petalka et al, 2020	1						X					X	
<i>Fagopyrum esculentum</i> Moench	Polygonaceae	-	w	0.01	0.11	Vitalini et al, 2015	1										X		
<i>Fagus sylvatica</i> L.	Fagaceae	Faggio	w	0.08	0.56	Leporatti et al, 1985b; Camangi et al, 2003; Vitalini et al, 2009; Di Novella et al, 2013; Petalka et al, 2020; Mattalia et al, 2021	6	X	X	X			X						
<i>Ferula communis</i> L.	Apiaceae	Ferula	w	0.04	0.67	Loi et al, 2005; Guarino et al, 2008; Tuttolomondo et al, 2014b	3		X	X			X				X	X	X
<i>Ficaria verna</i> Huds.	Ranunculaceae	Favagello	w	0.11	0.56	Maccioni et al, 2004; Guarrera et al, 2005; Guarrera and Lucia, 2007; Passalacqua et al, 2007; Guarino et al, 2008; Di Sanzo et al, 2013; Ranfa and Bodesmo, 2017; Lucchetti et al, 2019	8	X	X	X							X	X	
<i>Ficaria verna</i> subsp. <i>ficariiformis</i> (Rouy & Foucaud) B.Walln.	Ranunculaceae	Favagello	w	0.01	0.11	Menale et al, 2016	1	X											
<i>Ficus carica</i> L.	Moraceae	Fico	w/c	0.59	0.78	Leporatti et al, 1985a; Lokar and Poldrini, 1988; Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Ballero et al, 2001; Leporatti and Corradi, 2001; Palmese et al, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Maccioni et al, 2004; Pieroni et al, 2004a; Guarrera et al, 2005; Guarrera et al, 2005a; Loi et al, 2005; Pieroni and Quave, 2005; Scherrer et al, 2005; Menale et al, 2006; De Natale and Pollio, 2007; Passalacqua et al,	44	X	X	X	X		X	X			X	X	

<i>Geum montanum</i> L.	Rosaceae	Cariofillata montana	w	0.01	0.33	Petalka et al, 2020	1					X		X		
<i>Geum reptans</i> L.	Rosaceae	Cariofillata montana	w	0.01	0.33	Petalka et al, 2020	1					X		X		
<i>Geum rivale</i> L.	Rosaceae	Cariofillata fluviale	w	0.01	0.33	Petalka et al, 2020	1					X		X		
<i>Geum urbanum</i> L.	Rosaceae	Cariofillata comune	w	0.08	0.67	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003; Guarino et al, 2008; Di Sanzo et al, 2013; Tuttolomondo et al, 2014a; Petalka et al, 2020	6	X	X	X		X		X		
<i>Glaucium flavum</i> Grantz	Papaveraceae	-	w	0.01	0.11	Leporatti and Ivancheva, 2003	1		X							
<i>Glebionis coronaria</i> (L.) Cass. ex Spach	Compositae	Crisantemo giallo	w	0.01	0.11	Tuttolomondo et al, 2014a	1							X		
<i>Glechoma hederacea</i> L.	Lamiaceae	Ellera comune	w	0.05	1.00	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003; Petalka et al, 2020; Galuzzo et al, 2021	4	X	X	X		X		X	X	X
<i>Globularia alypum</i> L.	Plantaginaceae	Vedovella cespitosa	w	0.03	0.22	Leporatti and Ghedira, 2009; Tuttolomondo et al, 2014a	2	X			X					
<i>Globularia cordifolia</i> L.	Plantaginaceae	Vedovella celeste	w	0.01	0.22	Petalka et al, 2020	1					X		X		
<i>Glycyrrhiza glabra</i> L.	Fabaceae	Liquirizia	w	0.20	0.78	Bruni et al, 1997; Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Pieroni and Quave, 2005; Menale et al, 2006; Passalacqua et al, 2007; Maxia et al, 2008; Leporatti and Ghedira, 2009; Leto et al, 2012; Montesano et al, 2012; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b; Sansanelli et al, 2017; Bruschi et al, 2019	15	X	X	X		X		X	X	
<i>Gratiola officinalis</i> L.	Plantaginaceae	Graziella	w	0.03	0.44	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003	2	X				X		X		X
<i>Gymnadenia nigra</i> (L.) Rchb.f.	Orchidaceae	Manina scura	w	0.03	0.33	Vitalini et al, 2012; Danna et al, 2022	2	X				X		X		
<i>Harpagophytum procumbens</i> (Burch.) DC. ex Meisn.	Pedaliaceae	-	w	0.01	0.22	Bruschi et al, 2019	1					X				X
<i>Hedera helix</i> L.	Araliaceae	Edera	w	0.47	0.89	Leporatti et al, 1985a; Leporatti et al, 1985b; Lokar and Poldrini, 1988; Bruni et al, 1997; Ballero et al, 2001; Leporatti and Corradi, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Loi et al, 2004; Pieroni et al, 2004b; Guarrera et al, 2005; Guarrera et al, 2005a; Loi et al, 2005; De Natale and Pollio, 2007; Passalacqua et al, 2007; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Maxia et al, 2008; Leporatti and Ghedira, 2009; Signorini et al, 2009; Vitalini et al, 2009; Idolo et al, 2010; Leto et al, 2012; Cornara et al, 2014; Tuttolomondo et al,	35	X	X	X		X		X	X	X

<i>Heracleum sphondylium</i> L.	Apiaceae	Panace comune	w	0.05	0.33	Leporatti and Ivancheva, 2003; Dei Cas et al, 2015; Vitalini et al, 2015; Petalka et al, 2020	4	X				X					
<i>Herniaria glabra</i> L.	Caryophyllaceae	Erniaria glabra	w	0.03	0.44	Leporatti and Ivancheva, 2003; Petalka et al, 2020	2					X	X				X
<i>Hesperis matronalis</i> L.	Brassicaceae	Violaciocca	w	0.01	0.56	Guarino et al, 2008	1		X	X	X	X	X				X
<i>Hippocrepis emerus</i> (L.) Lassen	Fabaceae	Sferracavallo comune	w	0.03	0.11	De Natale and Pollio, 2007; Guarino et al, 2008	2								X		
<i>Hordeum vulgare</i> L.	Poaceae	Orzo selvatico	w	0.36	0.67	Bruni et al, 1997; Pieroni, 2000; Ballero et al, 2001; Palmese et al, 2001; Camangi et al, 2003; Loi et al, 2004; Maccioni et al, 2004; Pieroni et al, 2004a; Guarrera et al, 2005; Pieroni and Quave, 2005; Scherrer et al, 2005; De Natale and Pollio, 2007; Passalacqua et al, 2007; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Maxia et al, 2008; Leporatti and Ghedira, 2009; Idolo et al, 2010; Savo et al, 2011; Montesano et al, 2012; Menale and Muoio, 2014; Dei Cas et al, 2015; Vitalini et al, 2015; Menale et al, 2016; Motti and Motti, 2017; Menale et al, 2021; Danna et al, 2022	27	X	X	X	X	X	X			X	
<i>Humulus lupulus</i> L.	Cannabaceae	Luppolo	w	0.15	0.78	Lokar and Poldrini, 1988; Bruni et al, 1997; Pieroni, 2000; Leporatti and Ivancheva, 2003; Passalacqua et al, 2007; Mattalia et al, 2012; Di Novella et al, 2013; Vitalini et al, 2015; Bruschi et al, 2019; Mattalia et al, 2019; Petalka et al, 2020	11	X	X	X	X	X	X			X	
<i>Hylocomium splendens</i> (Hedw.) Schimp.	Hylocomiaceae	-	w	0.01	0.11	Dei Cas et al, 2015	1	X									
<i>Hyoscyamus albus</i> L.	Solanaceae	Giusquiamo bianco	w	0.11	0.67	Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Guarrera et al, 2015	8	X	X	X		X				X	
<i>Hyoscyamus niger</i> L.	Solanaceae	Giusquiamo nero	w	0.11	0.78	Lokar and Poldrini, 1988; Bruni et al, 1997; Leporatti and Ivancheva, 2003; Guarino et al, 2008; Vitalini et al, 2009; Di Sanzo et al, 2013; Vitalini et al, 2015; Petalka et al, 2020	8	X	X	X	X	X	X			X	X
<i>Hyoseris radiata</i> L.	Compositae	Radicchio selvatico	w	0.04	0.22	Cornara et al, 2009; Tuttolomondo et al, 2014; Geraci et al, 2018	3	X				X					
<i>Hypericum hircinum</i> L.	Hypericaceae	Erba di san Giovanni	w	0.04	0.22	Pieroni et al, 2004a; Pieroni and Quave, 2005; Tuttolomondo et al, 2014a	3		X				X				
<i>Hypericum maculatum</i> Crantz	Hypericaceae	Erba di san Giovanni	w	0.01	0.44	Petalka et al, 2020	1		X	X	X		X	X		X	
<i>Hypericum montanum</i> L.	Hypericaceae	Erba di san Giovanni	w	0.03	0.22	Petalka et al, 2021	2								X	X	X

<i>Jacobaea delphinifolia</i> (Vahl) Pelsér & Veldkamp	Compositae	-	w	0.01	0.22	Leto et al, 2012	1		X					X			
<i>Jasminum officinale</i> L.	Oleaceae	Gelsomino	w	0.03	0.22	Lucchetti et al, 2019; Tuttolomondo et al, 2014b	2		X				X				
<i>Juglans regia</i> L.	Juglandaceae	Noce	c	0.45	1.00	Lokar and Poldrini, 1988; Bruni et al, 1997; Pieroni, 2000; Ballero et al, 2001; Leporatti and Corradi, 2001; Palmese et al, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Pieroni et al, 2004b; Guarnera et al, 2005; Menale et al, 2006; Passalacqua et al, 2007; Guarino et al, 2008; Leporatti and Ghedira, 2009; Vitalini et al, 2009; Idolo et al, 2010; Savo et al, 2011; Mattalia et al, 2012; Montesano et al, 2012; Di Novella et al, 2013; Di Sanzo et al, 2013; Cornara et al, 2014; Menale and Muoio, 2014; Bellia and Pieroni, 2015; Vitalini et al, 2015; Menale et al, 2016; Bruschi et al, 2019; Maruca et al, 2019; Mautone et al, 2019; Fontefrancesco and Pieroni, 2020; Mattalia et al, 2020a; Menale et al, 2021; Danna et al, 2022	33	X					X				
<i>Juniperus communis</i> L.	Cupressaceae	Ginepro	w	0.35	1.00	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Uncini Manganeli and Tomei, 1999; Pieroni, 2000; Leporatti and Corradi, 2001; Palmese et al, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Scherrer et al, 2005; Menale et al, 2006; Gonzalez-Tejero et al, 2008; Cornara et al, 2009; Pieroni and Giusti, 2009; Vitalini et al, 2009; Vitalini et al, 2012; Di Sanzo et al, 2013; Cornara et al, 2014; Tuttolomondo et al, 2014a; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Fortini et al, 2016; Bruschi et al, 2019; Mattalia et al, 2020a; Petalka et al, 2020; Danna et al, 2022	26	X					X		X	X	X
<i>Juniperus communis</i> var. <i>saxatilis</i> Pall.	Cupressaceae	Ginepro	w	0.03	0.56	Di Novella et al, 2013; Petalka et al, 2020	2	X		X			X	X			
<i>Juniperus oxycedrus</i> L.	Cupressaceae	Ginepro giallo	w	0.08	0.56	Bruni et al, 1997; Ballero et al, 2001; Loi et al, 2004; Cornara et al, 2009; Leporatti and Ghedira, 2009; Lucchetti et al, 2019	6	X		X			X			X	
<i>Juniperus phoenicea</i> L.	Cupressaceae	Ginepro fenicio	w	0.01	0.11	Leporatti and Ghedira, 2009	1					X					
<i>Juniperus sabina</i> L.	Cupressaceae	-	w	0.04	0.44	Vitalini et al, 2015; Petalka et al, 2020; Danna et al, 2022	3					X	X	X			
<i>Juniperus</i> sp.pl.	Cupressaceae	-	nd	0.01	0.22	Idolo et al, 2010	1							X		X	
<i>Kalanchoe</i> sp.pl.	Crassulaceae	-	nd	0.01	0.11	Menale et al, 2021	1						X				
<i>Kickxia elatine</i> (L.) Dumort.	Plantaginaceae	Cencio minore	w	0.01	0.11	Tuttolomondo et al, 2014	1		X								
<i>Kickxia spuria</i> (L.) Dumort.	Plantaginaceae	Cencio spurio	w	0.01	0.11	Tuttolomondo et al, 2014	1		X								

<i>Knautia arvensis</i> (L.) Coult.	Caprifoliaceae	Ambretta	w	0.03	0.33	Guarino et al, 2008; Mattalia et al, 2019	2	X	X										
<i>Laburnum anagyroides</i> Medik.	Fabaceae	Maggiociondolo	w	0.03	0.11	Leporatti and Ivancheva, 2003; Guarino et al, 2008	2	X											
<i>Lactuca sativa</i> L.	Compositae	Lattuga	c	0.26	0.78	Leporatti and Corradi, 2001; Pieroni et al, 2004a; Guarrera et al, 2005; Pieroni and Quave, 2005; Scherrer et al, 2005; Passalacqua et al, 2007; Guarino et al, 2008; Motti et al, 2009; Savo et al, 2011; Montesano et al, 2012; Menale and Muoio, 2014; Vitalini et al, 2015; Fortini et al, 2016; Menale et al, 2016; Motti and Motti, 2017; Geraci et al, 2018; Bruschi et al, 2019; Mautone et al, 2019; Menale et al, 2021	19	X	X	X	X	X	X	X	X				
<i>Lactuca serriola</i> L.	Compositae	Lattuga selvatica	w	0.08	0.78	Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Guarino et al, 2008; Savo et al, 2011; Mattalia et al, 2012; Geraci et al, 2018	6	X	X	X	X	X	X	X	X				
<i>Lactuca virosa</i> Habi.	Compositae	-	w	0.03	0.33	Guarino et al, 2008; Galuzzo et al, 2021	2		X	X									
<i>Lamium album</i> L.	Lamiaceae	Falsa ortica	w	0.14	0.89	Lokar and Poldrini, 1988; Bruni et al, 1997; Leporatti and Ivancheva, 2003; De Natale and Pollio, 2007; Guarino et al, 2008; Vitalini et al, 2012; Menale and Muoio, 2014; Bellia and Pieroni, 2015; Vitalini et al, 2015; Petalka et al, 2020	10	X	X	X	X	X	X	X	X				X
<i>Lamium galeobdolon</i> subsp. <i>flavidum</i> (F.Herm.) A.Löve & D.Löve	Lamiaceae	-	w	0.01	0.11	Petalka et al, 2020	1				X								
<i>Lamium maculatum</i> (L.) L.	Lamiaceae	Falsa Ortica a macchie	w	0.01	0.22	Guarino et al, 2008	1				X								
<i>Lamium purpureum</i> L.	Lamiaceae	Falsa ortica rossa	w	0.01	0.67	Petalka et al, 2020	1				X	X	X	X	X				X
<i>Lapsana communis</i> L.	Compositae	Lassana	w	0.01	0.56	Guarino et al, 2008	1	X	X		X								X
<i>Larix decidua</i> Mill.	Pinaceae	Larice	w	0.14	1.00	Lokar and Poldrini, 1988; Pieroni and Giusti, 2009; Vitalini et al, 2009; Vitalini et al, 2012; Cornara et al, 2014; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Petalka et al, 2020; Danna et al, 2022	10	X	X	X	X	X	X	X	X	X	X	X	X
<i>Lathraea squamaria</i> L.	Orobanchaceae	-	w	0.01	0.11	Guarino et al, 2008	1												
<i>Lathyrus vernus</i> (L.) Bernh.	Fabaceae	Cicerchia primaticcia	w	0.01	0.22	Guarino et al, 2008	1	X	X										
<i>Laurus nobilis</i> L.	Lauraceae	Alloro	w/c	0.76	0.89	Lokar and Poldrini, 1988; Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Ballero et al, 2001; Leporatti and Corradi, 2001; Palmese et al, 2001; Camangi et al, 2003; Loi et al, 2004; Pieroni et al, 2004a; Pieroni et al, 2004b; Guarrera et al, 2005; Guarrera et al, 2005a; Loi et al, 2005; Pieroni and Quave, 2005; Scherrer et al, 2005; Guarrera and	56	X	X	X	X	X	X	X	X	X	X	X	X

<i>Leopoldia comosa</i> (L.) Parl.	Asparagaceae	Lampascione	w	0.15	0.67	Guarrera et al, 2005; Pieroni and Quave, 2005; Guarrera and Lucia, 2007; Guarino et al, 2008; Motti et al, 2009; Savo et al, 2011; Di Novella et al, 2013; Di Sanzo et al, 2013; Tuttolomondo et al, 2014b; Sansanelli et al, 2017; Maruca et al, 2019	11	X	X	X	X	X	X	X				
<i>Lepidium graminifolium</i> L. subsp. <i>graminifolium</i>	Brassicaceae	Lepidio graminifolio	w	0.01	0.11	Savo et al, 2011	1					X						
<i>Lepidium latifolium</i> L.	Brassicaceae	Lepidio grande	w	0.01	0.11	Ranfa and Bodesmo, 2017	1						X					
<i>Lepidium sativum</i> L.	Brassicaceae	Lepidio	w	0.01	0.56	Petalka et al, 2020	1	X	X	X			X	X				
<i>Leucanthemopsis alpina</i> (L.) Heywood	Compositae	-	w	0.03	0.67	Vitalini et al, 2015; Petalka et al, 2020	2	X		X	X	X	X	X				
<i>Leucanthemum vulgare</i> (Vail.) Lam.	Compositae	Margherita selvatica	w	0.03	0.33	Cornara et al, 2009; Petalka et al, 2020	2				X			X				
<i>Leucogium vernum</i> L.	Amaryllidaceae	Camapanella	w	0.01	0.33	Lokar and Poldrini, 1988	1		X					X	X			
<i>Levisticum officinale</i> W.D.J.Koch	Apiaceae	Levistico	w/c	0.01	0.22	Leporatti and Ivancheva, 2003	1	X				X						
<i>Ligustrum vulgare</i> L.	Oleaceae	Ligustro	w	0.03	0.33	Scherrer et al, 2005; Guarino et al, 2008	2	X	X	X								
<i>Lilium bulbiferum</i> L.	Liliaceae	Giglio arancio	w	0.04	0.67	Camangi et al, 2003; Guarino et al, 2008; Petalka et al, 2020	3	X	X	X	X			X				
<i>Lilium candidum</i> L.	Liliaceae	Giglio bianco	c	0.05	0.44	Bruni et al, 1997; Pieroni, 2000; Cornara et al, 2009; Danna et al, 2022	4		X	X	X	X						
<i>Lilium martagon</i> L.	Liliaceae	Giglio martagone	w	0.03	0.67	Vitalini et al, 2015; Petalka et al, 2020	2		X	X				X	X			
<i>Linaria vulgaris</i> Mill.	Plantaginaceae	Linaria comune	w	0.11	0.56	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Pieroni et al, 2004b; Pieroni and Quave, 2005; Guarino et al, 2008	8	X	X	X	X							
<i>Linum bienne</i> Mill.	Linaceae	Lino	w	0.01	0.11	Pieroni et al, 2004b	1		X									
<i>Linum catharticum</i> L.	Linaceae	Lino purgante	w	0.01	0.44	Guarino et al, 2008	1	X	X	X	X							
<i>Linum usitatissimum</i> L.	Linaceae	Lino	c	0.55	0.89	Lokar and Poldrini, 1988; Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Ballero et al, 2001; Palmese et al, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Guarrera et al, 2005; Loi et al, 2004; Maccioni et al, 2004; Pieroni et al, 2004b; Guarrera et al, 2005; Loi et al, 2005; Scherrer et al, 2005; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Maxia et al, 2008; Leporatti and Ghedira, 2009; Motti et al, 2009; Pieroni and Giusti, 2009; Idolo et al, 2010; Savo et al, 2011; Vitalini et al, 2012; Cornara et al, 2014; Menale and Muoio, 2014; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Guarrera et al, 2015; Vitalini et al, 2015;	41	X	X	X	X	X	X	X	X	X	X	X

<i>Melittis melissophyllum</i> L.	Lamiaceae	Erba limona	w	0.01	0.22	Idolo et al, 2010	1	X											
<i>Mentha aquatica</i> L.	Lamiaceae	Menta acquatica	w	0.12	0.78	Bruni et al, 1997; Loi et al, 2004; De Natale and Pollio, 2007; Guarino et al, 2008; Leto et al, 2012; Di Sanzo et al, 2013; Tuttolomondo et al, 2014; Vitalini et al, 2015; Petalka et al, 2020	9	X	X	X				X	X	X	X		X
<i>Mentha arvensis</i> L.	Lamiaceae	Menta dei prati	w	0.05	0.67	Dei Cas et al, 2015; Vitalini et al, 2015; Mattalia et al, 2019; Petalka et al, 2020	4	X	X	X				X	X	X			
<i>Mentha longifolia</i> (L.) L.	Lamiaceae	-	w	0.12	0.89	Lokar and Poldrini, 1988; Uncini Manganelli and Tomei, 1999; Vitalini et al, 2009; Vitalini et al, 2012; Bellia and Pieroni, 2015; Vitalini et al, 2015; Motti et al, 2020; Petalka et al, 2020; Danna et al, 2022	9	X	X	X				X	X	X	X	X	
<i>Mentha piperita</i> L.	Lamiaceae	Menta piperita	c	0.16	0.56	Uncini Manganelli and Tomei, 1999; Leporatti and Corradi, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Menale et al, 2006; Gonzalez-Tejero et al, 2008; Maxia et al, 2008; Dei Cas et al, 2015; Bruschi et al, 2019; Lucchetti et al, 2019; Maruca et al, 2019; Mautone et al, 2019	12	X	X	X				X					
<i>Mentha pulegium</i> L.	Lamiaceae	Menta poggio	w	0.15	0.89	Leporatti and Ivancheva, 2003; Guarino et al, 2008; Maxia et al, 2008; Leporatti and Ghedira, 2009; Di Sanzo et al, 2013; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Motti et al, 2020; Petalka et al, 2020; Menale et al, 2021	11	X	X	X				X	X	X	X	X	
<i>Mentha rotundifolia</i> (L.) Huds.	Lamiaceae	-	c	0.03	0.33	Loi et al, 2005; Mautone et al, 2019	2	X		X				X					
<i>Mentha</i> sp.pl.	Lamiaceae	-	nd	0.23	0.89	Leporatti et al, 1985b; Bruni et al, 1997; Pieroni, 2000; Loi et al, 2005; Scherrer et al, 2005; Cornara et al, 2009; Leporatti and Ghedira, 2009; Savo et al, 2011; Cornara et al, 2014; Menale and Muoio, 2014; Sansanelli and Tassoni, 2014; Menale et al, 2016; Motti and Motti, 2017; Bruschi et al, 2019; Mattalia et al, 2020a; Mattalia et al, 2020b; Galuzzo et al, 2021; Mattalia et al, 2021	18	X	X	X				X	X	X	X	X	
<i>Mentha spicata</i> L.	Lamiaceae	Menta comune	w	0.20	0.67	Ballero et al, 2001; Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Pieroni et al, 2004b; Pieroni and Quave, 2005; Passalacqua et al, 2007; Guarino et al, 2008; Leporatti and Ghedira, 2009; Pieroni and Giusti, 2009; Leto et al, 2012; Dei Cas et al, 2015; Fortini et al, 2016; Mautone et al, 2019; Fontefrancesco and Pieroni, 2020; Motti et al, 2020	15	X	X	X				X	X	X	X		
<i>Mentha suaveolens</i> Ehrh.	Lamiaceae	-	w	0.11	0.67	Uncini Manganelli and Tomei, 1999; Camangi et al, 2003; Pieroni et al, 2004b; De Natale and Pollio,	8	X	X	X				X	X	X	X	X	

<i>Opuntia ficus-indica</i> (L.) Mill.	Cactaceae	Fico d'india	w/c	0.31	0.78	Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Ballero et al, 2001; Palmese et al, 2001; Loi et al, 2004; Loi et al, 2005; Scherrer et al, 2005; Passalacqua et al, 2007; Maxia et al, 2008; Cornara et al, 2009; Leonti et al, 2009; Signorini et al, 2009; Savo et al, 2011; Leto et al, 2012; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Menale et al, 2016; Motti and Motti, 2017; Gargano et al, 2018; Maruca et al, 2019; Mautone et al, 2019; Menale et al, 2021	23	X	X	X	X	X	X	X	X	X
<i>Orchis</i> sp.pl.	Orchidaceae	-	nd	0.01	0.33	Lokar and Poldrini, 1988	1	X	X	X	X	X	X	X	X	X
<i>Origanum majorana</i> L.	Lamiaceae	Maggiorana	w/c	0.12	0.67	Leporatti et al, 1985b; Uncini Manganelli and Tomei, 1999; Loi et al, 2004; Loi et al, 2005; Guarino et al, 2008; Leporatti and Ghedira, 2009; Ranfa and Bodesmo, 2017; Lucchetti et al, 2019; Mattalia et al, 2020a	9	X	X	X	X	X	X	X	X	X
<i>Origanum</i> sp.pl.	Lamiaceae	-	nd	0.01	0.44	Galuzzo et al, 2021	1	X	X	X	X	X	X	X	X	X
<i>Origanum vulgare</i> L.	Lamiaceae	Origano	w/c	0.27	0.89	Lokar and Poldrini, 1988; Pieroni, 2000; Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; De Natale and Pollio, 2007; Guarino et al, 2008; Cornara et al, 2009; Idolo et al, 2010; Cornara et al, 2014; Tuttolomondo et al, 2014b; Bellia and Pieroni, 2015; Vitalini et al, 2015; Fortini et al, 2016; Bruschi et al, 2019; Lucchetti et al, 2019; Mautone et al, 2019; Petalka et al, 2020; Mattalia et al, 2021	20	X	X	X	X	X	X	X	X	X
<i>Origanum vulgare</i> subsp. <i>viridulum</i> (Martini-Donos) Nyman	Lamiaceae	Origano	w/c	0.11	0.56	Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Pieroni and Quave, 2005; Scherrer et al, 2005; Passalacqua et al, 2007; Guarino et al, 2008; Savo et al, 2011; Leto et al, 2012; Montesano et al, 2012; Menale et al, 2016; Menale et al, 2021	8	X	X	X	X	X	X	X	X	X
<i>Ornithogalum umbellatum</i> L.	Asparagaceae	Ornitogallo	w	0.01	0.11	Petalka et al, 2020	1								X	
<i>Orobancha crenata</i> Forssk.	Orobanchaceae	Succiamelle delle fave	w	0.01	0.22	Tuttolomondo et al, 2014b	1	X				X				
<i>Oryza sativa</i> L.	Poaceae	Riso	c	0.07	0.33	Pieroni et al, 2004b; Gonzalez-Tejero et al, 2008; Maxia et al, 2008; Bruschi et al, 2019; Danna et al, 2022	5	X	X	X						
<i>Onyropsis miliacea</i> (L.) Asch. & Schweinf.	Poaceae	Miglio	w	0.01	0.11	Tuttolomondo et al, 2014b	1	X								
<i>Ostrya carpinifolia</i> Scop.	Betulaceae	Carpino nero	w	0.04	0.11	Savo et al, 2011; Lucchetti et al, 2019; Mautone et al, 2019	3					X				

<i>Oxalis acetosella</i> L.	Oxalidaceae	Acetosella dei boschi	w	0.07	0.56	Lokar and Poldrini, 1988; Pieroni, 2000; Leporatti and Ivancheva, 2003; Dei Cas et al, 2015; Vitalini et al, 2015	5	X	X	X	X	X						
<i>Oxalis corniculata</i> L.	Oxalidaceae	Acetosella dei campi	w	0.01	0.22	Bruschi et al, 2019	1			X							X	
<i>Oxalis pes-caprae</i> L.	Oxalidaceae	Acetosella gialla	w	0.01	0.11	Tuttolomondo et al, 2014b	1	X										
<i>Paeonia officinalis</i> L.	Paeoniaceae	Peonia selvatica	w	0.03	0.22	Leporatti and Ivancheva, 2003; Vitalini et al, 2015	2					X	X					
<i>Pallenis spinosa</i> (L.) Cass.	Compositae	Asterisco spinoso	w	0.01	0.33	Tuttolomondo et al, 2014	1		X		X						X	
<i>Panax ginseng</i> C.A.Mey.	Araliaceae	Ginseng	c	0.01	0.11	Bruschi et al, 2019	1			X								
<i>Panicum miliaceum</i> L.	Poaceae	Panico	w	0.04	0.56	Pieroni, 2000; Guarino et al, 2008; Vitalini et al, 2015	3	X	X	X	X	X						
<i>Papaver rhoeas</i> L.	Papaveraceae	Papavero	w	0.59	0.89	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Bruni et al, 1997; Pieroni, 2000; Leporatti and Corradi, 2001; Palmese et al, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Loi et al, 2004; Pieroni et al, 2004b; Guarrera et al, 2005; Loi et al, 2005; Pieroni and Quave, 2005; Scherrer et al, 2005; Menale et al, 2006; De Natale and Pollio, 2007; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Maxia et al, 2008; Cornara et al, 2009; Leporatti and Ghedira, 2009; Motti et al, 2009; Signorini et al, 2009; Vitalini et al, 2009; Idolo et al, 2010; Savo et al, 2011; Leto et al, 2012; Di Novella et al, 2013; Menale and Muoio, 2014; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b; Guarrera et al, 2015; Vitalini et al, 2015; Fortini et al, 2016; Menale et al, 2016; Motti and Motti, 2017; Gargano et al, 2018; Bruschi et al, 2019; Lucchetti et al, 2019; Maruca et al, 2019; Mautone et al, 2019; Mattalia et al, 2020a; Petalka et al, 2020; Menale et al, 2021	44	X	X	X	X	X					X	
<i>Papaver somniferum</i> L.	Papaveraceae	Papavero domestico	w/c	0.16	0.44	Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Pieroni et al, 2004a; Pieroni and Quave, 2005; Guarino et al, 2008; Montesano et al, 2012; Tuttolomondo et al, 2014; Tuttolomondo et al, 2020b; Mattalia et al, 2019; Mattalia et al, 2020b; Mattalia et al, 2021; Danna et al, 2022	12	X	X					X				
<i>Papaver somniferum</i> subsp. <i>setigerum</i> (DC.) Arcang.	Papaveraceae	Papavero domestico	w/c	0.05	0.33	Uncini Manganelli and Tomei, 1999; Palmese et al, 2001; Camangi et al, 2003; Motti et al, 2009	4	X						X				
<i>Parietaria judaica</i> L.	Urticaceae	Vetriola giudaica	w	0.49	0.89	Leporatti et al, 1985a; Lokar and Poldrini, 1988; Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Ballero et al, 2001; Leporatti and Corradi, 2001; Palmese et al, 2001; Loi et al,	36	X			X	X	X	X			X	

<i>Persicaria bistorta</i> (L.) Samp.	Polygonaceae	-	w	0.04	0.44	Leporatti and Ivancheva, 2003; Mattalia et al, 2012; Bellia and Pieroni, 2015	3	X	X	X	X							
<i>Persicaria hydropiper</i> (L.) Delarbre	Polygonaceae	Persicaria pepe d'acqua	w	0.01	0.22	Guarino et al, 2008	1				X				X			
<i>Persicaria lapathifolia</i> (L.) Delarbre	Polygonaceae	Persicaria	w	0.01	0.22	Guarino et al, 2008	1				X				X			
<i>Petasites albus</i> (L.) Gaertn.	Compositae	Farfaraccio bianco	w	0.01	0.11	Leporatti et al, 1985b	1		X									
<i>Petasites hybridus</i> (L.) G.Gaertn., B.Mey. & Scherb.	Compositae	Farfaraccio maggiore	w	0.12	0.89	Lokar and Poldrini, 1988; Pieroni, 2000; Leporatti and Ivancheva, 2003; Guarino et al, 2008; Vitalini et al, 2012; Cornara et al, 2014; Ranfa and Bodesmo, 2017; Petalka et al, 2020; Danna et al, 2022	9	X	X	X	X	X	X	X	X	X	X	
<i>Petroselinum crispum</i> (Mill.) Fuss	Apiaceae	Prezzemolo	c	0.54	0.89	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Ballero et al, 2001; Leporatti and Corradi, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Loi et al, 2004; Maccioni et al, 2004; Pieroni et al, 2004a; Guarrera et al, 2005; Loi et al, 2005; Pieroni and Quave, 2005; Scherrer et al, 2005; Guarrera and Lucia, 2007; Passalacqua et al, 2007; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Maxia et al, 2008; Cornara et al, 2009; Leporatti and Ghedira, 2009; Motti et al, 2009; Savo et al, 2011; Montesano et al, 2012; Cornara et al, 2014; Menale and Muoio, 2014; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Dei Cas et al, 2015; Vitalini et al, 2015; Fortini et al, 2016; Menale et al, 2016; Motti and Motti, 2017; Bruschi et al, 2019; Lucchetti et al, 2019; Mautone et al, 2019; Galuzzo et al, 2021; Menale et al, 2021; Danna et al, 2022	40	X	X	X	X	X	X	X	X	X	X	X
<i>Petroselinum</i> sp.pl.	Apiaceae	-	nd	0.01	0.22	Idolo et al, 2010	1	X	X									
<i>Petunia</i> sp.pl.	Solanaceae	-	nd	0.01	0.11	Menale et al, 2016	1	X										
<i>Peucedanum officinale</i> L.	Apiaceae	-	w	0.03	0.44	Leporatti and Ivancheva, 2003; Guarino et al, 2008	2	X		X	X							
<i>Peucedanum oreoselinum</i> (L.) Moench	Apiaceae	-	w	0.01	0.22	Guarino et al, 2008	1			X	X							
<i>Peucedanum ostruthium</i> (L.) W.D.J.Koch	Apiaceae	-	w	0.05	0.89	Lokar and Poldrini, 1988; Vitalini et al, 2015; Petalka et al, 2020; Danna et al, 2022	4	X	X	X	X	X	X	X	X	X	X	
<i>Phaseolus vulgaris</i> L.	Fabaceae	Fagiolo	c	0.12	0.44	Pieroni, 2000; Camangi et al, 2003; Guarrera et al, 2005; Guarrera and Lucia, 2007; Passalacqua et al, 2007; Guarino et al, 2008; Menale et al, 2016; Lucchetti et al, 2019; Menale et al, 2021	9		X		X				X		X	
<i>Phlomis fruticosa</i> L.	Lamiaceae	Salvione giallo	w	0.01	0.11	Guarrera and Lucia, 2007	1								X			
<i>Phragmites australis</i> (Cav.) Trin. ex Steud.	Poaceae	Cannarella	w	0.03	0.11	Bruni et al, 1997; Guarino et al, 2008	2		X									

<i>Physalis alkekengi</i> L.	Solanaceae	Alkekengi	w/c	0.01	0.33	Leporatti and Ivancheva, 2003	1		X	X				X
<i>Phytolacca americana</i> L.	Phytolaccaceae	Fitolacca	w	0.04	0.33	Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Gonzalez-Tejero et al, 2008	3	X	X			X		
<i>Picea abies</i> (L.) H.Karst.	Pinaceae	Abete rosso	w	0.09	0.89	Lokar and Poldrini, 1988; Vitalini et al, 2009; Vitalini et al, 2012; Dei Cas et al, 2015; Vitalini et al, 2015; Petalka et al, 2020; Danna et al, 2022	7	X	X	X	X	X	X	X
<i>Picris hieracioides</i> Sibth. & Sm.	Compositae	Aspraggine comune	w	0.03	0.22	Scherrer et al, 2005; Lucchetti et al, 2019	2		X					
<i>Pilosella officinarum</i> Vaill.	Compositae	Pelosella comune	w	0.11	0.78	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003; Maccioni et al, 2004; Guarino et al, 2008; Bellia and Pieroni, 2015; Vitalini et al, 2015; Bruschi et al, 2019; Petalka et al, 2020	8	X	X	X	X	X	X	X
<i>Pilosella piloselloides</i> (Vill.) Soják	Compositae	Pelosella fiorentina	w	0.01	0.11	Menale et al, 2016	1			X				
<i>Pimpinella anisum</i> L.	Apiaceae	Anice verde	w	0.18	0.67	Lokar and Poldrini, 1988; Bruni et al, 1997; Ballero et al, 2001; Leporatti and Ivancheva, 2003; Guarrera et al, 2005; Passalacqua et al, 2007; Leporatti and Ghedira, 2009; Idolo et al, 2010; Savo et al, 2011; Dei Cas et al, 2015; Vitalini et al, 2015; Bruschi et al, 2019; Lucchetti et al, 2019	13	X	X	X	X	X	X	X
<i>Pimpinella major</i> (L.) Huds.	Apiaceae	Tragoselino maggiore	w	0.01	0.44	Petalka et al, 2020	1			X	X	X	X	
<i>Pimpinella saxifraga</i> L.	Apiaceae	Tragoselino comune	w	0.04	0.44	Leporatti and Ivancheva, 2003; Di Novella et al, 2013; Petalka et al, 2020	3	X		X	X	X	X	
<i>Pinguicula alpina</i> L.	Lentibulariaceae	Erba unta alpina	w	0.04	0.44	Vitalini et al, 2009; Vitalini et al, 2015; Petalka et al, 2020	3		X	X		X	X	
<i>Pinguicula grandiflora</i> Lam.	Lentibulariaceae	-	w	0.01	0.11	Fontefrancesco and Pieroni, 2020	1		X					
<i>Pinguicula leptoceras</i> Rchb.	Lentibulariaceae	-	w	0.01	0.22	Vitalini et al, 2015	1		X	X				
<i>Pinguicula</i> sp.pl.	Lentibulariaceae	-	w	0.01	0.11	Danna et al, 2022	1		X					
<i>Pinguicula vulgaris</i> L.	Lentibulariaceae	Erba unta comune	w	0.03	0.11	Cornara et al, 2014; Bellia and Pieroni, 2015	2		X					
<i>Pinus cembra</i> L.	Pinaceae	Pino cembro	w	0.08	0.89	Pieroni and Giusti, 2009; Mattalia et al, 2012; Bellia and Pieroni, 2015; Vitalini et al, 2015; Petalka et al, 2020; Danna et al, 2022	6	X	X	X	X	X	X	X
<i>Pinus halepensis</i> Mill.	Pinaceae	Pino d'aleppo	w	0.07	0.33	Maccioni et al, 2004; Scherrer et al, 2005; Passalacqua et al, 2007; Menale and Muoio, 2014; Motti and Motti, 2017	5		X			X		X
<i>Pinus mugo</i> Turra	Pinaceae	Pino mugo	w	0.16	0.67	Lokar and Poldrini, 1988; Vitalini et al, 2009; Idolo et al, 2010; Mattalia et al, 2012; Vitalini et al, 2012; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Bruschi et al, 2019; Fontefrancesco and	12	X	X	X	X	X	X	

<i>Potentilla reptans</i> L.	Rosaceae	Cinquefoglia comune	w	0.14	0.67	Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Pieroni and Quave, 2005; De Natale and Pollio, 2007; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Di Novella et al, 2013; Bellia and Pieroni, 2015; Menale et al, 2016; Petalka et al, 2020	10	X	X	X	X	X	X	X	X
<i>Potentilla</i> sp.pl.	Rosaceae	-	nd	0.01	0.44	Lokar and Poldrini, 1988	1	X		X				X	X
<i>Primula auricula</i> L.	Primulaceae	Primula orecchia d'orso	w	0.01	0.22	Petalka et al, 2020	1		X			X			
<i>Primula elatior</i> (L.) Hill	Primulaceae	-	w	0.04	0.78	Lokar and Poldrini, 1988; Bellia and Pieroni, 2015; Petalka et al, 2020	3	X	X	X	X	X	X	X	X
<i>Primula farinosa</i> L.	Primulaceae	Primula farinosa	w	0.01	0.11	Petalka et al, 2020	1								X
<i>Primula glutinosa</i> Wulfen	Primulaceae	Primula vischiosa	w	0.01	0.33	Petalka et al, 2020	1					X	X	X	
<i>Primula matthioli</i> (L.) K.Richt.	Primulaceae	-	w	0.01	0.33	Petalka et al, 2020	1		X					X	X
<i>Primula</i> sp.pl.	Primulaceae	-	nd	0.03	0.11	Bruschi et al, 2019; Mattalia et al, 2020a	2					X			
<i>Primula veris</i> L.	Primulaceae	Primula odorosa	w	0.08	1.00	Lokar and Poldrini, 1988; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Petalka et al, 2021; Danna et al, 2022	6	X	X	X	X	X	X	X	X
<i>Primula vulgaris</i> Huds.	Primulaceae	Primula comune	w	0.12	0.89	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Guarino et al, 2008; Mattalia et al, 2012; Cornara et al, 2014; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Bellia and Pieroni, 2015; Petalka et al, 2021	9	X	X	X	X	X	X	X	X
<i>Prunella laciniata</i> (L.) L.	Lamiaceae	Prunella gialla	w	0.01	0.22	Guarino et al, 2008	1	X				X			
<i>Prunella vulgaris</i> L.	Lamiaceae	Prunella comune	w	0.01	0.22	Guarino et al, 2008	1	X				X			
<i>Prunus armeniaca</i> L.	Rosaceae	Rusticano	c	0.04	0.33	Scherrer et al, 2005; Guarrera et al, 2015; Menale et al, 2016	3	X	X	X	X	X			
<i>Prunus avium</i> (L.) L.	Rosaceae	Ciliegio	w/c	0.27	0.89	Pieroni, 2000; Maccioni et al, 2004; Loi et al, 2005; Scherrer et al, 2005; Menale et al, 2006; Motti et al, 2009; Savo et al, 2011; Di Novella et al, 2013; Cornara et al, 2014; Bellia and Pieroni, 2015; Vitalini et al, 2015; Fortini et al, 2016; Menale et al, 2016; Motti and Motti, 2017; Bruschi et al, 2019; Lucchetti et al, 2019; Mautone et al, 2019; Fontefrancesco and Pieroni, 2020; Petalka et al, 2020; Danna et al, 2022	20	X	X	X	X	X	X	X	X
<i>Prunus cerasus</i> L.	Rosaceae	Amarena	c	0.14	0.56	Lokar and Poldrini, 1988; Pieroni, 2000; Ballero et al, 2001; Leporatti and Corradi, 2001; Menale et al, 2006; De Natale and Pollio, 2007; Guarino et al, 2008; Idolo et al, 2010; Menale and Muoio, 2014; Menale et al, 2021	10	X		X	X	X			X
<i>Prunus domestica</i> L.	Rosaceae	Prugno	c	0.23	0.56	Lokar and Poldrini, 1988; Uncini Manganeli and Tomei, 1999; Maccioni et al, 2004; Pieroni et al,	17	X	X	X	X	X		X	X

<i>Ranunculus acris</i> L.	Ranunculaceae	Ranuncolo	w	0.04	0.33	Camangi et al, 2003; Dei Cas et al, 2015; Petalka et al, 2020	3	X						X	X	X
<i>Ranunculus bulbosus</i> L.	Ranunculaceae	Ranuncolo	w	0.09	0.56	Pieroni et al, 2004b; Guarino et al, 2008; Cornara et al, 2009; Leporatti and Ghedira, 2009; Signorini et al, 2009; Vitalini et al, 2015; Lucchetti et al, 2019	7	X	X	X					X	
<i>Ranunculus glacialis</i> L.	Ranunculaceae	Ranuncolo glaciale	w	0.01	0.33	Vitalini et al, 2015	1	X	X	X						
<i>Ranunculus paludosus</i> Poir.	Ranunculaceae	Ranuncolo di palude	w	0.01	0.22	Guarino et al, 2008	1							X	X	
<i>Ranunculus sceleratus</i> L.	Ranunculaceae	-	w	0.01	0.11	Pieroni et al, 2004b	1								X	
<i>Ranunculus</i> sp.pl.	Ranunculaceae	-	nd	0.03	0.33	Lokar and Poldrini, 1988; Leporatti and Corradi, 2001	2	X	X						X	
<i>Ranunculus thora</i> L.	Ranunculaceae	-	w	0.01	0.22	Vitalini et al, 2009	1			X					X	
<i>Ranunculus velutinus</i> Ten.	Ranunculaceae	Ranuncolo vellutato	w	0.01	0.11	Lucchetti et al, 2019	1								X	
<i>Raphanus raphanistrum</i> L.	Brassicaceae	Ravanello selvatico	w/c	0.07	0.56	Guarino et al, 2008; Maxia et al, 2008; Leporatti and Ghedira, 2009; Ranfa and Bodesmo, 2017; Petalka et al, 2020	5	X		X	X	X	X			
<i>Raphanus raphanistrum</i> subsp. <i>sativus</i> (L.) Domin	Brassicaceae	Ravanello	w/c	0.11	0.67	Bruni et al, 1997; Leporatti and Ivancheva, 2003; Loi et al, 2004; Pieroni et al, 2004b; Guarino et al, 2008; Leporatti and Ghedira, 2009; Menale and Muoio, 2014; Menale et al, 2016	8	X	X			X	X			X
<i>Rapistrum rugosum</i> (L.) All.	Brassicaceae	Miagro	w	0.01	0.11	Pieroni et al, 2004b	1		X							
<i>Reichardia picroides</i> (L.) Roth	Compositae	Grattalingua	w	0.09	0.78	Pieroni, 2000; Loi et al, 2004; Cornara et al, 2009; Savo et al, 2011; Tuttolomondo et al, 2014a; Geraci et al, 2018; Lucchetti et al, 2019	7	X	X	X	X	X	X			X
<i>Reseda alba</i> L.	Resedaceae	Reseda bianca	w	0.01	0.11	Leporatti and Ghedira, 2009	1		X							
<i>Reseda luteola</i> L.	Resedaceae	Reseda comune	w	0.01	0.22	Leporatti and Ghedira, 2009	1			X	X					
<i>Rhamnus alaternus</i> L.	Rhamnaceae	Ramno	w	0.04	0.22	Ballero et al, 2001; Leporatti and Ghedira, 2009; Leto et al, 2012	3	X	X							
<i>Rhamnus alpina</i> subsp. <i>fallax</i> (Boiss.) Maire & Petitm.	Rhamnaceae	Ramno alpino	w	0.01	0.33	Fortini et al, 2016	1	X	X					X		
<i>Rheum officinale</i> Baill.	Polygonaceae	Rabarbaro	w	0.01	0.11	Bruschi et al, 2019	1	X								
<i>Rheum palmatum</i> L.	Polygonaceae	-	w	0.01	0.22	Leporatti and Ivancheva, 2003	1	X		X						
<i>Rheum rhabarbarum</i> L.	Polygonaceae	Rabarbaro	c	0.04	0.33	Dei Cas et al, 2015; Vitalini et al, 2015; Danna et al, 2022	3	X			X			X		
<i>Rhododendron ferrugineum</i> L.	Ericaceae	Rododendro	w	0.05	0.78	Vitalini et al, 2012; Bellia and Pieroni, 2015; Vitalini et al, 2015; Petalka et al, 2020	4	X	X		X	X	X	X	X	X
<i>Rhododendron hirsutum</i> L.	Ericaceae	Rododendro irsuto	w	0.03	0.33	Lokar and Poldrini, 1988; Fontefrancesco and Pieroni, 2020	2	X		X	X					

<i>Rhus coriaria</i> L.	Anacardiaceae	Sommacco maggiore	w	0.04	0.33	Leto et al, 2012; Tuttolomondo et al, 2014; Gargano et al, 2018	3	X	X	X							
<i>Ribes alpinum</i> L.	Grossulariaceae	Ribes alpinum	w	0.01	0.22	Vitalini et al, 2015	1	X					X				
<i>Ribes nigrum</i> L.	Grossulariaceae	Ribes nero	c	0.01	0.44	Leporatti and Ivancheva, 2003	1	X		X	X				X		
<i>Ribes petraeum</i> Wulfen	Grossulariaceae	Ribes rosso	w	0.03	0.44	Vitalini et al, 2015; Petalka et al, 2020	2	X	X				X				
<i>Ribes rubrum</i> L.	Grossulariaceae	Ribes rosso	w/c	0.04	0.56	Lokar and Poldrini, 1988; Menale et al, 2006; Dei Cas et al, 2015	3	X		X	X	X	X			X	
<i>Ribes uva-crispa</i> L.	Grossulariaceae	Uva spina	w/c	0.05	0.44	Leporatti et al, 1985b; Menale et al, 2006; Fortini et al, 2016; Mattalia et al, 2021	4	X		X	X					X	
<i>Ricinus communis</i> L.	Euphorbiaceae	Ricino	w/c	0.16	0.33	Uncini Manganello and Tomei, 1999; Ballero et al, 2001; Leporatti and Corradi, 2001; Camangi et al, 2003; Loi et al, 2005; Guarino et al, 2008; Maxia et al, 2008; Leporatti and Ghedira, 2009; Leto et al, 2012; Tuttolomondo et al, 2014b; Menale et al, 2016; Menale et al, 2021	12	X	X								
<i>Ridolfia segetum</i> (L.) Moris	Apiaceae	-	w	0.01	0.11	Tuttolomondo et al, 2014	1	X									
<i>Robinia pseudoacacia</i> L.	Fabaceae	Robinia	w	0.12	0.56	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Pieroni and Quave, 2005; Guarino et al, 2008; Lucchetti et al, 2019; Mattalia et al, 2019; Mattalia et al, 2020a; Mattalia et al, 2020b	9	X	X				X	X			
<i>Rosa alba</i> L.	Rosaceae	Rosa chiara	w	0.01	0.11	Danna et al, 2022	1		X								
<i>Rosa canina</i> L.	Rosaceae	Rosa canina	w	0.51	1.00	Leporatti et al, 1985b; Pieroni, 2000; Leporatti and Corradi, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Pieroni et al, 2004b; Loi et al, 2005; Pieroni and Quave, 2005; Menale et al, 2006; Passalacqua et al, 2007; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Leporatti and Ghedira, 2009; Pieroni and Giusti, 2009; Vitalini et al, 2009; Idolo et al, 2010; Savo et al, 2011; Vitalini et al, 2012; Di Novella et al, 2013; Cornara et al, 2014; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Fortini et al, 2016; Bruschi et al, 2019; Lucchetti et al, 2019; Mattalia et al, 2019; Mautone et al, 2019; Bottoni et al, 2020; Fontefrancesco and Pieroni, 2020; Mattalia et al, 2020a; Petalka et al, 2020; Mattalia et al, 2021; Danna et al, 2022	38	X	X				X	X			X
<i>Rosa centifolia</i> L.	Rosaceae	-	w	0.03	0.11	Bellia and Pieroni, 2015; Danna et al, 2022	2							X			
<i>Rosa corymbifera</i> Borkh.	Rosaceae	-	w	0.01	0.11	Petalka et al, 2020	1		X								
<i>Rosa damascena</i> Herrm.	Rosaceae	-	w	0.01	0.11	Pieroni et al, 2004b	1		X								

<i>Rosa gallica</i> L.	Rosaceae	Rosa strisciante	w	0.01	0.33	Leporatti and Ghedira, 2009	1	X	X	X								X		
<i>Rosa montana</i> Chaix ex Vill.	Rosaceae	Rosa alpina	w	0.01	0.11	Petalka et al, 2020	1		X											
<i>Rosa pendulina</i> L.	Rosaceae	Rosa alpina	w	0.01	0.33	Petalka et al, 2020	1			X	X			X						
<i>Rosa sempervirens</i> L.	Rosaceae	Rosa di san Giovanni	w	0.05	0.44	Palmease et al, 2001; Maxia et al, 2008; Menale et al, 2016; Menale et al, 2021	4	X	X					X	X		X			
<i>Rosa</i> sp.pl.	Rosaceae	-	nd	0.08	0.78	Lokar and Poldrini, 1988; Uncini Manganelli and Tomei, 1999; Pieroni et al, 2004a; Idolo et al, 2010; Menale and Muio, 2014; Menale et al, 2016	6	X	X	X	X	X	X	X	X	X				
<i>Rosmarinus officinalis</i> L.	Lamiaceae	Rosmarino	w/c	0.68	1.00	Leporatti et al, 1985a; Leporatti et al, 1985b; Lokar and Poldrini, 1988; Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Ballero et al, 2001; Leporatti and Corradi, 2001; Palmese et al, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Loi et al, 2004; Maccioni et al, 2004; Pieroni et al, 2004a; Pieroni et al, 2004b; Guarrera et al, 2005; Guarrera et al, 2005a; Loi et al, 2005; Pieroni and Quave, 2005; Scherrer et al, 2005; Passalacqua et al, 2007; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Maxia et al, 2008; Cornara et al, 2009; Leonti et al, 2009; Leporatti and Ghedira, 2009; Idolo et al, 2010; Savo et al, 2011; Leto et al, 2012; Montesano et al, 2012; Cornara et al, 2014; Menale and Muio, 2014; Sansanelli and Tassoni, 2014; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Fortini et al, 2016; Menale et al, 2016; Motti and Motti, 2017; Bruschi et al, 2019; Lucchetti et al, 2019; Mattalia et al, 2019; Mautone et al, 2019; Bottoni et al, 2020; Galuzzo et al, 2021; Mattalia et al, 2021; Menale et al, 2021; Danna et al, 2022	51	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Rubia peregrina</i> L.	Rubiaceae	Robbia	w	0.01	0.11	Leporatti et al, 1985a	1										X			
<i>Rubia tinctorium</i> L.	Rubiaceae	Robbia colorata	c	0.01	0.33	Leporatti and Ivancheva, 2003	1	X	X		X			X						
<i>Rubus bifrons</i> Vest	Rosaceae	-	w	0.01	0.44	Petalka et al, 2020	1		X		X	X	X							
<i>Rubus caesius</i> L.	Rosaceae	Rovo bluastro	w	0.03	0.22	Di Novella et al, 2013; Mautone et al, 2019	2	X	X	X										
<i>Rubus idaeus</i> L.	Rosaceae	Lampone	w	0.19	0.89	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Pieroni, 2000; Leporatti and Ivancheva, 2003; Vitalini et al, 2009; Idolo et al, 2010; Vitalini et al, 2012; Di Novella et al, 2013; Dei Cas et al, 2015; Vitalini et al, 2015; Fortini et al, 2016; Geraci et al, 2018; Mattalia et al, 2019; Petalka et al, 2020	14	X	X	X	X	X	X	X	X	X	X	X		

<i>Rubus</i> sp.pl.	Rosaceae	-	nd	0.04	0.67	Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; Di Sanzo et al, 2013	3	X	X	X	X	X	X	X	X	X
<i>Rubus ulmifolius</i> Schott	Rosaceae	Rovo	w	0.53	0.89	Leporatti et al, 1985a; Leporatti et al, 1985b; Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Ballero et al, 2001; Camangi et al, 2003; Loi et al, 2004; Pieroni et al, 2004a; Guarrera et al, 2005; Guarrera et al, 2005a; Pieroni and Quave, 2005; Scherrer et al, 2005; Menale et al, 2006; De Natale and Pollio, 2007; Passalacqua et al, 2007; Guarino et al, 2008; Leonti et al, 2009; Leporatti and Ghedira, 2009; Signorini et al, 2009; Idolo et al, 2010; Savo et al, 2011; Leto et al, 2012; Menale and Muoio, 2014; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Bellia and Pieroni, 2015; Guarrera et al, 2015; Fortini et al, 2016; Menale et al, 2016; Motti and Motti, 2017; Gargano et al, 2018; Lucchetti et al, 2019; Maruca et al, 2019; Mattalia et al, 2019; Fontefrancesco and Pieroni, 2020; Mattalia et al, 2020a; Mattalia et al, 2020b; Menale et al, 2021	39	X	X	X	X	X	X	X	X	X
<i>Rubus vulgaris</i> Weihe & Nees	Rosaceae	rovo	w	0.08	0.44	Lokar and Poldrini, 1988; Pieroni et al, 2004b; Loi et al, 2005; Leonti et al, 2009; Di Novella et al, 2013; Vitalini et al, 2015	6	X	X	X	X	X	X	X	X	X
<i>Rumex acetosa</i> L.	Polygonaceae	Romice	w	0.20	1.00	Lokar and Poldrini, 1988; Bruni et al, 1997; Pieroni, 2000; Leporatti and Ivancheva, 2003; Menale et al, 2006; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Leporatti and Ghedira, 2009; Vitalini et al, 2012; Cornara et al, 2014; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Fontefrancesco and Pieroni, 2020; Petalka et al, 2020	15	X	X	X	X	X	X	X	X	X
<i>Rumex acetosella</i> L.	Polygonaceae	Romice minore	w	0.04	0.44	Bruni et al, 1997; Loi et al, 2004; Mattalia et al, 2019	3	X	X	X	X	X	X	X	X	X
<i>Rumex alpinus</i> L.	Polygonaceae	Rabarbaro di monte	w	0.12	0.67	Pieroni and Giusti, 2009; Mattalia et al, 2012; Vitalini et al, 2012; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Vitalini et al, 2015; Fontefrancesco and Pieroni, 2020; Petalka et al, 2020; Danna et al, 2022	9	X	X	X	X	X	X	X	X	X
<i>Rumex bucephalophorus</i> L.	Polygonaceae	-	w	0.01	0.22	Loi et al, 2004	1	X	X	X	X	X	X	X	X	X
<i>Rumex conglomeratus</i> Murray	Polygonaceae	-	w	0.01	0.11	Scherrer et al, 2005	1	X	X	X	X	X	X	X	X	X
<i>Rumex crispus</i> L.	Polygonaceae	Romice screspo	w	0.20	0.56	Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Leporatti and Corradi, 2001; Loi et al, 2004; Pieroni et al, 2004b; Guarrera et al, 2005; Menale et al, 2006; Cornara et al, 2009; Motti	15	X	X	X	X	X	X	X	X	X

<i>Scolopendrium officinale</i> DC.	Aspleniaceae	Lingua cervina	w	0.01	0.44	Leporatti and Ivancheva, 2003	1	X	X	X	X						
<i>Scrophularia canina</i> L.	Scrophulariaceae	Scrophularia comune	w	0.08	0.56	Pieroni et al, 2004a; Pieroni and Quave, 2005; Guarrera and Lucia, 2007; Passalacqua et al, 2007; Guarino et al, 2008; Idolo et al, 2010	6	X	X	X					X		X
<i>Scrophularia nodosa</i> L.	Scrophulariaceae	Scrophularia nodosa	w	0.04	0.56	Guarino et al, 2008; Petalka et al, 2020; Danna et al, 2022	3	X	X	X				X			
<i>Scrophularia trifoliata</i> L.	Scrophulariaceae	Scrophularia a tre foglie	w	0.04	0.22	Bruni et al, 1997; Loi et al, 2004; Loi et al, 2005	3		X								
<i>Secale cereale</i> L.	Poaceae	Segale	c	0.04	0.44	Cornara et al, 2014; Dei Cas et al, 2015; Vitalini et al, 2015	3	X	X	X			X				
<i>Sedum acre</i> L.	Crassulaceae	Borracina gialla	w	0.01	0.22	Leporatti and Ivancheva, 2003	1		X						X		
<i>Sedum album</i> L.	Crassulaceae	Borracina bianca	w	0.03	0.11	Pieroni and Giusti, 2009; Danna et al, 2022	2		X								
<i>Sedum atratum</i> L.	Crassulaceae	Borracina verde	w	0.01	0.22	Petalka et al, 2020	1		X						X		
<i>Sedum caeruleum</i> L.	Crassulaceae	Borracina azzurra	w	0.01	0.22	Loi et al, 2004	1		X							X	
<i>Sedum maximum</i> (L.) Suter	Crassulaceae	Borracina maggiore	w	0.07	0.44	Lokar and Poldrini, 1988; Guarino et al, 2008; Vitalini et al, 2015; Menale et al, 2021; Danna et al, 2022	5	X	X						X	X	
<i>Sedum roseum</i> (L.) Scop.	Crassulaceae	-	w	0.01	0.33	Petalka et al, 2020	1		X						X		
<i>Sedum rupestre</i> L.	Crassulaceae	Borracina delle rupi	w	0.03	0.11	Pieroni et al, 2004a; Pieroni and Quave, 2005	2			X			X				
<i>Sedum</i> sp.pl.	Crassulaceae	-	nd	0.01	0.11	Ballero et al, 2001	1		X								
<i>Sedum telephium</i> L.	Crassulaceae	-	c	0.14	0.44	Uncini Manganelli and Tomei, 1999; Leporatti and Corradi, 2001; Maccioni et al, 2004; Pieroni et al, 2004a; Pieroni and Quave, 2005; Menale et al, 2006; Di Novella et al, 2013; Vitalini et al, 2015; Fontefrancesco and Pieroni, 2020; Petalka et al, 2020	10		X	X					X		
<i>Sempervivum arachnoideum</i> L.	Crassulaceae	Semprevivo ragnateloso	w	0.01	0.22	Leporatti et al, 1985b	1		X		X						
<i>Sempervivum montanum</i> L.	Crassulaceae	Semprevivo montano	w	0.05	0.89	Vitalini et al, 2012; Bellia and Pieroni, 2015; Vitalini et al, 2015; Petalka et al, 2020	4	X	X	X	X	X	X	X	X		X
<i>Sempervivum</i> sp.pl.	Crassulaceae	-	nd	0.01	0.11	Idolo et al, 2010	1		X								
<i>Sempervivum tectorum</i> L.	Crassulaceae	Semprevivo dei tetti	w	0.11	0.78	Lokar and Poldrini, 1988; Camangi et al, 2003; Guarrera et al, 2005; Guarino et al, 2008; Di Sanzo et al, 2013; Fortini et al, 2016; Mattalia et al, 2020a; Petalka et al, 2020	8	X	X	X	X			X		X	X
<i>Senecio delphinifolius</i> Vahl.	Compositae	-	w	0.01	0.11	Tuttolomondo et al, 2014	1								X		
<i>Senecio vulgaris</i> L.	Compositae	Senecio comune	w	0.09	0.44	Camangi et al, 2003; Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Pieroni and Quave, 2005;	7	X	X	X		X	X	X			

<i>Solanum dulcamara</i> L.	Solanaceae	Morella rampicante	w	0.15	0.78	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003; Guarino et al, 2008; Idolo et al, 2010; Leto et al, 2012; Tuttolomondo et al, 2014a; Tuttolomondo et al, 2014b; Vitalini et al, 2015; Menale et al, 2016; Fontefrancesco and Pieroni, 2020; Petalka et al, 2020	11	X	X	X	X	X	X	X	X	X	X	X
<i>Solanum linnaeanum</i> Hepper & P.-M.L.Jaeger	Solanaceae	-	w	0.01	0.44	Palmese et al, 2001	1	X	X	X	X	X	X	X	X	X	X	X
<i>Solanum lycopersicum</i> L.	Solanaceae	Pomodoro	c	0.05	0.44	Maxia et al, 2008; Savo et al, 2011; Motti and Motti, 2017; Menale et al, 2021	4	X	X	X	X	X	X	X	X	X	X	X
<i>Solanum melongena</i> L.	Solanaceae	Melanzana	c	0.04	0.44	Di Sanzo et al, 2013; Menale et al, 2016; Mautone et al, 2019	3	X	X	X	X	X	X	X	X	X	X	X
<i>Solanum tuberosum</i> L.	Solanaceae	Patata	c	0.46	0.78	Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Leporatti and Corradi, 2001; Palmese et al, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Maccioni et al, 2004; Pieroni et al, 2004b; Guarrera et al, 2005; Guarrera et al, 2005a; Pieroni and Quave, 2005; Scherrer et al, 2005; Menale et al, 2006; Passalacqua et al, 2007; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Maxia et al, 2008; Cornara et al, 2009; Motti et al, 2009; Idolo et al, 2010; Savo et al, 2011; Cornara et al, 2014; Menale and Muoio, 2014; Tuttolomondo et al, 2014b; Dei Cas et al, 2015; Guarrera et al, 2015; Vitalini et al, 2015; Fortini et al, 2016; Menale et al, 2016; Motti and Motti, 2017; Bruschi et al, 2019; Lucchetti et al, 2019; Mautone et al, 2019; Danna et al, 2022	34	X	X	X	X	X	X	X	X	X	X	X
<i>Soldanella alpina</i> L.	Primulaceae	Soldanella	w	0.01	0.11	Petalka et al, 2020	1										X	
<i>Solidago virgaurea</i> L.	Compositae	Verga d'oro	w	0.07	0.78	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003; Guarino et al, 2008; Petalka et al, 2020	5	X	X	X	X	X	X	X	X	X	X	X
<i>Sonchus arvensis</i> L.	Compositae	Grespino comune	w	0.03	0.22	Guarrera et al, 2005; Sansanelli and Tassoni, 2014; Fortini et al, 2016	3	X	X	X	X	X	X	X	X	X	X	X
<i>Sonchus asper</i> (L.) Hill	Compositae	Grespino spinoso	w	0.15	0.67	Pieroni, 2000; Guarrera et al, 2005; Pieroni and Quave, 2005; Savo et al, 2011; Tuttolomondo et al, 2014; Dei Cas et al, 2015; Fortini et al, 2016; Ranfa and Bodesmo, 2017; Geraci et al, 2018; Lucchetti et al, 2019; Menale et al, 2021	12	X	X	X	X	X	X	X	X	X	X	X
<i>Sonchus oleraceus</i> (L.) L.	Compositae	Grespino comune	w	0.30	0.78	Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Loi et al, 2004; Pieroni et al, 2004a; Guarrera et al, 2005; Guarrera et al, 2005a; Pieroni and Quave, 2005; Pieroni et al, 2005; Menale et al, 2006; Guarrera and Lucia, 2007; Guarino et al,	22	X	X	X	X	X	X	X	X	X	X	X

<i>Taraxacum</i> sp.pl.	Compositae	-	nd	0.01	0.22	Bottoni et al, 2020	1												
<i>Taxus baccata</i> L.	Taxaceae	Tasso	w/c	0.03	0.22	Bruni et al, 1997; Menale et al, 2006	2		X		X								
<i>Teucrium capitatum</i> L.	Lamiaceae	Camedrio capitato	w	0.01	0.22	Leporatti and Ghedira, 2009	1		X	X									
<i>Teucrium chamaedrys</i> L.	Lamiaceae	Camedrio	w	0.18	0.67	Pieroni, 2000; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Maccioni et al, 2004; Pieroni et al, 2004a; Pieroni and Quave, 2005; Guarino et al, 2008; Cornara et al, 2009; Savo et al, 2011; Di Sanzo et al, 2013; Cornara et al, 2014; Bellia and Pieroni, 2015; Danna et al, 2022	13	X	X	X	X							X	
<i>Teucrium flavum</i> L.	Lamiaceae	Camedrio doppio	w	0.01	0.11	Gargano et al, 2018	1											X	
<i>Teucrium fruticans</i> L.	Lamiaceae	-	w	0.01	0.11	Tuttolomondo et al, 2014a	1	X											
<i>Teucrium marum</i> L.	Lamiaceae	-	w	0.03	0.22	Bruni et al, 1997; Uncini Manganelli and Tomei, 1999	2					X	X						
<i>Teucrium montanum</i> L.	Lamiaceae	Camedrio montano	w	0.01	0.22	Guarino et al, 2008	1	X											X
<i>Teucrium polium</i> L.	Lamiaceae	Camedrio femmina	w	0.04	0.33	Bruni et al, 1997; Maccioni et al, 2004; Fortini et al, 2016	3	X	X				X						
<i>Teucrium scordium</i> L.	Lamiaceae	-	w	0.03	0.22	Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b	2		X				X						
<i>Thalictrum aquilegifolium</i> L.	Ranunculaceae	Pigamo	w	0.01	0.11	Guarino et al, 2008	1							X					
<i>Thalictrum flavum</i> L.	Ranunculaceae	Pigamo giallo	w	0.01	0.22	Guarino et al, 2008	1	X				X							
<i>Thapsia garganica</i> L.	Apiaceae	-	w	0.04	0.33	Bruni et al, 1997; Guarino et al, 2008; Leporatti and Ghedira, 2009	3							X				X	X
<i>Thlaspi rotundifolium</i> (L.) Gaudin	Brassicaceae	-	w	0.01	0.11	Petalka et al, 2020	1						X						
<i>Thuja occidentalis</i> L.	Cupressaceae	Tunia	c	0.01	0.22	Leporatti and Ivancheva, 2003	1		X			X							
<i>Thymbra capitata</i> (L.) Cav.	Lamiaceae	Timo arbustivo	w	0.07	0.44	Loi et al, 2005; Leporatti and Ghedira, 2009; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b; Gargano et al, 2018	5	X			X			X					
<i>Thymus herba-barona</i> Loisel.	Lamiaceae	Timo del barone	w	0.03	0.67	Bruni et al, 1997; Loi et al, 2004	2	X			X		X	X			X	X	
<i>Thymus longicaulis</i> C.Presl	Lamiaceae	-	w	0.07	0.22	Scherrer et al, 2005; Savo et al, 2011; Menale and Muolo, 2014; Fortini et al, 2016; Menale et al, 2016	5	X						X					
<i>Thymus praecox</i> subsp. <i>polytrichus</i> (A.Kern. ex Borbás) Jalas	Lamiaceae	Timo	w	0.01	0.67	Vitalini et al, 2015	1	X	X		X	X	X	X			X		
<i>Thymus pulegioides</i> L.	Lamiaceae	Timo	w	0.15	1.00	Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Menale et al, 2006; Cornara et al, 2009; Pieroni and Giusti, 2009; Vitalini et al, 2009; Vitalini et al, 2012; Vitalini et al, 2015; Fortini et al, 2016; Petalka et al, 2020; Danna et al, 2022	11	X	X		X	X	X	X			X	X	X

<i>Thymus serpyllum</i> L.	Lamiaceae	Timo serpillio	w	0.14	0.78	Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Mattalia et al, 2012; Di Novella et al, 2013; Bellia and Pieroni, 2015; Bellia and Pieroni, 2015; Dei Cas et al, 2015; Bruschi et al, 2019; Mattalia et al, 2019; Fontefrancesco and Pieroni, 2020	10	X	X	X	X	X	X	X	X
<i>Thymus</i> sp.pl.	Lamiaceae	-	nd	0.07	0.44	Lokar and Poldrini, 1988; Leporatti and Ivancheva, 2003; Bottoni et al, 2020; Mattalia et al, 2020a; Mattalia et al, 2021	5	X	X	X	X	X	X	X	X
<i>Thymus vulgaris</i> L.	Lamiaceae	Timo comune	w/c	0.11	0.78	Maccioni et al, 2004; Cornara et al, 2009; Idolo et al, 2010; Leto et al, 2012; Cornara et al, 2014; Lucchetti et al, 2019; Mautone et al, 2019; Galuzzo et al, 2021	8	X	X	X	X	X	X	X	X
<i>Thymus zygis</i> L.	Lamiaceae	-	w	0.01	0.11	Tuttolomondo et al, 2014a	1					X	X		
<i>Tilia americana</i> L.	Malvaceae	Tiglio americano	c	0.03	0.44	Guarrera et al, 2005; Tuttolomondo et al, 2014	2	X	X	X	X	X	X	X	
<i>Tilia cordata</i> Mill.	Malvaceae	Tiglio	w	0.26	0.78	Leporatti et al, 1985b; Uncini Manganelli and Tomei, 1999; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Maccioni et al, 2004; Pieroni et al, 2004a; Pieroni et al, 2004b; Pieroni and Quave, 2005; Menale et al, 2006; Mattalia et al, 2012; Di Sanzo et al, 2013; Bellia and Pieroni, 2015; Lucchetti et al, 2019; Mattalia et al, 2019; Fontefrancesco and Pieroni, 2020; Mattalia et al, 2020a; Mattalia et al, 2020b; Petalka et al, 2020; Mattalia et al, 2021	19	X	X	X	X	X	X	X	X
<i>Tilia platyphyllos</i> Scop.	Malvaceae	Tiglio nostrano	w	0.23	0.78	Pieroni, 2000; Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Vitalini et al, 2009; Idolo et al, 2010; Savo et al, 2011; Vitalini et al, 2012; Cornara et al, 2014; Vitalini et al, 2015; Fortini et al, 2016; Menale et al, 2016; Maruca et al, 2019; Mautone et al, 2019; Petalka et al, 2020; Danna et al, 2022	17	X	X	X	X	X	X	X	X
<i>Tilia</i> sp.pl.	Malvaceae	-	nd	0.05	0.67	Lokar and Poldrini, 1988; Guarrera et al, 2015; Motti and Motti, 2017; Bruschi et al, 2019	4	X	X	X	X	X	X	X	X
<i>Tordylium apulum</i> L.	Apiaceae	-	w	0.01	0.11	Ranfa and Bodesmo, 2017	1	X	X						
<i>Tragopogon dubius</i> Scop.	Compositae	Barba di becco	w	0.01	0.11	Petalka et al, 2020	1	X	X						
<i>Tragopogon porrifolius</i> L.	Compositae	Barba di becco viola	w	0.03	0.44	Geraci et al, 2018; Galuzzo et al, 2021	2	X	X	X	X	X	X	X	X
<i>Tragopogon pratensis</i> L.	Compositae	Barba di becco comune	w	0.05	0.44	Guarrera et al, 2005; Mattalia et al, 2012; Ranfa and Bodesmo, 2017; Danna et al, 2022	4	X	X	X	X	X	X	X	X
<i>Tribulus terrestris</i> L.	Zygophyllaceae	Tribolo	w	0.01	0.11	Bruni et al, 1997	1	X							
<i>Trifolium campestre</i> Schreb.	Fabaceae	Trifoglio campestre	w	0.01	0.33	Lokar and Poldrini, 1988	1	X	X			X	X	X	
<i>Trifolium medium</i> L.	Fabaceae	Trifoglio	w	0.01	0.22	Vitalini et al, 2015	1						X	X	
<i>Trifolium pallidum</i> Waldst. & Kit.	Fabaceae	Trifoglio	w	0.01	0.22	Guarino et al, 2008	1	X	X	X	X	X	X	X	

<i>Typha angustifolia</i> L.	Typhaceae	Lisca minore	w	0.03	0.22	Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b	2	X	X												
<i>Typha latifolia</i> L.	Typhaceae	Lisca maggiore	w	0.01	0.22	Motti et al, 2009	1		X								X				
<i>Ulmus minor</i> Mill.	Ulmaceae	Olmo	w	0.19	0.78	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Pieroni et al, 2004a; Pieroni et al, 2004b; Guarrera et al, 2005; Pieroni and Quave, 2005; Menale et al, 2006; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Savo et al, 2011; Guarrera et al, 2015; Menale et al, 2016	14	X	X	X	X	X	X	X	X	X	X	X	X		
<i>Ulmus</i> sp.pl.	Ulmaceae	-	nd	0.01	0.33	Idolo et al, 2010; Mattalia et al, 2019	1	X	X								X				
<i>Umbilicus horizontalis</i> (Guss.) DC.	Crassulaceae	-	w	0.09	0.33	Palme et al, 2001; Passalacqua et al, 2007; Guarino et al, 2008; Signorini et al, 2009; Leto et al, 2012; Tuttolomondo et al, 2014; Motti and Motti, 2017	7	X	X												
<i>Umbilicus rupestris</i> (Salisb.) Dandy	Crassulaceae	Ombelico di Venere	w	0.24	0.56	Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Ballero et al, 2001; Palmese et al, 2001; Loi et al, 2004; Maccioni et al, 2004; Pieroni et al, 2004a; Guarrera et al, 2005; Loi et al, 2005; Pieroni and Quave, 2005; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Cornara et al, 2009; Leto et al, 2012; Di Novella et al, 2013; Di Sanzo et al, 2013; Tuttolomondo et al, 2014b; Maruca et al, 2019	18	X	X	X	X	X	X	X	X						
<i>Urospermum dalechampii</i> (L.) Scop. ex F.W.Schmidt	Compositae	Boccione maggiore	w	0.04	0.33	Cornara et al, 2009; Tuttolomondo et al, 2014; Geraci et al, 2018	3	X	X			X	X								
<i>Urtica atrovirens</i> Req. ex Loisel.	Urticaceae	Ortica verde	w	0.01	0.11	Loi et al, 2005	1	X													
<i>Urtica dioica</i> L.	Urticaceae	Ortica comune	w	0.76	1.00	Leporatti et al, 1985b; Lokar and Poldrini, 1988; Bruni et al, 1997; Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Leporatti and Corradi, 2001; Camangi et al, 2003; Leporatti and Ivancheva, 2003; Loi et al, 2004; Maccioni et al, 2004; Pieroni et al, 2004b; Guarrera et al, 2005a; Loi et al, 2005; Pieroni and Quave, 2005; Scherrer et al, 2005; De Natale and Pollio, 2007; Guarrera and Lucia, 2007; Passalacqua et al, 2007; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Maxia et al, 2008; Cornara et al, 2009; Pieroni and Giusti, 2009; Signorini et al, 2009; Vitalini et al, 2009; Leto et al, 2012; Mattalia et al, 2012; Montesano et al, 2012; Vitalini et al, 2012; Di Novella et al, 2013; Di Sanzo et al, 2013; Cornara et al, 2014; Menale and Muoio, 2014; Tuttolomondo et al, 2014; Tuttolomondo et al, 2014b; Bellia and	56	X	X	X	X	X	X	X	X	X	X	X	X	X	X

<i>Viola alba</i> subsp. <i>dehnhardtii</i> (Ten.) W. Becker	Violaceae	Viola bianca	w	0.01	0.33	Guarino et al, 2008	1	X		X		X		X
<i>Viola arvensis</i> Murray	Violaceae	-	w	0.03	0.33	Guarino et al, 2008; Menale et al, 2016	2	X		X				X
<i>Viola biflora</i> L.	Violaceae	Viola gialla	w	0.03	0.33	Vitalini et al, 2012; Petalka et al, 2020	2	X		X		X		
<i>Viola calcarata</i> L.	Violaceae	-	w	0.04	0.33	Bellia and Pieroni, 2015; Fontefrancesco and Pieroni, 2020; Danna et al, 2022	3	X		X		X		
<i>Viola eugeniae</i> Parl.	Violaceae	-	w	0.01	0.11	Fortini et al, 2016	1			X				
<i>Viola odorata</i> L.	Violaceae	Viola comune	w	0.16	0.78	Leporatti and Ivancheva, 2003; Pieroni et al, 2004b; De Natale and Pollio, 2007; Guarino et al, 2008; Pieroni and Giusti, 2009; Idolo et al, 2010; Mattalia et al, 2012; Di Novella et al, 2013; Fortini et al, 2016; Mattalia et al, 2019; Mautone et al, 2019; Petalka et al, 2020	12	X	X	X	X	X	X	
<i>Viola pseudogracilis</i> Strobl	Violaceae	-	w	0.01	0.33	Guarino et al, 2008	1	X		X		X		X
<i>Viola reichenbachiana</i> Jord. ex Boreau	Violaceae	-	w	0.04	0.56	Leporatti et al, 1985b; Guarino et al, 2008; Fortini et al, 2016	3	X	X	X		X		X
<i>Viola</i> sp.pl.	Violaceae	-	nd	0.05	0.67	Lokar and Poldrini, 1988; Guarrera et al, 2005; Passalacqua et al, 2007; Bruschi et al, 2019	4	X	X	X	X	X	X	
<i>Viola tricolor</i> L.	Violaceae	Viola a tre colori	w	0.07	0.78	Leporatti and Ivancheva, 2003; Bellia and Pieroni, 2015; Vitalini et al, 2015; Petalka et al, 2020; Danna et al, 2022	5	X	X	X	X			X
<i>Viscum album</i> L.	Santalaceae	Vischio	w	0.11	0.67	Leporatti and Corradi, 2001; Leporatti and Ivancheva, 2003; Menale et al, 2006; Passalacqua et al, 2007; Guarino et al, 2008; Leto et al, 2012; Tuttolomondo et al, 2014b; Petalka et al, 2020	8	X	X	X	X	X	X	X
<i>Vitex agnus-castus</i> L.	Lamiaceae	Agnocasto	c	0.03	0.33	Guarino et al, 2008; Geraci et al, 2018	2	X		X		X		
<i>Vitis vinifera</i> L.	Vitaceae	Vite	w/c	0.38	1.00	Uncini Manganelli and Tomei, 1999; Pieroni, 2000; Ballero et al, 2001; Leporatti and Corradi, 2001; Palmese et al, 2001; Camangi et al, 2003; Maccioni et al, 2004; Pieroni et al, 2004a; Pieroni et al, 2004b; Guarrera et al, 2005; Guarrera et al, 2005a; Loi et al, 2005; Pieroni and Quave, 2005; Passalacqua et al, 2007; Gonzalez-Tejero et al, 2008; Guarino et al, 2008; Maxia et al, 2008; Leporatti and Ghedira, 2009; Pieroni and Giusti, 2009; Savo et al, 2011; Cornara et al, 2014; Fortini et al, 2016; Menale et al, 2016; Motti and Motti, 2017; Lucchetti et al, 2019; Mautone et al, 2019; Menale et al, 2021; Danna et al, 2022	28	X	X	X	X	X	X	X
<i>Xanthium spinosum</i> L.	Compositae	Nappola spinosa	w	0.04	0.33	Palmese et al, 2001; De Natale and Pollio, 2007; Guarino et al, 2008	3	X		X		X		
<i>Xanthium strumarium</i> L.	Compositae	Nappola	w	0.01	0.33	Leporatti and Ghedira, 2009	1			X	X	X		

Appendix 2: Complete dataset. Original and normalized (see Methods section) spectrophotometric data on water and methanol extracts. Polyphenols (Poly), reducing sugars (RedSug), proteins (Prot) and antioxidant activity (Antiox); Poly_norm: normalized polyphenols values; RedSug_norm: normalized reducing sugars values; Prot_norm: normalized proteins values; fl: flowers; le: leaves; st: stems; ro: roots; ba: bark; L: Liguria; P: Apulia; T: Tuscany. Antiox_norm: normalized antioxidant activity values; AA: ascorbic acid; GA: gallic acid; GLU: D-glucose; BSA: bovine serum albumin; eq: equivalent, DW: dry weight.

Species	Organ	Region	Solvent extraction	Antiox (mgAAeq/gDW)	Poly (mgGAeq/gDW)	RedSug (mgGLUeq/gDW)	Prot (mgBSAeq/gDW)	Antiox_norm	Poly_norm	RedSug_norm	Prot_norm
<i>B. officinalis</i>	fl	P	water	74.05	15.10	137.55	163.47	0.84	0.45	0.57	0.62
<i>B. officinalis</i>	fl	P	water	75.49	16.34	158.57	180.19	0.85	0.49	0.66	0.68
<i>B. officinalis</i>	fl	P	water	62.81	17.19	149.91	196.67	0.71	0.51	0.62	0.74
<i>B. officinalis</i>	le	P	water	97.62	33.65	121.26	199.04	1.10	1.00	0.50	0.75
<i>B. officinalis</i>	le	P	water	64.53	33.97	107.60	188.83	0.73	1.01	0.45	0.71
<i>B. officinalis</i>	le	P	water	58.61	36.72	116.05	187.16	0.66	1.09	0.48	0.71
<i>B. officinalis</i>	st	P	water	19.35	7.27	174.39	62.68	0.22	0.22	0.72	0.24
<i>B. officinalis</i>	st	P	water	19.55	7.90	161.68	81.89	0.22	0.24	0.67	0.31
<i>B. officinalis</i>	st	P	water	14.07	9.00	168.01	88.45	0.16	0.27	0.70	0.33
<i>B. officinalis</i>	ro	P	water	17.56	10.09	66.97	105.03	0.20	0.30	0.28	0.40
<i>B. officinalis</i>	ro	P	water	14.35	7.09	37.97	66.22	0.16	0.21	0.16	0.25
<i>B. officinalis</i>	ro	P	water	13.82	7.29	47.71	69.46	0.16	0.22	0.20	0.26
<i>B. officinalis</i>	fl	T	water	67.76	21.58	144.78	228.30	0.78	0.59	0.58	0.71
<i>B. officinalis</i>	fl	T	water	50.15	22.21	148.27	183.09	0.58	0.60	0.59	0.57
<i>B. officinalis</i>	fl	T	water	63.18	20.96	134.09	214.25	0.73	0.57	0.53	0.66
<i>B. officinalis</i>	le	T	water	99.46	35.72	112.95	297.29	1.15	0.97	0.45	0.92
<i>B. officinalis</i>	le	T	water	80.20	34.40	118.24	278.77	0.92	0.93	0.47	0.86
<i>B. officinalis</i>	le	T	water	79.70	38.05	108.59	299.99	0.92	1.03	0.43	0.93
<i>B. officinalis</i>	st	T	water	13.73	8.84	198.07	71.11	0.16	0.24	0.79	0.22
<i>B. officinalis</i>	st	T	water	12.46	7.59	214.52	73.68	0.14	0.21	0.85	0.23
<i>B. officinalis</i>	st	T	water	13.29	7.90	234.72	63.01	0.15	0.21	0.93	0.20
<i>B. officinalis</i>	ro	T	water	12.79	7.68	31.79	69.59	0.15	0.21	0.13	0.22
<i>B. officinalis</i>	ro	T	water	13.82	7.17	29.47	73.73	0.16	0.19	0.12	0.23
<i>B. officinalis</i>	ro	T	water	14.46	9.07	33.02	84.99	0.17	0.25	0.13	0.26
<i>B. officinalis</i>	fl	L	water	49.31	17.50	131.77	163.97	0.83	0.62	0.70	0.68
<i>B. officinalis</i>	fl	L	water	50.00	17.70	154.86	149.68	0.84	0.63	0.83	0.62
<i>B. officinalis</i>	fl	L	water	51.50	19.49	143.38	183.43	0.87	0.70	0.77	0.76
<i>B. officinalis</i>	le	L	water	38.51	20.18	54.62	213.58	0.65	0.72	0.29	0.88

<i>B. officinalis</i>	le	L	water	41.75	21.81	98.36	241.46	0.70	0.78	0.53	1.00
<i>B. officinalis</i>	le	L	water	45.27	16.80	68.76	185.31	0.76	0.60	0.37	0.76
<i>B. officinalis</i>	st	L	water	16.15	13.07	155.15	59.27	0.27	0.47	0.83	0.24
<i>B. officinalis</i>	st	L	water	13.56	9.47	135.51	39.33	0.23	0.34	0.72	0.16
<i>B. officinalis</i>	st	L	water	18.14	12.40	113.35	78.73	0.31	0.44	0.61	0.32
<i>B. officinalis</i>	ro	L	water	12.74	6.97	22.12	44.59	0.21	0.25	0.12	0.18
<i>B. officinalis</i>	ro	L	water	10.72	6.28	21.71	43.70	0.18	0.22	0.12	0.18
<i>B. officinalis</i>	ro	L	water	8.46	6.34	22.65	52.63	0.14	0.23	0.12	0.22
<i>C. dactylon</i>	le	P	water	12.50	8.20	80.27	108.89	0.60	0.79	0.67	0.73
<i>C. dactylon</i>	le	P	water	17.04	8.01	87.91	115.60	0.82	0.77	0.73	0.78
<i>C. dactylon</i>	le	P	water	13.13	8.49	80.96	117.69	0.63	0.81	0.67	0.79
<i>C. dactylon</i>	ro	P	water	6.74	2.13	43.85	39.68	0.32	0.20	0.37	0.27
<i>C. dactylon</i>	ro	P	water	6.92	2.47	37.28	31.13	0.33	0.24	0.31	0.21
<i>C. dactylon</i>	ro	P	water	6.28	2.02	29.98	32.11	0.30	0.19	0.25	0.22
<i>C. dactylon</i>	le	T	water	14.70	10.71	53.68	152.11	0.70	0.80	0.63	0.82
<i>C. dactylon</i>	le	T	water	17.05	12.61	62.93	180.21	0.81	0.95	0.74	0.97
<i>C. dactylon</i>	le	T	water	14.05	10.26	46.88	144.46	0.67	0.77	0.55	0.78
<i>C. dactylon</i>	ro	T	water	6.83	2.15	36.96	33.22	0.32	0.16	0.43	0.18
<i>C. dactylon</i>	ro	T	water	5.35	2.07	28.35	15.03	0.25	0.16	0.33	0.08
<i>C. dactylon</i>	ro	T	water	5.13	2.19	26.17	32.72	0.24	0.16	0.31	0.18
<i>C. dactylon</i>	le	L	water	10.49	9.69	30.37	109.39	0.55	0.82	0.54	0.78
<i>C. dactylon</i>	le	L	water	11.33	8.90	27.04	107.52	0.59	0.75	0.48	0.76
<i>C. dactylon</i>	le	L	water	11.60	10.13	27.29	113.83	0.60	0.86	0.48	0.81
<i>C. dactylon</i>	ro	L	water	7.80	2.36	29.84	31.74	0.41	0.20	0.53	0.23
<i>C. dactylon</i>	ro	L	water	8.31	2.19	26.87	29.43	0.43	0.18	0.47	0.21
<i>C. dactylon</i>	ro	L	water	8.06	2.28	28.35	30.58	0.42	0.19	0.50	0.22
<i>F. vulgaris</i>	le	P	water	27.50	18.37	71.93	215.20	1.06	1.04	0.41	1.08
<i>F. vulgaris</i>	le	P	water	24.95	20.46	61.61	180.24	0.96	1.16	0.35	0.90
<i>F. vulgaris</i>	le	P	water	23.98	20.73	68.93	222.14	0.92	1.18	0.39	1.11
<i>F. vulgaris</i>	st	P	water	11.29	5.17	171.57	71.06	0.43	0.29	0.97	0.36
<i>F. vulgaris</i>	st	P	water	7.17	4.88	189.23	74.44	0.28	0.28	1.07	0.37
<i>F. vulgaris</i>	st	P	water	6.56	5.07	181.02	66.89	0.25	0.29	1.03	0.34
<i>F. vulgaris</i>	ro	P	water	5.60	1.56	12.88	23.51	0.22	0.09	0.07	0.12
<i>F. vulgaris</i>	ro	P	water	5.02	1.42	22.87	22.00	0.19	0.08	0.13	0.11

<i>F. vulgaris</i>	ro	P	water	4.75	1.51	13.45	22.23	0.18	0.09	0.08	0.11
<i>F. vulgaris</i>	le	T	water	19.76	16.45	114.74	193.80	0.83	0.94	0.45	0.94
<i>F. vulgaris</i>	le	T	water	14.53	15.42	126.88	170.74	0.61	0.89	0.50	0.83
<i>F. vulgaris</i>	le	T	water	17.55	18.93	126.47	195.49	0.74	1.09	0.50	0.95
<i>F. vulgaris</i>	st	T	water	8.83	6.31	215.35	82.62	0.37	0.36	0.84	0.40
<i>F. vulgaris</i>	st	T	water	6.36	5.80	242.82	79.93	0.27	0.33	0.95	0.39
<i>F. vulgaris</i>	st	T	water	5.34	5.30	192.76	82.55	0.23	0.30	0.76	0.40
<i>F. vulgaris</i>	ro	T	water	7.33	3.43	42.98	31.78	0.31	0.20	0.17	0.15
<i>F. vulgaris</i>	ro	T	water	15.48	3.36	43.28	51.01	0.65	0.19	0.17	0.25
<i>F. vulgaris</i>	ro	T	water	11.41	3.39	43.13	41.40	0.48	0.19	0.17	0.20
<i>F. vulgaris</i>	le	L	water	27.00	25.97	91.36	231.16	0.91	1.41	0.72	1.40
<i>F. vulgaris</i>	le	L	water	31.19	23.10	91.19	156.67	1.05	1.26	0.72	0.95
<i>F. vulgaris</i>	le	L	water	27.40	16.02	74.87	164.91	0.92	0.87	0.59	1.00
<i>F. vulgaris</i>	st	L	water	10.96	3.00	90.65	39.40	0.37	0.16	0.71	0.24
<i>F. vulgaris</i>	st	L	water	8.72	4.16	86.62	42.72	0.29	0.23	0.68	0.26
<i>F. vulgaris</i>	st	L	water	8.37	4.29	92.59	37.71	0.28	0.23	0.73	0.23
<i>F. vulgaris</i>	ro	L	water	7.51	2.25	19.55	23.56	0.25	0.12	0.15	0.14
<i>F. vulgaris</i>	ro	L	water	7.73	2.23	16.93	24.83	0.26	0.12	0.13	0.15
<i>F. vulgaris</i>	ro	L	water	4.47	1.70	9.76	20.22	0.15	0.09	0.08	0.12
<i>H. perforatum</i>	fl	P	water	230.49	72.49	309.45	503.54	0.77	0.66	0.69	0.70
<i>H. perforatum</i>	fl	P	water	322.08	66.53	322.63	523.78	1.07	0.61	0.72	0.73
<i>H. perforatum</i>	fl	P	water	208.36	79.78	295.44	466.07	0.69	0.73	0.66	0.65
<i>H. perforatum</i>	le	P	water	204.04	73.19	367.14	678.46	0.68	0.67	0.82	0.94
<i>H. perforatum</i>	le	P	water	211.67	109.82	412.32	681.57	0.70	1.00	0.93	0.94
<i>H. perforatum</i>	le	P	water	232.49	107.92	388.10	667.15	0.77	0.99	0.87	0.92
<i>H. perforatum</i>	st	P	water	75.40	26.55	113.15	140.01	0.25	0.24	0.25	0.19
<i>H. perforatum</i>	st	P	water	78.68	33.97	149.49	179.72	0.26	0.31	0.34	0.25
<i>H. perforatum</i>	st	P	water	56.20	28.78	125.85	153.97	0.19	0.26	0.28	0.21
<i>H. perforatum</i>	ro	P	water	64.09	18.91	62.23	118.89	0.21	0.17	0.14	0.16
<i>H. perforatum</i>	ro	P	water	62.66	19.72	62.96	105.98	0.21	0.18	0.14	0.15
<i>H. perforatum</i>	ro	P	water	56.29	19.45	63.62	109.38	0.19	0.18	0.14	0.15
<i>H. perforatum</i>	fl	T	water	175.02	105.47	355.13	429.29	0.79	0.91	0.97	0.88
<i>H. perforatum</i>	fl	T	water	180.39	104.11	337.30	331.22	0.82	0.90	0.93	0.68
<i>H. perforatum</i>	fl	T	water	190.45	107.39	328.52	426.09	0.86	0.93	0.90	0.87

<i>H. perforatum</i>	le	T	water	164.88	81.15	217.51	363.07	0.75	0.70	0.60	0.74
<i>H. perforatum</i>	le	T	water	141.79	91.08	235.02	398.03	0.64	0.79	0.64	0.82
<i>H. perforatum</i>	le	T	water	92.70	95.44	228.06	409.53	0.42	0.82	0.63	0.84
<i>H. perforatum</i>	st	T	water	71.80	29.48	129.64	147.31	0.33	0.25	0.36	0.30
<i>H. perforatum</i>	st	T	water	90.78	28.30	136.94	166.05	0.41	0.24	0.38	0.34
<i>H. perforatum</i>	st	T	water	86.37	29.56	134.86	139.51	0.39	0.26	0.37	0.29
<i>H. perforatum</i>	ro	T	water	45.79	7.77	27.26	38.05	0.21	0.07	0.07	0.08
<i>H. perforatum</i>	ro	T	water	39.26	7.79	28.82	41.07	0.18	0.07	0.08	0.08
<i>H. perforatum</i>	ro	T	water	42.52	7.78	28.04	39.56	0.19	0.07	0.08	0.08
<i>H. perforatum</i>	fl	L	water	178.92	85.31	276.28	526.01	0.58	0.84	0.82	0.92
<i>H. perforatum</i>	fl	L	water	258.92	71.31	308.18	462.28	0.84	0.70	0.91	0.81
<i>H. perforatum</i>	fl	L	water	217.21	80.72	300.63	402.78	0.71	0.80	0.89	0.70
<i>H. perforatum</i>	le	L	water	318.03	95.65	254.76	524.74	1.04	0.94	0.75	0.92
<i>H. perforatum</i>	le	L	water	200.30	107.10	260.85	569.02	0.65	1.06	0.77	1.00
<i>H. perforatum</i>	le	L	water	272.08	85.99	275.49	476.65	0.89	0.85	0.81	0.83
<i>H. perforatum</i>	st	L	water	106.93	21.18	93.72	119.82	0.35	0.21	0.28	0.21
<i>H. perforatum</i>	st	L	water	72.72	15.00	69.40	85.66	0.24	0.15	0.20	0.15
<i>H. perforatum</i>	st	L	water	95.81	17.47	84.83	103.84	0.31	0.17	0.25	0.18
<i>H. perforatum</i>	ro	L	water	31.49	10.61	37.86	77.60	0.10	0.10	0.11	0.14
<i>H. perforatum</i>	ro	L	water	45.99	11.81	34.98	46.59	0.15	0.12	0.10	0.08
<i>H. perforatum</i>	ro	L	water	43.90	6.15	35.22	35.13	0.14	0.06	0.10	0.06
<i>M. sylvestris</i>	fl	P	water	29.52	16.24	107.65	156.28	0.60	0.75	0.73	0.73
<i>M. sylvestris</i>	fl	P	water	45.49	17.18	125.16	195.35	0.93	0.79	0.85	0.91
<i>M. sylvestris</i>	fl	P	water	37.52	18.10	106.58	177.44	0.77	0.84	0.72	0.83
<i>M. sylvestris</i>	le	P	water	49.28	22.63	53.44	202.79	1.01	1.05	0.36	0.95
<i>M. sylvestris</i>	le	P	water	39.65	16.51	29.93	184.88	0.81	0.76	0.20	0.87
<i>M. sylvestris</i>	le	P	water	35.24	14.14	18.16	157.07	0.72	0.65	0.12	0.73
<i>M. sylvestris</i>	st	P	water	14.06	5.13	123.71	29.03	0.29	0.24	0.84	0.14
<i>M. sylvestris</i>	st	P	water	9.81	6.09	124.65	49.28	0.20	0.28	0.84	0.23
<i>M. sylvestris</i>	st	P	water	11.73	5.83	116.06	45.06	0.24	0.27	0.78	0.21
<i>M. sylvestris</i>	ro	P	water	7.62	2.65	28.12	28.45	0.16	0.12	0.19	0.13
<i>M. sylvestris</i>	ro	P	water	6.78	2.56	26.59	28.29	0.14	0.12	0.18	0.13
<i>M. sylvestris</i>	ro	P	water	7.20	2.61	27.35	28.37	0.15	0.12	0.18	0.13
<i>M. sylvestris</i>	fl	T	water	31.54	16.14	189.95	158.52	0.73	0.75	1.13	0.71

<i>M. sylvestris</i>	fl	T	water	31.16	17.17	159.29	174.22	0.73	0.79	0.95	0.78
<i>M. sylvestris</i>	fl	T	water	26.93	15.24	150.59	173.73	0.63	0.70	0.90	0.78
<i>M. sylvestris</i>	le	T	water	29.80	21.76	42.63	237.47	0.69	1.01	0.25	1.07
<i>M. sylvestris</i>	le	T	water	52.96	20.43	42.38	218.50	1.23	0.95	0.25	0.98
<i>M. sylvestris</i>	le	T	water	44.55	18.47	37.66	131.48	1.04	0.85	0.22	0.59
<i>M. sylvestris</i>	st	T	water	7.62	4.26	109.20	46.25	0.18	0.20	0.65	0.21
<i>M. sylvestris</i>	st	T	water	9.22	4.50	112.30	53.76	0.21	0.21	0.67	0.24
<i>M. sylvestris</i>	st	T	water	8.45	4.56	103.38	44.98	0.20	0.21	0.61	0.20
<i>M. sylvestris</i>	ro	T	water	5.84	2.57	20.81	36.73	0.14	0.12	0.12	0.16
<i>M. sylvestris</i>	ro	T	water	4.94	2.10	21.30	35.90	0.11	0.10	0.13	0.16
<i>M. sylvestris</i>	ro	T	water	4.70	2.49	19.28	25.71	0.11	0.12	0.11	0.12
<i>M. sylvestris</i>	fl	L	water	23.02	21.74	128.63	222.72	0.58	0.76	0.88	0.79
<i>M. sylvestris</i>	fl	L	water	28.61	21.61	129.35	210.82	0.72	0.76	0.88	0.74
<i>M. sylvestris</i>	fl	L	water	37.39	23.12	116.43	209.18	0.94	0.81	0.79	0.74
<i>M. sylvestris</i>	le	L	water	31.37	28.79	59.67	268.88	0.79	1.01	0.41	0.95
<i>M. sylvestris</i>	le	L	water	42.28	29.48	62.95	329.04	1.07	1.04	0.43	1.16
<i>M. sylvestris</i>	le	L	water	37.18	26.30	67.40	281.34	0.94	0.92	0.46	0.99
<i>M. sylvestris</i>	st	L	water	6.56	4.54	99.08	46.07	0.17	0.16	0.68	0.16
<i>M. sylvestris</i>	st	L	water	9.21	4.19	101.37	34.07	0.23	0.15	0.69	0.12
<i>M. sylvestris</i>	st	L	water	7.99	4.90	72.58	38.82	0.20	0.17	0.49	0.14
<i>M. sylvestris</i>	ro	L	water	3.33	2.23	16.25	20.90	0.08	0.08	0.11	0.07
<i>M. sylvestris</i>	ro	L	water	5.10	1.72	10.80	17.38	0.13	0.06	0.07	0.06
<i>M. sylvestris</i>	ro	L	water	5.65	2.12	15.53	19.16	0.14	0.07	0.11	0.07
<i>S. nigra</i>	fl	P	water	73.53	36.78	201.87	358.22	0.52	0.42	0.71	0.61
<i>S. nigra</i>	fl	P	water	68.02	33.53	175.96	367.70	0.48	0.38	0.62	0.63
<i>S. nigra</i>	fl	P	water	77.86	40.32	167.38	376.49	0.55	0.46	0.59	0.64
<i>S. nigra</i>	le	P	water	112.19	72.48	153.83	367.79	0.80	0.82	0.54	0.63
<i>S. nigra</i>	le	P	water	103.66	81.74	173.62	365.08	0.73	0.92	0.61	0.62
<i>S. nigra</i>	le	P	water	117.80	86.63	175.14	416.39	0.84	0.98	0.61	0.71
<i>S. nigra</i>	ba	P	water	28.29	15.35	72.89	135.51	0.20	0.17	0.26	0.23
<i>S. nigra</i>	ba	P	water	27.30	17.19	77.79	115.89	0.19	0.19	0.27	0.20
<i>S. nigra</i>	ba	P	water	26.02	14.58	86.63	141.39	0.18	0.16	0.30	0.24
<i>S. nigra</i>	fl	T	water	71.20	31.01	188.07	156.21	0.53	0.47	0.67	0.53
<i>S. nigra</i>	fl	T	water	123.91	37.91	182.84	161.69	0.92	0.57	0.65	0.55

<i>S. nigra</i>	fl	T	water	102.75	36.20	162.35	166.74	0.77	0.55	0.57	0.57
<i>S. nigra</i>	le	T	water	81.43	50.08	186.73	119.57	0.61	0.76	0.66	0.41
<i>S. nigra</i>	le	T	water	61.34	51.39	186.93	135.89	0.46	0.78	0.66	0.46
<i>S. nigra</i>	le	T	water	83.42	45.97	184.66	114.66	0.62	0.70	0.65	0.39
<i>S. nigra</i>	ba	T	water	28.20	14.84	60.46	166.38	0.21	0.22	0.21	0.57
<i>S. nigra</i>	ba	T	water	25.77	15.72	59.92	134.78	0.19	0.24	0.21	0.46
<i>S. nigra</i>	ba	T	water	26.23	14.04	60.47	167.88	0.20	0.21	0.21	0.57
<i>S. nigra</i>	fl	L	water	79.90	28.78	100.86	249.13	0.79	0.53	0.45	0.60
<i>S. nigra</i>	fl	L	water	63.65	27.19	120.62	182.63	0.63	0.50	0.53	0.44
<i>S. nigra</i>	fl	L	water	64.14	26.28	102.97	242.80	0.64	0.49	0.45	0.59
<i>S. nigra</i>	le	L	water	52.54	47.99	197.27	284.18	0.52	0.89	0.87	0.69
<i>S. nigra</i>	le	L	water	65.77	44.08	172.96	277.65	0.65	0.82	0.76	0.67
<i>S. nigra</i>	le	L	water	67.46	41.02	193.59	275.82	0.67	0.76	0.86	0.67
<i>S. nigra</i>	ba	L	water	19.91	10.14	46.68	125.71	0.20	0.19	0.21	0.30
<i>S. nigra</i>	ba	L	water	18.69	8.72	42.81	127.97	0.19	0.16	0.19	0.31
<i>S. nigra</i>	ba	L	water	21.73	8.52	41.09	100.20	0.22	0.16	0.18	0.24
<i>U. dioica</i>	le	P	water	55.12	12.85	49.77	178.77	1.19	0.89	0.42	0.93
<i>U. dioica</i>	le	P	water	56.87	14.40	60.51	148.38	1.23	1.00	0.52	0.77
<i>U. dioica</i>	le	P	water	42.71	13.26	50.83	197.43	0.92	0.92	0.43	1.02
<i>U. dioica</i>	st	P	water	10.42	5.05	71.07	67.56	0.22	0.35	0.61	0.35
<i>U. dioica</i>	st	P	water	9.01	4.88	78.94	64.17	0.19	0.34	0.67	0.33
<i>U. dioica</i>	st	P	water	7.29	4.73	72.89	63.35	0.16	0.33	0.62	0.33
<i>U. dioica</i>	ro	P	water	7.85	3.11	42.55	51.21	0.17	0.22	0.36	0.27
<i>U. dioica</i>	ro	P	water	10.03	3.20	52.99	62.55	0.22	0.22	0.45	0.32
<i>U. dioica</i>	ro	P	water	9.27	3.64	48.99	36.05	0.20	0.25	0.42	0.19
<i>U. dioica</i>	le	T	water	64.49	16.92	116.29	269.13	1.16	0.94	0.64	1.01
<i>U. dioica</i>	le	T	water	65.45	18.97	135.51	232.14	1.18	1.05	0.74	0.87
<i>U. dioica</i>	le	T	water	66.27	19.62	129.77	275.73	1.20	1.09	0.71	1.04
<i>U. dioica</i>	st	T	water	9.62	5.38	112.67	75.41	0.17	0.30	0.62	0.28
<i>U. dioica</i>	st	T	water	7.03	4.46	101.98	65.07	0.13	0.25	0.56	0.24
<i>U. dioica</i>	st	T	water	9.31	4.68	94.77	65.71	0.17	0.26	0.52	0.25
<i>U. dioica</i>	ro	T	water	9.28	4.09	45.56	74.74	0.17	0.23	0.25	0.28
<i>U. dioica</i>	ro	T	water	7.40	3.30	45.04	72.17	0.13	0.18	0.25	0.27
<i>U. dioica</i>	ro	T	water	10.33	3.53	38.71	66.64	0.19	0.20	0.21	0.25

<i>U. dioica</i>	le	L	water	59.01	16.42	61.48	195.62	1.26	1.01	0.72	0.93
<i>U. dioica</i>	le	L	water	38.77	17.86	64.33	173.65	0.83	1.10	0.76	0.83
<i>U. dioica</i>	le	L	water	57.56	15.86	57.27	206.94	1.23	0.97	0.67	0.99
<i>U. dioica</i>	st	L	water	9.90	4.71	49.97	51.52	0.21	0.29	0.59	0.25
<i>U. dioica</i>	st	L	water	6.50	4.43	41.53	56.98	0.14	0.27	0.49	0.27
<i>U. dioica</i>	st	L	water	8.73	4.67	43.80	64.07	0.19	0.29	0.51	0.31
<i>U. dioica</i>	ro	L	water	12.71	3.21	18.57	62.11	0.27	0.20	0.22	0.30
<i>U. dioica</i>	ro	L	water	7.82	2.81	23.37	63.71	0.17	0.17	0.27	0.30
<i>U. dioica</i>	ro	L	water	9.70	3.29	22.59	70.20	0.21	0.20	0.27	0.33
<i>B. officinalis</i>	fl	P	methanol	18.29	10.91	79.77	64.00	0.33	0.24	0.59	0.39
<i>B. officinalis</i>	fl	P	methanol	19.01	12.55	100.50	68.63	0.35	0.27	0.74	0.41
<i>B. officinalis</i>	fl	P	methanol	21.36	14.32	88.08	72.67	0.39	0.31	0.65	0.44
<i>B. officinalis</i>	le	P	methanol	59.65	58.49	67.01	172.94	1.09	1.28	0.50	1.04
<i>B. officinalis</i>	le	P	methanol	52.29	38.41	48.57	147.23	0.96	0.84	0.36	0.89
<i>B. officinalis</i>	le	P	methanol	49.58	49.06	60.14	160.42	0.91	1.07	0.44	0.97
<i>B. officinalis</i>	st	P	methanol	16.01	13.43	125.62	50.91	0.29	0.29	0.93	0.31
<i>B. officinalis</i>	st	P	methanol	14.18	15.02	97.54	53.93	0.26	0.33	0.72	0.33
<i>B. officinalis</i>	st	P	methanol	19.21	16.03	106.03	53.15	0.35	0.35	0.78	0.32
<i>B. officinalis</i>	ro	P	methanol	21.33	16.16	16.99	57.13	0.39	0.35	0.13	0.34
<i>B. officinalis</i>	ro	P	methanol	19.29	15.39	8.91	49.42	0.35	0.34	0.07	0.30
<i>B. officinalis</i>	ro	P	methanol	17.75	14.11	12.67	44.79	0.32	0.31	0.09	0.27
<i>B. officinalis</i>	fl	T	methanol	42.60	43.13	105.48	67.84	0.52	0.55	0.55	0.47
<i>B. officinalis</i>	fl	T	methanol	42.24	41.39	112.81	78.10	0.51	0.53	0.59	0.54
<i>B. officinalis</i>	fl	T	methanol	52.14	40.47	111.47	73.23	0.63	0.52	0.58	0.51
<i>B. officinalis</i>	le	T	methanol	70.74	64.80	85.18	128.66	0.86	0.83	0.45	0.89
<i>B. officinalis</i>	le	T	methanol	78.90	73.44	86.32	142.87	0.96	0.94	0.45	0.99
<i>B. officinalis</i>	le	T	methanol	77.64	72.90	72.71	150.17	0.94	0.93	0.38	1.04
<i>B. officinalis</i>	st	T	methanol	16.81	18.45	179.67	28.23	0.20	0.24	0.94	0.19
<i>B. officinalis</i>	st	T	methanol	20.85	18.59	179.07	29.95	0.25	0.24	0.94	0.21
<i>B. officinalis</i>	st	T	methanol	18.01	18.82	175.74	31.02	0.22	0.24	0.92	0.21
<i>B. officinalis</i>	ro	T	methanol	24.05	26.35	12.30	46.49	0.29	0.34	0.06	0.32
<i>B. officinalis</i>	ro	T	methanol	22.93	28.73	11.17	44.48	0.28	0.37	0.06	0.31
<i>B. officinalis</i>	ro	T	methanol	26.70	21.08	12.25	48.21	0.32	0.27	0.06	0.33
<i>B. officinalis</i>	fl	L	methanol	47.45	16.94	86.91	85.66	0.72	0.63	0.83	0.62

<i>B. officinalis</i>	fl	L		methanol	45.15	14.56	80.68	83.52	0.69	0.54	0.77	0.61
<i>B. officinalis</i>	fl	L		methanol	44.83	17.12	70.92	96.67	0.68	0.64	0.68	0.70
<i>B. officinalis</i>	le	L		methanol	51.35	21.78	43.98	134.26	0.78	0.81	0.42	0.97
<i>B. officinalis</i>	le	L		methanol	50.67	23.76	47.71	144.73	0.77	0.89	0.45	1.05
<i>B. officinalis</i>	le	L		methanol	44.00	18.34	48.48	116.97	0.67	0.68	0.46	0.85
<i>B. officinalis</i>	st	L		methanol	12.01	6.18	74.33	21.09	0.18	0.23	0.71	0.15
<i>B. officinalis</i>	st	L		methanol	17.88	8.00	78.45	27.56	0.27	0.30	0.75	0.20
<i>B. officinalis</i>	st	L		methanol	14.94	7.09	76.39	24.32	0.23	0.26	0.73	0.18
<i>B. officinalis</i>	ro	L		methanol	19.07	9.18	7.37	31.22	0.29	0.34	0.07	0.23
<i>B. officinalis</i>	ro	L		methanol	22.35	8.48	6.92	29.72	0.34	0.32	0.07	0.22
<i>B. officinalis</i>	ro	L		methanol	24.36	9.64	7.72	32.05	0.37	0.36	0.07	0.23
<i>C. dactylon</i>	le	P		methanol	5.80	3.36	24.68	55.45	0.70	0.66	0.94	0.62
<i>C. dactylon</i>	le	P		methanol	4.08	3.26	22.31	60.46	0.49	0.64	0.85	0.67
<i>C. dactylon</i>	le	P		methanol	4.09	2.97	19.82	58.67	0.50	0.58	0.75	0.65
<i>C. dactylon</i>	ro	P		methanol	3.96	1.96	5.18	31.23	0.48	0.38	0.20	0.35
<i>C. dactylon</i>	ro	P		methanol	3.91	2.03	4.94	33.98	0.47	0.40	0.19	0.38
<i>C. dactylon</i>	ro	P		methanol	2.90	1.75	2.18	29.38	0.35	0.34	0.08	0.33
<i>C. dactylon</i>	le	T		methanol	6.71	9.32	28.71	40.61	0.50	0.81	0.96	0.76
<i>C. dactylon</i>	le	T		methanol	9.40	9.34	31.54	43.36	0.71	0.81	1.06	0.82
<i>C. dactylon</i>	le	T		methanol	9.70	8.11	27.52	37.06	0.73	0.70	0.92	0.70
<i>C. dactylon</i>	ro	T		methanol	4.42	2.68	0.78	12.51	0.33	0.23	0.03	0.24
<i>C. dactylon</i>	ro	T		methanol	5.13	2.52	0.82	13.80	0.39	0.22	0.03	0.26
<i>C. dactylon</i>	ro	T		methanol	4.60	2.63	0.28	12.14	0.35	0.23	0.01	0.23
<i>C. dactylon</i>	le	L		methanol	23.35	9.06	7.32	30.88	1.22	1.30	0.41	0.45
<i>C. dactylon</i>	le	L		methanol	9.73	3.52	14.72	53.63	0.51	0.50	0.83	0.77
<i>C. dactylon</i>	le	L		methanol	9.12	3.84	19.95	61.30	0.48	0.55	1.13	0.88
<i>C. dactylon</i>	ro	L		methanol	5.47	1.57	4.60	21.14	0.29	0.22	0.26	0.31
<i>C. dactylon</i>	ro	L		methanol	4.76	1.46	2.82	20.17	0.25	0.21	0.16	0.29
<i>C. dactylon</i>	ro	L		methanol	5.12	1.51	3.71	20.66	0.27	0.22	0.21	0.30
<i>F. vulgaris</i>	le	P		methanol	10.93	14.03	36.56	94.42	0.81	1.01	0.41	0.93
<i>F. vulgaris</i>	le	P		methanol	14.19	14.07	39.18	106.85	1.05	1.02	0.44	1.05
<i>F. vulgaris</i>	le	P		methanol	14.10	16.77	44.25	130.22	1.04	1.21	0.50	1.28
<i>F. vulgaris</i>	st	P		methanol	3.68	4.30	77.27	30.36	0.27	0.31	0.88	0.30
<i>F. vulgaris</i>	st	P		methanol	4.74	4.61	77.16	33.42	0.35	0.33	0.87	0.33

<i>F. vulgaris</i>	st	P	methanol	3.96	3.97	65.75	28.63	0.29	0.29	0.74	0.28
<i>F. vulgaris</i>	ro	P	methanol	3.39	1.81	19.92	14.35	0.25	0.13	0.23	0.14
<i>F. vulgaris</i>	ro	P	methanol	2.82	1.32	18.21	8.53	0.21	0.10	0.21	0.08
<i>F. vulgaris</i>	ro	P	methanol	2.95	1.40	19.06	9.47	0.22	0.10	0.22	0.09
<i>F. vulgaris</i>	le	T	methanol	21.73	49.10	47.84	141.55	0.84	1.02	0.47	1.07
<i>F. vulgaris</i>	le	T	methanol	21.48	50.80	49.18	119.69	0.83	1.06	0.48	0.91
<i>F. vulgaris</i>	le	T	methanol	21.64	48.70	48.51	130.22	0.83	1.02	0.48	0.99
<i>F. vulgaris</i>	st	T	methanol	7.41	13.98	88.89	35.87	0.28	0.29	0.88	0.27
<i>F. vulgaris</i>	st	T	methanol	6.99	13.33	87.70	36.02	0.27	0.28	0.86	0.27
<i>F. vulgaris</i>	st	T	methanol	6.71	11.39	77.31	31.73	0.26	0.24	0.76	0.24
<i>F. vulgaris</i>	ro	T	methanol	10.15	10.04	19.92	34.44	0.39	0.21	0.20	0.26
<i>F. vulgaris</i>	ro	T	methanol	10.61	8.99	18.21	31.92	0.41	0.19	0.18	0.24
<i>F. vulgaris</i>	ro	T	methanol	10.38	9.52	19.06	33.18	0.40	0.20	0.19	0.25
<i>F. vulgaris</i>	le	L	methanol	18.54	12.18	40.67	95.73	0.82	0.92	1.16	0.86
<i>F. vulgaris</i>	le	L	methanol	16.67	11.28	38.61	89.44	0.74	0.85	1.10	0.80
<i>F. vulgaris</i>	le	L	methanol	18.27	13.10	40.17	97.14	0.81	0.99	1.15	0.87
<i>F. vulgaris</i>	st	L	methanol	6.32	4.20	7.94	35.00	0.28	0.32	0.23	0.31
<i>F. vulgaris</i>	st	L	methanol	8.61	5.68	7.71	47.88	0.38	0.43	0.22	0.43
<i>F. vulgaris</i>	st	L	methanol	8.74	4.99	6.67	41.38	0.39	0.38	0.19	0.37
<i>F. vulgaris</i>	ro	L	methanol	8.60	2.72	8.24	31.01	0.38	0.21	0.24	0.28
<i>F. vulgaris</i>	ro	L	methanol	9.51	3.32	6.53	36.81	0.42	0.25	0.19	0.33
<i>F. vulgaris</i>	ro	L	methanol	6.38	2.26	1.06	27.65	0.28	0.17	0.03	0.25
<i>H. perforatum</i>	fl	P	methanol	143.78	99.20	210.41	987.04	0.76	0.71	0.64	0.76
<i>H. perforatum</i>	fl	P	methanol	133.98	100.91	220.49	1014.63	0.71	0.72	0.67	0.78
<i>H. perforatum</i>	fl	P	methanol	143.48	106.16	238.00	1041.13	0.76	0.76	0.73	0.80
<i>H. perforatum</i>	le	P	methanol	144.95	98.71	241.94	910.37	0.77	0.70	0.74	0.70
<i>H. perforatum</i>	le	P	methanol	133.98	105.11	220.95	999.47	0.71	0.75	0.68	0.77
<i>H. perforatum</i>	le	P	methanol	150.89	121.49	257.01	1073.39	0.80	0.87	0.79	0.82
<i>H. perforatum</i>	st	P	methanol	51.66	30.63	80.13	238.71	0.27	0.22	0.25	0.18
<i>H. perforatum</i>	st	P	methanol	72.28	45.73	118.29	383.71	0.38	0.33	0.36	0.29
<i>H. perforatum</i>	st	P	methanol	58.53	38.93	95.52	293.89	0.31	0.28	0.29	0.23
<i>H. perforatum</i>	ro	P	methanol	29.44	27.76	78.86	239.62	0.16	0.20	0.24	0.18
<i>H. perforatum</i>	ro	P	methanol	36.49	32.57	94.24	311.44	0.19	0.23	0.29	0.24
<i>H. perforatum</i>	ro	P	methanol	36.02	33.04	104.11	318.56	0.19	0.24	0.32	0.24

<i>H. perforatum</i>	fl	T	methanol	169.00	106.39	181.31	592.98	0.67	0.52	0.60	0.80
<i>H. perforatum</i>	fl	T	methanol	191.79	121.84	247.30	749.32	0.76	0.60	0.81	1.01
<i>H. perforatum</i>	fl	T	methanol	200.84	120.90	230.69	691.74	0.80	0.59	0.76	0.94
<i>H. perforatum</i>	le	T	methanol	153.71	209.46	236.42	506.56	0.61	1.02	0.78	0.69
<i>H. perforatum</i>	le	T	methanol	158.03	202.88	225.79	509.49	0.63	0.99	0.74	0.69
<i>H. perforatum</i>	le	T	methanol	146.96	178.19	228.30	483.77	0.59	0.87	0.75	0.65
<i>H. perforatum</i>	st	T	methanol	90.02	70.63	120.71	177.49	0.36	0.35	0.40	0.24
<i>H. perforatum</i>	st	T	methanol	116.74	76.35	134.60	225.61	0.47	0.37	0.44	0.31
<i>H. perforatum</i>	st	T	methanol	113.44	84.01	138.22	227.22	0.45	0.41	0.46	0.31
<i>H. perforatum</i>	ro	T	methanol	58.78	14.87	18.71	77.41	0.23	0.07	0.06	0.10
<i>H. perforatum</i>	ro	T	methanol	51.61	22.21	33.98	101.87	0.21	0.11	0.11	0.14
<i>H. perforatum</i>	ro	T	methanol	55.20	18.54	26.34	89.64	0.22	0.09	0.09	0.12
<i>H. perforatum</i>	fl	L	methanol	246.80	124.62	174.64	743.25	0.79	0.82	0.61	0.80
<i>H. perforatum</i>	fl	L	methanol	219.01	126.07	223.92	708.17	0.70	0.83	0.78	0.77
<i>H. perforatum</i>	fl	L	methanol	192.32	124.30	171.58	677.44	0.61	0.82	0.60	0.73
<i>H. perforatum</i>	le	L	methanol	234.15	127.28	279.77	628.08	0.74	0.84	0.97	0.68
<i>H. perforatum</i>	le	L	methanol	231.86	129.87	291.10	598.74	0.74	0.85	1.01	0.65
<i>H. perforatum</i>	le	L	methanol	233.00	128.58	285.44	613.41	0.74	0.85	0.99	0.66
<i>H. perforatum</i>	st	L	methanol	96.37	34.77	75.51	288.08	0.31	0.23	0.26	0.31
<i>H. perforatum</i>	st	L	methanol	114.19	21.90	35.77	316.72	0.36	0.14	0.12	0.34
<i>H. perforatum</i>	st	L	methanol	105.28	27.00	41.14	302.40	0.33	0.18	0.14	0.33
<i>H. perforatum</i>	ro	L	methanol	66.55	23.35	47.09	211.03	0.21	0.15	0.16	0.23
<i>H. perforatum</i>	ro	L	methanol	75.37	26.54	49.92	233.82	0.24	0.17	0.17	0.25
<i>H. perforatum</i>	ro	L	methanol	70.96	17.47	48.51	222.43	0.23	0.11	0.17	0.24
<i>M. sylvestris</i>	fl	P	methanol	5.50	6.01	54.27	57.16	0.56	0.82	0.87	0.85
<i>M. sylvestris</i>	fl	P	methanol	6.89	6.25	56.36	55.21	0.70	0.85	0.91	0.82
<i>M. sylvestris</i>	fl	P	methanol	7.06	6.48	46.94	59.59	0.72	0.88	0.76	0.88
<i>M. sylvestris</i>	le	P	methanol	6.93	5.78	18.64	55.60	0.70	0.79	0.30	0.82
<i>M. sylvestris</i>	le	P	methanol	7.91	5.91	28.28	58.46	0.80	0.80	0.46	0.86
<i>M. sylvestris</i>	le	P	methanol	7.32	5.34	20.05	55.53	0.74	0.73	0.32	0.82
<i>M. sylvestris</i>	st	P	methanol	2.39	1.53	48.93	8.64	0.24	0.21	0.79	0.13
<i>M. sylvestris</i>	st	P	methanol	2.80	1.43	46.03	8.60	0.28	0.19	0.74	0.13
<i>M. sylvestris</i>	st	P	methanol	2.45	1.49	46.42	9.44	0.25	0.20	0.75	0.14
<i>M. sylvestris</i>	ro	P	methanol	3.07	1.27	2.16	10.97	0.31	0.17	0.03	0.16

<i>M. sylvestris</i>	ro	P		methanol	3.57	1.34	2.43	13.99	0.36	0.18	0.04	0.21
<i>M. sylvestris</i>	ro	P		methanol	3.32	1.30	2.29	12.48	0.34	0.18	0.04	0.18
<i>M. sylvestris</i>	fl	T		methanol	10.72	12.50	101.23	58.76	0.72	0.92	1.07	1.09
<i>M. sylvestris</i>	fl	T		methanol	11.08	11.47	75.51	57.76	0.75	0.85	0.80	1.07
<i>M. sylvestris</i>	fl	T		methanol	11.31	11.65	82.33	56.86	0.76	0.86	0.87	1.05
<i>M. sylvestris</i>	le	T		methanol	10.77	8.70	39.76	28.16	0.73	0.64	0.42	0.52
<i>M. sylvestris</i>	le	T		methanol	9.57	9.01	41.16	27.20	0.65	0.67	0.44	0.50
<i>M. sylvestris</i>	le	T		methanol	8.50	9.30	44.12	33.66	0.57	0.69	0.47	0.62
<i>M. sylvestris</i>	st	T		methanol	3.69	3.16	61.21	7.90	0.25	0.23	0.65	0.15
<i>M. sylvestris</i>	st	T		methanol	4.13	3.10	56.26	8.71	0.28	0.23	0.59	0.16
<i>M. sylvestris</i>	st	T		methanol	4.29	3.30	59.30	9.85	0.29	0.24	0.63	0.18
<i>M. sylvestris</i>	ro	T		methanol	4.96	3.18	2.16	13.19	0.34	0.23	0.02	0.24
<i>M. sylvestris</i>	ro	T		methanol	5.10	2.55	2.43	9.86	0.34	0.19	0.03	0.18
<i>M. sylvestris</i>	ro	T		methanol	4.65	3.20	2.29	12.48	0.31	0.24	0.02	0.23
<i>M. sylvestris</i>	fl	L		methanol	12.00	6.85	82.41	73.95	0.90	1.02	0.94	0.94
<i>M. sylvestris</i>	fl	L		methanol	11.44	6.68	69.42	88.02	0.85	1.00	0.79	1.11
<i>M. sylvestris</i>	fl	L		methanol	8.13	4.95	69.48	50.76	0.61	0.74	0.80	0.64
<i>M. sylvestris</i>	le	L		methanol	8.42	4.99	38.82	63.47	0.63	0.75	0.44	0.80
<i>M. sylvestris</i>	le	L		methanol	7.28	4.41	36.42	62.53	0.54	0.66	0.42	0.79
<i>M. sylvestris</i>	le	L		methanol	7.53	4.33	40.88	58.77	0.56	0.65	0.47	0.74
<i>M. sylvestris</i>	st	L		methanol	3.29	1.52	65.06	15.16	0.25	0.23	0.74	0.19
<i>M. sylvestris</i>	st	L		methanol	3.82	1.51	60.98	17.36	0.29	0.23	0.70	0.22
<i>M. sylvestris</i>	st	L		methanol	5.44	1.87	57.71	15.35	0.41	0.28	0.66	0.19
<i>M. sylvestris</i>	ro	L		methanol	4.21	1.06	1.39	9.82	0.31	0.16	0.02	0.12
<i>M. sylvestris</i>	ro	L		methanol	4.20	0.96	0.80	8.56	0.31	0.14	0.01	0.11
<i>M. sylvestris</i>	ro	L		methanol	4.66	1.02	0.92	10.30	0.35	0.15	0.01	0.13
<i>S. nigra</i>	fl	P		methanol	85.38	53.53	83.65	258.93	0.70	0.61	0.79	0.61
<i>S. nigra</i>	fl	P		methanol	77.38	54.40	79.74	276.77	0.63	0.62	0.76	0.65
<i>S. nigra</i>	fl	P		methanol	94.63	52.22	66.02	285.90	0.78	0.60	0.63	0.67
<i>S. nigra</i>	le	P		methanol	78.69	66.26	65.66	254.52	0.65	0.76	0.62	0.60
<i>S. nigra</i>	le	P		methanol	82.69	70.15	60.08	291.58	0.68	0.80	0.57	0.69
<i>S. nigra</i>	le	P		methanol	76.54	61.96	54.61	267.80	0.63	0.71	0.52	0.63
<i>S. nigra</i>	ba	P		methanol	15.22	12.06	18.59	87.98	0.12	0.14	0.18	0.21
<i>S. nigra</i>	ba	P		methanol	20.94	12.56	24.08	109.91	0.17	0.14	0.23	0.26

<i>S. nigra</i>		ba	P	methanol	16.96	11.10	21.33	81.94	0.14	0.13	0.20	0.19
<i>S. nigra</i>		fl	T	methanol	89.53	85.14	71.95	268.59	0.78	0.64	0.66	0.73
<i>S. nigra</i>		fl	T	methanol	89.50	111.90	68.07	297.79	0.78	0.84	0.63	0.81
<i>S. nigra</i>		fl	T	methanol	93.22	94.47	66.29	307.95	0.82	0.71	0.61	0.84
<i>S. nigra</i>		le	T	methanol	60.96	72.22	81.41	168.58	0.53	0.54	0.75	0.46
<i>S. nigra</i>		le	T	methanol	52.55	92.17	72.01	200.40	0.46	0.69	0.66	0.54
<i>S. nigra</i>		le	T	methanol	52.65	79.94	79.92	174.97	0.46	0.60	0.74	0.48
<i>S. nigra</i>		ba	T	methanol	23.57	24.73	17.87	89.44	0.21	0.18	0.16	0.24
<i>S. nigra</i>		ba	T	methanol	25.69	20.45	15.79	72.95	0.22	0.15	0.15	0.20
<i>S. nigra</i>		ba	T	methanol	26.59	20.77	14.97	76.16	0.23	0.16	0.14	0.21
<i>S. nigra</i>		fl	L	methanol	61.31	32.51	70.57	266.20	0.52	0.55	0.69	0.63
<i>S. nigra</i>		fl	L	methanol	86.08	40.82	66.06	323.30	0.73	0.70	0.65	0.77
<i>S. nigra</i>		fl	L	methanol	77.80	35.26	57.97	279.84	0.66	0.60	0.57	0.66
<i>S. nigra</i>		le	L	methanol	85.52	42.66	76.63	241.73	0.72	0.73	0.75	0.57
<i>S. nigra</i>		le	L	methanol	75.10	40.92	64.59	239.82	0.63	0.70	0.64	0.57
<i>S. nigra</i>		le	L	methanol	84.18	39.91	57.70	238.36	0.71	0.68	0.57	0.56
<i>S. nigra</i>		ba	L	methanol	22.88	12.44	21.82	115.82	0.19	0.21	0.21	0.27
<i>S. nigra</i>		ba	L	methanol	17.55	9.27	19.44	92.43	0.15	0.16	0.19	0.22
<i>S. nigra</i>		ba	L	methanol	22.83	9.94	22.80	102.04	0.19	0.17	0.22	0.24
<i>U. dioica</i>		le	P	methanol	11.33	8.87	41.54	45.22	0.65	0.87	0.61	0.65
<i>U. dioica</i>		le	P	methanol	14.69	9.05	46.13	55.02	0.84	0.89	0.68	0.80
<i>U. dioica</i>		le	P	methanol	15.49	9.21	47.85	49.70	0.89	0.90	0.71	0.72
<i>U. dioica</i>		st	P	methanol	5.95	3.60	48.13	26.11	0.34	0.35	0.71	0.38
<i>U. dioica</i>		st	P	methanol	5.34	3.67	57.36	28.36	0.31	0.36	0.85	0.41
<i>U. dioica</i>		st	P	methanol	4.89	3.29	50.50	25.74	0.28	0.32	0.75	0.37
<i>U. dioica</i>		ro	P	methanol	6.19	2.74	5.71	25.28	0.36	0.27	0.08	0.37
<i>U. dioica</i>		ro	P	methanol	7.75	2.93	4.19	28.74	0.44	0.29	0.06	0.42
<i>U. dioica</i>		ro	P	methanol	6.74	2.60	3.31	26.85	0.39	0.25	0.05	0.39
<i>U. dioica</i>		le	T	methanol	29.46	49.07	47.37	89.74	0.83	1.22	0.70	0.91
<i>U. dioica</i>		le	T	methanol	33.45	42.07	46.65	92.93	0.94	1.05	0.69	0.94
<i>U. dioica</i>		le	T	methanol	41.39	47.85	44.36	100.83	1.16	1.19	0.66	1.02
<i>U. dioica</i>		st	T	methanol	8.64	9.57	57.55	31.20	0.24	0.24	0.86	0.32
<i>U. dioica</i>		st	T	methanol	8.08	7.72	50.72	30.47	0.23	0.19	0.75	0.31
<i>U. dioica</i>		st	T	methanol	10.11	7.05	40.06	28.25	0.28	0.18	0.60	0.29

<i>U. dioica</i>	ro	T	methanol	10.42	6.07	6.54	24.28	0.29	0.15	0.10	0.25
<i>U. dioica</i>	ro	T	methanol	9.59	5.94	7.15	22.27	0.27	0.15	0.11	0.23
<i>U. dioica</i>	ro	T	methanol	9.00	5.27	2.09	24.33	0.25	0.13	0.03	0.25
<i>U. dioica</i>	le	L	methanol	26.68	12.91	50.54	74.30	1.00	1.03	0.85	0.77
<i>U. dioica</i>	le	L	methanol	13.53	11.41	50.69	82.02	0.51	0.91	0.85	0.85
<i>U. dioica</i>	le	L	methanol	25.56	11.99	51.02	88.99	0.96	0.96	0.86	0.92
<i>U. dioica</i>	st	L	methanol	7.65	3.75	35.76	28.00	0.29	0.30	0.60	0.29
<i>U. dioica</i>	st	L	methanol	8.11	4.14	37.42	34.55	0.31	0.33	0.63	0.36
<i>U. dioica</i>	st	L	methanol	9.24	3.85	34.26	35.31	0.35	0.31	0.58	0.36
<i>U. dioica</i>	ro	L	methanol	7.18	2.84	1.24	28.70	0.27	0.23	0.02	0.30
<i>U. dioica</i>	ro	L	methanol	13.66	2.72	3.44	32.62	0.51	0.22	0.06	0.34
<i>U. dioica</i>	ro	L	methanol	7.90	2.63	2.52	31.17	0.30	0.21	0.04	0.32

Appendix 3.

Enzyme activity assays (protocols taken from INFOGEST [Brodkorb, A., et al. (2019) INFOGEST static in vitro simulation of gastrointestinal food digestion. Nature Protocols, **14**, 991–1014 <http://dx.doi.org/10.1038/s41596-018-0119-1>]. TCA: trichloroacetic acid; TAME: p-toluene-sulfonyl-L-arginine methyl ester.

Enzyme	Principle	Unit definition	Substrate
α -amylase	Starch + water produce reducing groups (e.g. maltose) when α -amylase is applied.	1 unit releases 1 mg of maltose equivalent from starch in 3 min at pH 6.9 and 20°C.	1% (w/v) soluble potato starch in 20 mM sodium phosphate buffer with 6.7 mM NaCl, adjusted to pH 6.9.
Pepsin	Hemoglobin + water produces TCA-soluble tyrosine peptides when pepsin is applied.	1 unit produces a ΔA_{280} of 0.001 per min at pH 2.0 and 37°C, measured as TCA-soluble products	2% (w/v) hemoglobin in water at pH 2.0.
Pancreatin	TAME + water produces p-toluene-sulfonyl-L-arginine plus methanol when pancreatin is applied.	1 unit hydrolyses 1 μ mol of TAME per min at pH 8.1 and 25°C.	10 mM TAME in H ₂ O.

α -AMYLASE ACTIVITY ASSAY

Conditions: T= 20°C, pH= 6.9, A_{540nm}

Preparation of substrate solution:

- Prepare a 20mM sodium phosphate buffer containing 6.7 mM NaCl
- Adjust pH to 6.9 at 20°C with 1 M NaOH to obtain SOL. A
- Dissolve 0.25 g soluble potato starch (SPS) in 20 ml of SOL. A
- Heat while stirring (not boil) for 15 min
- Cool to room temperature and up to 25 ml with H₂O

Preparation of standard solution:

- 0.02g maltose in 10 ml H₂O

Preparation of enzyme solution:

- Estimated activity of 1 U/ml H₂O of α -amylase

Preparation of assay reagent:

- 0.8 g NaOH in 10 ml H₂O and heat at 50-70°C to obtain SOL. 1
- Add 12 g NaK tartrate tetrahydrate in 8 ml SOL. 1, and maintain the temperature constant while stirring (not boil) to obtain SOL. 2
 - Add 438 mg of 3,5-Dinitrosalicylic acid (DNS) in 20 ml H₂O and heat at 50-70°C (not boil) to obtain SOL. 3
- Heat 12 ml H₂O at 60°C and add slowly 8 ml of SOL. 2
- Add 20 ml of SOL.3 and stir until complete dissolution

Assay procedure:

- standard curve of maltose 0.2%:

Volume (μl)	0	5	20	40	60	80	100	200
H₂O (μl)	200	195	180	160	140	120	100	0
Concentration (μg)	0	1	4	8	12	16	20	40

- in 1.5 ml Eppendorf, dilute the maltose solution in H₂O according to the scheme
- add 100 μl DNS reagent and boil 15 min
- cool in ice and add 900 μl H₂O
- read at 540 nm – 20°C
- samples:

Volumes (μl)	1st enzyme concentration	2nd enzyme concentration	3rd enzyme concentration	Blank
Substrate	100	100	100	100
Enzyme solution	50	70	100	-
DNS reagent	100	100	100	100
Enzyme solution	50	30	0	100
H ₂ O	900	900	900	900

- prepare 3 different enzyme concentrations (+ blank) in 1.5 ml Eppendorf tubes by following the pipetting scheme above
- pipette the substrate, mix and incubate at 20°C – 5 min
- add the enzyme solution, mix and incubate at 20°C – 3 min
- add DNS reagent to stop the reaction
- up to 100 μl of enzyme, mix and incubate at 100°C – 15 min
- cool in ice and add 900 μl H₂O
- read at 540 nm – 20°C

PEPSIN ACTIVITY ASSAY

Conditions: T= 37°C, pH= 2.0, A_{280nm}

Preparation of substrate solution:

- add 0.5 g haemoglobin in 20 ml H₂O

- adjust to pH 2.0 with HCl 300 mM
- up to 25 ml with H₂O

Preparation of enzyme solution:

- prepare a Tris buffer with 10mM Tris HCl and 150 mM NaCl in H₂O
- add 1 mg pepsin each ml of Tris buffer (pepsin stock solution)

Preparation of assay reagent:

- 5 g trichloroacetic acid (TCA) in 100 ml H₂O

Assay procedure:

Pepsin stock solution (μl)	5	10	15	20	25	30	35	40
10 mM HCl (μl)	995	990	985	980	975	970	965	960

- Prepare a range of different concentration of pepsin in 10 mM HCl by following the scheme above
- Pipette 500 μl haemoglobin solution in 2 ml Eppendorf tubes and shake at 37°C for 3-4 min
- Add 100 μl each pepsin concentration and incubate at 37°C for 10 min
- Add 1 ml TCA to stop the reaction
- Centrifuge at 6000 g at 20°C for 30 min to precipitate the remaining hemoglobin
- Read supernatant in quartz cuvette at 280 nm

PANCREATIN ACTIVITY ASSAY

Conditions: T= 25°C, pH= 8.1, A_{247nm}

Preparation of substrate solution:

- 10 mM TAME (0.3789 g in 100 ml H₂O)

Preparation of enzyme solution:

- Dissolve 10 mg pancreatin in 1 ml HCl 1 mM
- Dilute the enzyme solution to have 4 different enzyme concentrations (scheme below)

Pancreatin concentration (μg/ml HCl)	10	20	50	100
Dilution (1:x)	1000	500	200	100

Preparation of assay solution:

- Add 46 mM Tris HCl and 11.5 mM CaCl₂ in 50 ml H₂O
- Adjust pH 8.1 at 25°C

Assay procedure:

- Pipette 1.3 ml Tris buffer and 150 μ l TAME in a quartz cuvette and mix by inversion
- Incubate 25°C for 3-4 min
- Add 50 μ l pancreatin solution and mix
- Read at 247 nm (10 second each, for 10 min)
- Repeat for each enzyme concentration