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Modeling the Morphogenetic and Ontogenetic Changes in Essential Oil Composition of Hypericum Perforatum

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Abstract

In this research, prediction models for the content of several volatile chemical classes, namely, oxygened hydrocarbons, monoterpenes, oxygened monoterpenes and sesquitterpenes hydrocarbons were developed for Hypericum perforatum growing in Northern Turkey. Wild growing plant samples were harvested at their vegetative, floral budding, full flowering, and fresh fruiting stages. The EO composition of the selected plant materials was defined by Gas Chromatography-FID (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS) analyses. Multiple linear regression analysis (MLR) was performed for each volatile group to develop the models. R2 values varied with 0.56 and 0.96 depending of the volatile constituents examined. All R^2 values and standard errors were found to be significant at the levels of $P<0.05$ and $P<0.001$.

Keywords: *Hypericum perforatum, essential oils, GC-MS, modeling, Multiple Linear Regression*

Introduction

Hypericum perforatum L. (Guttiferae) (St. John's wort) is a well-known traditional medicinal plant that has been used for centuries for the treatment of several diseases, such as skin lesions, eczema, burns and microbial, inflammatory and psychological disorders [1,2]. The crude extract of H. perforatum is now widely used in Europe as a drug for the treatment of depression [3]. Proven photodynamic, antiviral, antiretroviral, and antitumoral activities of Hypericum extracts also suggests using of this plant in Acquired Immune Deficiency Syndrome (AIDS) and cancer treatments [4].

H. perforatum contains a number of different secondary metabolites namely naphthodianthrones, phloroglucinols, flavonoids, phenylpropanes, essential oils, amino acids, xanthones, tannins, procyanidins and other water-soluble components [3]. Furthermore, the species is especially rich, as are other members of the genus Hypericum, in volatile compounds [5].

Although the ecology importance of volatile compounds in the direct interaction with the habitat and their proven contribution to bioactivities of Hypericum spp. EOs, [6,7], only a few studies on volatile chemistry of H. perforatum have been undertaken so far [8-11] due to the low essential oil (EO) yields [12]. In previous reports, ontogenetic [10, 13] and morphogenetic changes [14] in the essential oil composition of wild and field grown H. perforatum plants were detected.

Developmental models are commonly explored using computational or simulation techniques [15,16]. The simulation software may be general-purpose, intended to capture a variety of developmental processes depending on the input files, or special-purpose, intended to capture a specific phenomenon. Input data range from a few parameters in models capturing a fundamental mechanism to thousands of measurements in calibrated descriptive models of specific plants (species or individuals). Standard numerical outputs (i.e. numbers or plots) may be complemented by computer-generated images and animations [17].

Most of the researches have focused on the investigation of plant developmental periods as different physiological processes have occurred in different periods of plant growth stage [18-20]. In our previous report, we found significant variations in the content of various volatile constituents of H. perforatum, belonging to oxygened hydrocarbons, monoterpenes, oxygened monoterpenes and sesquitterpenes hydrocarbons [14]. In the present study, the statistical modelling for prediction of the contents of aforesaid volatiles in H. perforatum var. perforatum of Turkish origin were performed by Multiple Linear Regression (MLR) for the first time.

Material And Methods

Brief Description of Plant Material

The plant material was described in our previous study [11]. The species were identified by Dr. Hasan Korkmaz, Faculty of Science and Art, Department of Biology, University of 19 Mayis, Samsun-Turkey. Voucher specimen was deposited in the herbarium of Ondokuz Mayis University Faculty of Agriculture (OMUZF # 61/10).

Experimental Procedures

The procedures were described in our previous study in detail [14]. Briefly, plant material was collected in dry grassland within the Çakallı district of Samsun province, Turkey (41°04' N; 36°01' E; 470 m above sea level) at different stages of plant development. The material represented 30 randomly gathered plants in four phenological stages: vegetative, floral budding, full flowering and fresh fruiting. The top of 2/3 plant, was harvested between 12:00 am and 13:00 pm. After collected, plant materials were dissected into floral, leaf and stem tissues, dried at room temperature (20 ± 2 °C) and subsequently assayed for volatile constituents.

Sample Preparation

Air-dried plant samples (200 g) were hydrodistilled by Clevenger apparatus for 2 h. The essential oil were diluted in n-hexane (HPLC solvent grade, 10%) and injected in GC-FID (injection volume 1 µl) and GC- MS (injection volume 0.1 µl).

Gas Chromatography-FID and Gas Chromatography-Mass Spectrometry Analyses

The analyses were described in our previous study in detail [14]. Briefly, GC analyses were accomplished by HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m x 0.25 mm, 0.25 µm film thicknesses). GC/EIMS analyses were performed by a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector.

Multiple Linear Regression Analyses (Mlr)

MLR is a statistical method used to investigate the relationship between several independent variables and a dependent variable. A linear regression model assumes that the relationship between the dependent variable and the p-vector of regressors is linear, where p is the number of independent variables. Thus the model takes the form $y_i = \beta_1\chi_{i1} + \ldots + \beta_p\chi_{ip} + \varepsilon_i = \chi_i{}'\beta + \varepsilon_i$ $i = 1, \ldots, n$, where ′ denotes the transpose, so that $x_i'\beta$ is the inner product between vectors x_i and β. The y_i is called the dependent variable and the χ_i is called regressor or independent variable. The decision as to which variable in a data set is modeled as the dependent variable and which are modeled as the independent variables may be based on a presumption that the value of one of the variables is caused by, or directly influenced by the other variables.

Result And Discussion

Significant differences in the EOs yield and volatile composition were observed during the ontogenetic cycle of the selected plant material. The analyzed samples were collected from different plant organs of the same species H. perforatum var. perforatum during its phenological cycle: leaves at the stages of vegetative, floral budding, flowering and green capsule; buds, full opened flowers and green capsules. The highest and comparable values in terms of the EO yields were obtained for the leaf at vegetative stage (1), leaf at fruiting (4), and full opened flowers (6) (0.44, 0.61, and 0.53 % v/w dry plant, respectively). The lowest yield resulted from the floral buds (5) and green capsules (7) (0.04 and 0.07 % v/w dry plants, respectively). Several peculiar tendencies in specific variations of the typical volatiles were observed during the phenological cycle (Table 1)

Table1. *Typical chemical classes of volatile constituents in the essential oils of Turkish H. perforatum samples collected in different phonological stages (1-7)**

Sample	1	2	3	4	5	6	7
Chemical classes							
Monoterpene hydrocarbons	2.30	2.28	1.24	1.93	2.40	2.55	2.58

Oxygenated hydrocarbons	37.67	33.39	25.15	24.03	19.46	12.81	12.85
Monoterpenes	0.52	0.99	0.80	0.48	2.55	2.56	5.78
Oxygenated monoterpenes	1.99	2.17	0.85	2.09	1.97	4.12	4.67
Sesquiterpenes hydrocarbons	26.42	25.59	33.60	26.76	32.02	40.15	50.39
Oxygenated sesquiterpenes	28.77	31.63	33.73	40.17	33.31	33.34	19.85

**1-leaf at vegetative stage, 2-leaf at floral budding, 3-leaf at full flowering, 4-leaf at fruiting, 5-floral buds, 6-full opened flowers, 7-green capsules*

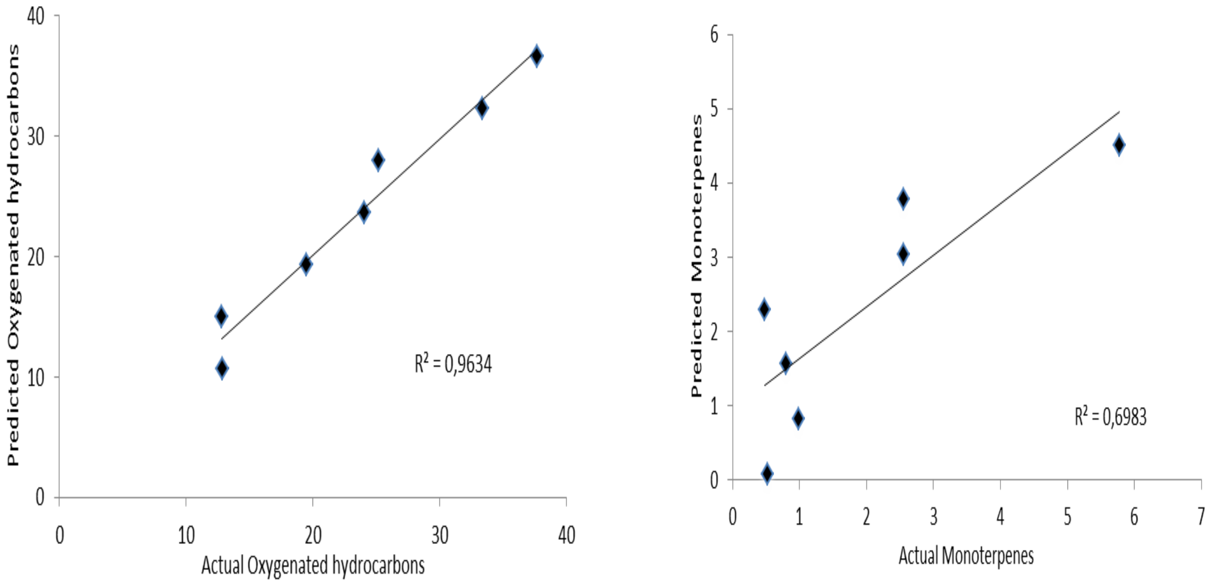
- oxygenated derivatives of monoterpenes and sesquiterpenes showed high levels at each phenological stage
- oxygenated hydrocarbons showed a decrease from the first up to the last phenological stage
- oxygenated sesquiterpenes were the only volatiles which displayed the lowest production at the last phenological stage compared with the other constituents
- monoterpene hydrocarbons and sesquiterpene hydrocarbons showed higher percentages comparing the first and last phenological stages after increase/decrease fluctuations.

The variation explained by the parameters in H. perforatum was 9% for oxygened hydrocarbons, 69% for monoterpenes, 56% for oxygened monoterpenes, and 72% for sesquiterpenes hydrocarbons. The produced prediction equations were OH= (40.95) - (4.33 x S); MT= (-0.65) + (0.73 x S); OM= (0.68) + (0.46 x S); and SH= (19.35) + (3.55 x S) where OH: oxygened hydrocarbons, MT: monoterpenes, OM: oxygened monoterpenes, SH: sesquiterpenes hydrocarbons, and S: stages (Table 2). Relationships between predicted and actual oxygenated hydrocarbons, monoterpenes, oxygenated monoterpenes, and sesquiterpenes hydrocarbons were shown in Figure 1.

Table2. *The coefficients, their standard errors and R^2 values of the predicting chemical classes in Turkish H. perforatum*

	Coefficients and Standard Errors (SE)		
Chemical Classes	a	b	R^2
Oxygened hydrocarbons	40.95±1.687***	-4.33±0.377***	0.96
Monoterpenes	-0.65±0.970**	0.73±0.217**	0.69
Oxygened monoterpenes	0.68±0.825*	0.46±0.184*	0.56
Sesquiterpenes hydrocarbons	19.35±4.416**	3.55±0.987**	0.72

*R^2: regression coefficiency, SE: standard error; a and b are coefficients of produced equations, *significant at the level of $p<0.05$, **significant at the level of $p<0.01$, ***significant at the level of $p<0.001$*



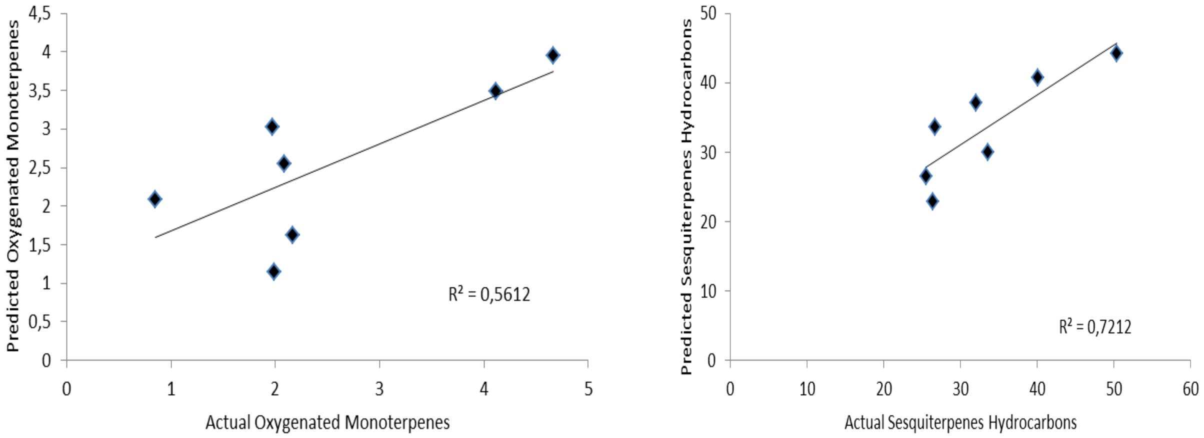


Figure1. *Relationships between predicted and actual oxygenated hydrocarbons, monoterpenes, oxygenated monoterpenes, and sesquiterpenes hydrocarbons*

Conclusions

In the present study, we have developed for the first time prediction models for the content of several H. perforatum constituents belonging to different chemical classes of volatile compounds (oxygened hydrocarbons, monoterpenes, oxygened monoterpenes and sesquiterpenes hydrocarbons). As the understanding of plant growth and development has been increasing, such mathematical models, performed in the present study could be very useful tools for the prediction of secondary metabolite profiles for many plants without using of expensive analytical experimental plans. Due to the complexity of Hypericum spp. chemistry, prediction of secondary metabolite composition by using simple equations instead of expensive and time-consuming analytical procedures may represent a complementary important topic for phytochemical and taxonomical studies on the Hypericum genus. Hence, the models produced in the present study can be used safely by Hypericum perforatum researchers. On the other hand, different models can be developed for other Hypericum species and their relative phytochemicals which can be very different from those used for H. perforatum in the present study.

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