SEVERAL IMPORTANT MEDICINAL PLANTS, COMPARATIVE ANALYSIS OF BIOACTIVE COMPOUNDS OF TOTAL PHENOLIC AND FLAVONOIDS CONTENT

Oksana Sytar
Taras Shevchenko National university of Kyiv, Ukraine
Slovak university of agriculture in Nitra

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Contents

Summary

This chapter presents scientific data analyis and results of the author's own scientific research on some important medicinal plants, comparative analysis of bioactive compounds of total phenolic and flavonoids content. Based on the prescreenig experiments regarding plants with possible high content of phenolics and flavonoids were chosen to describe *Stachys bythanthyna (Lamiaceae), Calendula officinalis* L. (*Asteraceae*), *Fagopyrum sp. (Polygonaceae*), *Rosa canina* and *Rosa rubiginosa (Rosaceae)*. The current results indicate that profiles of phenolic acids are valuable specific chemical markers and can be authenticity indicators for plant-based preparations involving species. For example, the significant presence of syringic acid in the leaves of representative's family *Asteraceae* which is higher than 50% of total amount of identified phenolic acids can be used as a species-specific biochemical marker. It is described that peonidin 3-O-glucoside is the main anthocyanin in the leaves and stems of buckwheat seedlings, which provides a pink color of the vegetative organs in plant buckwheat seedlings. The results are new and can have a practical application, as the knowledge of the content of the major phenolic acids in known herbs in the current study makes it possible to determine the optimal management practice of leaf harvest of selected herbs to obtain more top-quality raw material.

1. General Introduction

Changes in lifestyle and the increase of chronic diseases, increased affluence and urbanization are linked also to a lifestyle in which our daily routine requires less physical activity and there is greater access to foods with higher energy densities. We now have a real challenge to balance energy intake and expenditure as more of us are becoming overweight. In 2008, across the 27 countries of the EU, 59% of adult men and 48 % of adult women were either overweight or obese. The problem of overweight and obesity is main challenge to chronic disease prevention and health across the life course around the world (Hruby and Hu, 2015).

For much of its history, nutrition science has focused on the role of essential nutrients in preventing deficiencies. However, there is now a need to ensure unaltered levels of key nutrients or functional components in the context of declining energy expenditures.

Further, the prevalence of chronic non-communicable diseases such as CVD, high blood pressure and both Types I and II of diabetes has increased, leading to concerns about escalating costs of healthcare and reduced quality of life. Modern lifestyles and longer life are also linked to various mental health problems such as depression, poor concentration, and loss of memory (Velten et al., 2014).

Awareness of links between food and health increased interest in the relationship between diet, health and well-being has grown substantially in the developed and developing countries of the world. Most of us know about the importance of a healthy lifestyle, including diet and its role in reducing risk of our illness and disease. Alongside growing affluence across the world, this knowledge has allowed easier access to a safer, more varied diet, all of which should ensure longevity.

Nowadays there is considerable evidence from data suggesting benefits of intake of phenolic nature compounds through plant foods against chronic diseases, including cardiovascular disease, neurodegeneration, and cancer (Del Rio et al., 2013; Karakaya et al., 2019, 2020, Gabr et al., 2019, Salehi et al., 2020). In the scientific literature studies reflect great interest in the potential health benefits of dietary plant polyphenols as antioxidant agents (Pandey & Rizvi, 2009; Siddique et al., 2010). From pharmacological and therapeutic points of view, the antioxidant properties of polyphenols, such as free radical scavenging and inhibition of lipid peroxidation, are the most crucial.

At the same time, phenolic compounds, and plant phenolic extracts can be used as natural antioxidants in the prevention of lipid oxidation in meat and seafood products due to delaying oxidation in meat muscle fish muscle, fish oil, and fish oil ‐ in ‐ water emulsions (Maqsood et al., 2014, Kalogianni et al., 2020). The natural phenolic compounds and antimicrobial antioxidant substances can be tested for the creation of novel commercial food additives that will simplify production of high-quality meat and fish products ensurung their safety. In future, natural phenolic compounds are expected to constitute to an innovative tool integrated into food production systems for meat and fish industry to satisfy the ever-increasing demands for natural, safe, and healthy food of good quality as well.

We observe nowadays the use of natural plant compounds in many ways to meet human needs. But due their large presence in the plants and also plant biodiversity, there needs to be a detailed analysis of the existing data of some important medicinal plants, with comparative analysis of bioactive compounds of total content of phenolic and flavonoids.

2. Plant Phenolics Classification

Polyphenols are naturally present secondary metabolites in plants which are known to be involved in defense reactions of plants against ultraviolet radiation or actions of bacterial pathogens (e.g., *Staphylococcus areus, Pseudomonas aeroginosa, Bacillus cereus and Esherishia coli*) (Beckman, 2000; Ghasemi et al., 2011; Haq et al., 2011). More than 8,000 polyphenolic compounds have been identified in various plant species. All plant phenolic compounds arise from a common intermediate, phenylalanine, or a close precursor-shikimic acid. Primarily they occur in conjugated forms, with one or more sugar residues linked to hydroxyl groups, although direct linkages of the sugar (polysaccharide or monosaccharide) to an aromatic carbon also exist. Association with other compounds, like carboxylic and organic acids, amines, lipids and linkage with other phenol is also common (Kondratyuk et al., 2004).

Several classes of phenols have been categorized based on the following of their components (Harborne, 1980; Aoki et al., 2000; Hättenschwiler and Vitousek, 2000; Iwashina, 2000; Lattanzio and Ruggiero, 2003):

C6 (simple phenol, benzoquinones),
C6-C1 (phenolic acid),
C6-C2 (acetophenone, phenylacetic acid),
C6-C3 (hydroxycinnamic acids, coumarins, phenylpropanes, chromones),
C6-C4 (naphthoquinones),
C6-C1-C6 (xanthones),
C6-C2-C6 (stilbenes, anthraquinones),
C6-C3-C6 (flavonoids, isoflavonoids), (C6-C3)2 (lignans, neolignans),
(C6-C3-C6)2 (bioflavonoids),
(C6-C3)n (lignins),
(C6)n (catechol melanins),
(C6-C3-C6)n (condensed tannins)

King and Young (1999) selected 3 most important groups of dietary phenols: flavonoids, phenolic acids, and polyphenols.

Several classes of phenols can be distinguished according to the number of phenol rings and to the structural elements that join these rings (Stalikas, 2007). The structural features of 3 typical and most important groups of phenols (flavonoids, phenolic acids, and polyphenols) are based on the presence of benzoic and phenolic functional groups on a core monocyclic carbon skeleton. Despite different source and various biosynthetic origins of phenolic compounds, many of these molecules have been shown in experimental studies to have similar biological functions.

Flavonoids are the largest group of plant phenols that are most studied. Flavonoids are secondary plant metabolites belonging to the phenylpropanoid group of compounds. The basic flavonoid skeleton consists of two aromatic rings joined by a three-carbon bridge. Two separate biosynthetic pathways contribute to the formation of this skeletal structure; the three-carbon bridge and one aromatic ring are derived from the shikimic acid pathway via phenylalanine, and the other aromatic ring comes from the condensation of three acetate units produced in the malonate pathway.

Phenolic acids form a diverse group that includes the widely distributed and checked hydroxybenzoic and hydroxycinnamic acids. Two main groups of polyphenols, termed flavonoids, and non-flavonoid have been adopted in the literature (De la Rosa et al., 2010). The flavonoid group, including flavanones, flavones, dihydroflavonols, flavonols, flavan-3-ols, isoflavones, anthocyanidins, proanthocyanidins and chalcones, comprises those compounds with a C6-C3-C6 structure.

Phenolic compounds are potential antioxidants because there is a relation between antioxidant activity and presence of phenols in common vegetables and fruits (Cai et al., 2004; Fu et al., 2011). A positive linear correlation between antioxidant capacities and total phenolic contents implied that phenolic compounds in 50 tested medicinal plants could be the main components contributing to the observed activities. The results showed that *Geranium wilfordii, Loranthus parasiticus, Polygonum aviculare, Pyrrosia sheaeri, Sinomenium acutum* and *Tripterygium wilfordii* possess the highest antioxidant capacities and total phenolic content among 50 tested plants and could be rich potential sources of natural antioxidants (Gan et al., 2010).

Most qualitative and quantitative analyses of phenolics are usually traditional methods such as HPLC-UV for qualitative analysis and Folin-Ciocalteu method, which is the only procedure used to measure total phenolics through the reduction capacity of the components of the extract. Such comparative analysis of biological active compounds and antioxidant activity in known east central Europe medicinal and industrial crop plants of 26 species of families *Asteraceae, Rosaceae and Lamiaceae* are found to have the highest total phenolic, total flavonoid contents and antioxidant activity has been seen in *Stachys byzantine L.* (*Lamiaceae*), *Calendula officinalis L.* (*Asteraceae*) and for *Potentilla recta L.* (*Rosaceae*) (Sytar et al., 2018). Among the studied edible food plants high total phenolics content and antioxidant potential have been seen in buckwheat plants (*Polygonaceae*) (Sytar et al., 2015), *Rosa canina* L. *Rosa rubiginosa* L. (*Rosaceae*). A detailed analysis of these medicinal herbs regarding presence of flavonoids and phenolics together with their use as herbal tea and food will be presented in present review.

3. *Stachys* sp. (*Lamiaceae*) - Biochemical Characteristics and Possible Use

Stachys genus is one of the largest genera of the Labiatae (*Lamiaceae*) family and is widely distributed across Europe and East Asia, as well as in America. The origin of species name is Greek which means “an ear of grain” referring to the inflorescence spike found in many representatives. Many *Stachys* species are used in decoctions or infusions for the treatment of asthma, skin diseases, stomach, rheumatic disorders, ulcer and vaginal tumors (Gören et al., 2011a, 2011b). Some members of genus have been seen to be used as anti-inflammatory and antibacterial agents (Gören, 2014). In Iran, S. byzantina (known in Farsi as "lamb's ear" or "lamb's tongue" or "sonbolehe noghrehi" or "zabanehe bare") are applied as traditional therapeutic agents in various conditions (Aminfar et al., 2019).

In the author's experimental work among investigated methanolic extracts of leaves of representative family *Lamiaceae Stachys byzantine* K. Koch leaves have been found to have the highest total phenolics, total flavonoid contents (11.1±0.003 mg QE mg^{-1}DW) and antioxidant activity (94.56±0.35) (Sytar et al., 2018).

	mg g^{-1}DW
4-Hydroxybenzoic acid	0.006 ± 0.002
Vanillic acid	0.113 ± 0.037
Chlorogenic acid	0.002 ± 0.000
Syringic acid	2.368 ± 0.311
o-Coumaric acid	0.013 ± 0.004
p- Coumaric acid	0.006 ± 0.001
ferulic acid	0.030 ± 0.006
p-anisic acid	0.053 ± 0.004
salicylic acid	0.168 ± 0.023
cinnamic acid	0.045 ± 0.004
methoxycinnamic acid	0.056 ± 0.025
Total phenolics	18.64±0.699

Table 1. Phenolic acids and their amounts in methanolic extract of *Stachys byzantine* K. Koch (mg g^{-1}DW).

Estimates of phenolic acids in methanolic extract of *Stachys byzantina* revealed that the highest percentage of syringic acid in the studied leaves extracts is about 81.88%. On the second place in leaf methanolic extract was salicylic acid - 5.81%. Third place with 3.91% was identified for vanillic acid. Other identified phenolic acids were in lower range from 0.21% to 1.93%.

Previously, syringic acid was identified in several plants including *Ardisia elliptica* (*Primulaceae*) and *Schumannianthus dichotomus* (*Marantaceae*) (Rob et al., 2020). Syringic acid is one of the intermediate products of malvidin (O-methylated anthocyanidin). Malvidin is a plant pigment responsible for the color of red wine (Malaj et al., 2013). Syringic acid is also found in red wine and vinegar (Gálvez et al., 1994). Therapeutic activity of syringic acid is attributed to the presence of methoxy groups onto the aromatic ring at positions 3 and 5. Syringic acid is able to modulate the changes of some biological targets such as transcriptional and growth factors, proteins, signaling molecules involved in progression of different diseases (cerebral ischemia, diabetes, CVDs, cancer, neuro and liver damage). At the same time, available data shows that syringic acid has greater industrial applications including photocatalytic ozonation and bioremediation (Wang et al., 2011; Srinivasulu et al., 2018). The experimental data has revealed that, presence of syringic acid in *Quercus infectoria* plant extract is responsible for its anesthetic and sedative activity (Dar and Ikram, 1979).

The ethyl acetate, methanol, and water extracts of *S. byzantina* contained considerable number of flavonoid compounds (31.99, 47.70, and 34.66 mg REs/g extract, respectively). The inhibitory activity on acetylcholineserase, butrylcholinesterase, α-amylase is shown by extracts of *S. byzantine* (Sarikurkcu et al., 2016).

The most important and predominant phenolic compound in the investigated samples of seven Croatian Stachys taxa (*S. alpina, S. officinalis, S. palustris, S. recta subsp. recta, S. recta subsp. subcrenata, S. salviifolia, and S. sylvatica*) was found to be the chlorogenic acid. In samples of *S. recta subsp. recta* and *S. sylvatica*, the amounts of isoquercitrin, luteolin-7-O-glucoside, and quercitrin were estimated as well. Rutin was determined in samples of *S. recta* subsp. recta, *S. recta* subsp. *subcrenata, S. sylvatica*, and *S. salviifolia*. Interestingly *Stachys recta* subsp. *recta* didn’t contain chlorogenic acid but is characterized by the richest flavonoids content and composition (Bilušic Vundac et al., 2005, 2007)

Most of genera of the *Lamiaceae* among which is also *Stachys byzantine* K. Koch are rich sources of terpenoids and they also contain a considerable amount of various iridoid glycosides, flavonoids, and phenolic acids such as rosmarinic acid and other phenolic compounds (Naghibi et al., 2005). Main component groups found in the essential oil from aerial parts of *Stachys* species are monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. β-Caryophyllene, germacrene D, α-pinene, β-pinene, α- cadinene, spathulenol were major terpenes identified essential oil from aerial parts of *Stachys* species (Bilušić Vundać et al., 2019).

Furthermore, from the aerial parts of *S. byzantina* three phenylethanoid glycosides, including verbascoside, 2′-O-arabinosyl verbascoside and aeschynanthoside C were reported (Aminfar et al., 2019).

According to ethnobotanical studies and literature survey, a similar pattern of consumption of *Stachys* species throughout Europe to the East Asia has been documented. From Europe to Iran, it is generally consumed as herbal tea due to its volatile components and phenolic species. Similarly, this type of consumption of the species is observed in South America. For example, in Peru, decoctions of the aerial parts of Stachys sp. LAM have been consumed as traditional medicine for thousands of years (Monigatti et al., 2013). This is a very interesting example of similar behaviors of people living in different geographic regions utilizing an opportunity offered by the nature. However, it is generally consumed as a rich carbohydrate source in East Asia for their tich carbohydrate content and they are called *Chinese artichoke*.

In Poland, *Stachys palustris* is used as a food source because of the presence of large number of edible tubers which are used as soup and vodka additives, in salads and raw snacks (Luczaj et al., 2011). Similar consumptions were also observed in Sweden, Ukraine and Great Britain. During food shortages in Europe the dried powder of the Stachys species was used as an additive for bread (Luczaj et al., 2011). The species in China, *Stachys geobombycis*, known as DongChongXiaCao, has been used for medical applications and as tonic for thousands of years. Interestingly, this species is also used in Europe, China and Japan for similar properties.

The herbal preparations of *Stachys* spp. are widely consumed in folk medicine to treat a broad array of disorders and diseases, including stress, skin inflammations, stomach disorders and genital tumors (Karioti et al., 2010). Specially, the herbal teas of these plants, known as “mountain tea”, are used for skin and stomach disorders (Öztürk et al., 2009, Delazar et al., 2011).

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Biographical Sketch

Dr. Oksana Sytar is writing habilitation work at the Department of Plant Biology, Institute Biology and Medicine, Kiev national university of Taras Shevchenko, Ukraine. The development of research interests of Dr. Sytar is based on few international postdoctoral stays (India, Slovakia, Germany, France) in the different countries in the world and developed international activities. Dr. Oksana Sytar scientific interest is mainly focused on the biochemistry of secondary metabolites and metabolomics which are important tools in many disciplines, including research on plant resources for food and pharmaceutical use. Special interests are developing methods for obtaining and screening plant secondary metabolites (nathtodianthrones, phenolic acids, catechins, anthocyanins, sulpholipids, differ alliins), which are characterized by the health-promoting properties and can be used as functional food components or nutraceuticals. The main topic of research activity is biodiversity of useful plants which are a crucial player in the emerging field of functional food and nutrition industry and see themselves as especially dedicated to improving the quality of life as well as to ensuring a variability of products with high quality for the international food and drugs market what based on plants biodiversity. The scientific activity is documented by more than 90 research or conference papers, and 8 book chapters.