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Crop plants and herbs for the treatment of women disorders: Analytical methods for the bioactive compounds – A review

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Abstract

During last decades an increasing number of herbal and plant crops products specifically targeting women's disorders has appeared in the worldwide marketplace. Herbs are natural products and, for this reason, they do not have a consistent, standardized composition. So, it is a high need to evaluate the bioactive compounds of these products.

The analytical methods applied to determine and highlight the main bioactive compounds from crop plants (*Medicago sativa sprouts* and *Glycine max.beans)* and herbs (*Salvia officinalis aerial parts*) for women health (relief of premenopausal, menopausal and postmenopausal related symptoms) is the subject to this review.

The purpose of this review is to evidence their phytochemical composition which relief the use of the products from this plants as homeophatic therapy. So, these are classified on the basis of the chemical markers used for the quality control or the evidence that isoflavones are present in this two crop plants and the flavonoids in the aromatic herb. Also, a part of this review is the main chemical composition of these plants.

Keywords: bioactive compounds, *Medicago sativa*, Glycine *max*., *Salvia officinalis*, analytical methods, women's disorders

1. Introduction

During last decades an increasing number of herbal products specifically targeting women in menopause and with menstrual affections has appeared in the worldwide marketplace. This growth highlights the need for a critical evaluation of quality, safety and efficacy of these products.

Ensuring that plant-based products are of suitable quality is important for several reasons. Herbs are natural products and, for this reason, they do not have a consistent, standardized composition [1].

Plants contain numerous chemical constituents and if we analyze different parts of the plant (e.g. roots, leaves), we certainly find a different qualitative and quantitative profile of constituents. The reason of this variability is that the content and concentration of constituents can be influenced by several factors including climate, growing conditions, time of harvesting, and post-harvesting factors, such as storage conditions (e.g. light, temperature, humid-ity) and processing (e.g. extraction and drying).

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The quantity of a chemical marker can be an indicator of the quality of a herbal medicine. This point is very important, because the chemical marker approach for quality control is realized by means of both qualitative and quantitative analyses. The analysis of chemical markers requires specific analytical methods for qualitative analysis and validated, accurate, precise, and robust methods for quantitative analysis [2].

The purpose of this review is to assess the evidence for quality of the crop plants used as medicinal plants for women health (relief of menopause and menstrual related symptoms) with a specific focus on their phytochemical composition. Medicinal plants used with this purpose are classified on the basis of the chemical markers, not always corresponding to the active constituents, used for the quality control (*Table 5*).

2. Chemical markers.

Ideally, chemical markers should be components that contribute to the therapeutic effects of a medicinal plant. Considering that only a small number of chemical compounds were shown to have clear pharmacological actions, and a large number of plants are not studied for their bioactive metabolites, other chemical components can be used as markers. The European Medicines Agency (EMEA) [2] defines chemical markers as chemically defined constituents or groups of constituents of a medicinal plant which are of interest for quality control purposes regardless of whether they possess any therapeutic activity.

Chemical markers for soybeans, mature seeds, raw.

The soybean [*Glycine max(L.) Merrill*] have been extensively used as important source of dietary protein and oil throughout the world. Though, soybean is a widely cultivated crop, most of it is used as the raw material for oil milling, and the residue (soy meal) is mainly used as feedstuff for domestic animals [3]. Dry soybean contain 36% protein, 19% oil, 35% carbohydrate (17% of which dietary fiber), 5% minerals and several other components including vitamins [3].

Several years of rigorous scientific and clinical research has established that most of the components of soybean have beneficial health effects as characterized by its preventive potential for the so-called life-style-related diseases. The impact of most of the nutritionally and physiologically functional components of soybean [4] have been summarized in *Table 1*.

In summary, though the available human studies seem to show a few conflicting results in terms of the consistent efficacy of soy isoflavones in alleviating post menopausal symptoms, from the epidemiological studies it can be stated that isoflavone do help the regular soybean consumer to better manage their post menopausal symptoms.

Table 1. Functional components of soy and their impact [4].

α-Linolenic acid	Essential fatty acid, hypotriglyceridemic, improves heart health
Isoflavones	Estrogenic, hypocholesterolemic, improves digestive tract function, prevents breast, prostate, and colon cancer, bone health, improve lipid metabolism
Lecithins	Improve lipid metabolism, improve memory and learning abilities
Lectins	Anti-carcinogenic, immunostimulator
Linoleic acid	Essential fatty acid, hypocholesterolemic
Peptides	Readily absorbed, reduce body fat, anticancer
Phytosterols	Hypocholesterolemic, improves prostate cancer
Protein	Hypocholesterolemic, antiatherogenic, reduces body fat
Saponin	Regulates lipid metabolism, antioxidant

Table 2. Phenolic acids, flavones and flavonols of Glycine max, Vigna radiata and Medicago sativa sprouts (mg/kg) [7].

Compounds	RT	Glycine max	Vigna radiate	Medicago sativa
5-O-Caffeoylquinic acid	15.5	5.4±0.0	2.4±0.0	nd
Caffeic acid	17.0	25.9±0.9	21.2±0.1	nd
p-Coumaric acid	23.2	9.5±0.0	2.6±0.0	14.4±0.0
Ferulic acid	28.5	1.6±0.0	4.1±0.0	6.0±0.0
Quercetin-3-O-rutinoside	41.0	29.5±0.3	nd	nd
Cinammic acid	41.8	nd	nd	7.9±0.2
Luteolin-7-O-glucoside	43.5	nd	nd	1.5±0.0
Kaempferol-3-O-glucoside	44.9	1.9±0.0	1.0±0.0	nd
Kaempferol-3-O-rutinoside	45.3	12.9±0.5	3.2±0.1	nd
Luteolin-4′-O-glucoside	47.7	nd	nd	17.4±0.1

nd, not detected.
RT, retention time (min).

Tabel 3. Isoflavones *of Glycine max, Vigna radiata* and *Medicago sativa* sprouts (mg/kg) [7].

Isoflavones	RT	Glycine max	Vigna radiata	Medicago sativa
Daidzin	49.5	556.4±12.8	187.8±10.9	664.3±0.4
Genistin	50.3	52.9±2.4	48.6±5.1	123.7±0.4
Daidzein	53.1	9.8±0.4	26.3±1.1	18.9±0.1
Genistein	56.0	5.7±0.1	15.7±0.4	7.6±0.1

RT, retention time (min).

Table 4. Essential oil composition (% of major components) of sage *Salvia officinalis* collected as a sample.

Compound*	*S.officinalis***	*S.officinalis****
(1R)-(+)-α-Pinene	3.70	4.50
(-)-Camphene	2.60	5.00
β-Pinene	6.00	5.20
Sabinene	-	0.30
β-Mircene	3.00	3.50
α-Tirpene	-	0.40
P-Cimene	0.60	0.60
Terpinolene	-	0.20
(-)-α-Thujone	1.38	1.80
B-Thujone	0.72	1.50
Camphor	8.00	10.00
(-)-Linalool	0.80	0.80
Linalyl acetate	0.60	0.30
(-)-*Trans*-caryophyllene	2.00	1.00
Monoterpene	1.26	1.10
(+)-Menthol	-	-
Borneol	5.00	4.50.
α-Terpineol	0.20	-
Geranyl acetate	0.30	-
Geranyol	0.10	0.25
Phytol	0.18	-
Thymol	0.80	0.70
Carvacrol	0.20	0.40
Farnesol	0.20	-
Trans, trans-Farnesol	0.06	0.15
Total components	45	30

*Compounds of essential oil extracted from fresh green leaves and flowering top;** S.officinalis collected at 100m above the sea level; *** S.officinalis collected at 500m above the sea level

Table 5. Medicinal plants discussed in the present review with relative chemical markers, analytical techniques and references

Plants	Chemical markers	Analytical techniques	References
Glycine max	Isoflavones	HPLC-UV, LC–MS, UPLC, UFLC, NMR	[16-24]
Medicago sativa	Isoflavones	HPLC-UV, HPLC-DAD, HPLC-FLD, LC–MS, GC-IT/MS	[25-32]
Salvia officinalis	Phenolic compounds, diterpenes	LC–MS/MS, HPLC-DAD, TLC,	[33-35]

Chemical markers for alfalfa (Medicago sativa) sprouts.

Sprouts are believed to be rich in health-promoting phytochemicals compared with their mature counterparts. Germination (sprouting) has been suggested as an inexpensive and effective way to improve the quality of legumes. Sprouting mobilizes polymerized forms, such as concentrated starch and protein, into carbohydrates and free amino acids, respectively. This significantly improves the nutritional value of sprouts, which can be readily used by the human body [5].

Leaves and seeds of alfalfa (M. sativa) are also sold as bulk powdered herb, capsules, and tablets for nutritional supplement in health food stores. The sprouts are often consumed as salad and are known for their high content of phenolic compounds, with correspondent high antioxidant activity [6]. The phenolic compounds found in the extracts contribute, to some extent, to the observed effects. Antioxidants are one of the most common active ingredients of nutritionally functional foods, which can play an important role in the prevention of oxidation and cellular damage by inhibiting or delaying the oxidative process. HPLC-DAD analysis of the sprouts aqueous extracts allowed the identification of ten phenolic compounds (*Table 2*) [7].

Four isoflavones, including two aglycones (daidzein and genistein) and their glucosides (daidzin and genistin, respectively), were identified in the analyzed sprouts (*Table 3*) [7].

Chemical markers for sage.

The genus *Salvia*, commonly known as sage, is the largest member of *Lamiacea* or mint family containing over 900 species throughout the world.[8,9] The plants are mostly aromatic and perennial, with flowers in different colors [10]. Many species of *Salvia*, including *Salvia officinalis* (common sage), are native to the Mediterranean region and some of the *Salvia* species have been used worldwide as flavoring spices as well as traditional herbal medicine [10,11].

Sage is also a natural source of flavonoids and polyphenolic compounds (e.g., carnosic acid, rosmarinic acid and caffeic acid) possessing strong antioxidant, radical-scavenging, and antibacterial activities [12]. The majority of the phenolic acids in *Salvia* species are derivatives of caffeic acid which is the building block of a variety of plant metabolites [13]. Caffeic acid plays a central role in the biochemistry of the *Lamiaceae* plants, and occurs mainly in a dimer form as rosmarinic acid [13]. Carnosic acid and rosmarinic acid, which are present at high concentrations in the extract of sage plants, have shown strong antioxidant properties [14]. Ursolic acid, also a component of sage, has strong anti-inflammatory properties, and in sage preparations, it is considered as a quality control measurement for the anti-inflammatory effects of different solutions [15]. In *tabel 4* we can enumerate the most important polyphenols content.

3. Analytical methods for plants that are used in treatment of women disorders.

The four common steps for any analytical method are sampling, sample preservation, sample preparation and analysis. *Fig. 1* presents a general overview of the most common steps for sample preparation for the determination of soy isoflavones. The initial step in any analysis is sampling, where a representative sample is collected from the entire sample matrix that needs to be analyzed. The entire food-stuff should be represented in the sample that will be used for the analysis. Sample preservation is an important step as there is often some delay between sample collection

and/or preparation and analysis. Proper sample preservation ensures that the sample retains its physical and chemical characteristics from the time it is collected to the time it is analyzed. Sample preparation may consist of multiple steps such as drying, homogenization, sieving, extraction of target compounds, pre-concentration, hydrolysis and derivatization. Sample preparation can seek several objectives: to increase the efficiency of an assay procedure, to eliminate or reduce potential interferences, to enhance the sensitivity of the analytical procedure by increasing the concentration of the analyte in the assay mixture, and sometimes to transform the analyte of interest to a more suitable form that can be easily separated, detected, and/or quantified. Isoflavone determination is complex since its concentration in the sample depends of several variables which may difficult the determination. Overall, the ultimate goal is to obtain a concentrated extract with all isoflavones and free of interfering compounds from the matrix [36-38].

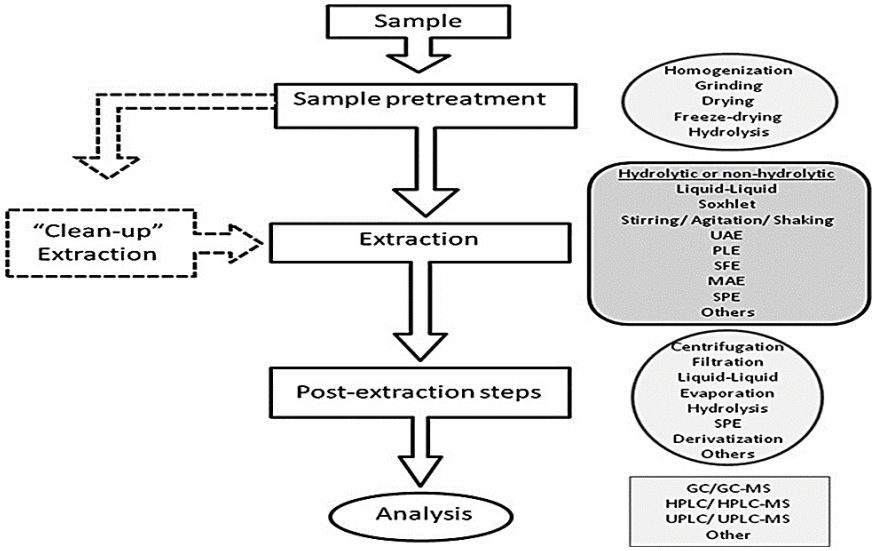

Figure 1. Most common steps for sample preparation for the determination of soy isoflavones.

Soy (Glycine max (L.) Merrill, Fabaceae), a legume originating from Asia, is widely distributed throughout the world, for preparing food products.

More recently its consumption is linked, among different properties, to the reduction of menopausal symptoms. This finding has resulted in the development and commercialization of many functional foods and food supplements based on soy ingredients. Soy extracts contain a mixture of isoflavones belonging to the group of phytoestrogens. The major isoflavones in soybeans include daidzein, glycitein, genistein, their glycosides, glycoside malonates and glycoside acetates, in which the predominant isoflavone forms in soybeans and non-fermented soy products are the glycoside malonates, 6"-O-malonylgenistin and 6"-O-malonyldaidzin [39]. Studies have shown that they play an important role in reducing climacteric symptoms in menopausal and postmenopausal women [40].

A recent review reports the sample preparation and analysis for the quantification of isoflavones in soybeans and soyfoods. Modern techniques including ultrasound-assisted extrac-tion, pressurized liquid extraction, supercritical fluid extraction and microwave-assisted extraction, and analysis by HPLC are reported [41]. In the quality control of soy the amount of isoflavones, both aglycones and glycosides, is usually determined by means of reversed-phase HPLC-U [42]. Although the HPLC methods have some advantages when they are applied to the analysis of isoflavones in terms of

specificity, sensitivity, and straight forward operation, they require a relatively long period of time, normally from 20 min to 65 min. So, the use of high-throughput liquid chromatography technologies has been reported in the last decade for isoflavone analysis, reducing the chromatographic time to less than 10 min. These techniques allow the use of short columns, packed with 3 µm particles, supporting elevated pressures, thus reducing analysis time, solvent consumption, and, consequently, environmental impact [43]. Among these studies, Apers and coworkers developed an HPLC method using two linked monolithic silica-based reversed-phase C18 columns. This method for determination of isoflavones in soy extracts needs less than 25 min. Among the high-throughput methods reported in the literature for isoflavones analysis, UPLC and ultra-fast liquid chromatography (UFLC) are cited for their determination in soybeans.

Alfalfa (Medicago sativa L., Fabaceae) is the main Medicago species widely grown throughout the world, predominantly as a source of high quality forage for livestock, renewable energy production, phyto remediation and as a source of phytochemicals [44]. It is also used as a human food ingredient, consumed as sprouts in salads, sandwiches or soups, as leaf protein concentrates or as food supplements [45]. Despite this use, alfalfa have pharmacological activities, being used in some human health disfunctions, and among these anemia, endometriosis, osteoporosis and menopausal symptoms [41].The plant contains many important constituents including saponins, sterols, coumarins, flavonoids, phenolics, vitamins, proteins, minerals, and other nutrients [46]. As above reported for Glycine max, to isoflavones present in M. sativa are ascribable some of the pharmacological activity of alfalfa. Murphy and coworkers analyzed isoflavones in retail and institutional foods (alfalfa and soy) by HPLC [47]. Further HPLC [48–50], ESI-MS [48, 49], and CE [51] methods were employed to analyze flavonoid content in M.sativa. More recently, two glycosides (daidzin and genistin) and six aglycones (daidzein, glycitein, genistein, formononetin, prunetin and biochanin A) were determined by HPLC-DAD in three different extracts (aqueous, hydroalcoholic and alcoholic) of M. sativa [45]. Abo Markeb quantified 5- and 7-hydroxyflavone in the M.sativa samples by HPLC-FLD [52]. A study compared the isoflavone production of the callus cell suspension cultures of M. sativa to the original plants. The extracts were analyzed by LC–MS for their isoflavones, mainly formononetin, biochanin A, daidzein, and genistein [53].

In the sprouts of M. sativa and G. max, phenolic compounds, sterols and triterpenes were determined by HPLC-DAD, organic acids by HPLC-UV and fatty acids and volatile compounds by GC-IT/MS [44]. The metabolic profiling of triterpene saponins in M.sativa was also investigated using HPLC-ESI-MS [54].

Sage (Salvia officinalis) is a popular kitchen herb and is a member of the mint (Labiatae) family. It has been used in a variety of food preparations since ancient times. From its Latin name, ‘‘Salvia’’ meaning to cure and ‘‘Officinalis’’ meaning medicinal, it is clear that sage has a historical reputation for promotion of health and treatment of ailments [55].

Phenolic compounds are well-known phytochemicals found in all plants. They consist of simple phenols, benzoic and cinnamic acid, coumarins, tannins, lignins, lignans and flavonoids. Substantial developments in research focused on the extraction, identification and quantification of phenolic compounds as medicinal and/or dietary molecules have occurred over the last 25 years. Organic solvent extraction is the main method used to extract phenolics. Chemical procedures are used to detect the presence of total phenolics, while spectrophotometric and chromatographic techniques are utilized to identify and quantify individual phenolic compounds [56].

Several separation techniques [e.g., gas chromatography (GC), capillary electrophoresis (CE) and LC] have been applied in analysis of flavonoids, their derivatives and metabolites, yet HPLC is used most commonly. HPLC separates components of a mixture on the basis of their differential distribution between the mobile and the stationary phase.

Using the stationary phases described above, separation is carried out by reversed-phase LC (RP-HPLC or RP-UHPLC) [57, 58]. Separation can also be achieved using hydrophilic interactive liquid

chromatography (HILIC) with properly selected ion stationary phases and excess of acetonitrile as a component of the mobile phase [59]. The detectors used most often are based on UV absorbance or, less frequently, visible light absorbance. In analysis of flavonoids, UV detectors can be used, due to the presence of aromatic rings in the molecule. These rings absorb light in the wavelength range 230–280 nm [57, 60]. Flavonoids have two characteristic ranges of absorption:

- range II with absorption maximum at λ= 240– 285 nm associated with the ring A; and,
- range I, with the maximum occurring at λ = 300– 550 nm, associated with the ring B.

HPLC-MS offers higher sensitivity, selectivity and versatility. This type of detection is especially useful for reliable identification of flavonoids in complex matrices. The use of HPLC-MS obtains simultaneously information regarding not only the type, the amount and the RT of the particular compounds, but also their molecular weight and fragmentation pathways [61].

4.Conclusions

Advanced methods have been proposed for the quality control of these species in order to obtain specificity, sensitivity, and straight forward operation, reducing the analysis time. Often, powerful analytical techniques such as HPLC-DAD-MS, UPLC-MS, HPLC–MS/MS, RP-UHPLC were employed to obtain a metabolic fingerprint.

This classification allows to select the most suitable analytical technique on the basis of the chemical composition of the medicinal plant.

All the methods proposed for the determination of selected flavonoids and their metabolites appear to be the latest trends in bioanalysis, aiming at selecting a method capable of extracting and analyzing the highest possible number of samples and different flavonoids at one time, with the additional purpose of shortening sample preparation and analysis time.

The analytical strategies here reported can be used during the standardization process with the aim to assure quality, safety and efficacy to herbal products.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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