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Facets of the elaboration of the *Salvia sclarea* L. extracts

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In this article we presented some facets of the development of liquid ethanolic extracts of *Salvia sclarea* L. The primary purpose was to elaborate the analytical procedure of the quantitative determination of the total flavonoid content with the establishing dominating group of flavonoids in the extracts of *Salvia sclarea* by colorimetric aluminum chloride method. The second purpose of the work was to study the influence of pharmaceutical factors like temperature of extraction, particle size of the herbal raw material and ultrasound on the extraction of flavonoids. It was studied that the higher temperatures and ultrasound induced higher extraction of flavonoids from the herb of *Salvia sclarea*. The TFC was in the range of 6.4 to 14.7 mg rutin-equivalents per one gram of the herb and depended on temperature of extraction, particle size of herbal raw material and ultrasound on the extraction of flavonoids. Rutin was chosen as a commercially available marker. The calibration curve was plotted in the concentration range of 10 to 102 mg.L^{-1} of rutin. The wavelength of maximum absorption of four extracts was in the range of 390–391 nm that indicated that flavons were dominating group of flavonoids in these extracts. These studies can be basis for the development of extracts of *Salvia sclarea* for pharmaceutical and cosmetic industries.

Keywords: *Salvia sclarea*, ethanolic extracts, total flavonoid content

Introduction

Currently, the role of medicinal plants as a source of herbal medicinal preparations has become a topic of global importance (Zengin et al., 2018, Kivrak et al., 2019). Plants from the Lamiaceae family containing terpenes and polyphenols have interesting biological activities (Valant-Vetschera et al., 2003; Tavakkoli et al., 2014, Kivrak et al., 2019; Yezerska et al., 2021). This family consists of about 200 genera and from 3200 to 6500 species and is known as a family of aromatic plants (Valant-Vetschera et al., 2003; Schmiderer et al., 2008; Asadi et al., 2010; Hanganu et al., 2018;

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Hudz et al., 2021). Most genera of Lamiaceae are rich in terpenoids, iridoid glycosides and flavonoids (Valant-Vetschera et al., 2003; Asadi et al., 2010; Aćimović et al., 2018; Zengin et al., 2018; Yezerska et al., 2021). The characteristic feature of plants from the Lamiaceae family is good antioxidant capacity, which is connected to many medicinal properties. Among these activities are anti-inflammatory, antidiabetic, antiviral and antitumor ones (Asadi et al., 2010; González-Chávez et al., 2017). It was stated that the dichlorometane extract of *Salvia connivens* had an anti-inflammatory effect as it reduced the levels of the proinflammatory cytokines IL-1b, Il-6 and TNF-a and increased the level of the antiinflammatory cytokine IL-10 in the culture medium of macrophages stimulated with lipopolysaccharides (González-Chávez et al., 2017).

Clary sage (*Salvia sclarea* L.) belongs to the most popular species in the genus *Salvia*, which includes more than 900 species (Kintzios, 2000; Valant-Vetschera et al., 2003; Asadi et al., 2010; Šućur et al., 2016b; González-Chávez et al., 2017; Zengin et al., 2018; Kivrak et al., 2019; Afonso et al., 2021; Svydenko et al., 2022). *Salvia sclarea* is an aromatic biennial or perennial plant, 20 to 120 cm tall with a thick square stem (Angelova et al., 2016). It comes from Southern Europe, but it is bred all over the world for the health-promoting properties of the essential oil and as an ornamental plant often used in perfumery (Schmiderer et al., 2008; Goncerariuc et al., 2016; Angelova et al., 2016; Aćimović et al., 2018). The essential oil is obtained from the fresh parts of the sage which is in full flowering stage. The content of essential oil in fresh inflorescences ranges from 0.15 to 0.20% (Kuzma et al., 2009; Angelova et al., 2016).

Clary sage is well known for its high value essential oil, widely used in perfumery. This oil possesses high biological activity and it can be used for the treatment of patients with stress, tension, depression, insomnia (Kamatou et al., 2008; Yaseen et al., 2014, Hao et al., 2015). Linalool (18%), linalyl acetate (63%) and sclareol (6%) are among the major components of the essential oil from leaves and inflorescences of *Salvia sclarea* (Schmiderer et al., 2008). The chemical composition of essential oil significantly depends on the part of the plant (leaf, corola, or calyx) and type of glandular trichomes (Schmiderer et al., 2008).

The aqueous extract of *Salvia sclarea* exhibits allelopathic and insecticidal properties valued in agriculture because allelochemicals are attractive as new classes of herbicides (Šućur et al., 2015; Šućur et al., 2016a; Šućur et al., 2016b). Clary sage seeds are rich in fatty acids and have high levels of antioxidant and antiradical activities making them suitable for use as nutraceuticals (Aćimović et al., 2018). *S. sclarea* is used not only in medicine, but also as additive in the food industry, where the interest in this plant is constantly growing due to the search for healthy and natural food (Goncerariuc et al., 2016). This species is used widely as an ornamental and landscape plant (Çetinkale Demirkan and Akat, 2018).

Recent studies demonstrate analgesic, antimicrobial (Kuzma et al., 2009; Hristova et al., 2013), anti-anxiety (Gross et al., 2013; Yang et al., 2014), antidiabetic (Raafat and Habib, 2018; Afonso et al., 2021) and cytotoxic effects (Kuzma et al., 2009; Gulcin et al., 2004), insecticidal activity (Šućur et al., 2015) of essential oil and extracts of *S. sclarea*. The inhalation of clary oil may be useful for inducing relaxation in female urinary incontinence patients, as well as in menstrual and digestive problems (Szentmihalyi et al., 2009; Verma, 2010; Seol et al., 2013).

Polyphenols are secondary metabolites that are broadly spread in the plans. They are known for their antioxidative capacity as they neutralize free radicals which are responsible for cell damage. Phenolic acids are largely responsible for antioxidant properties of the extracts of *Salvia sclarea*. Caffeic acid, compared to other species of sage (*S. aetiopsis*, *S. austriaca*, *S. nutans*, *S. verticillatta*, *S. nemorosa*), is most abundant in *Salvia sclarea*. Apart from caffeic acid, HPLC analysis showed that compounds such as *p*-coumaric or ferulic acids can be isolated from clary sage. Rosmarinic acid was previously isolated from the methanolic extracts of some *Salvia* species leaves (*S. officinalis*, *Salvia glutinosa*, *Salvia aethiopis* and *S. sclarea*) (Bandoniene et al., 2005, Kostic et al., 2017).

Kharazian (2013) identificated flavonoids in leaves of seven wild growing *Salvia* species from Iran. Flavons were the most frequent flavonoid in seven *Salvia* species (35.7%) and dihydroflavonoles were in the least concentrations (5.3%). The highest flavonoid content was identified in *S. multicaulis* and *S. hydrangea*. It can be concluded that the flavonoid constituents seem to be a suitable indicator in chemotaxonomic studies in *Salvia* genus. Hanganu et al. identified such flavonoids like isoquercitrin (2208 $\mu g.g^{-1}$ of the plant dry weight), luteolin (780 $\mu g.g^{-1}$ the plant dry weight), luteolin (293 $\mu g.g^{-1}$ the plant dry weight) (Hanganu et al., 2018).

In general, there are a few studies directed at the approach of the elaboration of the standardized procedure of the determination of the total flavonoid content with the justification of choosing a marker for

the calculation the content of flavonoids, establishing the dominating group of flavonoids on the base of the absorption maximum and repeatability of results in different time of the reaction with aluminum chloride with the simultaneous study of the influence of pharmaceutical factors on the extraction of flavonoid (Hudz et al., 2017). Therefore, the goal of the work was to provide the justified approach to the elaboration of the standardized procedure of the determination of the total flavonoid content (TFC) for the fluid extract of *S. sclarea*.

Material and methodology

While carrying out this study, such methods were used: analysis, synthesis, systematization, and comparison for processing published scientific data; technological method (maceration); spectrophotometric method for the elaboration of the analytical procedure of the determination of the TFC by aluminum spectrophotometric method.

Plant material

The aerial parts of *Salvia sclarea* L. were collected at late flowering stage in August 2017 in the Sector of mobilization and saving herbal resources of the Rice Institute of the National Agrarian Academy of Sciences of Ukraine located in Plodove of Kherson region of Ukraine (latitude: 46° 39‘ 20.92“ N, longitude: 32° 37‘ 4.08“ E).

Reagents

The following reagents were used: ethanol 96% (manufacturer “Centrachem“ (Slovakia)), aluminium chloride (’Sigma Aldrich‘), and rutin hydrate (’Sigma Aldrich‘).

Extraction

The plant material was dried at room temperature, then crushed (to particle size 0.5–5.0 mm) and subjected to extraction by ethanol of different concentrations (65 and 70%). The crushed particles of *Salvia sclarea* were extracted with 65 and 70% ethanol. The characteristic of the extracts used in this study is provided in Table 1.

Total flavonoid content (TFC)

TFC was determined using the slightly modified analytical procedures of differential spectrometry provided by Hudz et al. (2017). This procedure is considered a main procedure for the measuring the TFC in herbal preparations and beekeeing products (Pękal and Pyrzynska, 2014; Hudz et al., 2017). Rutin trihydrate was used to build the calibration curve in the concentration range of 10 to 102 mg.L^{-1}. Rutin trihydrate dissolution in 50% ethanol was carried out with the aid of ultrasound. The results were expressed as rutin equivalents: mg eq-rutin.L^{-1} of an extract and mg eq-rutin.g^{-1} of the *S. sclarea* herb.

The TFC was calculated as follows. 10, 30, 50, 70 and 100 µL of the stock solution of rutin trihydrate (1016 mg.L^{-1}) were diluted with 50% ethanol up to 1.0 mL. The obtained dilutions of rutin trihydrate were mixed with 1.0 ml of 2% aluminum chloride hexahydrate in 50% ethanol. After the incubation at room temperature for 75 ±15 min the spectra of the reaction mixtures were measured in the range of 360 nm to 460 nm with the spectrophotometer «Photometry Hitachi U-2810». The volume of 2% aluminum chloride hexahydrate in 50% ethanol was substituted by the same volume of 50% ethanol in the blank for each dilution of rutin trihydrate. In a like manner, 50 or 100 µL of the developed extracts of the *S. sclarea* herb were mixed with 1.0 ml of aluminum chloride hexahydrate. The mixture was mixed by vortex and incubation was performed at room temperature for 75 ±15 minutes. The volume of 2% solution of aluminum chloride was substituted by the same amount of 50% ethanol in blank. The test was carried out for each extract in triplicate. The TFC was calculated

Table 1 Characteristics of the *Salvia sclarea* L. extracts

Identification number of extract	Particle size	Ratio of raw material to the solvent	Maceration time and conditions	Yield of an extract, ml
E-1	0.5–5.0 mm	5.0 g to 110 ml of 70% ethanol	200 min at ultrasound and a temperature of (40–46 °C) plus 21 hour of maceration at room temperature	89.5
E-2	0.5–5.0 mm	5.0 g to 108 ml of 65% ethanol	6 days at room temperature	82.5
E-3	0.5–5.0 mm	5.0 g to 108 ml of 65% ethanol	100 min at (36–41 °C) plus 6 days at room temperature	81.5
E-4	2.0–5.0 mm	5.05 g to 50 ml of 70% ethanol	7 days at room temperature	32.0

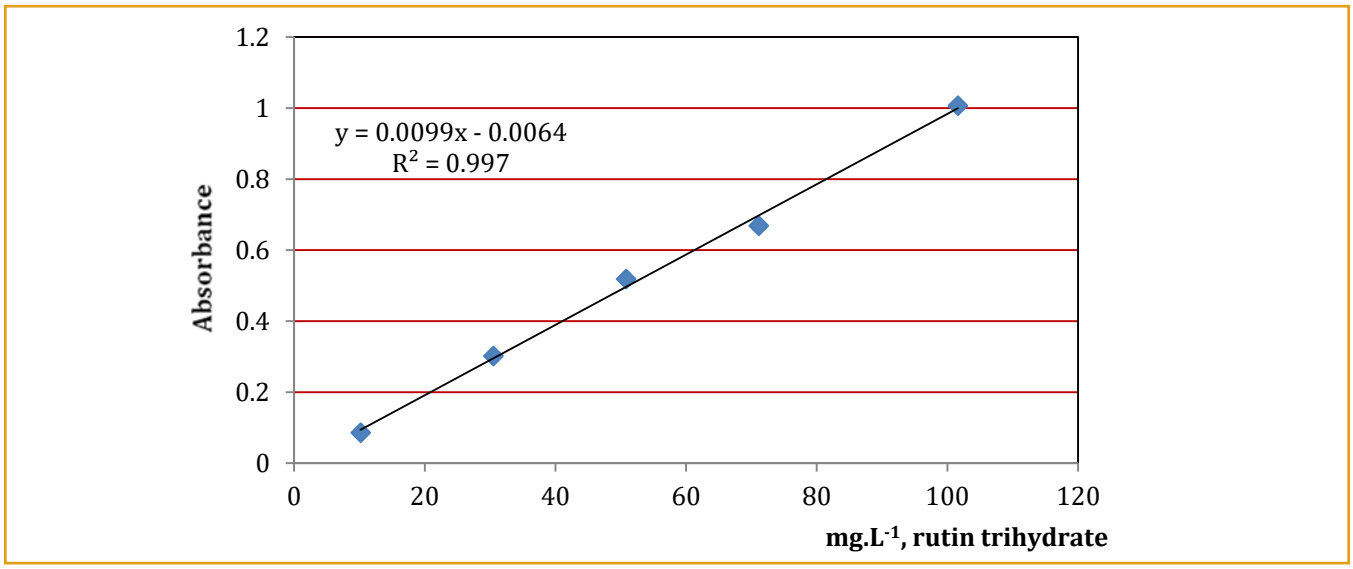

Figure 1 Calibration curve of rutin trihydrate

using expression C = c × 20(10) × k, where C is TFC of the tested extract, c is TPC taken from the calibration curve, 20 for 50 µL of an extract or 10 for 100 µL of an extract is coefficient of dilution of the extracts for their testing E-4, E-3, E-1 and E-2 respectively, k is coefficient for the recalculation of rutin trihydtate into rutin (0.917).

Results and discussion

Flavonoids are considered as principal substances in plants. In this paper, quantitative determination of TFC was carried out by colorimetric aluminum chloride method. The principle of aluminum chloride colorimetric method is that aluminum chloride forms complexes with flavones and flavonols wherein it reacts with the C-4 keto group and hydroxyl groups of the ring C, and/or ring A and/or ring B (Pękal and Pyrzynska, 2014; Hudz et al., 2017). In this work four extracts of *Salvia sclarea* were investigated. The TFC in the tested extracts was in the range of 596 to 1150 mg.L^{-1} depending on the particle size of the herbal raw material, heating and ultrasound in the extraction. The TFC in the herbal raw material was in the range of 6.6 to 14.7 mg per one gram. Our study confirmed that complex of rutin with aluminum chloride had the maximum absorption at 413 nm in differential spectra in the range of rutin concentrations of 10.0–102.0 mg.L^{-1} (50% ethanol was as a solvent). The wavelength of maximum absorption of four extracts was in the range of 390–391 nm that indicated that flavons were dominating group of flavonoids in these extracts. According to literature data, flavones (chrysin, apigenin, and luteolin) and glycosides of flavonols have maximum absorption less 415 nm (Hudz et al., 2017). In our studies published earlier the solutions of complexes aluminum chloride with quercetin (20 mg.L^{-1}), rutin (50.2 mg.L^{-1}), and chrysin (80 mg.L^{-1}) had the maximum absorption at the wavelengths of 425.9 ±0.3 nm at 77 min of the reaction, 412.3 ±0.3 nm at 82 min, 388.4 ±0.7 nm at 81 min, respectively. The tinctures of *Monarda fistulosa*, *Satureja hortensis*, *Thymus vulgaris*, and *Mentha piperita* had the maximum absorption at 391.2 ±0.5 nm at 91 min, 389.9 ±0.5 nm at 76 min, 391.8 nm at 83 min, 394.9 ±1.1 nm at 78 min, respectively (Yezerska at al., 2021). Repeatability of the position of an absorption maximum of the extracts is very good at performing analyses in the different times of the reaction of forming complexes flavonoids with aluminum chloride or in different days of performing analysis (Table 2). We also can conclude that all the four extracts had the absorption maximum about 390 nm.

In addition, we used 50 or 100 µL of the extracts in order to obtain the values of absorbance of the solutions of the extracts or rutin with aluminum chloride not more 0.80 according to rules ofspectrophotometry. In general, if the absorbance of a reaction mixture is less than 0.05 or significantly higher 1.0, it is necessary to correct the volume of a sample for the determination of TFC (Hudz et al., 2017). Moreover, we selected rutin as a commercially available marker for constructing its calibration curve (Hudz et al., 2021). Furthermore, it was detected as a phenolic compounds in some specis of *Salvia* (*S. potentillifolia*, *S. albimaculata* and *S. nydeggeri*) (Kivrak et al., 2019). Hanganu et al. employed also rutin for measuring TFC in some *Salvia* species, including *S. sclarea*.

According to literature data flavons are identified in many Lamiaceae species, including *Salvia* genus. Veličkovič et al. (2007) analyzed extracts from garden (*Salvia officinalis* L.) and glutinous (*Salvia glutinosa* L.) sage by ultrasonic and classical maceration. The flavonoids were also detected in considerable quantities in the plant material from which the essential oils had been already removed. Apigenin and its derivatives (e.g., apigenin 4'-methyl ether), scutellarein 6-methyl ether, isoscutellarein 8-methyl ether, luteolin and 6-OH-luteolin-6-methyl ether were distinctive for *S. officinalis*. Apigenin, luteolin, 6-OH-luteolin-6-methyl ether, kaempherol 3-methyl ether, kaempherol 3,7-dimethyl ether, quercetin 3,7,3'-trimethyl ether and quercetin 3,7,3',4'-tetramethyl ether were distinctive for *S. glutinosa*.

Valant-Vetschera et al. (2003) studied chemodiversity of various species of Lamiaceae family, in particular flavonoid profile, and they recognized for *Salvia sclarea* some flavonoids such as apigenin (5,7,4'-triOH flavone), genkwanin (5,4'-diOH-7-OMe flavone), 5-OH-7,4'-diOMe flavone, salvigenin (5-OH-6,7,4'-triOMe flavone).

Flavonoids display a wide range of pharmacological activities, including neuroprotective, anti-inflamatory one (Asadi et al., 2010; Tavakkoli et al., 2014). In study performed by Asadi et al. (2010), the methanolic extracts of *S. hydrangea* and *S. sclarea* (≤50 μg.ml^{-1}) were shown to fight DNA oxidative damage of PC12 neural cells in rats induced by Fe(II)–H_2O_2. Additionally, the neuroprotective effects of *Salvia* extracts from *S. hydrangea* and *S. sclarea* against oxidative stress (using H_2O_2 as oxidative agent), were observed in PC12 neural cells, for which pretreatment with 100 μg.mL^{-1} significantly protected cell survival (76 to 93%) with respect to the control. This order *Salvia hydrangea*, *Salvia macilenta* > *Salvia multicalis*, *Salvia sclarea* > *Salvia xanthocheila* > *Salvia lachnocalyx* has shown the scavenging ability of six species methanolic extracts reported by Asadi et al. (2010). Asadi et al. consider above mentioned plants as a source of preparations for treating neurodegenerative diseases (Asadi et al., 2010). Tavakkoli et al. (2014) studied the methanolic extracts of *Salvia santolinifolia* Boiss. and *S. sclarea* L. (100 μg.mL^{-1}). These extracts reduced the H_2O_2-stimulated ROS production in neuronal PC12 cells by 61.9 and 61.4%, and showed significant neuroprotection in the MTT assay by 34.7 and 39.5%, respectively. In the same study, the *S. santolinifolia* extract significantly reduced H_2O_2-induced apoptosis (Tavakkoli et al., 2014). Recent studies demonstrate anti-inflamatory activity of the ethanolic extract of *S. sclarea*. The treatment with the extract, compared to the untreated group of the rats, significantly decreased the inflammation diminishing the levels of IL-1β, IL-6 and TNF-α, reducing the gingival tissue lesions and preserving bone alveolar resorption (Kostic et al., 2017).

As can be seen from Table 2, the TFC was in the ramge of 6.4 to 14.7 mg per one gram of the herb. The highest flavonoid content was observed for extracts 1 and 3 in reference to one gram of the herb. For their preparation heating and ultrasond were used. Our data are in line with results of Jasicka-Misiak et al. (2018). These authors studied phenolic compounds in *S. officinalis* and *S. sclarea* growing in different habitats and determined the total phenolic content in the range of 63.9 (*S. officinalis*) to 134.4 (*S. sclarea*) mg.GAE.g^{-1}. The yield of flavonoids from herb of *Salvia sclarea* was the highest in extracts for the preparation of which higher temperature (36–46 °C) and ultrasonic were used. The TFC was the highest in the extract in which the solvent-herb ratio was the least (10 : 1) and particle size was in the range of 2 to 5 mm, however the extraction of flavonoids from the herb (depletion degree of the raw material) was the least.

Hanganu et al. (2019) determined only 2.0 mg rutin eqvivalets per one gram of the herb of *Salvia sclarea*. Hovewer, these cauthors used shortlasted extraction. The material was extracted at a temperature of 60 °C (on a water bath) with 70% ethanol for 30 min.

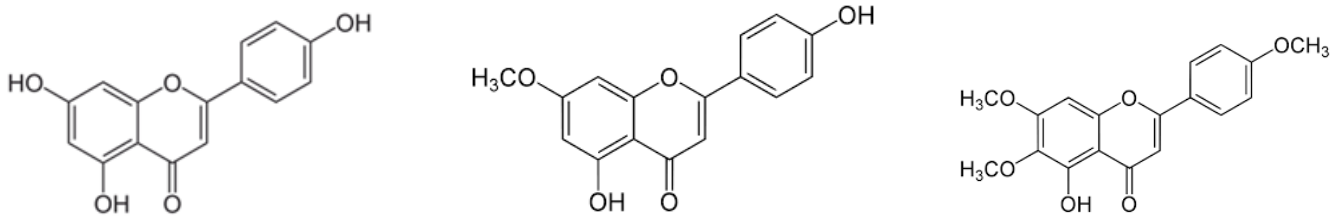

Figure 2 Structures of some identified flavonoids in *Salvia sclarea* L.

Table 2 Calculations of the TFC in the developed tinctures and precision of the analytical procedure

Identification of extracts	Absorption maximum/mean value ± SD (nm)	Volume of the extract (µL)	Absorbance/mean absorbance ± SD	TFC ±SD (extract)	TFC (1 g of the herb)	Time of the reaction
E-1	390.2, 390.2, 390.0/390.1 ±0.1	50	0.404, 0.382, 0.428/0.405 ±0.023	831.1 ±59.4 mg.L^{-1}	14.7 mg.g^{-1}	62 min
			in 57 min			
	390.1, 389.1, 389.7/389.6 ±0.5	50	0.365, 0.404 0.422/0.405 ±0.023	815.0 ±71.5 mg.L^{-1}	14.6 mg.g^{-1}	119 min
E-2	391.9, 392.3, 391.1/391.8 ±0.6	100	0.587, 0.587, 0.577/0.584 ±0.006	596.4 ±12.5 mg.L^{-1}	9.8 mg.g^{-1}	68 min
			in 16 min			
	391.9, 392.1, 391.1/391.7 ±0.5	100	0.592, 0.596, 0.585/0.591 ±0.006	603.4 ±12.5 mg.L^{-1}	10.0 mg.g^{-1}	84 min
E-3	391.3, 391.0, 391.1/391.1 ±0.2	50	0.306, 0.329, 0.318/0.318 ±0.012	655.4 ±37.2 mg.L^{-1}	10.7 mg.g^{-1}	80 min
			in 5 days			
	391.1, 391.1, 390.7/391.0 ±0.2	50	0.330, 0.305, 0.336/0.324 ±0.016	667.5 ±45.3 mg.L^{-1}	10.9 mg.g^{-1}	88 min
E-4	391.0, 391.1, 390.5/390.9 ±0.3	50	0.566, 0.523, 0.599/0.563 ±0.038	1150.3 ±89.7 mg.L^{-1}	7.4 mg.g^{-1}	86 min
			in 1 day			
	391.4, 391.0, 391.1/391.2 ±0.2	50	0.481, 0.520, 0.524/0.508 ±0.024	1039.2 ±61.4 mg.L^{-1} 90.34% from initial value	6.6 mg.g^{-1}	78 min

Conclusion

The analytical procedure of the TFC determination of the *Salvia sclarea* extracts by the colometric aluminum chloride method was developed from a point of view of choosing a volume and dilution of the extracts (50 or 100 µL), marker for the calculation of the antioxidant activity of the extracts. The calibration curve was plotted in the concentration range of 10 to 102 mg.L^{-1} of rutin. The results suggest that the herb of *Salvia sclarea* is a valuable source of flavonoids. The wavelength of maximum absorption of four extracts ranged from 390 nm to 391 nm. Such an absorption maximum indicated that flavons were dominating group of flavonoids in the extracts of *Salvia sclarea*. The influence of pharmaceutical factors like temperature, particle size of herbal raw material and ultrasound on the extraction of flavonoids was established. It was revealed that the higher temperatures induced higher extraction of flavonoids. These studies can be basis for the development of extract of *Salvia sclarea* for pharmaceutical and cosmetic industries

Conflict of interest

The authors declare no conflict of interest.

Ethical Statement

This article does not contain any studies that would require an ethical statement.

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