Biological activity and profiling of *Salvia sclarea* essential oil obtained by steam and hydrodistillation extraction methods via chemometrics tools

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Abstract

Salvia sclarea L. or clary sage is cultivated worldwide in temperate and sub-tropical climates, as an ornamental and essential oil (EO) bearing plant. EO is obtained from fresh spikes in full flowering stage, and is recognized as an important commercial product for food, beverage and cosmetic industries. This study investigated the EO composition of *S. sclarea* grown in Serbia (Southeast Europe) obtained by two different methods, steam (SD) and hydrodistillation (HD). GC-MS analysis identified oxygenated monoterpenes as the main class of compounds for all EOs (between 81.8 and 88.2% depending on the distillation process). The most abundant oxygenated monoterpenes were linalyl acetate and linalool. In addition, *in vitro* antimicrobial (modified resazurin microtitre-plate assay) and antioxidant activities (DPPH$^{\bullet}$ assay) and total polyphenol content of obtained EOs were also evaluated. According to the assay used for the evaluation of the antibacterial activity, Gram negative bacteria were more sensitive to *S. sclarea* EO in comparison to Gram positive bacteria. EOs exhibited low antioxidant capacity, below 3% neutralized DPPH$^{\bullet}$ radicals, reaching up to approximately 400 µg AAE mL^{-1}. This study also investigated a possibility for predicting retention indices (*RI*s) of compounds isolated from EOs. In total, 78 experimentally obtained *RI*s were applied to construct the prediction model. The quantitative structure-chromatographic retention relationship (*QSRR*) model was used to anticipate the experimentally obtained *RI*s. Five molecular descriptors were selected by factor analysis and genetic algorithm to predict *RI*s. The obtained accuracy of the *QSRR* model reached r^2=0.912, which showed that these models might be applied for predicting retention indices.

KEYWORDS: antibacterial activity; antioxidant activity, artificial neural network; clary sage; *QSRR*.

1. INTRODUCTION

Salvia sclarea L., also known as clary sage, belongs to the Lamiaceae family and is native to Southern Europe and is cultivated worldwide in temperate and sub-tropical climates, as an ornamental and essential oil (EO) bearing plant. The plant reproduces from the brown, round to triangular seeds. It is usually a biennial or a perennial plant, with a thick, square, erect stem, 20-120 cm high, branched toward the top. Some plants bloom in the first year if sown in early spring. Annual leaves are arranged in a rosette, while biennial are arranged along the stem in pairs. Simple and multicellular glandular trichomes are present on both sides of the leaf. The plant reaches a height of up to 130 cm, with flowering spikes averaging up to 40 cm. The cymose

inflorescence of *S. sclarea* represents an assemblage of lilac to whitish axillary flowers in clusters subtended by bracts.[1,2,3] In the agro-ecological conditions of SE Europe (Serbia, Hungary), *S. sclarea* is often harvested twice per year. The first harvest is usually performed during June or July, and the second one in September. However, the chemical composition of the obtained EO is significantly different. In the first harvest, a high content of linalyl acetate is reported. Conversely, in the second harvest, linalyl acetate, 1,8-cineole and myrcene content decreases while α- and β- pinene disappears. Consequently, the scent of the oil is affected.[4]

S. sclarea EO is obtained from fresh spikes in full flowering stage, and the content ranges from 0.01%, v/w (plants regenerated *in vitro*)[5] to 0.83 % (v/w). This depends on the distillation method (traditional or advanced)[6,7,8] and analysis technique[9], origin or population and growing conditions[10,11,12], plant development phase (full blooming, phase of growing fruit and full maturity of the seeds)[3], or sample amount, particle size, extraction time and temperature[13]. *S. sclarea* EO is an important commercial oil, characterized as a colorless, brownish-yellow or pale yellow liquid with a characteristic odor.[14] It originates from linalyl acetate content and is usually described as sweet, green, floral and spicy with clean, woody, terry and citrus nuances.[15] In general, the second most abundant compound in EO is linalool, which is characterized by a floral odor. The most valuable commercial *S. sclarea* EO is linalool/linalyl acetate chemotype.[16] Other significant volatile compounds are geranyl acetate, α-terpineol and sclareol.[17] However, in fragranced cosmetic products some of these compounds with low allergenic potency turn into stronger allergens after autoxidation. These compounds such as linalool, linalyl acetate and geraniol cause contact allergy and dermatitis.[18,19,20] Therefore, it is very important to keep and store EO without air exposure.

S. sclarea EO is used as aromatic agent in the food industry[21], especially in condiments, frozen desserts, puddings, gelatins, pastries and in alcoholic beverages. Apart from flavoring food, *S. sclarea* EO can also be used for preventing food spoilage due to its antimicrobial properties.[22] Furthermore, sclareol is a highly valuable compound in the fragrance industry.[23,24] Due to its characteristics, it is considered to be an important starting material for a number of commercial substances and a replacement for ambergris used in the formulation of exclusive perfumes. Most of the commercially-produced sclareol is derived from cultivated *S. sclarea*.[25]

. *sclarea* is commercially cultivated on a large scale in Europe, especially in Bulgaria and France, through Russia and Morocco.[2,21] It is widely used in perfume industry and aromatherapy against stress, tension, depression and insomnia.[26] Traditionally, *S. sclarea* EO was used as an agent against inflammatory conditions of oral cavity such as gingivitis, stomatitis and aphthae.[27] Apart from this, recent studies reported anti-inflammatory, antimicrobial and analgesic, as well as antidiabetic and cytotoxic effects.[2] In addition to biological activities, *S. sclarea* is one of the most economically important plants for phytoextraction and phytostabilization of zinc and cadmium contaminated soils[28,29], and because of this there is growing interest in cultivation of this plant.

The extraction of EOs is generally carried out by hydro or steam distillation processes, nonetheless, there is a number of novel techniques such as solvent extraction, supercritical CO_2, microwave-assisted extraction, vacuum extraction and other.[7,8,30,31] These techniques are developed because heat inevitably causes thermal degradation of the natural fragrance, because several EO components may re-arranged when exposed to heat and several artifacts could be produced.[13,32,33]

One of the most important steps in postharvest procedures in *S. sclarea* production is immediate distillation which has to be performed immediately after the harvest due to the loss of some

volatiles by evaporation.[34,35] Apart from this, the developmental stage of the plant at harvest time is very important for EO content, as well as distillation kinetic.[36] If distillation time increases, it causes partial hydrolysis of linalyl acetate followed by a partial acid catalyzed degradation of linalool resulting in an increase in myrcene content, as well as *cis*- and *trans*-*β*-ocimene, limonene, terpinolene, *α*-terpineol, geraniol, neryl acetate and geranyl acetate.[37]

Quantitative structure-chromatographic retention relationship (*QSRR*) depicts the chemical structure according to the molecular descriptors (*MD*s).[38,39] Gas Chromatography-Mass Spectometry (GC-MS) data are broadly used in previous *QSRR* models.[40,41,42,43,44]

The main goal of this investigation was to determine the difference in EO quality depending on the distillation conditions (a commercial distillation unit with steam and a laboratory with Clevenger apparatus) of *S. sclarea*. Furthermore, chemical compounds found in *S. sclarea* EO using the GC-MS technique were the main focus in establishing the new *QSRR* model for anticipating the retention indices (*RI*s), applying factor analysis and genetic algorithm (*GA*) for *MD*s selection. Also, the artificial neural network (*ANN*) model was enforced in this investigation.[45,46]

2. Material and method

2.1. Plant material

Domestic fragrant variety of *S. sclarea* called "Domaća mirisna" (voucher number 2-1560, Herbarium BUNS) was commercially cultivated at the Institute of Field and Vegetable Crops Novi Sad, at the Department of Alternative Crops and Organic Agriculture Bački Petrovac (45°21′N; 19°35′E). *S. sclarea* was sown in spring 2018, in continuous rows with row spacing of 70 cm. Only mechanical weeding and digging was performed during vegetation period in all three years. In the first year, *S. sclarea* was in vegetative stage, followed by generative (blossom) stage in the second year (2019) when plants were harvested, between June 25th and July 1st during 2019. During full blossom stage, the upper 50-60 cm of the plant with inflorescence was picked early in the morning. The fresh material was immediately distilled.

2.2. Steam distillation

The steam distillation (SD) was performed in a small scale distillation unit at the Institute of Field and Vegetable Crops Novi Sad. The fresh upper parts with flowers of *S. sclarea* (100 kg) were placed in a stainless steel distillation vessel (volume 0.8 m^3) constructed by the Inox Ltd. Bački Petrovac, Serbia. Steam was supplied through a manifold pipe into the bottom of the vessel from a high-pressure boiler (Vaporax, Ventilator Ltd. Zagreb, Croatia) and routed upward through a plumbing system to the vessel with plant material being extracted. The steam, water vapor, and entrained volatiles exited the tank near the top via a 10 cm diameter pipe and were carried to a water-cooled condenser that is mounted vertically, it acts as a pipe heat exchanger (the distillate flows through a pipe system and is immersed into a cooling fluid – water in with the re-circulation flow rate of 2.5 $m^3\ h^{-1}$). Heat exchange surface in the condensator (10.8 m^2) was chosen so that only the latent heat of evaporation of the distillate was subtracted. Cooler was horizontal, one pipe held concentrically inside of a larger pipe (heat exchange surface of 4.3 m^2). The inner pipe acts as the conductive barrier, where one fluid flows through this inner pipe while the cooling fluid flows around it through the outer pipe (0.6 $m^3\ h^{-1}$), forming an annulus shape. The oil and water condensate was separated in a glass florentine flask (1 m height, 20 cm diameter) which enables efficient separation of the compounds into EO and water (hydrolate).

2.3. Hydro-distillation

Hydrodistillation (HD) was performed in laboratory using a Clevenger-type apparatus. Fresh plant material (100 g) was placed in 1 L conical flask and connected to the Clevenger apparatus. Distilled water (approx. 500 mL) was added to the flask and heated to the boiling point. The vapor phase was collected into a graduated cylinder. After 3 h EO was separated from aqueous layer, according to the method outlined by the European Pharmacopoeia.[10]

2.4. Essential oil (EO) analysis

Obtained EOs used for GC/FID and GC-MS analysis was dried over anhydrous sodium sulfate and stored at 4-6 °C. Analysis were carried out with an Agilent 7890A apparatus equipped with an 5975 C MSD, FID and a HP-5MS fused-silica capillary column (30m×0.25mm, film thickness 0.25 μm). The carrier gas was helium, and its inlet pressure was 19.6 psi and linear velocity of 1 mL min^{-1} at 210 °C. The injector temperature was 250 °C, injection volume was 1 μL, split ratio, 10:1. MS detection was carried out under source temperature conditions of 230 °C and interface temperature of 315 °C. The EI mode set at electron energy, 70 eV with mass scan range of 40–600 amu. Temperature was programmed from 60 °C to 300 °C at a rate of 3 °C min^{-1}. The components were identified based on their linear retention index relative to C_8-C_{32} *n*-alkanes, comparison with data reported in the literature (Adams4 and NIST17 databases). The relative percentage of the oil constituents was expressed as percentages by FID peak area normalization.

2.5. Antimicrobial activity

Antimicrobial activity of the tested EOs was evaluated using laboratory control bacterial strains obtained from the American Type Culture Collection: Gram-negative *Escherichia coli* (ATCC 8739) and *Salmonella enteritidis* (ATCC 13076) and Gram-positive *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212). Antimicrobial activity of *S. sclarea* EO was determined according to the CLSI with slight modifications in determination of end point.[47,48]

2.6. Total polyphenolics content and antioxidant activity

Total polyphenols content (TPC) was determined using modified Folin-Ciocalteu's method described by Makkar.[49] Diluted (EO:MeOH=0.1:4.9; v/v) EO (200 μL) was added to a mixture of distilled water (5 mL), Folin-Ciocalteu's reagent (500 μL, diluted with distilled water 1:2, v/v) and after 1 min, 1 mL of sodium carbonate (20 %) was added and tubes were covered with parafilm and left in a dark place for an hour. After incubation, absorption were measured spectrophotometrically (Perkin Elmer, UV/VIS Lambda Bio 20) at λ=765 nm. Results were calculated from gallic acid calibration curve and expressed as gallic acid equivalents (GAE) in mL of EO.

Antioxidant activity was determined by DPPH$^{\bullet}$ test as ability of diluted EO to neutralize 1.1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) free radicals.[50] The working solution was produced by diluting stock DPPH$^{\bullet}$ solution with methanol (24 mg DPPH$^{\bullet}$ in 100 mL MeOH) to obtain an absorbance of about 0.998 (±0.002) at 517 nm. A 100 μL of varying concentrations of EO (25–250 μg mL^{-1}) diluted in MeOH were added to a 3 ml DPPH$^{\bullet}$ solution and after incubation in the dark (30 min), at room temperature, the absorbance was measured at 517 nm. Results of DPPH$^{\bullet}$ radical scavenging activity (DPPH$^{\bullet}$ test) was expressed as % inhibition and ascorbic acid

equivalents (AAE) in mL EO, based on calculations from ascorbic acid standard curve performed in the same manner.

2.7. QSRR analysis

The molecular structures data was introduced using .smi files, obtained from PubChem database. The investigation of *MD*s was done by exploring the PaDel-descriptor database.[51] The selection of the *MD*s for *RI*s anticipation was performed using factor analysis and *GA*[52,53] using Heuristic Lab software. Statistica 10 software was used for statistical analysis of the data.

2.8. Artificial neural network (ANN)

Multi-layer perceptron (*MLP*) was used for the construction of the *ANN* model for prediction of *RI*s for compounds found in *S. sclarea* EOs identified using GC-MS data.[54] Broyden–Fletcher–Goldfarb–Shanno (*BFGS*) algorithm was used to speed-up the calculation of weight coefficients of the *ANN*.[21] The observed data were randomly separated to 60%, 20% and 20% of data used for training, testing and validations, respectively.[55,56]

2.9. Global sensitivity analysis

Yoon's global sensitivity equation was utilized to calculate the relative impact of the chosen *MD*s on *RI*s.[57]

3. RESULTS AND DISCUSSION

3.1. Chemical composition of EOs

Totals of 39 and 40 compounds were characterized, corresponding to 95.3% of the total for EO obtained by SD and 97.5% of the total for EO obtained by HD (Table 1). Oxygenated monoterpenes were identified as the major class of compounds for all EOs (81.1 and 88.2% depending on the distillation technique). The most abundant among the oxygenated monoterpenes were linalyl acetate (with 40.3% and 43.6% in EO obtained by SD and HD, respectively) and linalool (with 28.3 and 25.3% obtained by SD and HD, respectively), followed by α-terpineol and geranyl acetate.

Monoterpene hydrocarbons were present in the amounts of 0.5 and 3.1% (in the oil obtained by SD and by HD, respectively), while sesquiterpene hydrocarbons were present with 0.8 and 9.1% in EOs obtained by SD and HD, respectively. These two classes of compounds (monoterpene and sesquiterpene hydrocarbons) were the most abundant in EO obtained by HD. Oxygenated sesquiterpenes were also the most abundant in SD (1.7%) in comparison with HD (1.4%), as well as oxygenated diterpenes (3.2 and 1.6% in EOs obtained by SD and HD, respectively). Monoterpenes are also predominant in comparison to sesquiterpenes in the EOs of *Salvia leriifolia* and *S. multicaulis* flowers.[58] In case of *S. mirzayanii*, it is established that the flower and leaf mainly contain monoterpene hydrocarbons, while the stem mostly contains oxygenated monoterpenes. Additionally, a larger sample amount can cause some changes in the chemical composition of volatile compounds.[13] In our study, a larger amount of plant material in SD sample could have caused these differences.

Table 1

According to cluster analysis based on chemical compositions of 39 samples of *S. sclarea* EO from literature, it is concluded that most of the samples belong to the chemotype rich in linalyl

acetate and linalool.[59,60] Linalyl acetate content increases from full blossom through seed formation, and is highest during full seed maturity, while linalool content decreases.[3]
Similarly to *S. sclarea*, linalyl acetate and linalool are the quality determining constituents of lavender EO. However, investigations showed higher amounts of linalyl acetate in the EO produced by HD (30.0%) than by SD method (35.28%).[61] These differences could be attributed to the degradation of linalyl acetate (when in contact with water) into linalool.[62] The main reason for the change of linalool:linalyl acetate ratio in case of *S. sclarea* are most probably enzymatic and acidic degradation reactions which are occur during crushing of fresh plants before extraction.[63] In addition, it is reported that linalyl acetate changes into linalool by thermal hydrolysis during steam distillation[64], as well as that linalool:linalyl acetate ratio may change in distillation times and flowering phenophase.[65]

The current experimental findings reveal that laboratory obtained EO by HD using Clevenger apparatus produced better quality EO in terms of higher linalyl acetate content than the SD method in industrial conditions. In addition, other techniques such as water-steam distillation provide the highest content of linalyl acetate.[61] However, it is well-known that *S. sclarea* EO is mainly obtained by SD on commercial scale. Vegetal waste material after processing of *S. sclarea* could be converted into "green" bioactive particles with high biomedical value[66], as well as into hydrolate, which as by-product during SD also has commercial value on the market.[67,68]

3.2. Antibacterial activity

According to the assay, Gram negative bacteria were more sensitive to the EO of *S. sclarea* than Gram positive bacteria (Table 2). Distillation method did not affect *S. sclarea* EO antimicrobial activity. Antimicrobial activity of *S. sclarea* EOs obtained by SD and HD showed the highest effectiveness against Gram negative bacteria: *E. coli* (MIC/MBC= 28.40/28.40 µL mL^{-1}) and *S. enteritidis* (MIC/MBC= 3.55/3.55µL mL^{-1}). EO obtained by SD was slightly less effective against *E. coli* (MIC/MBC=14.20/28.40 µL mL^{-1}) and *S. enteritidis* (MIC/MBC= 56.81/113.63 µL/mL^{-1}). Tested EOs exhibited lower effectiveness against Gram-positive bacteria. Results of antimicrobial activity of *S. sclarea* EO (SD) against *B. cereus*, *S. aureus*, and *E. faecalis* indicated equal MIC and MBC (>454.50 µL mL^{-1}). EO obtained by HD exhibited slightly higher effectiveness against Gram-positive bacteria.

Table 2

In addition, investigations by Kuzma et al.[5] showed that *E. coli* was the most sensitive bacteria to *S. sclarea* essential oil (MIC=2.5 mg mL^{-1}), followed by *S. epidermidis* (MIC=5.0 mg mL^{-1}). Both of these bacteria are Gram negative. These findings are in agreement with a study conducted by Cui et al.[22] within which bactericidal effectiveness of *S. sclarea* EO against Gram-negative and Gram-positive bacteria was investigated. Based on scanning electron microscopy (SEM) analysis as well as measurements of cellular ATP concentration and DNA after treatment with EO it was concluded that *S. sclarea* EO damages the cell membrane and changes the cell membrane permeability, leading to the release of the material inside the cell such as ATP and DNA. The antimicrobial activity of *S. sclarea* can be attributed to the significant amounts of linalyl acetate, linalool and geranyl acetate. Thus, it may be assumed that these components play a crucial role in the antimicrobial activity of the tested EOs. Obtained results of the chemical composition (Table 1) and exhibition of different antibacterial activity toward tested bacteria

(Table 2) could be explained by the synergistic or additive effects caused by minor components in the EO, which was previously confirmed in other researches.[5,69]
Additionally, the results for antibacterial activities of *S. sclarea* EO showed that *E. coli*, *Pseudomonas fluorescens*, *Kocuria marina* and *B. cereus* are sensible bacterial strains.[68] Furthermore, *S. sclarea* caused a dose-dependent inhibition of mycelial growth.[17] It is posible that applying higher doses of *S. sclarea* essential oil could be effective against other bacteria.

3.3. Total polyphenolics and antioxidant activity

According to TPC, EOs show slight difference between methods used for distillation conditions (Table 3). As for DPPH$^{\bullet}$ test, EOs had low antioxidant capacity, below 3% neutralized DPPH$^{\bullet}$ radicals, reaching up to approx. 400 µg AAE mL^{-1}. Obtained results are much lower than for some other commonly used EOs: thyme, oregano, clove, sage and rosemary (62.8, 51.8, 97.8, 51.2, 47.5 % neutralized DPPH$^{\bullet}$ radicals, respectively).[70] However, research shows that methanol, chloroform and acetone extract of *S. sclarea* are effective antioxidant[71,72,73], in comparison to essential oil.[74]
In a study by Ovidi et al.,[68] *S. sclarea* EO with a high content of linalyl acetate (62.6%) displayed good antioxidant activity. Furthermore, it is known that linalyl acetate can reduce oxidative changes.[75] In addition, some species from genus *Salvia* such as *S. limbata* and *S. bracteata* have good antioxidant effects.[7,76] It is clear that EO compounds act synergistically, antagonistically and additively.[77]

Table 3

3.4. QSRR models

Retention indices (as dependent variables) are calculated by *QSRR* model using the independent variable matrix of molecular descriptors.[43] PaDel-descriptor software was used for evaluation of *MD*s. A large set of *MD*s was determined, and only the most significant descriptors were chosen to build the predictive *RI*s model. The factor analysis was used to exclude the descriptors with practically equivalent correlations, and the uncorrelated *MD*s were used in the *GA* calculation. As a result of this preliminary consideration only cca. 150 descriptors remained for *GA* calculation. *GA* was applied to choose between *MD*s, for the most appropriate variables for *RI*s prediction.[56,78,79] Five most important molecular descriptors were chosen; four 2D Autocorrelation descriptors (*ATSC4s*, *AATSC1v*, *MATS2s*, *GATS6v*) which explain how the considered property is distributed along the topological structure, and one Barysz matrix descriptor (*VE2_Dze*) which was calculated by using weighted molecular graphs, and the weighting scheme based on the atomic weight Z and polarizability.[80,81] The predicted *RI*s and *MD*s are presented in Table 1. The anticipated *RI*s are displayed in Fig. 1 confirming the sufficient expectation abilities of the developed *ANN*, by demonstrating the connection between the anticipated and experimentally gained retention values.

Fig. 1

Based on the Pearson's correlation analysis, there was a rather poor correlation between all molecular descriptors (Table 4). Subsequently, the used *MD*s were appropriate to foresee the *RI*s of compounds in *S. sclarea* by applying the multivariate *ANN* model.[82] Definite clarifications about the descriptors were found in the Handbook of Molecular Descriptors.[80,81] These

descriptors encode various points of the molecular structure and were applied to build the *QSRR* model. Table 6 represents the correlation matrix among these descriptors.

Table 4

3.5. Artificial neural network (ANN)

To investigate the relationship between *MD*s selected by factor analysis and *GA*, *ANN* model was used, as one of the most commonly used mathematical tool in agriculture research.[83] The MLP 6-5-1 neural network was constructed to foresee the retention time of compounds isolated from *S. sclarea.* The coefficient of determination (r^2) during training was 0.912, showing the good predicting abilities of the model for predicting *RI*s. The statistical results of this network were displayed in Table 5.

Table 5

The accuracy indices of the model were presented in Table 6. The lower χ^2, *MBE*, *RMSE* and *MPE* values showing the better fit to the experimental results.[84] The predicted *RI*s are presented in Table 1, confirming the good quality of the constructed *ANN*, by showing the relationship between the predicted and experimental *RI*s values. Graphical comparison between: experimentally obtained retention indices of *S. sclarea* EOs composition in 2019 (RI^a), and the retention time indices predicted by the *ANN* model ($RI_{pred.}$) are presented in Fig. 2. The calculated results show that the *ANN* models results could be applied for predicting of the *RI*s in *S. sclarea* EOs obtained by GC-MS analysis.

Table 6

Fig. 2

3.6. Global sensitivity analysis-Yoon's interpretation method

The impact of five most significant *MD*s, chosen by factor analysis and *GA* on *RI*s, were explored. According to the Fig. 3, *Ve2Dze* was the most important *MD* for chemical compounds in *S. sclarea*, with relative importance of 50.63%.

Fig. 3

4. CONCLUSION

The major compounds in Serbian domestic fragrant variety of *S. sclarea* EOs were oxygenated monoterpens, linalool and linalyl acetate. Slight differences were observed in the content of the major EO compounds (oxygenated monoterpens) and antimicrobial activity when different distillation techniques were concerned, however monoterpene and sesquiterpene hydrocarbons and antioxidant activity were greatly affected by mentioned factors. Chemical compounds in *S. sclarea* EO were identified by GC-MS analysis and were used for *QSRR* analysis. The following eight molecular descriptors were suggested by *GA*: *ATSC4s*, *AATSC1v*, *MATS2s*, *GATS6v* and *Ve2_Dze* that characterize *RI*s of identified compounds. The chosen molecular descriptors were not correlated statistically significant to other molecular descriptors, and thus they could be

applied for *QSRR* model building, for estimating the retention indices using a set of GC-MS data from a series of 78 compounds identified in *S. sclarea* EOs.

The *QSRR* model results explained that the selected molecular descriptors were accurate enough for predicting the *RI*s of the observed chemical compounds. The value of r^2 during training reached 0.912, which is a good indication that the model could be appropriate tool for prediction of retention indices, due to a high r^2.

Acknowledgments: This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number: 451-03-68/2020-14/200032.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figure captions

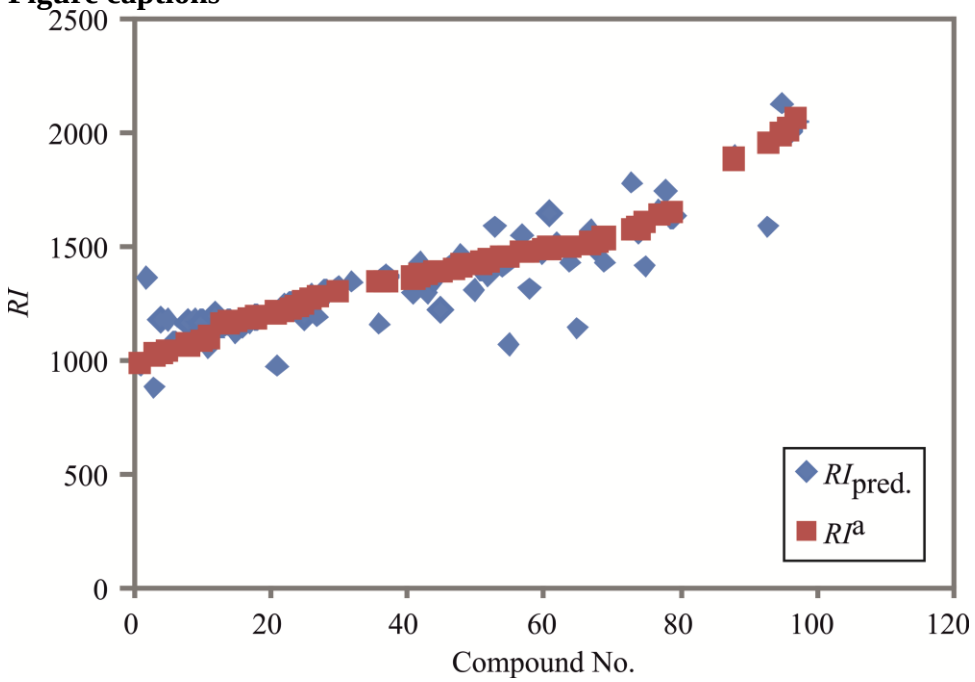

Fig. 1. Retention indices (RI^a) of the *S. sclarea* EOs composition from experimentally obtained GC-MS data and predicted by the *ANN* ($RI_{pred.}$).

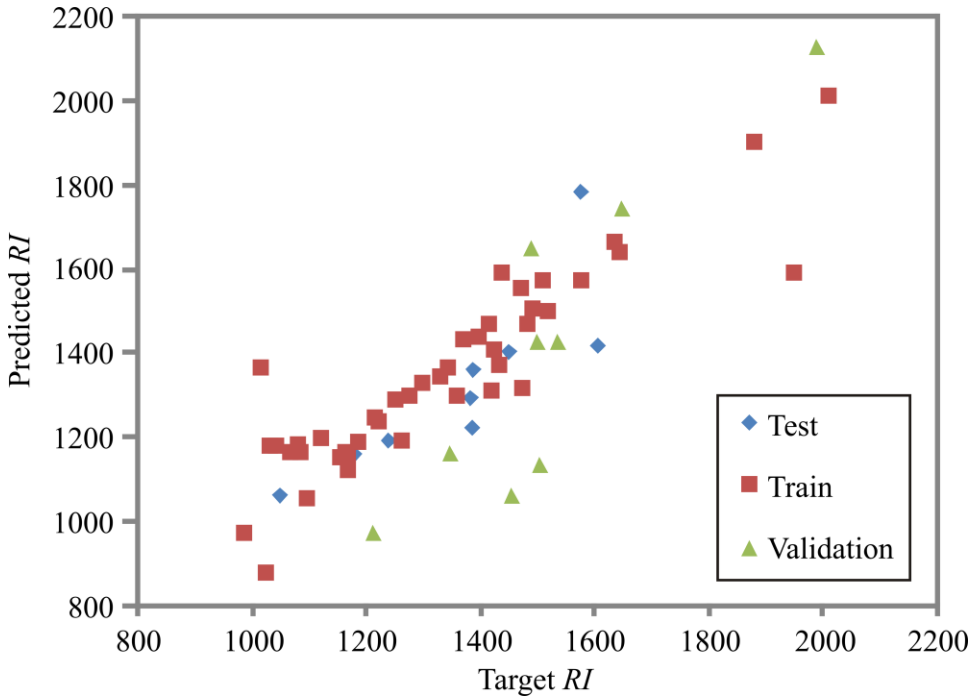

Fig. 2. Comparison of retention indices (*RI*s) of *S. sclarea* EOs with *ANN* predicted values ($RI_{pred.}$).

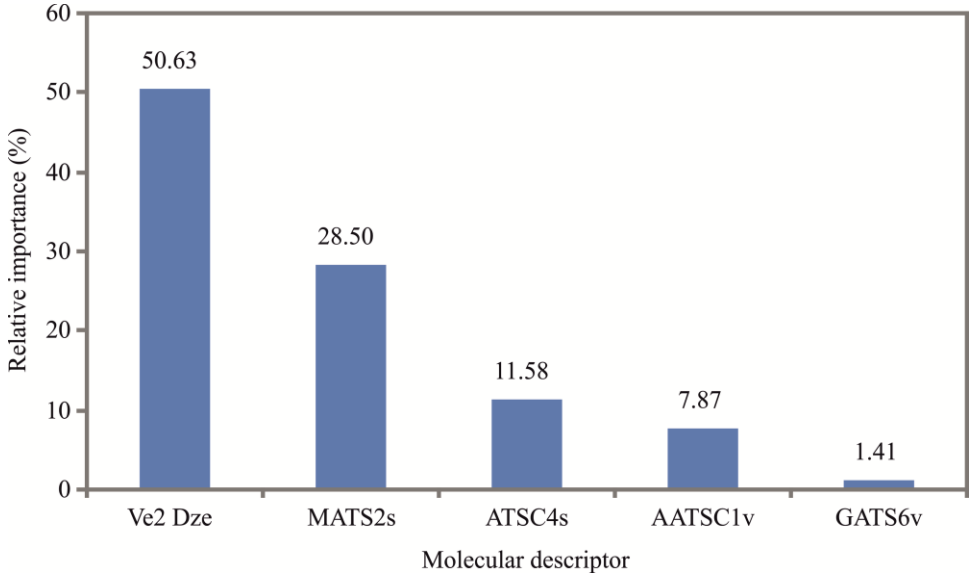

Fig. 3. Yoon's global sensitivity equation: the relative importance of the five molecular descriptors (*MD*s) on retention indices (*RI*s).

656 **Table 1.** Chemical composition and prediction retention indices (RI_{pred}) of *S. sclarea* EOs obtained by different methods

No	Compound	Cycle	$RI_{pred.}$	*RI*	SD	HD	*ATSC4s*	*AATSC1v*	*MATS2s*	*GATS6v*	*VE2_Dze*
1	Myrcene [MT]	Train	970.1	992	0.2	1.4	-1.664	-3.410	0.088	0.677	0.002
2	Limonene [MT]	Train	875.8	1029	0.3	0.4	-0.875	0.000	0.091	0.580	0.004
3	*Z*-*β*-Ocimene [MT]	Train	1176.5	1036	nd	0.5	-0.837	-3.410	0.116	0.825	0.005
4	*E*-*β*-Ocimene [MT]	Train	1176.5	1047	nd	0.8	-0.837	-3.410	0.116	0.825	0.005
5	*Z*-Linalool oxide (furanoid) [OMT]	Train	1162.6	1073	1.0	nd	-12.718	-0.740	0.131	1.113	0.004
6	*E*-Linalool oxide (furanoid) [OMT]	Train	1162.6	1090	1.0	0.2	-12.718	-0.740	0.131	1.113	0.004
7	**Linalool [OMT]**	**Train**	**1052.9**	**1103**	**28.6**	**25.3**	-5.013	-2.083	0.140	0.735	0.008
8	4-(acetyloxy)-4-methyl-5-Hexenal [O]	Train	1149.8	1161	0.1	nd	-9.368	-5.123	0.115	0.747	0.017
9	*Z*-Linalool oxide (pyranoid) [OMT]	Train	1162.6	1168	0.1	nd	-12.718	-0.740	0.131	1.113	0.004
10	*E*-Linalool oxide (pyranoid) [OMT]	Train	1117.1	1173	0.1	nd	-13.397	-3.044	0.237	0.797	0.003
11	*p*-Cymen-8-ol [O]	Test	1160.1	1184	0.1	nd	-0.253	1.495	0.183	0.769	0.006
12	***α*-Terpineol [OMT]**	**Train**	**1185.0**	**1190**	**8.4**	**5.0**	-1.226	1.111	0.145	0.592	0.001
13	Linalool formate [OMT]	Validation	969.6	1214	0.3	0.1	2.963	-4.533	0.154	0.706	0.014
14	2-Oxabicyclo[2.2.2]octan-6-ol [O]	Train	1244.7	1221	0.1	nd	-4.667	2.921	-0.080	0.000	0.003
15	Nerol [OMT]	Train	1235.7	1227	0.6	1.1	5.925	-2.083	-0.005	0.684	0.003
16	Neral [OMT]	Train	1234.0	1240	0.3	nd	7.180	-3.191	0.030	0.711	0.006
17	**Linalyl acetate [OMT]**	**Test**	**1189.4**	**1257**	**40.3**	**43.6**	4.609	-4.180	0.261	0.741	0.015
18	Geranial [OMT]	Train	1286.2	1269	0.8	nd	7.180	-3.191	0.030	0.711	0.006
19	Nerylformate [OMT]	Train	1189.4	1281	0.1	nd	14.037	-0.528	0.196	0.987	0.002
20	Cyclohexene. 3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methyl [O]	Train	1294.1	1282	0.2	nd	4.257	-4.533	0.118	0.900	0.004
21	Geranyl formate [OMT]	Train	1325.7	1303	0.1	nd	-0.806	0.000	0.085	0.858	0.007
22	NI-1	-	-	1303	0.2	nd	-	-	-	-	-
23	NI-2	-	-	1339	0.4	nd	-	-	-	-	-
24	NI-3	-	-	1340	0.1	nd	-	-	-	-	-
25	*α*-Terpinyl acetate [OMT]	Validation	1158.7	1349	0.1	nd	-0.578	4.159	0.065	1.102	0.009
26	*α*-Cubebene [ST]	Train	1364.2	1349	nd	0.4	3.205	-4.180	0.231	0.845	0.005
27	NI-4*	-	-	1351	1.1	0.1	-	-	-	-	-
28	NI-5*	-	-	1354	1.0	0.1	-	-	-	-	-
29	Neryl acetate [OMT]	Train	1293.5	1364	2.1	2.2	-0.002	4.159	0.102	0.777	0.013
30	*α*-Copaene [ST]	Train	1430.9	1375	0.4	1.1	3.205	-4.180	0.231	0.845	0.005
31	**Geranyl acetate [OMT]**	**Test**	**1293.5**	**1383**	**4.0**	**4.3**	-0.578	4.159	0.065	1.102	0.009
32	*β*-Cubebene [ST]	Test	1364.2	1389	nd	0.1	-2.676	0.000	0.082	0.742	0.008
33	*β*-Elemene [ST]	Test	1218.8	1391	nd	0.1	-5.197	0.000	-0.071	0.954	0.024
34	NI-6	-	-	1397	0.1	nd	-	-	-	-	-
35	Benzenebutanal [O]	Train	1434.8	1401	0.1	nd	-0.221	2.131	0.116	0.944	0.001
36	*E*-Caryophyllene [ST]	Train	1464.5	1419	nd	1.9	-18.169	1.260	0.081	0.918	0.008
37	Carvone hydrate [OMT]	Train	1306.0	1424	0.3	nd	0.253	4.159	0.065	0.784	0.012
38	*β*-Copaene [ST]	Train	1403.7	1429	0.1	0.1	-1.188	4.159	0.087	1.065	0.004
39	Aromadendrene [ST]	Train	1366.6	1439	nd	0.2	-3.055	2.131	0.141	1.079	0.008
40	*α*-Humulene [ST]	Test	1407.1	1453	nd	0.1	-1.337	-2.244	0.080	0.748	0.001

41	*E*-*β*-Farnesene [ST]	Validation	1065.6	1457	nd	0.2	-0.185	2.131	0.056	0.915	0.008
42	NI-7	-	-	1467	0.1	nd	-	-	-	-	-
43	*γ*-Muurolene [ST]	Train	1550.3	1477	nd	0.9	-1.672	0.000	0.103	0.934	0.003
44	Germacrene D [ST]	Train	1315.3	1480	0.1	1.9	-2.350	2.131	0.086	0.650	0.008
45	NI-8	-	-	1487	0.1	0.2	-	-	-	-	-
46	*β*-Selinene [ST]	Train	1467.3	1488	0.1	0.2	-5.484	2.131	0.107	0.852	0.008
47	Valencene [ST]	Validation	1646.1	1495	nd	0.4	-0.586	2.131	0.158	1.111	0.004
48	Bicyclogermacrene [ST]	Train	1500.7	1496	nd	0.2	0.628	2.131	0.086	0.970	0.008
49	NI-9	-	-	1500	nd	0.1	-	-	-	-	-
50	*α*-Muurolene [ST]	Validation	1428.6	1501	0.1	nd	-0.800	-2.244	0.101	0.789	0.004
51	NI-10	-	-	1509	nd	0.1	-	-	-	-	-
52	*Z*-Dihydroagarofuran [OST]	Train	1571.5	1514	0.1	0.4	0.222	2.131	0.127	0.980	0.012
53	*δ*-Cadinene [ST]	Train	1495.4	1524	nd	1.1	0.628	2.131	0.086	0.970	0.008
54	*α*-Cadinene [ST]	Validation	1428.6	1538	nd	0.2	-5.071	4.804	0.095	1.199	0.005
55	Spathulenol [OST]	Test	1778.7	1577	0.4	0.3	-2.343	2.708	0.152	0.942	0.000
56	Caryophyllene oxide [OST]	Train	1570.9	1582	0.8	0.4	-0.311	0.732	0.187	1.134	0.000
57	Humulene epoxide II [OST]	Test	1413.3	1611	nd	0.1	-1.592	2.828	0.095	0.913	0.009
58	NI-11	-	-	1638	nd	0.2	-	-	-	-	-
59	*epi*-*α*-Cadinol (=tau-cadinol) [OST]	Train	1658.9	1641	nd	0.1	-4.833	2.828	0.148	0.668	0.009
60	*β*-Eudesmol [OST]	Train	1634.7	1650	0.4	0.2	2.558	2.325	0.328	0.597	0.003
61	NI-12	-	-	1654	0.1	0.2	-	-	-	-	-
62	NI-13	-	-	1668	nd	0.2	-	-	-	-	-
63	NI-14	-	-	1676	nd	0.1	-	-	-	-	-
64	NI-15	-	-	1682	nd	0.2	-	-	-	-	-
65	NI-16	-	-	1706	0.2	0.1	-	-	-	-	-
66	NI-17	-	-	1786	0.1	nd	-	-	-	-	-
67	NI-18	-	-	1837	0.2	0.1	-	-	-	-	-
68	Sclareoloxide [O]	Train	1899.6	1884	0.1	0.1	-0.843	0.000	0.225	0.955	0.015
69	NI-19	-	-	1920	nd	0.1	-	-	-	-	-
70	NI-20	-	-	1920	nd	0.1	-	-	-	-	-
71	NI-21	-	-	1941	0.1	0.3	-	-	-	-	-
72	Geranyl-*p*-cymene [O]	Train	1587.2	1955	0.2	0.4	-0.677	2.122	0.274	0.655	0.001
73	Manool oxide [OD]	Validation	2129.1	1991	0.2	0.1	-6.496	2.161	0.134	0.656	0.011
74	13-*epi*-Manool oxide [OD]	Train	2008.0	2014	0.1	nd	-0.933	0.850	0.315	0.835	0.021
75	13-*epi*-Manool [OD]	Train	2050.0	2061	0.3	0.1	-0.817	1.932	0.424	0.715	0.019
76	NI-22	-	-	2071	0.1	nd	-	-	-	-	-
77	NI-23	-	-	2095	0.1	nd	-	-	-	-	-
78	Sclareol [OD]	Train	2220.9	2232	2.6	1.4	-5.826	2.321	0.254	0.726	0.020
	Monoterpene hydrocarbons (MT)				0.5	3.1					
	Oxygenated monoterpenes (OMT)				88.2	81.8					
	Sesquiterpene hydrocarbons (SH)				0.8	9.1					
	Oxygenated sesquiterpenes (OST)				1.7	1.4					
	Oxygenated diterpenes (OD)				3.2	1.6					

	Other (O)				0.9	0.5					
	NI				4.0	2.2					
	Total identified				95.3	97.5					

RI – Retention Index; SD – steam distillation; HD – hydrodistillation; ATSC4s – Centered Broto-Moreau autocorrelation - lag 4; AATSC1v – Average centered Broto-Moreau autocorrelation - lag 1; MATS2s – Moran autocorrelation - lag 2; GATS6v – Geary autocorrelation - lag 6; VE2_Dze – Average coefficient sum of the last eigenvector from Barysz matrix; NI – not identified compounds, nd – not detected, *mass spectrometric fragmentation of not identified compound (1.0% and higher) *m/z* (relative intensity):

NI-4: 94(24), 81(33), 79(56), 71(34), 68(26), 67(26), 59(20), 55(21), 43(100), 41(26),

NI-5: 94(23), 81(33), 79(58), 71(37), 68(26), 67(26), 59 (21), 55(19), 43(100), 41(27)

663 **Table 2.** Antibacterial activity of *S. sclarea* EOs obtained by different methods

Bacterial strain	SD		HD	
	MIC (µL mL^{-1})	**MBC (µL mL^{-1})**	**MIC (µL mL^{-1})**	**MBC (µL mL^{-1})**
Escherichia coli	14.20	28.40	28.40	28.40
Bacillus cereus	>454.50	>454.50	7.10	7.10
Salmonella enteritidis	56.81	113.63	3.55	3.55
Staphylococcus aureus	>454.50	>454.50	14.20	14.20
Enterococcus faecalis	454.50	454.5	56.81	56.81

664 SD – steam distillation; HD – hydrodistillation; MIC – minimal inhibitory concentration; MBC – minimal
665 bactericidal concertation
666

667 **Table 3.** Total polyphenolics content and antioxidant activity (DPPH•-test) of *S. sclarea* EOs
668 obtained by different methods

		SD	HD
Total polyphenolics	(mg GAE mL^{-1})	2.83	2.41
DPPH• test	%	0.72	1.87
	(µg AAE mL^{-1})	414.20	426.32

669 SD – steam distillation; HD – hydrodistillation
670

671

672 **Table 4.** The correlation coefficient matrix for the five selected descriptors by *GA*

	AATSC1v	***MATS2s***	***GATS6v***	***VE2 Dze***
ATSC4s	-0.185	0.114	-0.124	-0.074
	p=0.142	p=0.373	p=0.331	p=0.560
AATSC1v		-0.159	0.163	0.007
		p=0.214	p=0.205	p=0.960
MATS2s			0.124	-0.188
			p=0.339	p=0.143
GATS6v				0.025
				p=0.841

673 *ATSC4s* – Centered Broto-Moreau autocorrelation - lag 4; *AATSC1v* – Average centered Broto-Moreau
674 autocorrelation - lag 1; *MATS2s* – Moran autocorrelation - lag 2; *GATS6v* – Geary autocorrelation - lag 6;
675 *VE2_Dze* – Average coefficient sum of the last eigenvector from Barysz matrix

676

677 **Table 5.** Summary ANN model for training, testing and validation cycles*

Net. name	Performance			Error			Train. algor.	Error funct.	Hidden activat.	Output activat.
	Train.	Test.	Valid.	Train.	Test.	Valid.				
MLP 5-10-1	0.912	0.837	0.899	5091.120	6872.825	25100.73	BFGS 85	SOS	Exponential	Identity

678 *Performance term represent the coefficients of determination, while error terms indicate a lack
679 of data for the ANN model; Train. – training; Test. – testing; Valid. – validation; algor. –
680 algorithm; funct. – function; activat. – activation.
681

682 **Table 6.** The "goodness of fit" tests for the developed ANN model

χ^2	*RMSE*	*MBE*	*MPE*	r^2
1.7E+04	129.315	19.498	6.491	0.840

683 χ^2 – reduced chi-square; *RMSE* – root mean square error; *MBE* – mean bias error; *MPE* – mean
684 percentage error; r^2 – coefficient of determination
685