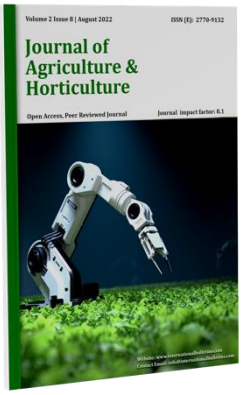

ANALYSIS OF VARIOUS BIOACTIVE SUBSTANCES IN THE PLANT SALVIA OFFICINALIS

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Abstract

Salvia officinalis L., also known as the “Salvation Plant”, has been long used and well-documented in traditional medicine around the globe. Its bioactive compounds, and especially its polyphenol profile, have been extensively researched and reviewed. However, sage’s beneficial effects reach much further, and nowadays, with a range of new extraction techniques, we are discovering new components with new therapeutic effects, especially in the context of neurodegenerative diseases and various carcinomas. This review describes the bioactive profile of various sage preparations depending on the extraction techniques and extraction parameters, and this review lists the newest research findings on its health effects.

Introduction Salvia officinalis L. is a plant in the mint family Lamiaceae, subfamily Nepetoideae, tribe Mentheae, and genus Salvia [1]. Salvia is the largest genus of the Lamiaceae family, containing around 1000 species [2], and can be found in Europe around the Mediterranean, in Southeast Asia, and Central and South America [3]. S. officinalis grows in the form of an outcrossing, perennial subshrub up to 60 cm high. The leaves are opposite and simple with white hairs on the lower leaf surface and greenish or greenish-grey on the upper surface. Stems are erect or procumbent with abundant hairy dark green branches. Leaves are elongated and petiolate with a serrate margin, rugose surface, and sometimes with basal lobes. The flowers are 2–4 mm long from the pedicel, and they are in pseudoverticillasters with 5–10 violet-blue color flowers that form spurious, composed spikes. They bloom from March to July depending on habitat and climatic conditions [4,5]. Historically, sage is known as the “Salvation Plant”, originating from the old Latin word “salvarem”, which means save or cure. It has been used to reduce perspiration, as a gargle for sore throat, to improve regularity of a menstrual cycle and to reduce hot flashes in menopause, to fight gastroenteritis and other infections, to improve lipid status and liver function in general, to improve appetite and digestion, and to improve mental capacity [6]. Recently, focus has been put on the link between specific bioactive compounds in sage and specific health effects. Additionally, more effort Plants 2019, 8, 55; doi:10.3390/plants8030055 www.mdpi.com/journal/plants Plants 2019, 8, 55 2 of 30 has been put in to determine the best extraction method, conditions, and parts of a plant to be used in order to get the most effective preparation. This is the first review to encompass the extraction techniques, composition, and health effects of various sage preparations. Previous review articles covered the topic of pharmacological properties

and components [7,8], biochemical studies [9], medical properties, and genetic diversity of Dalmatian sage [10], as well as chemistry, pharmacology and medicinal properties for the prevention and treatment of various health conditions [11], chemistry and antioxidative factors in sage [12], and polyphenolics of Salvia [13].

Methodology of Review

Hereby, we used scientific databases, such as PubMed, ScienceDirect, Scopus, ResearchGate, and Google Scholar, with emphasis on ScienceDirect and Scopus. Review of the literature was carried out using the keywords “Sage”, “Salvia officinalis”, and “extraction”. According to these key words we obtained 511 references in ScienceDirect and 101 references in Scopus during February and March 2017. During the search of literature and writing of the review, the time period in which the papers were published had not been selected, since the focus was on significant works selected for the areas covered in this review. All data were analyzed in corresponding articles.

Production of Sage Extracts

Today, a number of various techniques are used for the production of various sage products. The techniques are chosen depending on the desired profile of sage’s bioactive compounds in an extract.

1. Hydrodistillation There are several processes for obtaining sage products mentioned in the scientific literature. Most of the research involving this plant is focused on the production of essential oil and its chemical composition. The most commonly used methods are conventional processes including hydrodistillation, using a Clevenger-type apparatus for 30 min [14], 1 h [14], 2 h [14–19], 3 h [20–26] or 4 h [27–31], the modified Clevenger apparatus for 2 h [32,33], and an Unger-type apparatus for 3 h [34]. The hydrodistillation apparatus can be placed inside a microwave oven to obtain essential oil without any addition of solvents, including water, as described by Koubaa et al. [35]. Hydrodistillation was performed using a microwave power of 500 W and a temperature of 100 ◦C for 30 min.

2. Soxhlet Extraction Certain sage extracts were obtained by Soxhlet extraction with different solvents, as well as the time of the extraction [29,36,37]. In Farhat et al. [36], methanol was used as the extraction solvent, and the extraction was performed for 2 h, while in the case of Kontogianni et al. [37], two solvents of different polarity, hexane and ethyl acetate, were used for an extraction that lasted for 6 h. Soxhlet extraction of sage leaves was also conducted by using mixture of ethanol and water (70:30 v/v) for 4 h [29].

3. Infusion Infusion of sage leaves, or so-called production of sage tea, is a very popular preparation in folk medicine. This process is very simple, but is conducted quite differently in the research. According to Radulescu, Chiliment, and Oprea [38], 100 mL of boiling water are poured over 5 g of leaves of Salvia officinalis L. and filtered after 30 min. In Martins et al. [39], 200 mL of boiling water was poured over 1 g of sample, left for 5 min, and then filtered under reduced pressure. In Zimmermann et al. [40], 150 mL of boiling water was poured over 1.5 g of sage leaves or tea bags from 16 different brands, steeped for 15 min, and then a filtered 1 mL sample was used for further analysis.

Solid–Liquid Extraction

In addition to hydrodistillation, a significant number of studies of Salvia officinalis L. were conducted on the products obtained by solid–liquid extraction by using different solvents and comparing both classical and innovative extraction techniques.

Dent et al. [41] used three different aqueous solutions of ethanol (30%, 50%, or 70%), acetone (30%, 50%, or 70%) and distilled water to extract polyphenols, which were determined by the Folin-Ciocalteu method and the HPLC UV/PDA (High-Performance Liquid Chromatography Ultraviolet/photodiode array) method. In addition to various solvents, the extraction took place at different temperatures (60 and 90 ◦C) for different times (30, 60, and 90 min) to show whether these parameters influenced the amount of total and individual polyphenols. The results showed that extraction with an aqueous solution of ethanol or acetone (30%) at 60 ◦C for 30 min was the most effective method for extracting polyphenols from dried sage leaves.

The influence of temperature, extraction time, solvent composition, sage particle size, and solvent-to-sage ratio was examined by Durling et al. [42]. The authors studied the efficacy of extraction of carnosic and rosemary acid as well as the yield and composition of sage essential oil. The optimum conditions for the highest yield of carnosic compounds, rosemary acid, and essential oil, which were 10.6%, 6.9%, and 7.3% respectively, were a 1 mm sample size of dried sage, 55–75% ethanol, and a solvent-to-sage ratio of 6:1 at 40 ◦C for 3 h. It is well-known that diffusion of the solvent is better when the plant material is ground to the smallest particle size, but grinding can cause dust generation and heat production, which may affect the composition of the plant material itself. The increase in temperature increases yield, but at a certain temperature, in this case up to 40 ◦C, it can cause a reduction in the yield and the evaporation of the volatile components. By increasing the time of extraction, yields and total polyphenols did not increase, i.e., they were constant, but the yield of carnosic components, rosemary acid, and essential oil increased, with the conclusion that other components were unstable for a longer duration of extraction. Therefore, the authors recommended the duration of the extraction for no longer than 3 h. As the extraction of the bioactive components was limited by the solubility in the solvent mixture used, it is important to find a suitable solvent ratio and solvent-to-sage ratio to reduce the cost of solvents and the energy associated with solvent evaporation. Duleti´c-Lauševi´c et al. [43] extracted the sage material with dichloromethane (DCM), chloroform, ethyl acetate, and ethanol for 24 h at 30 ◦C before and after the ultrasound treatment for 1 h. According to their results, yield was higher in plants that originated from Serbia (2.50%) than from those in Montenegro (2.03%), and yield was higher in plants that were extracted with dichloromethane (3.23%). Harvest season did not influence the yields. The content of total polyphenols and flavonoids depended on the extraction solvent and harvest season, and was higher in those plants originally from Serbia and harvested in the summer. When ethanol was used as a solvent it exhibited the highest influence on content of polyphenols, unlike ethyl acetate, which had the highest influence on content of flavonoids. Extracts of sage harvested in the summer and extracted with ethanol showed a better antioxidant activity, proving correlation between total polyphenols and antioxidant activity, which confirms the fact that polyphenols have more effect on antioxidant activity than flavonoids.

In the research by Roby et al. [44], sage extracts were prepared with solvents of different polarity (methanol, ethanol, diethyl ether, and hexane) with shaking at room temperature for 72 h. The yield differed depending on the solvent used, and the highest yield of 23.41% ± 2.65% was achieved with methanol, whereas the lowest yield (4.63% ± 1.73%) was obtained with hexane. Likewise, the highest number of total polyphenols was found in the extracts with methanol and ethanol (5.95% ± 2.65%; 5.80% ± 1.00%), while the lowest

amount was found in hexane samples (4.25% ± 1.00%). These results were expected since the polar solvents are more effective in extracting phenolic components than less polar solvents.

Ultrasound-Assisted Extraction (UAE)

Application of UAE is expanding, so Sališová, Toma, and Mason [45] compared conventional and ultrasound assisted extraction in the content of active components including cineole, thujone, and borneol using 65% ethanol as solvent. They studied the effect of temperature, stirring, and ultrasound (ultrasonic bath or horn system) for 12 h, and the concentration of the components was measured not only during this 12 h period, but also after 7 days. The results showed that ultrasonic extraction for 12 h at room temperature with stirring had better results compared with convectional techniques. Stirring is an important factor since it was observed that almost the same results were obtained at 30 ◦C without stirring and at a temperature of 20 ◦C with stirring. Even better results were obtained with an ultrasound horn, where a 2 h extraction resulted in the same amount of bioactive components as a 12 h extraction, but the main disadvantage of this method is the inability to control the temperature. Veli˘ckovi´c et al. [46] examined the effect of ultrasound and classical maceration on the extraction yield and composition by selection of suitable solvent. UAE was performed on an ultrasonic bath for 20 min and 40 ◦C, while maceration was carried out for a period of 6 h at 20 ◦C, with petroleum ether, 70% ethanol, and water as a solvent. Among these solvents, a 70% solution of ethanol appeared to be the most appropriate because the yield had the largest number of typical components. As far as the yields were concerned, a dependence on the solvent polarity was observed, where the yields increased with the solvent polarity. Therefore, the greatest yield was in the case of water and ethanol, respectively.

Sage and Health Benefits

Along with some of the traditional uses of sage mentioned in the introduction [6], many recent studies report on anti-inflammatory and antinociceptive effects related to pain relief, antioxidant and antidementia effects related to Alzheimer's disease, antimicrobial effects related to various infections including worm infestations and gastroenteritis, anticancer and antimutagenic effects related to various cancers such as colon or breast cancer, and very important hypoglycemic and hypolipidemic effects related to metabolic diseases such as non-alcoholic fatty liver or diabetes [8,11,87]. However, the main obstacle when assessing the relevance of reported results remains the variable extracts used (e.g., tea, essential oils, ethanolic extracts, etc.), with different compositions of bioactive compounds. For example, the anti-inflammatory effect of the methanolic extract is associated with a higher content of polar components such as rosmarinic, ursolic, caffeic, and oleanolic acids [88]. In the chloroform extract, ursolic and oleanolic acid are present in the highest amounts, and they have been proven to have the best dose-dependent topical anti-inflammatory activity [89].

A lot of attention has been put on specific components found in different sage extracts that are being analyzed, mostly in vitro or in animal studies. For example, Juhás et al. [90] found that borneol, one of the key ingredients in sage essential oil, significantly suppresses pro-inflammatory cytokine mRNA expression characteristic of colonic inflammation. Carnosic acid, from the methanolic sage leaf extract, especially at a dose of 20 mg kg−1 , significantly inhibited triglyceride elevation, reduced body weight gain, and inhibited activity against pancreatic lipase [91].

According to Ghorbania and Esmaeilizadeh [8], confirmed clinical pharmacological effects of sage on humans so far include improvement of memory and cognitive functions,

pain relief, especially for sore throat, and significant improvement in blood glucose (including HbA1c and post-prandial glucose) and lipid profile (especially an increase of high-density lipoprotein, HDL). Especially interesting are the beneficial effects on memory and cognitive functions. Every year about 8 million people are newly diagnosed with dementia, 60%–80% of all dementia is Alzheimer's, and the highest risk for developing any dementia is among the elderly. The world's population is getting older, so the burden of Alzheimer's and other progressive, incurable neurodegenerative diseases is a major public health issue. Finding a way to prevent or cope with the degenerative nature of the disease is the best way we can determine striking predictions for the near future. Traditionally, sage has been used to improve memory and reduce age-related cognitive decline. Besides well-documented antioxidant effects, major components in sage have shown to decrease the inflammation resulting from the neurotoxic effects of accumulated amyloid-β peptide, which is a characteristic of Alzheimer's disease [87]. Even the sage aroma shows a positive effect on memory [92].

Metabolic improvements in terms of glucose and lipid profile are interesting for the fact that alterations in these parameters are directly related to diabetes, obesity, non-alcoholic liver disease, metabolic syndrome, and cardiovascular diseases, i.e., diseases with the highest mortality and morbidity rates with immense individual and societal burdens. Hernandez-Saavedra et al. [93] observed the effect of sage infusion on obesity-related metabolic alterations in rats during a 12-week period. Significant reductions in the total cholesterol, triglycerides, low-density lipoprotein, and C-reactive protein was found, along with a decrease in body weight and abdominal fat mass [93]. Several other studies illustrated various positive metabolic changes in animal studies [94,95]. A non-randomized crossover trial on six women who consumed 600 mL of sage infusion per day during 4 weeks, followed by 2 weeks of a wash-out period, found no effect on plasma glucose, but the level of LDL and the total cholesterol lowered, while HDL increased [96]. Importantly, no adverse hepatotoxic effects were observed. Colorectal cancer draws a lot of scientific interest because of its strikingly high correlation with the diet. Sage infusion has been found to prevent colorectal cancer in rats [97], but also has cytotoxic effects on cancer cell lines [11,98] and diminishes the negative effects of radiotherapy used for the cancer treatment [99].

Conclusion

Sage has been long used and has well-documented benefits for various health conditions, but continues to elicit interest by researchers around the globe. The most commonly used and tested is sage essential oil, but recent studies, especially in the field of oncology and neurodegenerative diseases, show that other sage products offer a huge potential. New extraction techniques, such as UAE and MAE or SC-CO2 extraction, allow us to determine new compounds in sage extracts that are unable to get by hydrodistillation or infusion. By optimization of the extraction techniques, i.e., extraction parameters, we are able to get the desired composition with the highest activity for a specific purpose. As in all other cases when the processing material is a plant, harvesting conditions, geographical area, and the plant itself affect the yield and the final bioactive composition of the extract. We are yet to evidence the number of extracts and their benefits, especially for the treatments of cancers and neurological diseases.

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