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Chemical Composition of Essential Oil of *Stachys pilifera* Benth by Hydrodistillation, Head Space-Solid Phase Microextraction and Solvent Free Microwave Extraction Methods and QSRR Evaluation

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Composition of the essential oils of *Stachys pilifera* (*Lamiaceae*) that obtained by hydrodistillation, solid phase microextraction and solvent free microwave extraction were analyzed by GC and GC/MS. In these methods *cis*-chrysanthenyl acetate was the main compound in the oils. The recognized compounds in the oil by hydrodistillation, solvent free microwave extraction and solid phase microextraction were depicted (92.52, 95.1 and 97.95 %) respectively. Furthermore, a simple, descriptive and interpretable model, based on a quantitative structure-retention relationship, was developed using the stepwise-multiple linear regression approach for the prediction of the retention indices of essential oil components. A MLR model with five selected descriptors was obtained. Then, the MLR model was validated using the leave-one-out (LOO) and leave-group-out (LGO) cross validation. This model, with high statistical significance ($R^2_{calibration}$ = 0.953, F = 110.551, Q^2_{LOO} = 0.932, Q^2_{LGO} = 0.913 and $R^2_{prediction}$ = 0.966) could predict the retention indices of the essential oils with a percentage prediction error lower than 3 %.

Key Words: *Stachys pilifera*, *Cis*-chrysanthenyl acetate, *Lamiaceae*, Quantitative structure-retention relationship.

INTRODUCTION

Essential oils are utilized in the perfume industries, cosmetic industries, hair lotion, shampoo and as ingredients of disinfectants and insecticides[1]. Several methods exist for the extraction of essential oils such as hydrodistillation, supercritical-fluid extraction, microwave extraction and solid phase microextraction methods. Hydrodistillation is a traditional method used to extract essential oil from the herbal plants. It can be applied in industry and has no chemical pollution[2]. Nevertheless, in order to decrease the extraction time and possibly enhance the extraction efficiency, to improve the quality of extracts and also to reduce the operation costs, new methods such as microwave extraction, supercritical fluid extraction and solid phase extraction have been developed[3]. Headspace solid-phase microextraction is a sample extraction and simultaneous technique. It is used for the analysis of volatile organic compounds in different complicated matrices such as environmental, food and biomedical samples by the use of fused silica fiber coated with variety stationary phase. This technique has the advantages of simplicity, rapidness, low cost, free solvent, selectivity and sensitivity when combined with suitable detection method[4-7]. Microwave heating has been utilized for the separation and analysis of essential oils in recent years. Solvent free microwave extraction is a new green technique which combines microwave heating with dry distillation at atmospheric pressure for the separation of the essential oils in fresh natural products[8]. These techniques no need to add any water and solvent. Sufficient water exists within the fresh plant. Therefore essential oil can be evaporated using heating *in situ* water that can absorb microwave energy. If dry plant is applied, the sample is rehydrated by immersing in water and then removing the excess water[8]. An advantage of technique rather than hydrodistillation involves rapidity in achieving the extraction temperature, high efficiency of essential oil, lower energy necessity and high purity of the essential oil[9].

Stachys is a fruticose, perennial and pregnant plant which belongs to Labiate family. It involves about 200 species found in mild regions of the Mediterranean and Southwest Asia. It is exhibited by 34 species in the flora of Iranica, of which 18 are endemic species[10]. This plant often grows in the slop of the

mountainous elevation which is affected by spring and subterranean Ganats. *Stachy pilifera* has short stem covered by fluff, simple and slender leaves, pink to white flowers and all of the shoots organs with very effective odor. The common apparent habitats of this plant are in spot and in rather humid regions beside of rivers and around the subterranean Ganats.

On the other hand, one of the most successful approaches to the prediction of chemical properties starting only with molecular structural information is modeling of quantitative structure-activity/property relationships (QSAR/QSPR). Quantitative structure-property relationships are mathematical equations relating chemical structure to a wide variety of physical, chemical, biological and technological properties. Quantitative structure property relationship models, once established, can be used to predict properties of compounds as yet unmeasured or even unknown.

A QSRR study involves the prediction of chromatographic retention parameters using molecular structure. Chromatographic retention is a physical phenomenon that is primarily dependent on the interactions between the solute and the stationary phase. QSRRs are statistical models which quantify the relationship between the structure of a molecule and its chromatographic retention index, enabling prediction of the retention indices of novel compounds. Such correlations can provide profound theoretical insight into the interactions between the compounds and the mobile and stationary phases. They can also provide very important information about the effect of the chemical structure on retention behaviour and possible mechanisms of absorption and elution. QSRR models have been successfully developed for a large number of compound classes[11-17].

The aim of this work is use of three different extraction methods for composition of essential oil of *Stachys pilifera* and search for an efficient model to quantitative relationship between the molecular structure and the retention indices of the essential oils by stepwise-MLR.

EXPERIMENTAL

Stachys pilifera was collected from Yasuj, Province of Kohgiluyeh va Boyer ahmad, Iran in May 2010.

Yield: Essential oil yield was proclaimed in terms of the weight of the oil obtained per gram of plant material.

Gas chromatography: GC analysis was utilized on a Shimadzu 15A gas chromatography equipped with a split/splitless injector (250 ºC) and a flame ionization detector (250 ºC). N_2 was used as a carrier gas (1 mL/min) and the capillary applied was DB-5 (30 m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60 ºC for 3 min and then heated to 220 ºC with a 5 ºC/min rate and kept constant at 220 ºC for 5 min. Relative percentage amounts were calculated from peak area using a Shimadzu C-R 4 A chromatopac without the use of correction factors.

Gas chromatography-mass spectrometry: GC-MS analysis was carried out by Hewlett-Packard 6890/5973 GC-MS instrument with a HP-5MS column (30 m × 0.2 mm, film thickness 0.32 µm). The column temperature was as like as GC condition. Helium was used as carrier gas (1 mL/min). Mass spectra were taken at 70 eV. The components of oil were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and those authentic sample[18].

Hydrodistillation: 100 g of air-dried plant material were hydrodistilled using a Clevenger type apparatus for 4 h. The essential oil was collected and analyzed.

Solvent free microwave extraction: a Milestone srl was operated at 2450 MHz. the maximum power of the oven was 1000 w which was measured by ATC-EO sensor.

Solvent free microwave extraction was carried out at atmospheric pressure. 80 g of fresh plant material was heated by an optimize fixed power of 800 w for optimize time 0.5 h without added any solvent or water. A Clevenger system outside the microwave cavity condensed the distillate continuously. Condensed water was refluxed to the extraction vessel in order to provide uniform condition of temperature and humidity for extraction. The essential oil was collected, dried on anhydrous sodium sulphate and stored at until analyzed.

Headspace solid phase micro extraction (SPME): A manual solid phase micro extraction holder and 65 µM PDMS-DVB fiber from supelo (Bellefonte, USA) and were applied for solid phase micro extraction method. The fiber was condition at 250 ºC for 0.5 h in GC injector. 1.5 g of powdered plant material was inserted in 20 mL sample vials sealed with septum -type caps from supelco (Bellefonte, USA) and heated for 10 min at 70 ºC then the solid phase micro extraction needle was penetrated the septum, the SPME fiber was extended through the needle and subjected to the head space above the sample for 15 min. Afterward the fiber was drown into the needle and the needle was removed from the septum and placed on to the injection port of GC. The desorption of analytes from the fiber coating was carried out using heating the fiber in the split less (250 ºC) injection port at for 3 min.

Computer hardware and software: A Pentium IV personal computer (CPU at 3.06 GHz) with the Windows XP operating system was used. The geometry optimization was performed with HyperChem (Version 7.0 Hypercube, Inc). For the calculation of the molecular descriptors, the Dragon 2.1 software was used. The SPSS software (version 11.50, SPSS, Inc.) was employed for the MLR analysis, other calculations were performed in the MATLAB (version 6.5, Math Works, Inc.) environment.

Determination of molecular descriptors: Molecular descriptors are defined as numerical characteristics associated with chemical structures. The molecular descriptor is the final result of a logic and mathematical procedure which transforms chemical information encoded within a symbolic representation of a molecule into a useful number applied to correlate physical properties.

The Dragon software was used to calculate the descriptors in this research and a total of 1481 molecular descriptors, from 18 different types of theoretical descriptor, were calculated for each molecule. Since the values of many descriptors are related to the bonds length and bonds angles *etc.*, the chemical structure of every molecule must be optimized before calculating its molecular descriptors. For this reason, chemical structure of the 44 studied molecules were drawn with the Hyperchem software and saved with the HIN extension. To

optimize the geometry of these molecules, the AM1 geometrical optimization was applied. After optimizing the chemical structures of all compounds, the molecular descriptors were calculated using Dragon. A wide variety of descriptors have been reported in the literature, having been used in the QSRR analysis[19-24]. Descriptors with constant or almost constant values for all molecules were eliminated. Also, pairs of variables with a correlation coefficient greater than 0.90 were classified as intercorrelated and only one of them was considered in developing the model. Then, the remaining descriptors were collected in an n × m data matrix, where n = 44 and m = 415 are the numbers of the compounds and the descriptors, respectively.

RESULTS AND DISCUSSION

The identities of extracted essential oil from three techniques are presented in Table-1. *Cis*-chrysanthenyl acetate was the major compound in the oils of *Stachys pilifera*. The highest percentage of *cis*-chrysanthenyl acetate was (21.76, 28.65 and 26.04 %) in hydrodistillation, solvent free micro-

TABLE-1
PERCENTAGE COMPOSITION OF *Stachys pilifera* BY VARIOUS METHODS AND THE DATA SET AND THE CORRESPONDING OBSERVED AND PREDICTED RI VALUES BY SW-MLR FOR THE TRAINING AND TEST SET

Compounds	HD (%)	SFME (%)	HD-SPME (%)	Exp. (RI)	Pred. (RI)	E (%)
α-Tthujene	0.56	-	-	930	963.9343	3.64884
α-Pinene	4.89	2.58	7.65	939	975.1068	3.845236
Sabinene	0.79	0.43	-	975	942.7164	-3.31114
Myrcene	1.71	1.60	4.12	991	999.1647	0.823886
δ-2-Carene	-	1.07	2.32	1003	1037.775	3.46713
α-Phellandrene	0.36	-	-	1009	1034.589	2.536121
Sylvestrene (iso)	1.97	-	-	1025	1060.743	3.487094
p-Cymene	1.55	0.98	-	1029	1075.527	4.521607
Limonene	3.12	1.91	6.17	1031	1006.603	-2.36635
β-Phellandrene	1.46	0.84	6.72	1037	1002.961	-3.28244
1,8-Cineol	1.24	0.79	-	1060	1038.449	-2.03315
z-Ocimene	0.50	0.40	-	1089	1013.143	-6.96578
γ-Terpinene	0.50	-	-	1123	1174.83	4.61531
Terpinolene	0.62	0.17	1.88	1126	1087.5678	-3.41223
Linalool	6.04	4.27	-	1145	1156.184	0.97673
Mentha-2,8-Dien-1-ol(*trans-para*)	-	1.37	-	1164	1195.705	2.723773
α-Compholnal	0.40	-	-	1183	1083.042	-8.44956
E-Verbenol	1.11	-	-	1189	1180.909	-1.29832
z-Chrysantheanol	2.84	3.27	-	1189	1173.563	-1.29832
Terpinene-4-ol	0.78	0.75	10.81	1216	1250.399	2.828865
Acetophenon(para-methyl	-	-	-	1221	1220.822	-0.01459
Menthe-1(7),8-dien-2-ol(*tran-para*)	3.76	4.46	-	1265	1350.772	6.780422
α-Terpineol	2.25	-	-	1290	1253.029	-2.86597
Linalyl formate	-	0.47	-	1327	1364.607	2.834021
z-Sabinen hydrate acetate	7.84	-	-	1338	1368.33	2.266795
Linalyl acetate	-	15.51	4.89	1362	1395.582	2.465602
Chrysanthenyl acetate	21.76	28.65	26.04	1381	1402.722	1.572914
Thymol	4.08	0.27	-	1471	1467.99	-0.20461
Myrtenyl acetate	0.36	-	-	1481	1519.708	2.613611
δ-Elemene	-	-	1.44	1498	1433.352	-4.31559
α-Terpinyl acetate	0.54	0.54	-	1500	1473.498	-1.76704
Neryl acetate	1.11	0.65	0.94	1522	1439.889	-5.39492
Granyl acette	-	-	1.75	1578	1567.522	-0.66399
E-caryophyllene	2.47	2.18	8.76	159*3	1553.822	-2.45941
β-Acoradiene	0.48	-	1.51	1002	996.9633	-0.50266
Ar-curcumene	2.28	2.47	5.12	1030	1030.142	0.013768
α-Zingiberene	-	-	1.36	1097	1126.45	2.684613
α-Selinene	0.71	0.63	1.01	1177	1134.37	-3.62193
Bicyclogermacrene	1.39	1.50	-	1257	1295.898	3.094526
Myristicin	0.53	-	-	1349	1270.593	-5.81226
α-Selinene(7-epi)	0.92	0.86	2.29	1419	1461.456	2.991951
Spathulenol	3.68	4.95	3.17	1494	1500.016	0.402705
Caryophyllen oxide	3.29	3.57	-	1519	1520.626	0.107018
Viridiflorol	5.03	7.13	-	1583	1547.691	-2.23054
Total (%)	92.52	95.1	97.95			
Monoterpene (%)	72.27	71.34	62.48			
Sesquitepene (%)	20.25	23.76	24.66			
Oxygenated compound (%)	62.49	72.06	45.37			
Yeild (%)	1.4	2.3	-			

TABLE- 2
SELECTED DESCRIPTORS OF MULTIPLE LINEAR REGRESSION

Descriptor	Type of descriptor	Notation	Coefficient
Mean information content on the distance degree magnitude	Topological descriptors	IDDM	1056.31
3D-MoRSE –signal 05/unweighted	3D-MoRSE descriptors	Mor05u	26.15
Number of OH groups	Constitutional descriptors	nOH	100.36
Topological charge index of order 1.	Galvez topological charge indices	GGI1	-65.33
Leverage-weighted autocorrelation of lag 2/ weighted by atomic polarizabilities	GETAWAY descriptors	HATS2p	1256.43
Constant			-2346.40
$R^2_{calibration}$ = 0.953, $R^2_{prediction}$ = 0.966, Q^2_{LOO} = 0.932, Q^2_{LGO} = 0.913, REP (%) = 2.83, RMSEP = 36.68			

wave extraction and headspace solid-phase microextraction respectively.

Fig. 1 indicates that the essential oil obtained by hydrodistillation, solvent free microwave extraction and solid phase micro extraction methods have maximum amount of oxygenated monoterpenes rather than other classes. The higher amount of oxygenated compounds in solvent free microwave extraction oil than in hydrodistillation oil is due to the rapid heating of polar substances by microwave and to the smaller amount of water used, which prohibited the decomposition of principal oxygenated compounds by hydrolytic reaction[25].

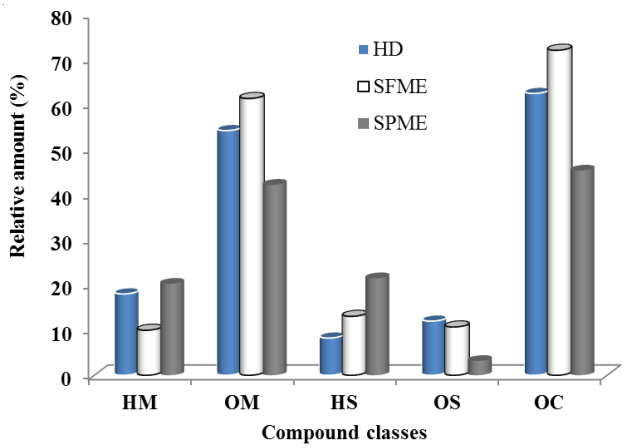

Fig. 1. Comparison of relative amount of different compound classes by various methods

Theoretical results: A stepwise multiple linear regression procedure was used for variable selection. This method has been used for variable selection or model development in variety systems[26,27]. For regression analysis, data set was separated into two groups *i.e.*, training and prediction sets. The molecules included in these sets were selected randomly. The training set, consisted of 34 molecules, was used for the model generation using the SPSS software package. The prediction set, consisted of 10 molecules, was used to evaluate the generated model. It is clear that many MLR models will result using stepwise multiple regression procedure; among them we have to choose the best one. It is common to consider four statistical parameters for this purpose. These parameters are the number of descriptors, coefficient of determination (R^2) for training and prediction sets, standard error for training and prediction sets and F statistic. A reliable MLR model is one that has high R^2 and F values, low standard error and least number of descriptors. In addition to these, the model should have a high predictive ability. Consequently, among different models, the best model as following was chosen.

RI = -2346.40 + 1056.31 (IDDM) + 26.15 (Mor05u) + 100.36 (nOH)-65.33 (GGI1) + 1256.43 (HATS2p)

It is obvious that as the number of descriptors increase the R^2 will increase. Increasing the number of parameters only up to five has a large influence on improving correlation. Therefore, we have chosen five descriptors as optimum number of parameters.

The descriptors appearing in this model are IDDM, Mor05u, nOH, GGI1and HATS2p, whose definitions are given in Table-2. Dragon software can easily calculate these descriptors and their equations are not given here for the sake of brevity[19]. As it can be seen from the correlation matrix (Table-3) there is no significant correlation between the selected descriptors.

TABLE- 3
CORRELATION MATRIX FOR THE FIVE SELECTED DESCRIPTORS

	IDDM	Mor05u	nOH	GGI1	HATS2p
IDDM	1				
Mor05u	-0.063	1			
nOH	-0.237	0.164	1		
GGI1	0.103	0.303	-0.172	1	
HATS2p	-0.157	0.690	0.406	0.435	1

The data set and the corresponding experimental and predicted RI values of all the molecules studied in this work are summarized in Table-1. Fig. 2 shows a plot of values predicted by the SW-MLR against experimental values of the retention indices of the training and prediction sets. The residuals (experimental RI-predicted RI) *versus* experimental RI value, obtained by the SW-MLR modeling, shown in Fig. 3. The distribution of the residuals on both sides of the zero line indicates there is no systematic error in the SW-MLR model.

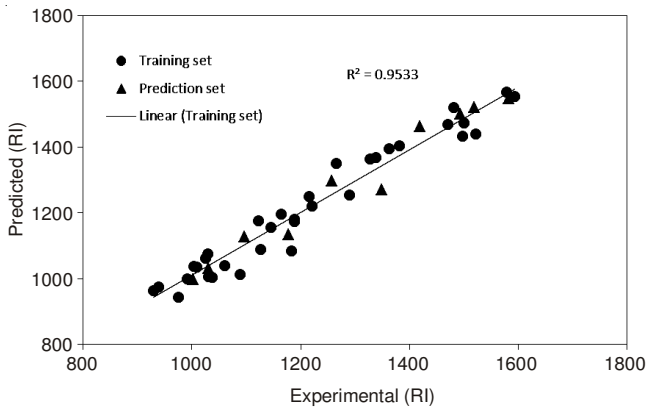

Fig. 2. Predicted RI values by the MLR modeling *vs.* the experimental RI values

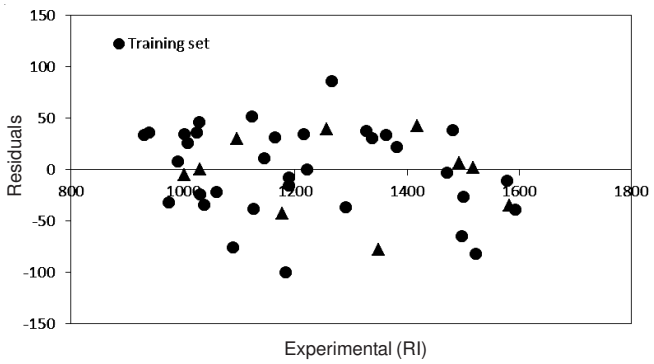

Fig. 3. Plot of the residuals against the experimental values of the retention indices

Statistical parameters: For evaluation of the predictive power of the generated MLR, the optimized model was applied for prediction of RI values of test compounds in the prediction set, which were not used in the optimization procedure. For the constructed models, two general statistical parameters were selected to evaluate the prediction ability of the model for RI. For this case, the predicted RI of each sample in prediction step was compared with the experimental RI.

Root mean square error of prediction (RMSEP) is a measurement of the average difference between predicted and experimental values, at the prediction stage. Root mean square error of prediction can be interpreted as the average prediction error, expressed in the same units as the original response values. The root mean square error of prediction was obtained by the following formula:

$$RMSEP = \left[\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2\right]^{0.5} \quad (1)$$

The second statistical parameter was relative error of prediction (REP) that shows the predictive ability of each component and is calculated as:

$$REP\ (\%) = \frac{100}{y}\left[\frac{1}{4}\sum_{i=1}^{n}(\hat{y}_i - y_i)^2\right]^{0.5} \quad (2)$$

where y_i is the experimental RI of the essential oil in the sample i, $\hat{y}_i$ represents the predicted RI of the essential oil in the sample i, $\bar{y}$, is the mean of experimental RI in the prediction set and n is the total number of samples used in the prediction set. The statistical parameters calculated for the SW-MLR model are listed in under Table-2.

Also the model obtained was validated using leave-one-out (LOO) and leave-group-out (LGO) cross-validation process. For LOO cross-validation, a data point is removed from the set and the model is recalculated. The predicted property for that point is then compared with its actual value. This is repeated until each data point is omitted once. For LGO, 20 % of the data points are removed from the dataset and the model is refitted, the predicted values for those points are then compared with its experimental values. Again, this is repeated until each data point has been omitted once. The cross-validation correlation coefficient (Q^2) is 0.913 for LGO and 0.932 for LOO. This confirms that the obtained regression model has a good internal- and external-predictive power.

Conclusion

In this study, the extraction of essential oil of *Stachys pilifera* through hydrodistillation, HS-SPME and solvent free microwave extraction methods was performed.the result show that the oxygenated compounds in solvent free microwave extraction is higher than hydrodistillation method. Forty -four compounds were identified by applied methods and GC/GC MS equipment. The stepwise-MLR analysis was followed to develop a model for predicting the retention indices of essential oil compounds. The QSRR model with simply calculated molecular descriptors could be employed to estimate the retention index for new compounds, even in the absence of the standard candidates.

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