European Medicinal and Edible Plants Associated with Subacute and Chronic Toxicity

Part I: Plants with Carcinogenic, Teratogenic and Endocrine-Disrupting Effects

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Abstract

In recent decades, the use of herbal medicines and food products has been widely embraced in many developed countries. These products are generally highly accepted by consumers who often believe that “natural” equals “safe”. This is, however, an oversimplification because several botanicals have been found to contain toxic compounds in concentrations harmful to human health. Acutely toxic plants are in most cases already recognised as dangerous as a result of their traditional use, but plants with subacute and chronic toxicity are difficult or even impossible to detect by traditional use or by clinical research studies. In this review, we systematically address major issues including the carcinogenicity, teratogenicity and endocrine-disrupting effects associated with the use of herbal preparations with a strong focus on plant species that either grow natively or are cultivated in Europe. The basic information regarding the molecular mechanisms of the individual subtypes of plant-induced non-acute toxicity is given, which is followed by a discussion of the pathophysiological and clinical characteristics. We describe the genotoxic and carcinogenic effects of alkenylbenzenes, pyrrolizidine alkaloids and bracken fern ptaquiloside, the teratogenicity issues regarding anthraquinone glycosides and specific alkaloids, and discuss the human health concerns regarding the phytoestrogens and licorice consummation in detail.

Key words: European plants; carcinogenic; teratogenic; alkenylbenzene; pyrrolizidine alkaloid; phytoestrogen
1. Introduction

The use of herbal medicinal products and phytonutrients is growing in popularity around the world, with many people now using these products for self-medication and culinaric purposes. It has been estimated that up to 80% of the world’s population living predominantly in developing countries use herbal medicines and traditional medical practices as a primary source of healthcare (Bodeker et al., 2005; Ekor, 2014). The high prevalence of people seeking herbal therapy in developing countries, especially in rural areas, is associated with tradition, availability and low cost. In recent decades, the use of herbal remedies, which are used in complementary or alternative approaches, has also been widely accepted in developed countries, including many European countries, especially the UK, France and Germany (Braun et al., 2010; Calapai, 2008; Ekor, 2014). In contrast to its use in developing countries, phytotherapy has gained popularity in developed countries as a result of the widespread public belief that it is safe, balanced and nature-friendly. Furthermore, it is associated with a healthy way of life and is viewed as a nonaggressive and holistic approach to healing. Thus, people may spend a substantial amount of money for various over-the-counter remedies and food supplements of herbal origin with an intention to improve their general well-being, appearance and health (Kong et al., 2003; WHO global atlas, 2005; WHO Monographs, 2002).

In light of the global growth in interest in phytotherapy and phytonutrition, which encompasses the use of exponentially increasing numbers of newly emerging herbal products, the public health concerns regarding their safety are also increasing. In spite of the promising medicinal and nutritional potential of many herbal species, we must remain aware of the fact that the majority of them have not been thoroughly studied in terms of their safety and that the adverse effects associated with their use have not been monitored in most cases as rigorously as conventional pharmaceuticals (Traditional Medicine Strategy, 2002). Although the systematic control of the quality and safety of industrially produced herbal products has undoubtedly been increased in Europe during recent decades, there is still room for improvement (Raynor et al., 2011).

Plants with substantial acute toxicity are in general already recognised as dangerous because of the historical incidents of poisonings. Subacute and chronic toxicity, on the other hand, are difficult or even impossible to detect in traditional use or even when using clinical research methods. Events that emerge only after long-term use or a lag time are not usually
connected to a causative agent. These types of dangers can therefore go unnoticed even when a plant is widely used.

Generally, the most poisonous plant substances are neurotoxins, which are followed by cytotoxins and metabolic poisons that disturb the structural integrity and functions of the internal organs, such as the liver, heart, kidneys, gastrointestinal system and lungs. In addition to the inherent properties of toxic chemicals, it is important to keep in mind that dose is a crucial parameter. The idea that it is the dose that causes a poison to be toxic was recognized by the first true toxicologist, Paracelsus, five centuries ago. Nevertheless, during the last few decades, it has become apparent that certain substances that originate in plants can cause serious disturbances, including carcinogenesis, hormonal dysregulation, and the disruption of reproductive and developmental processes, even at very low doses which are below those used for traditional toxicological studies (Vandenbergh et al., 2012). Therefore, in contrast to acute toxicity, the dose-effect relationship in cases of chronic toxicity is not always so straightforward.

Another important aspect of the possible intoxications with herbal products and plant food is their contamination or incorrect identification of plant in the product. A toxic *Senecio* species were, for instance, found as contaminant in rocket (*Eruca sativa*) salad (Wiedenfeld, 2011) and herbal teas (Rasenack, 2003; Schulz et al., 2015). Several dozens of Belgian women were intoxicated by herbal slimming pills contaminated (or possibly adulterated) with *Aristolochia* species (Cosyns, 2003). Herbal medicinal products often contain toxic amounts of heavy metals or synthetic medicines (Saper et al., 2004; Au et al., 2000). Furthermore, in folk medicine, people can incorrectly identify a plant and use a toxic one instead of medicinal or edible one (e.g. *Veratrum album* instead of *Gentiana lutea* (Verovnik, 1999)). Adulterations, contaminations and misidentification of herbal products are by themselves extensive topics and will not be covered in this review, nevertheless, they were dealt with to some extent elsewhere (Ekor, 2014; Farah et al., 2000).

In this review, we systematically address the issues of carcinogenicity, teratogenicity and the endocrine-disrupting effects that are associated with the use of herbal preparations. Our focus is on plant species that either grow natively or are cultivated in Europe. We sometimes widened our selection to species that are extensively used in Europe but not cultivated there, including pepper and ginger. In the beginning of each chapter, some basic concepts regarding the molecular mechanisms that have been implicated in individual subtypes of plant-induced non-acute toxicity are described. This is followed by a discussion about the pathophysiological, clinical and epidemiological characteristics of these substances.
2. Genotoxic and carcinogenic plants

Herbal products are not always safe. On the contrary, some of them have been found to contain substances that are potentially genotoxic or carcinogenic. In this respect, substances belonging to the groups of alkenylbenzenes and pyrrolizidine alkaloids are of special concern to human health.

Many secondary plant metabolites are known to attack DNA, either through covalent binding (alkylation) or intercalation (Schmeller et al., 1997; Wink, 2000; Wink, 2008). Plant-derived alkylating agents, which will be more thoroughly presented in the following chapters, include alkenylbenzenes, pyrrolizidine alkaloids, ptaquiloside, aristolochic acids and certain furanocoumarins. If the alkylated DNA bases are not adequately repaired, or if the cell is not directed into apoptosis, the cell may proliferate, thereby fixing the mutation, which represents the first step of carcinogenesis. While plant-derived alkylating agents are generally recognized as procarcinogenic substances, the relation between carcinogenesis and plant-derived intercalating compounds, such as β-carboline and certain other alkaloids, including berberine and sanguinarine (contained in Berberis and Chelidonium), and anthraquinones (found for instance in Senna and Frangula), is not so straightforward (Lu et al., 2012; Schmeller et al., 1997). It has been shown, that they are capable of inserting themselves between successive base pairs in DNA which can result in the disruption of DNA replication and the occurrence of frameshift mutations (Schmeller et al., 1997; Wink, 2000). Although they can undoubtedly have a profound impact on the cell cycle, these plant-derived intercalating agents have not been experimentally linked with carcinogenesis. Some of them have even been shown to have antineoplastic activity as a result of their antiproliferative and proapoptotic effects (Lu et al., 2012; Wink, 2007).

2.1 Alkenylbenzenes

Many alkenylbenzenes, i.e., estragole, eugenol, methyl eugenol, safrole, β-asarone and myristicin, have been demonstrated to have genotoxic and carcinogenic properties (Figure 1 and Table 1), and the EU Scientific Committee on Food (EU-SCF) has suggested restrictions for their use (Scientific Committee on Food, 2001a, 2001b, 2001c). They are present in a wide
variety of plants, including nutmeg, cinnamon, basil, fennel, anise, tarragon and black pepper (Table 1) (Scientific Committee on Food, 2001a, 2001b, 2001c). Alkenylbenzenes are used as flavours and fragrances in many foods and food products, perfumes, soaps, aromatic oils and detergents. Despite the existence of regulatory efforts in several countries (Van den Berg et al., 2011), their use in food supplements that contain them in higher levels is not sufficiently controlled. It has been namely shown, that the recommended use of some food supplements that contain high concentrations of alkenylbenzenes could lead to exposure levels that have caused malignant tumours in experimental animals (Scientific Committee on Food, 2001a, 2001b, 2001c; Van den Berg et al., 2011).

Figure 1. The comparison of the chemical structures of the most common alkenylbenzenes. The molecules typically consist of a benzene ring substituted with a propenyl and a methoxy groups.

Nevertheless, the industrial expert panel from the Flavour and Extract Manufacturers’ Association (FEMA) concluded that the consumption of flavours and the related exposure to methyl eugenol and estragole is generally too low to pose a significant cancer risk in humans because it has been shown in rodent studies that the activating biotransformation pathway does not play an important role when these drugs are used at low doses (Smith et al., 2002). Alkenylbenzenes must be metabolically activated to become genotoxic and to induce tumours (Miller et al., 1983; Wiseman et al., 1985). The relevant bio-activating pathways of all alkenylbenzenes are quite similar. In the most significant 1-hydroxylation pathway, 1-hydroxy-derivatives (the proximate carcinogen) are formed, which are subsequently transformed into ultimate carbocationic carcinogens through a process involving P450 cytochromes and sulfotransferases (Miller et al., 1983). In addition, highly reactive epoxide metabolites have been detected in rodent livers following the exposure to alkenylbenzenes,
but were found to be insignificant because they are rapidly converted to less toxic dihydrodiol or glutathione conjugates (Guenthner and Luo, 2001; Luo and Guenthner, 1996).

It has been suggested that the results obtained in studies of herbal extracts may be different from those obtained from isolated carcinogenic compounds (De Paula et al., 2007; Jeurissen et al., 2008). This discrepancy could be associated with the complex mixture of compounds present in the extracts that might influence either bio-activation (toxication) and/or detoxification pathways, the most significant of the later being glucuronidation and binding with glutathione (Guenthner and Luo, 2001; Iyer et al., 2003). For instance, in HepG2 human hepatoma cells the DNA alkylation mediated by the sulfotransferase-catalysed bio-activation of estragol was suppressed by a flavonoid substance nevadensin, present in the methanol basil extract (Alhusainy et al., 2010; Jeurissen et al., 2008). Therefore, the level of the bio-activation of alkenylbenzenes could be significantly diminished when they are consumed along with other herbal ingredients. The plants that are used in herbal medicine in Europe that contain genotoxic or carcinogenic alkenylbenzenes are listed in Table 1.

Table 1. List of plants species containing alkenyl benzene compounds with genotoxic and/or carcinogenic properties.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Compound</th>
<th>Genotoxic and/or Carcinogenic Activity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Foeniculum vulgare,</em></td>
<td>Estragole</td>
<td>Well established genotoxic (mutagenic and clastogenic) and carcinogenic (especially hepatocarcinogenic)</td>
<td>Drinkwater et al., 1976;</td>
</tr>
<tr>
<td><em>Ocimum basilicum,</em></td>
<td></td>
<td>effects in various rodent models.</td>
<td>EFSA, 2009a; Martins et al., 2012; SCF, 2001 a,b,c; Swanson et al., 1979</td>
</tr>
<tr>
<td><em>Artemisia dracunculus,</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Pimpinella anisum</em></td>
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<td></td>
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<tr>
<td><em>Laurus nobilis,</em></td>
<td>Eugenol</td>
<td>Genotoxicity was indicated in a number of <em>in vitro</em> assays (resulting from oxidative DNA damage). There is also modest evidence for genotoxic effects <em>in vivo</em> models (in mice).</td>
<td>EFSA, 2009b; NTP, 1983; Swanson et al., 1979</td>
</tr>
<tr>
<td><em>Ocimum basilicum,</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Geum rivale,</em></td>
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<td></td>
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<tr>
<td><em>Heracleum spp.</em></td>
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<tr>
<td><em>Angelica archangelica,</em></td>
<td>Isosafrole</td>
<td>A weak hepatocarcinogenic effect was found in rodents (a non-genotoxic mechanism is suspected). In vitro systems testing mutagenicity gave equivocal results.</td>
<td>SCF, 2003a; Swanson et al., 1979</td>
</tr>
<tr>
<td><em>Sassafras spp.</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Petroselinum crispum,</em></td>
<td>Apiole</td>
<td>DNA adducts were detected in HepG2 cells and laboratory animals (weaker effects than were observed for estragole and safrole).</td>
<td>Miller et al., 1983; Zhou et al., 2007</td>
</tr>
<tr>
<td><em>Anethum graveolens</em></td>
<td></td>
<td></td>
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</tbody>
</table>
\[ \begin{array}{|c|c|c|c|}
\hline
\textbf{Species} & \textbf{Isolated Compound} & \textbf{Genotoxicity} & \textbf{References} \\
\hline
\textit{Acorus calamus}, \textit{Asarum europaeum} & B-Asarone & \textit{In vitro} genotoxicity tests gave equivocal results. \textit{In vivo} tests demonstrated an increased incidence of hepatic adenomas and carcinomas in mice and leiomyosarcomas in rats. & Cartus and Schrenk, 2015; Cartus et al., 2015; SCF, 2002 \\
\hline
\textit{Petroselinum crispum}, \textit{Myristica fragrans} & Elemicin & The reactive hydroxyl metabolites of elemicin were found to bind covalently to DNA and to induce hepatocarcinogenesis in rodents. & Hasheminejad and Caldwell, 1994; Miller et al., 1983; Wiseman et al., 1987 \\
\hline
\textit{Myrrhis odorata} & Trans-Anethole & Several \textit{in vitro} and \textit{in vivo} experiments showed that trans-anethole is not genotoxic, but it was shown to induce hepatocellular carcinomas in rats. & Newberne, 1999; Swanson et al., 1979 \\
\hline
\textit{Foeniculum vulgare}, \textit{ Ocimum basilicum}, \textit{Artemisia dracunculus}, \textit{Laurus nobilis}, \textit{Zingiber officinale}, \textit{Cymbopogon spp.} & Methyl eugenol & Methyl eugenol is mutagenic (can induce DNA adducts through its 1-hydroxy-metabolite) and has been associated with liver tumours in rodents. & EFSA, 2009a; Zhou et al., 2007 \\
\hline
\textit{Petroselinum crispum}, \textit{Anethum graveolens}, \textit{Heracleum} spp., \textit{Pastinaca sativa}, \textit{Myristica fragrans} & Myristicin & Myristicin and its 1-hydroxy-metabolite have genotoxic and carcinogenic (especially hepatocarcinogenic) effects in various rodent models. & EFSA, 2009a; Zhou et al., 2007 \\
\hline
\textit{Ocimum basilicum}, \textit{Piper nigrum}, \textit{Myristica fragrans} & Safrole & Safrole is a well-documented genotoxic carcinogen. It was shown to be genotoxic and to have carcinogenic (especially hepatocarcinogenic) effects in rodents. & EFSA, 2009a; Swanson et al., 1979 \\
\hline
\end{array} \]

\subsection*{2.2 Pyrrolizidine Alkaloids}

Pyrrolizidine alkaloids (PAs) are secondary metabolites found in thousands of plant species from more than 12 unrelated botanical families that are distributed throughout many geographical regions in the world (Roeder, 1995; Roeder, 2000; Smith and Culvenor, 1981). It has been reported that approximately 3\% of the world’s flowering plants (roughly 6000 species) contain more than 600 PAs, roughly half of them exhibiting chronic toxicity (Smith and Culvenor, 1981) (Figure 2). Although toxic PA derivatives were detected also in certain
species of *Convolvulaceae, Poaceae* and *Lamiaceae*, they are primarily found in the members of the following four plant families (Reimann, 2004; Smith and Culvenor, 1981):

- **Asteraceae** – in plants of the *Senecioneae* subtribe (24 genera, the genus *Senecio* is prevalent) and the *Eupatorieae* subtribe (mainly in the genera *Eupatorium* and *Ageratum*),
- **Boraginaceae** – in virtually all plant species of this family,
- **Fabaceae** – in the subtribe *Crotalarieae* (not found in Europe), mainly in the genus *Crotalaria* but also in the genera *Chromolaena* and *Lotononis*, and
- **Orchidaceae** – for instance, in the genus *Liparis*.

![Chemical structures](image)

Figure 2. Some of the PAs which were characterized as mutagenic and/or carcinogenic in animal studies. PAs are derived from esters of basic alcohols and share a common chemical structure that consists of a necine base moiety.

In addition to the well described hepatotoxic effects of PAs, many of these substances have also been shown to have genotoxic and carcinogenic properties (EFSA, 2011). *In vitro* and *in vivo* studies demonstrated that PAs produce, upon metabolic activation, DNA adducts, cross-linking and breaks, leading to gene mutations (most frequently G:C → T:A transversions and tandem base substitutions) and chromosomal aberrations (Chen et al., 2010; EFSA, 2011). Studies in experimental animals that were exposed to different PAs or their
metabolites have demonstrated the development of cancers in the liver, lungs, intestines, bladder, kidneys, pancreas, skin, brain and spinal cord, adrenal glands, blood (leukaemia), and muscle (Chan et al., 2003; Chen et al., 2010; Hirono, 1993). It has been shown in both experimental animals and human cell cultures that one of the multiple known genetic targets of toxic PA metabolites is the gene TP53, which encodes the well-known tumour suppressor protein p53 (Chen et al., 2010; Couet, 1993; Ji et al., 2005). It has been hypothesized that the carcinogenesis is particularly favoured by extended, low-level, and intermittent periods of exposure to PAs since they have been found to be not only genotoxic but also antimitotic (Mattocks, 1968). Intermittent low-dose exposure to PAs, which is a most likely scenario in the case of PA-contaminated herbal preparation or food use and is associated with the DNA injury and the inhibition of mitoses, would, namely, allow intervening periods of normal or even increased mitotic activity, providing an opportunity for the tumours to evolve (McLean, 1970; Rai et al., 2008; Schoental, 1968). The importance of the antimitotic activity of PAs for the carcinogenic process has, however, not been thoroughly substantiated by the experimental studies.

A list of plants for which concerns have been raised regarding their PA levels is presented in Table 2. Some of them are foraged by animals, while others are weeds that grow among grains that are harvested for human use. These can therefore contaminate the food supply. Several of the listed herbs have been used as medicinals for many centuries (the principal medicinal genera that are currently in use are Senecio, Tussilago, Borago, Lithospermum, Heliotropium and Eupatorium).

Table 2. Plant species and specific PAs that may have genotoxic and carcinogenic effects.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Compounds</th>
<th>Genotoxicity and Carcinogenicity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tussilago farfara</td>
<td>Senkirkine, tussilagine,</td>
<td>Several in vitro genotoxicity studies have provided weak positive results for senkirkine. A</td>
<td>Chen et al., 2010; COC, 2008; EFSA, 2011;</td>
</tr>
<tr>
<td></td>
<td>senecionine</td>
<td>increased incidence of hepatic adenomas was observed in male rats after senkirkine administration. The Committee On Carcinogenicity (COC) and the IARC have concluded that the present data are not sufficient to ascertain the carcinogenicity of senkirkine.</td>
<td>Fu et al., 2004; Gree et al., 1981; Mori</td>
</tr>
<tr>
<td>Petasites spp.</td>
<td>Senkirkine, Senecionine,</td>
<td>Senecionine was found to form DNA adducts and was positive in several genotoxicity assays,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>seneciphylline</td>
<td>including UDS assays and a Wing spot test. In spite of a number of negative results for senecionine in the Ames test, it is most likely</td>
<td></td>
</tr>
<tr>
<td>Adenostyles spp.</td>
<td>Senecionine, seneciphylline,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>spartioidine, integerrimine</td>
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</table>


<table>
<thead>
<tr>
<th>Plant Family</th>
<th>Constituents</th>
<th>Toxicity</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucanthemum spp.</td>
<td>Spartioidine, integerrime, retrosine, Jacobine, senecionine</td>
<td>carcinogenic. Genotoxicity was indicated in a number of <em>in vitro</em> and <em>in vivo</em> tests of riddelliine and monocrotaline. Riddelliine demonstrated carcinogenicity in experimental animals and has been listed also as a probable human carcinogen. In male rats that were given monocrotaline, liver cell carcinomas developed.</td>
<td>et al., 1985b; Newberne and Rogers, 1973</td>
</tr>
<tr>
<td>Senecio spp.</td>
<td>Riddelliine, floridanine, monocrotaline, otosenine, senecionine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symphytum spp.</td>
<td>Symphytine, lycopsamine, echimidine, symglandine</td>
<td>Genotoxic potential was identified for symphytine in a Wing spot test. In experimental animals exposed to symphytine, an increased incidence of liver haemangiosarcomas and hepatic adenomas was observed.</td>
<td>Chen et al., 2010; COC, 2008; EFSA, 2011; Fu et al., 2004</td>
</tr>
<tr>
<td>Myosotis spp.</td>
<td>Symphytine, lasiocarpine, myoscorpine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eupatorium spp.</td>
<td>Lycopsamine, intermedine, supinine, rinderine, echinatine</td>
<td>Lycopsamine displayed no mutagenicity in Ames tests, but was found to be mutagenic in <em>Drosophila</em>. Lycopsamine and its intermediates demonstrated only weak hepatic toxicity in rats.</td>
<td>Chen et al., 2010; EFSA, 2011; Fu et al., 2004</td>
</tr>
<tr>
<td>Lithospermum spp.</td>
<td>Lycopsamine, intermedine, lithosenine, triangularine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cynoglossum officinale</td>
<td>Heliotrine, lasiocarpine, cynoglossophine</td>
<td>Many <em>in vitro</em> and an <em>in vivo</em> tests have demonstrated the genotoxic potential of lasiocarpine and heliotrine (including clastogenic effects). Hepatic angiosarcomas were observed in a 2-year study of lasiocarpine in rats. In a study of male rats that were exposed to heliotrine, increases in the incidence of pancreatic islet cell tumours, transitory cell papillomas of the urinary bladder and intestinal testicular tumours were observed.</td>
<td>Chen et al., 2010; EFSA, 2011; Fu et al., 2004; NTP, 1978; Schoental, 1975</td>
</tr>
<tr>
<td>Heliotropium spp.</td>
<td>Lasiocarpine, heliotrine, indicine heliosupine, echinatine</td>
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2.3 Ptaquiloside and Bracken Fern

Bracken fern (*Pteridium aquilinum*) is one of the most common fern species with a wide geographical and ecological distribution. It is present in all continents, except in Australia, from subtropic to subarctic areas (Taylor, 1990). Bracken fern is a very adaptable plant and is capable of forming dense, rapidly expanding populations in course of the first phases of the ecological succession in forest cleanings and other disturbed rural areas.
(Pakeman and Marrs, 1992). Its aggressive growth, characterized by an extensive rhizome system and rapidly growing fronds, sometimes enables it to be a dominant species in certain plant communities, for instance in the understorey of pine and oak forests, in heather (*Calluna vulgaris*) stands, acid grasslands and moors (Le Duc et al., 2003; Smith and Taylor, 1995).

Bracken contains different poisonous agents, including some cyanogen glycosides (e.g., prunasin), two types of thermolabile thiaminases, a few thermostable thiamine antagonists (e.g., caffeic acid, astragalin and isoquercetin), and carcinogenic compounds (e.g., ptaquiloside and shikimic acid) (Alonso-Amelot and Oliveros 2000; Konishi and Ichijo, 1984; Meyer, 1989) (Figure 3). Ptaquiloside is certainly the major carcinogen in bracken (Smith and Seawright, 1995). The distribution of ptaquiloside in a variety of ferns has been examined in Japan using chromatography and a modified Ames test (Saito et al., 1989). Nineteen of the 31 ferns that were tested were found to contain the potentially carcinogenic ptaquiloside and its close analogues, in addition to *Pteridium*, and especially the ferns in the genera *Pteris*, *Microlepia* and *Hypolepis* (Saito et al., 1989).

![Figure 3. Some of the toxic compounds isolated from bracken fern, prunasin (a cyanogen glycoside), ptaquiloside (the major carcinogen in bracken) and shikimic acid (a potential carcinogen-promoting agent).](image)

After the ingestion of the plant material or contaminated milk, both ptaquiloside and its aglycone ptaquilosin are converted by the hepatic cytochrome P-450 enzymes into the unstable dienone, which is regarded as the ultimate carcinogen (Kigoshi et al., 1993). The dienone metabolite is strongly electrophilic, and it rapidly reacts with amino acids and nucleotides, forming covalent adducts with DNA and causing DNA strand breaks (Ojika et al., 1987; Ojika et al., 1989). Crude bracken extracts that were incubated with liver microsomal systems have been found to be positive in Ames tests (Matsuoka et al., 1989).
Furthermore, it was found out that ptaquiloside can cause chromatid exchanges in Chinese hamster lung cells and unscheduled DNA synthesis in rat hepatocytes (Evans, 1986; Hirono et al. 1984; Matsuoka et al., 1989; Mori et al., 1985a; Saffhill et al., 1985). On the other hand, shikimic acid and its metabolite cyclohexane carboxylate were not mutagenic in Ames tests, and whether they are carcinogenic in experimental animals and humans remains controversial (Jones et al., 1983). Hence, although shikimic acid is unlikely to be a cancer-initiating agent, it may act as a carcinogen-promoting agent (Jones et al., 1983).

Bracken carcinogenicity in animals had already been demonstrated fifty years ago (Evans and Mason, 1965). Many subsequent studies performed in either the field or the laboratory have associated the ingestion of bracken or its extracts with diseases and cancers in many sites in different animal species, such as rats, hamsters, guinea pigs, cattle, sheep and toads (Evans, 1987). The consumption of bracken was linked to a number of different, generally well recognised syndromes, some of which are species specific (Smith, 2004): i) bovine enzootic haematuria and mixed tumours of the urinary bladder (in cattle), ii) acute haemorrhagic disease and upper alimentary carcinoma (in cattle and sheep), iii) thiamine deficiency (in horses and sheep), and iv) retinal atrophy (in sheep). Three main routes of exposure to the toxic effects of bracken fern have been proposed in humans, i.e., consummation of the plant, ingestion of milk from affected animals and inhalation of its spores.

Bracken ferns have been part of human diets from antiquity. Prehistoric man is thought to have regularly consumed bracken rhizomes in winter and the young fronds in the spring. During the First World War, the rhizomes of these plants were consumed by people in Scotland (Vetter, 2009). Today, its fronds are traditionally eaten in Japan and some parts of South America, while its rhizomes are a part of the diet of Aboriginal populations and Maori (Hirayama, 1979; Vetter, 2009). In certain parts of the world, especially in Russia, China and Japan, bracken fern is even grown commercially for human use (Vetter, 2009). The usual procedure that is performed before eating the plant (boiling in water in the presence of different chemicals, such as soda ash) appears to render their components less harmful, presumably by inactivating some of their toxic agents. Nevertheless, some carcinogenic activity persists (Hirayama, 1979). In some countries (e.g., Japan, Brazil and Venezuela), a close association was identified between the consumption of bracken fern and cancers of the upper gastrointestinal tract (Alonso-Amelot and Avendano, 2002; Hirayama, 1979). It has been found that the concentration of ptaquiloside in fronds increases with their maturation process, while it is almost absent in rhizomes (Alonso-Amelot et al., 1992; Vetter, 2009).
Ptaquiloside has been identified in the milk produced by bracken-fed cows, and the concentrations at which it was found were observed to be roughly linearly dependent on the dose that was ingested by the cow (Alonso-Amelot et al., 1996). The proportion of ptaquiloside that was excreted with the milk was estimated to be as high as 8.6% of the total consumed substance (Alonso-Amelot et al., 1996). It is quite plausible that the ptaquiloside in the milk of these animals is responsible for the association between bracken infestation and the incidence of gastric cancer in the populations of farmers that inhabit cattle-range areas in Costa Rica and other countries where bracken growth is dense (Villalobos-Salazar, 1995). Indeed, tumours have been demonstrated in rats that were fed with the milk of cows that had grazed bracken (Villalobos-Salazar, 1995). While this route of exposure may be relevant in small, geographically remote rural communities, the dilution resulting from the bulk processing of milk is thought to reduce any risk from milk in most developed countries to negligible levels (Vetter, 2009).

Because spores have been found to be carcinogenic in mice, there is a theoretical risk that the inhalation and subsequent ingestion of spores could also be carcinogenic in humans. To date, there have been no studies of the effects of exposure of animals to airborne spores, but studies have explored the effects of directly ingesting the spores (Povey et al., 1996). In mice, spores administered by gavage produced DNA adducts (Wilson et al., 1998). Nevertheless, the significance of these adducts requires further investigation.

Recently, ecotoxicological studies investigating the concentration of bracken fern ptaquilosides in the soil of the bracken-populated areas were performed. The investigation conducted in Denmark, has, for instance, detected the following concentrations: 0.008–40.6 µg ptaquiloside/g dry soil in the autumn and 1.56–212 µg ptaquiloside/g dry soil in late winter (Rasmussen et al., 2005). It has been established that the risk of ptaquiloside soil leaching depends on many environmental factors, such as the soil pH (it is the highest in slightly acidic to neutral soil), the clay content and the rate of microbiological degradation (Rasmussen et al., 2005; Vetter, 2009). However, the health risk implications of the presence of ptaquiloside in soil and in the groundwater for animals and humans are not well established and require further ecotoxicological research.

2.4 Other carcinogenic plants
The compendium of plants that are either wild-growing, cultivated or extensively consumed in Europe that may have carcinogenic potential has not yet been exhausted. In the following list (Table 3), an overview of the remainder of plants that might be of concern because they have genotoxic and/or carcinogenic potential is provided. For the majority of the selected botanical ingredients, carcinogenicity has been either reported or suspected.

Table 3. List of plant species with potentially genotoxic and/or carcinogenic properties.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Compound</th>
<th>Genotoxic and/or Carcinogenic Activity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rubia tinctorum</em></td>
<td>Lucidin, alizarin</td>
<td>Lucidin and alizarin have genotoxic properties (they were positive in a battery of short-term <em>in vitro</em> tests). Furthermore, carcinogenic activity was demonstrated in rats (intestinal and liver tumours).</td>
<td>Blömeke et al., 1992; EFSA, 2009a; Westendorf et al., 1988</td>
</tr>
<tr>
<td><em>Galium odoratum</em>, <em>Melittis melissophyllum</em>, <em>Verbascum spp.</em>, <em>Anthoxanthum spp.</em>, <em>Melilotus spp.</em>, <em>Cassia spp</em>, <em>Lavandula spp.</em></td>
<td>Coumarin</td>
<td>The epoxide metabolites of coumarin produced in the liver of rats and mice were shown to be carcinogenic, they caused adenomas and carcinomas of the liver, bile ducts and lungs, and adenomas in the kidneys. In contrary, hydroxy metabolites of coumarin produced in human liver are not classified as carcinogens (Group 3 by IARC).</td>
<td>Abraham et al., 2010; Born et al., 2003; NTP, 1993; SCF, 1999</td>
</tr>
<tr>
<td><em>Aristolochia spp.</em>, <em>Asarum spp.</em>, <em>Bragantia spp.</em></td>
<td>Aristolochic acids</td>
<td>Aristolochic acids are genotoxic and form DNA-adducts. They were indicated to be carcinogenic in rats, mice and rabbits, and also in humans. Urothelial cancers have namely been observed in patients suffering from aristolochic acid nephropathy.</td>
<td>Arlt et al., 2002; Chen et al., 2012; Jelaković et al; 2012</td>
</tr>
<tr>
<td><em>Aloysia citriodora</em>, <em>Melissa officinalis</em>, <em>Cymbopogon spp.</em></td>
<td>Citral</td>
<td>Several <em>in vitro</em> as well as <em>in vivo</em> tests for genotoxicity have shown negative results for citral (but positive results were found in sister chromatid exchange assays and comet tests). No evidence was found for the carcinogenic activity of citral in rodents (but lymphomas were induced in female mice). Citral is considered safe for human use.</td>
<td>EFSA, 2009c; NTP, 2003; Sinha et al., 2014</td>
</tr>
<tr>
<td>Plants</td>
<td>Compound</td>
<td>Effects</td>
<td>References</td>
</tr>
<tr>
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</tr>
<tr>
<td><em>Thymus</em> spp., <em>Humulus lupulus</em>, <em>Cannabis</em> spp., <em>Petroselinum crispum</em></td>
<td>Myrcene</td>
<td>No evidence of genotoxicity was found in <em>in vitro</em> and <em>in vivo</em> genotoxicity assays for myrcene. However, administration of myrcene to rodents induced liver and kidney tumours.</td>
<td>NTP, 2010</td>
</tr>
<tr>
<td><em>Citrus limon</em> and <em>Citrus reticulates</em> (essential oil and exocarp), <em>Levisticum officinale</em>, <em>Petroselinum crispum</em></td>
<td>8-Methoxypsoralen (methoxsalen)</td>
<td>8-Methoxypsoralen was demonstrated to have genotoxic activity in several <em>in vitro</em> and <em>in vivo</em> assays. Carcinogenicity was detected in male rats. It is classified as Group 1 carcinogen, but it has carcinogenic activity only in combination with UV radiation.</td>
<td>NTP, 1989 and 2010</td>
</tr>
<tr>
<td><em>Mentha</em> spp. (especially <em>M. pulegium</em>), <em>Agastache</em> spp.</td>
<td>Pulegone</td>
<td>Mutagenicity assays of pulegone have provided negative results. Carcinogenic activity was found in mice (liver tumours, osteomas, osteosarcomas) and in rats (urinary bladder tumours).</td>
<td>Da Rocha et al., 2012; EMA, 2014; NTP, 2011</td>
</tr>
<tr>
<td><em>Cycas</em> spp.</td>
<td>Cycasin</td>
<td>Cycasin is a mutagenic and a carcinogenic agent, but only when it is deglucosylated to its principal metabolite, methylazoxymethanol (interestingly, in adult mammals, the deglucosylation of cycasin is catalysed only by enzymes present in the microflora of the gut). Long-term administration of cycasin and methylazoxymethanol acetate by oral and intraperitoneal routes has been shown to be hepatotoxic and carcinogenic in old-world monkeys.</td>
<td>Sieber et al., 1980</td>
</tr>
</tbody>
</table>

### 3. Teratogenic plants

In the case of pregnant women, the use of any drug or product, either natural or synthetic, must be especially seriously considered in light of the risk-benefit ratio. Many substances can harm a developing embryo or foetus, especially when they are used during the critical periods corresponding to organogenesis in the first trimester of pregnancy. Nevertheless, throughout the world, many pregnant women consume a wide variety of herbs and herbal products during pregnancy for many reasons that may be related or not to pregnancy. For example, herbal products are used to treat nausea, vomiting, dyspepsia and constipation, to induce abortion, to treat infections of the urinary tracts, to prepare for labour,
Plant toxins that can efficiently cross the placenta and that can induce congenital malformations in a developing embryo or foetus when present at critical time periods during gestation are called to have teratogenic effects (Keeler, 1984). Therefore, exposure to these toxins in the prenatal stage can lead to teratogenesis, but many of them can also have a negative impact on the postnatal growth and maturation processes (developmental toxicity). Many teratogenic mechanisms have been associated with the use of herbal products. These include the disruption of the microtubule assembly process, the folate antagonism, the induction of oxidative stress, the specific receptor-mediated teratogenesis and DNA alkylation (Schmeller et al., 1997; Van et al., 2010; Wink, 2007 and 2008).

3.1 Anthraquinone Glycosides

Plant species containing anthraquinones (Figure 4), including senna glycosides or sennosides, cascarosides, emodine and rhein, are part of the formulations of numerous medicinal products that are mainly used for the treatment of constipation. Anthranoid laxatives act by exciting peristalsis and increasing fluid secretion in the colon. Worldwide, the most common such stimulant is made of sena species (Cassia angustifolia Vahl; Cassia senna L. – syn. Senna alexandrina Mill). However, many other plants containing anthraquinone glycosides, such as those derived from Frangula alnus L., Rhamnus spp., Aloe spp. and Rheum spp., are often included (Roberts and Tyler, 1999).

Figure 4. Chemical structures of two typical anthraquinone glycosides, found in Cassia spp. (sennoside A) and Rheum spp. (cascaroside A).
It is well known that constipation is very common in pregnant women. Although this is not the topic of the current article, in which we focus mainly on the chronic toxicity issues, it is appropriate to mention here that the use of laxatives containing anthraquinones might potentially be dangerous because they can induce acute unwanted effects, such as uterine contractions and increased blood flow to the uterus and its attachments, thereby increasing the risk of foetal loss (Acs et al., 2009; Conover, 2003). The anthraquinones may even pass into breast milk to cause unwanted effects, such as spasms, in an infant (Acs et al., 2009).

The potential teratogenic effects of senna were recently examined in a case-control epidemiological study. No significant risk was found for more than 20 different congenital abnormality groups in relation to treatment with senna during the second and third gestational month. It was also demonstrated, somewhat surprisingly, that the gestational age at delivery was somewhat longer (by 0.2 week) and that the rate of preterm birth was lower (6.6 % vs. 9.2 %) in the newborn infants of mothers who had used senna during pregnancy (Acs et al., 2009).

3.2 Alkaloids

The use of the herbs mentioned in Table 4 should be avoided during pregnancy because abortifacient and/or teratogenic potential (demonstrated mainly in animal studies) has been linked to their alkaloid content (Figure 5). Many of these plants can also induce acute (Lupinus spp., Veratrum spp., Conium spp., and the included members of the family Solanaceae) or chronic (neurotoxicity: certain species in the genera Astragalus and Oxytropus; carcinogenicity: Cycas spp.; hepatotoxicity: certain PA-containing species) toxicity.

Table 4. List of plant species containing alkaloids that are potentially teratogenic.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Active Constituents Responsible for Teratogenicity and their Toxicity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lupinus</em> spp.</td>
<td>Quinolizidine and piperidine alkaloids, such as lupinine (Figure 5), ammodendrine and anagyline, which cause congenital contracture-type skeletal malformations and cleft palates in foetuses (especially in cows, sheep and goats).</td>
<td>Bunch et al., 1992; Keeler, 1983 and 1984; Panter et al., 2013</td>
</tr>
<tr>
<td>Plant Family</td>
<td>Alkaloids/Toxins</td>
<td>References</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td><em>Nicotiana</em> spp.</td>
<td>Piperidine alkaloids, such as anatabine (Figure 5) and anabasine, which cause congenital contracture-type skeletal malformations and cleft palates in foetuses (especially in pigs).</td>
<td>Bunch et al., 1992; Crowe and Swerczek, 1974; Keeler, 1984; Panter et al., 2013</td>
</tr>
<tr>
<td><em>Oxytropis</em> spp. and <em>Astragalus</em> spp. (certain species of these two genera, toxicity varies)</td>
<td>The indolizidine alkaloid swainsonine, an alphamannosidase inhibitor that induces neurological defects of foetuses and vasoconstriction in the placentae of sheep and cattle.</td>
<td>Bunch et al., 1992; Keeler, 1984; Lather et al., 2011; Panter et al., 2013</td>
</tr>
<tr>
<td><em>Veratrum</em> spp.</td>
<td>Steroidal alkaloids, including veratridine (Figure 5), cyclopamine, cycloposine and jervine, which cause craniofacial malformations, including cyclopia, in the foetuses of sheep, horses, goats and cows.</td>
<td>Incardona et al., 1998; Keeler, 1984; Lather et al., 2011; Panter et al., 2013</td>
</tr>
<tr>
<td><em>Cycas</em> spp.</td>
<td>Quinolizidine and piperidine alkaloids.</td>
<td>Lather et al., 2011</td>
</tr>
<tr>
<td><em>Trigonella foenum-graecum</em></td>
<td>Alkaloids, such as trigonelline, that can cause a decrease in bone marrow cell proliferation and disrupt foetal development in rats and rabbits. In Morocco, cases of pronounced congenital malformations, including hydrocephalus, anencephaly and spina bifida, were linked to the consumption of fenugreek seeds during pregnancy. However, this causality remains questionable because fenugreek is widely used by people in Morocco and neighbouring countries.</td>
<td>Araee et al., 2009; Gaffield and Keeler, 1994</td>
</tr>
<tr>
<td><em>Ruta graveolens</em></td>
<td>Quinoline and quinolone alkaloids that induce changes in the formation of blastocysts, reducing the number and delaying the development of embryos in mice. Another study in mice also showed no significant difference in the loss of embryos before implantation between the treated and control groups, but deaths were observed in the foetuses of treated females, suggesting an embryotoxic effect.</td>
<td>De Freitas et al., 2005; Gutierrez-Pajares et al., 2003</td>
</tr>
<tr>
<td><em>Conium maculatum</em></td>
<td>Piperidine alkaloids, including coniine and γ-coniceine, which cause congenital contracture-type skeletal malformations, cleft palates, restricted foetal movements and arthrogrypotic limb deformities in calves.</td>
<td>Bunch et al., 1992; Keeler and Balls, 1978; Keller et al., 1980; Panter et al., 2013</td>
</tr>
<tr>
<td><em>Solanum</em> spp. (green parts of plants and unripe fruits)</td>
<td>Glycoalkaloides, including solanidanes and spirosolanes, which are capable of inhibiting certain cholinesterase enzymes and increasing the permeability of mitochondrial membranes (thereby promoting apoptosis). These can cause brain defects, spina bifida and cleft palate in the foetuses of sheep and cattle.</td>
<td>Gaffield and Keeler, 1994</td>
</tr>
<tr>
<td>Some PA-containing plant species, especially</td>
<td>PAs (especially heliotrin) have been found to be teratogenic in rats (e.g., growth retardation, abnormal skeletal development, and deficiencies in ossification). The exposure</td>
<td>Edgar et al., 2011; Wiedenfeld, 2011</td>
</tr>
</tbody>
</table>
Heliotropium spp. of pregnant women to PAs through the consumption of herbal teas, herbal medicines, and spices has been linked to fatal hepatic sinusoidal obstruction syndrome (HSOS) in human neonates.

Datura stramonium, Mandragora officinarum, Hyosciamus spp., Atropa belladonna

The tropane alkaloid hyoscyamine, which causes deformities in foals during foetal development.

Lather et al., 2011; Panter et al., 2013

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>Teratogenic Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>lupinine (from Lupinus spp.)</td>
<td>Anatabine (from Nicotiana spp.) and Veratridine (from Veratrum spp.)</td>
</tr>
</tbody>
</table>

Figure 5. Some of the alkaloids that were found to have teratogenic effects: lupinine (from Lupinus spp.), anatabine (from Nicotiana spp.) and veratridine (from Veratrum spp.).

3.3 Other Potentially Teratogenic plants

Other plant species in which the pharmacological potential to cause teratogenicity has been described but not studied in as much detail as the previously mentioned plants are listed in Table 5.
Table 5. List of other potentially teratogenic plants that grow (or are available) in Europe.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Active Constituents Responsible for Teratogenicity and their Toxicity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cannabis sativa</em></td>
<td>Causes brain defects in the foetuses.</td>
<td>Reece, 2009</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em></td>
<td>Cyanogenic glycosides that can cause contracture-type skeletal defects.</td>
<td>Lather et al., 2011; Panter et al., 2013</td>
</tr>
<tr>
<td><em>Podophyllum</em> spp.</td>
<td>Absent finger anomalies, septal defects of the heart, defects of the external ears and skin tags in human foetuses.</td>
<td>Lather et al., 2011</td>
</tr>
<tr>
<td><em>Prunus</em> spp. (especially seeds of <em>P. dulcis</em> and <em>P. armeniaca</em>)</td>
<td>Cyanogenic glycosides that can cause contracture-type skeletal defects and cleft palate.</td>
<td>Panter et al., 2013</td>
</tr>
<tr>
<td><em>Lathyrus</em> spp. (<em>L. cicera</em> and <em>L. odorata</em>)</td>
<td>Osteolathyrogens, including γ-aminopropionitrile, which cause congenital skeletal malformations in cows and sheep.</td>
<td>Panter et al., 2013</td>
</tr>
</tbody>
</table>

4. Plants with Endocrine-Disrupting Potential

4.1 Endocrine-Disrupting Chemicals with Plant Origins

In recent decades, great concern has arisen regarding the potential human health risks that may be associated with exposure to endocrine-disrupting chemicals (EDCs). Endocrine disruptors are defined as exogenous natural or synthetic substances that alter the function of the endocrine system and that consequently produce adverse health effects in an organism or its descendants. EDCs influence homeostasis, development, and cell proliferation by mimicking or inhibiting the actions of endogenous hormones or in other way altering the function of the endocrine system (Choi et al., 2004; Cooper and Kavlock, 1997). EDCs may interfere with the synthesis, storage, release, metabolism, transport, elimination, or receptor binding of endogenous hormones (Cooper and Kavlock, 1997). Many EDCs, including industrial chemicals, pesticides, environmental contaminants, heavy metals, and phytoestrogens, have been associated with reproductive disorders in both men and women. These include impaired fertility, decreases in sperm count and quality, cryptorchidism,
hypospadias, miscarriages, endometriosis and irregularities in the menstrual cycle (Balabanic et al., 2011).

In addition to the endocrine-disrupting potential of phytoestrogens, another well studied type of endocrine imbalance that has been linked to plant substances is presented in this review, i.e., pseudohyperaldosteronism, associated with the ingestion of products containing licorice (*Glycyrrhiza glabra*).

### 4.2 Phytoestrogens

Phytoestrogens are plant secondary metabolites that are structurally and/or functionally similar to mammalian oestrogens or their active metabolites. The main groups of phytoestrogen are the lignans, flavonoids (subgroups isoflavones, coumestans and prenyllflavonoids) and stilbenes (Limer and Speirs, 2004; Moon et al., 2006; Sirtoti et al., 2005). Lignans are the main phytoestrogens in a normal diet, and they are found in many cereals, fruits and vegetables. They can be subdivided into enterolactones and enterodiols, which have very weak oestrogenic properties (Limer and Speirs, 2004). Isoflavones are a subgroup of flavonoids that are most abundant in soybeans and other legumes but that are also present in berries, wine, grains and nuts (Kurzer and Xu, 1997). They include, for example, genistein, daidzein and biochanin A (Figure 6). Isoflavones display variable oestrogenic activity, and they are considered to be natural SERMs (selective oestrogen receptor modulators) (Zand et al., 2000). Isoflavones act as antioxidants *in vitro*, where they have been shown to have antiproliferative activities (Fotsis et al., 1993; Peterson, 1995). Compared to studies on isoflavones, significantly fewer studies have been performed using coumestans, prenyllflavonoids and stilbenes. Coumestans are potent activators of oestrogen receptor (ER) signalling pathways, but they are not as common in the diet as other phytoestrogens (Kurzer and Xu, 1997; Limer and Speirs, 2004). The chemopreventive potential of the most common stilbene, resveratrol, against breast cancer has been studied in rodent models (Limer and Speirs, 2004).

Phytoestrogens can be regarded as endocrine disruptors and could potentially be harmful to human health. However, many health benefits have been associated with their use, including the alleviation of menopausal symptoms, especially the reduction of the frequency of hot flushes, and a lowered risk of developing atherosclerosis, ischaemic heart disease, osteoporosis and breast cancer (Chen et al., 2015; Patisaul and Jefferson, 2010; Schmidt et al.,
As previously mentioned, we are exposed to phytoestrogens on a daily basis because they are widely distributed in plants that are components of our diets. In spite of their weak affinity for oestrogen receptors (ER), they therefore cannot be neglected as EDCs, and their consumption could have profound physiological effects (Patisaul and Jefferson, 2010). In the next subsection, we consider the mechanism of action of phytoestrogens and reveal that their interaction with oestrogen receptors is in some way similar to the SERM/ER interaction.

**Figure 6.** Structures of three isoflavones which are characteristic for soy and certain other legumes, such as red clover, alfalfa and peanuts.

Although the ER signalling is characterized as the principal mechanism of the phytoestrogen action, phytoestrogens do not exert their effects solely through ERs. Instead, they have the potential to affect a wide array of intracellular signalling mechanisms that are important for regulating cellular growth, proliferation and protection against harmful environmental influences (Oseni et al., 2008; Ruiz-Larrea et al., 1997). Several phytoestrogens are powerful antioxidants and antiinflammatory agents, including isoflavones and resveratrol (Ruiz-Larrea et al., 1997). Daidzein and genistein are the two best-characterized isoflavones. Human exposure to these compounds occurs primarily through the consumption of soy-based food and beverage products. Some isoflavones, most notably genistein, inhibit pathways that are important for cell growth and proliferation, thereby affecting multiple organ systems (Oseni et al., 2008; Piontek et al., 1993). Genistein, for instance, inhibits the activity of protein tyrosine kinases, which are very important regulatory proteins (Piontek et al., 1993).

*In vitro* assays have shown that although most phytoestrogens, including the isoflavones, bind both ERα and ERβ and activate ER-dependent gene transcription through both receptor subtypes, they generally have a higher relative binding affinity for ERβ than for ERα (Kuiper et al., 1997; Kuiper et al., 1998). Once bound, isoflavones do not act like typical oestrogen agonists; instead, they act like SERMS, such as tamoxifen, which acts as an ER
agonist in the uterus and bone but as an antagonist in the breast, where it has proven anticancer activity (Oseni et al., 2008). This differential activity by phytoestrogens and SERMS results, in part, from the profile of co-activator and corepressor proteins in the cell being affected (Kushner et al., 2000; Oseni et al., 2008). Once bound to ERs, phytoestrogens can initiate transcription either classically, through interactions with oestrogen response elements (ERE), or by binding to early immediate genes, such as Jun and Fos (Kushner et al., 2000).

Phytoestrogens, particularly the isoflavones, fit the definition of an endocrine disruptor. In animal models, they produce effects such as premature reproductive senescence, compromised fertility, disruption of the lactation and the timing of puberty, the inhibition of the sexual behaviour etc. (Jefferson et al., 2007; Patisaul et al., 2001; Patisaul and Jefferson, 2010; Setchell et al., 1987). The exposure to high levels of phytoestrogens could therefore pose a risk to human reproductive health, especially when taken by women diagnosed with endometriosis or those experiencing irregular menstrual cycles. In addition, phytoestrogens should be consumed in moderate amounts by women diagnosed with estrogen-sensitive cancers and during the critical periods, such as puberty and adolescence, pregnancy or lactation. Phytoestrogens have been namely shown to be able to perturb normal menstrual cycles in humans, for instance, their intake in high amounts can prolong the menstrual bleeding (Strom et al., 2001) or cause dysmenorrhea, worsen the clinical course of endometriosis, induce postmenopausal bleedings or elicit endometrial proliferation with polyps (Chandrareddy et al., 2008). Nevertheless, there is a relative lack of studies among humans regarding effects of the phytoestrogen-induced hormonal disbalance on fecundity and fertility. Some studies have even demonstrated that the phytoestrogen supplementation in women can be even associated with improved reproductive outcomes, such as higher ovulation and implantation rates (Kohama et al., 2005; Unfer et al., 2004), shorter time to pregnancy (Mumford et al., 2014), higher pregnancy rates among couples with unexplained infertility (Shahin et al., 2008). Studies in men mainly reported no harmful effects of phytoestrogens on their reproductive capacity (Beaton et al., 2010; Mitchell et al., 2001), except for two reports which associated their intake with lower sperm concentration (Chavarro et al., 2008) and with idiopathic male infertility (Xia et al., 2013).

Among researchers, there is still no definite arrangement whether the phytoestrogens increase or reduce the risk of developing breast cancer (Enderlin et al., 2009). While the majority of epidemiological studies and meta-analyses concluded that the high intake of soy isoflavones is associated with reduced breast cancer risk in pre- and postmenopausal women
(Boucher et al., 2013; Lee et al. 1991; Schmidt et al., 2016; Trock et al., 2006; Verheus et al., 2007; Wu et al., 2008), some studies state that the opposite might be true (Maskarinec et al., 2004; Padilla-Banks et al., 2006). Recently, it has been demonstrated that the use of soy isoflavones is safe in women with the history of breast cancer and is not contraindicated in women on hormone therapy with tamoxifen and anastrozole (Kang et al., 2010; Schmidt et al., 2016; Wu et al., 2007). Studies even indicate that the isoflavone intake improves the prognosis of breast cancer patients, prolonging their life and decreasing the cancer recurrence rates (Marini et al., 2008; Shu et al., 2009).

### 4.3 “Oestrogenic” Plant Species

Mainly through our diet, we are exposed to a continuous and complex mixture of plant substances that, as outlined above, probably have a substantial impact on our endocrine system. “Oestrogenic” plant compounds are widespread in food, including herbs and seasonings (e.g., garlic and parsley), grains (wheat, rye, and oat), vegetables (e.g., soybeans and beans), fruits (cherries and apples), and drinks (coffee). In Table 6, we present the plants that are known to contain very high levels of phytoestrogens (Geller and Studee, 2006; Poluzzi et al., 2014; Thompson et al., 2006); the list is not exhaustive.

Table 6. Plant species with potentially endocrine-disrupting potential as a result of their high level of phytoestrogens.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Major Phytoestrogenic Constituents</th>
<th>Chemical Group</th>
</tr>
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<tbody>
<tr>
<td><em>Glycine max</em></td>
<td>Genistein, daidzein, glycine, coumestrol</td>
<td>Isoflavones, coumestans</td>
</tr>
<tr>
<td><em>Arachis hypogea</em></td>
<td>Genistein, resveratrol</td>
<td>Isoflavones, stilbenes</td>
</tr>
<tr>
<td><em>Cicer arietinum</em></td>
<td>Biochanin A</td>
<td>Isoflavones</td>
</tr>
<tr>
<td><em>Psoralea corylifolia</em></td>
<td>Isobavachalcone, bavachin, corylin</td>
<td>Isoflavones</td>
</tr>
<tr>
<td><em>Bituminaria bituminosa</em></td>
<td>Bitucarpin A and B</td>
<td>Isoflavonoids (pterocarps)</td>
</tr>
<tr>
<td><em>Medicago sativa</em></td>
<td>Spinasterol, coumestrol, coumestan, formononetin</td>
<td>Phytosterols, coumestans, isoflavones</td>
</tr>
<tr>
<td>Plant Species</td>
<td>Compounds</td>
<td>Category</td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td><em>Trifolium pratense</em></td>
<td>Coumestrol, trifoliol, formononetin, biochanin A</td>
<td>Coumestans, isoflavones</td>
</tr>
<tr>
<td><em>Trifolium repens</em></td>
<td>Coumestrol, trifoliol</td>
<td>Coumestans</td>
</tr>
<tr>
<td><em>Linum usitatissimum</em></td>
<td>Seecoisolariciresinol, matairesinol</td>
<td>Lignans</td>
</tr>
<tr>
<td><em>Sesamum indicum</em></td>
<td>Sesamolin, sesamine, pinoresinol, lariciresinol, hydroxymatairesinol</td>
<td>Lignans</td>
</tr>
<tr>
<td><em>Secale cereale</em></td>
<td>Syringaresinol, lariciresinol</td>
<td>Lignans</td>
</tr>
<tr>
<td><em>Triticum spp.</em></td>
<td>Secoysolariciresinol, Syringaresinol, Secoisolariciresinol</td>
<td>Lignans</td>
</tr>
<tr>
<td><em>Avena sativa</em></td>
<td>Pinioresinol, lariciresinol, hydroxymatairesinol</td>
<td>Lignans</td>
</tr>
<tr>
<td><em>Brassica spp.</em></td>
<td>Pinioresinol, lariciresinol, coumestrol</td>
<td>Lignans, coumestans</td>
</tr>
<tr>
<td><em>Humulus lupulus</em></td>
<td>8-Prenylnaringenin (hopein), xanthohumol, isoaxanthohumol</td>
<td>Prenylflavonoids (flavanones)</td>
</tr>
<tr>
<td><em>Glycyrrhiza</em> spp.</td>
<td>Liquiritigenin, glabrene, glabridine</td>
<td>Prenylflavonoids (flavanones), isoflavones</td>
</tr>
<tr>
<td><em>Vitis</em> spp.</td>
<td>Resveratrol</td>
<td>Stilbenes</td>
</tr>
<tr>
<td><em>Morus</em> spp.</td>
<td>Resveratrol</td>
<td>Stilbenes</td>
</tr>
<tr>
<td><em>Fallopia japonica</em></td>
<td>Resveratrol</td>
<td>Stilbenes</td>
</tr>
</tbody>
</table>

* Found in medicinal products used to treat perimenopausal ailments.

### 4.4 Pseudohyperaldosteronism and *Glycyrrhiza* spp.

Pseudohyperaldosteronism is a condition that clinically mimics hyperaldosteronism, including the suppression of plasma renin activity and aldosterone levels. It can be a consequence of an overconsumption of licorice, carbenoxolone or grapefruit juice, but it can also be caused by genetic (Liddle syndrome) and endocrinal (congenital adrenal hyperplasia) defects. The excessive use of licorice is by far its most significant dietary cause (Obolentseva et al., 1999; Palermo et al., 2003).

The fabacean genus *Glycyrrhiza* consists of approximately 20 species. Two of them, *G. glabra* and *G. uralensis*, are generally recognized as a source of licorice because of the
distinctive sweet taste of their roots and stolons (Society of Japanese Pharmacopoeia, 2012). This taste can be attributed to the triterpenoid substance glycyrrhizin, which is a mixture of potassium, calcium and magnesium salts of glycyrrhizic acid (Obolentseva et al., 1999). The level of glycyrrhizin that is present in the roots of different *Glycyrrhiza* species varies from 2 to 25%, depending on growth conditions (Obolentseva et al., 1999).

Licorice is popular not only as a food and drink sweetener. It has also been used by tobacco companies as a flavouring agent and in herbal medicines, especially in China and Japan. There, it has been used for decades to treat gastric ulcers (Obolentseva et al., 1999). However, this particular use of licorice has become obsolete as a result of the emergence of newer, more powerful and safer prescription drugs. Nevertheless, licorice continues to be included in many health products and could even gain new medical interests as a result of recent research findings which show that licorice has antiviral, antiinflammatory, antithrombotic and hepatoprotective activities (Coon and Ernst, 2004; Marianecci et al., 2012; Mendes-Silva et al., 2003; Tsuruoka et al., 2009).

The traditional belief that licorice is a healthy, natural substance without side effects drives its broad consumption, which can in some cases be dangerous. The active compound in licorice extracts, that is the glycyrrhizic acid, and its metabolites that are produced in the intestine and in the liver, i.e., glycyrrhetinic acid and 3-monoglucuronyl-glycyrrhetinic acid have mineralocorticoid-like activities and can cause pseudohyperaldosteronism (Yoshino et al., 2014; Makino, 2014; Stewart et al., 1987). This results from its direct binding to mineralocorticoid receptors in the kidneys (minor reason) and the inhibition of two enzymes that are involved in the corticosteroid metabolism (major reason) (Calo et al., 2004; Yoshino et al., 2014; Whorwood et al., 1993). Licorice extract (especially 3-monoglucuronyl-glycyrrhetinic acid) specifically inhibits 11-ß-hydroxysteroid dehydrogenase (11-ß-HSD) type 2, which metabolises cortisol to cortisone. Subsequently, the activity of cortisol, which binds as avidly to the mineralocorticoid receptor as aldosterone, is increased (Funder et al., 1988). Furthermore, glycyrrhetinic acid also inhibits the hepatic metabolism of aldosterone by suppressing 5-ß reductase activity (Latif et al., 1990; Makino, 2014).

Licorice overconsumption is most often characterised by muscle weakness, pain and numbness, oedema of the face and lower extremities, and variously severe hypertension that is accompanied by a reduction in plasma renin and aldosterone levels. This differentiates its symptoms from those of primary or secondary hyperaldosteronism (Joshino et al., 2014; Lin et al., 2003; Makino, 2014; Stewart et al., 1987). In addition to low serum levels of potassium (due to its increased secretion in the kidneys), metabolic alkalosis (resulting from an increase
in renal proton secretion) and a raised cortisol to cortisone ratio in the peripheral venous plasma (resulting from the inhibition of 11-ß-HSD) are also found in most cases (Joshino et al., 2014; Stewart et al., 1987). In cases with rhabdomyolysis (due to severe and long lasting hypokalemia), creatine phosphokinase may be also elevated, and mioglobinuria with acute kidney failure may ensue (Joshino et al., 2014; Mumoli and Cei, 2008; Murphy et al., 2009; Omar et al., 2012; Stewart et al., 1987).

The main difficulty in dosing licorice lies in the fact that it is available in various food and medicinal items that contain highly variable levels of licorice. In 1991, the European Union proposed a provisional dose of 100 mg/day as the upper limit for the ingestion of glycyrrhizin (approximately the amount found in 60–70 g of licorice) (Murphy et al., 2009; Omar et al., 2012). Based on data obtained in studies of human volunteers, this upper limit was later confirmed by the Scientific Committee on Food, which also stated that human toxicity studies were too limited to define an exact safe average daily intake for glycyrrhizic acid (SCF, 2003b).

5. Conclusions

While there has long been widespread awareness among people regarding the acute toxicity of herbs and their preparations, their potential for inducing chronic toxicity has received the attention it deserves only in the last few decades. A number of compounds with the potential to induce chronic toxicity have been found in plants that are used in various botanical preparations. Among these, alkenylbenzenes and pyrrolizidine alkaloids present a special concern for human health because of their genotoxic, carcinogenic and teratogenic potential. The basic intention of this review was therefore to gather and systematically present what is currently known about these aspects of herbal toxicity and to describe how they could have a significant impact on public health.

Issues related to the adverse reactions that have been associated with different herbal products, the use of which is growing exponentially, have become increasingly exposed in recent times. Safety assessments of herbal medicines have become an important issue for consumers, regulatory authorities, and healthcare professionals, but analyses of the adverse events that are related to these products is much more complex than analyses of conventional pharmaceuticals (Ekor, 2014). This is especially true in the case of chronic toxicity, where causality can be very difficult to establish.
Botanicals are self-prescribed and widely available, which make them difficult to control. Furthermore, folklore and folk practices, and not scientific studies, are often used as a means of obtaining information on the indications, efficacy and safety of medicinal plants and herbal medicines (Lather et al., 2011). Many gatherers, manufacturers and consumers of herbal medicines lack the appropriate level of botanical and toxicological knowledge. They may be prone to misidentifying plant species, and they may be unfamiliar with the scientific binomial classification of plants. This lack of knowledge can lead to mistakes resulting from confusion that is created by the numerous common names that are in use among people (Ekor, 2014; Farah et al., 2000). In addition, the variability of secondary metabolites with medicinally valuable or toxic effects in herbal products, which are not under strict control, is very high, as it depends on a myriad of factors, e.g., the plant phenotype, the soil and climate characteristics, the harvest time, the preparation procedure etc. All of these constraints also limit the efficiency of monitoring the safety of herbal medicines, which essentially requires the close collaboration of different experts in the field. The same basic standards should therefore apply to the recognition and reporting of herbal toxicity that apply to prescription medications. The systematically collected data about the frequency of toxic reactions and the understanding of their etiopathogenesis and clinical manifestations are needed to enable improvements in the safety of herbal medicine use.

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29


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