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Prediction model of the effect of light intensity on phenolic contents in *hypericum triquetrifolium* turra

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The objective of the present study is to develop a prediction model for the phenolic contents in *Hypericum triquetrifolium* Turra with multiple regression analysis. The best estimating equations for the phenolic compounds (chlorogenic acid, rutin, hyperoside, isoquercetrin, quercitrin and quercetin) contents are formulized as PC = (a) + (b × L) + (c × L²), where PC is the phenolic content, L is light intensity (μmol m² s^{-1}) and a, b and c are coefficients. Multiple regression analysis was carried out until the least sum of squares (R^2) was obtained. R^2 value was 0.70 for rutin and 0.97 for chlorogenic acid. Standard errors were found to be significant at the $p<0.001$ level.

Keywords: *Hypericum triquetrifolium*, phenolic compounds, modeling, light intensity.

INTRODUCTION

The genus *Hypericum* L. includes, at the most recent count, 469 species of flowering plants classified into 36 taxonomic sections [1]. The species of this genus have been used as traditional medicinal plants due to their wound-healing, bactericide, anti-inflammatory, diuretic and sedative properties for hundreds of years [2]. Despite the large number of *Hypericum* species, undoubtedly, only *H. perforatum* has been studied in depth as a pharmaceutical important medicinal plant [3]. In particular, extracts of *H.perforatum* are now widely used in Europe as a drug for the treatment of depression [4]. In Turkey, the genus is represented by 89 species, of which 43 are endemic [5].

Hypericum triquetrifolium Turra. is an herbaceous perennial plant, which grows in open, dry stony, sandy ground and cultivated fields in Turkey [6]. It has been traditionally used by Turkish folk in the treatment of bile and intestine ailments [7]. The plant has great pharmaceutical potential with its well-documented antinociceptive, anti-inflammatory, antioxidant, antibacterial, antifungal and cytotoxic activities [8, 9].

Developmental models are commonly explored using computational or simulation techniques. 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 [10, 11]. Input data range from a few parameters in models capturing a fundamental mechanism to thousands of measurements in calibrated descriptive models of specific plants. Standard numerical outputs (i.e. numbers or plots) may be complemented by computer-generated images and animations [12].

The content of bioactive compounds in plants varies with internal factors, as organs, age of the plant, phenological stage or external environmental factors [13]. The light intensity within the crop environment strongly increases its growth and yield. The light intensity can be identified as a key environment variable related to crop yields, phenological development, etc. The relationship between chemical composition and light intensity in *H. perforatum* was investigated in a previous study [14], but to author's knowledge there is no report on *H. triquetrifolium* in terms of the same relationship. In the present study, the effect of light intensity on phenolic compounds accumulation in *H. triquetrifolium* was quantitatively examined for the first time.

MATERIAL AND METHODS

Plant material and culture conditions

H. triquetrifolium plantlets were established from 5-month-old seeds collected on plants growing wild in the Erbaa district of Tokat, Turkey. Seeds were germinated in a float system, commonly used for seedling production of broad-leaves tobacco Burley and Flue-Cured-Virginia under a 16 h light/8 h dark cycle. Newly emerged seedlings were transferred to pots filled with the commercial peat tray substrate, 30 cm in diameter and watered daily until they reached maturity. Then, the pots were transferred to a greenhouse, separated into shaded and unshaded parts. Polyethylene cover of 50% transparency was used for shading. Plants were watered daily until they reached

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maturity, then three times a week. Light intensities were measured at one-meter height over the plants with a Delta-T Sun Scan Canopy Analyzer. The measurements were performed daily in both shaded and unshaded parts separately. 100 pots were treated for each experimental part, thus a total of 200 pots were used. Ten pots (5 pots for shaded and un-shaded parts) were harvested weekly and 20 samples were taken between May-September, 2007. The aerial parts of the plants were dried at room temperature (20 ± 2°C) and subsequently assayed for the phenolic concentrations by HPLC.

Preparation of plant extracts

Air-dried plant material was mechanically ground with a laboratory mill to obtain a homogenous drug powder. Samples of about 0.5 g (weighed with 0.0001 g precision) were extracted in 50 ml of 100 % methanol by ultrasonication at 40º C for 30 min. in a Sonorex Super model RK 225H ultrasonic bath. The prepared extracts were filtered through a membrane filter with pore size of 0.22 µm (Carl Roth GmbH, Karlsruhe, Germany) and kept in a refrigerator until analysis, no longer than 3 h.

HPLC analysis

A Shimadzu Prominence LC-20A (Shimadzu Europa GmbH, Duisburg, Germany) chromatographic system equipped with two LC-20AD model pumps, a SIL-20AC auto-injector, a thermostat CTO-20AC and a SPD-M20A detector was used for HPLC analysis. Separation of all compounds was carried out using an YMC Pack Pro-C18 (YMC Europe GmbH, Dinslaken, Germany) column (150 mm × 4 mm i.d.; 3 µm particle sizes) with 10 mm guard-precolumn. For mobile phase, 0.1 % aqueous trifluoroacetic acid (TFA) was used as eluent A and acetonitrile containing 0.1 % TFA as eluent B. The following binary gradient elution program was used: 0–1 min (B 5→5%), 1–14 min (B 5→20%), 14–20 min (B 20→80 %), 20–30 min (B 80→100 %), 30–39 min (B 100→100 %), 39–39.5 min (B 100→5 %), 39.5–45 min (B 5–5 %). The mobile phase was delivered with a flow rate of 1.0 mL min^{-1}; volume of extract injected was 10 µL. Detection was performed in the 210–790 nm wavelength range with a constant column temperature at 40º C. The eluted compounds were identified by comparison of their retention times and UV spectra with those of reference standards. The maximal absorption on the UV spectra of the compounds was obtained as follows: chlorogenic acid – 325 nm, rutin, hyperoside and isoquercetin – 353 nm, quercetrin – 347 nm and quercetin – 368 nm wavelength.

The quantities of compounds were calculated from external standard calibration curves. The stock solutions of reference standards were prepared by dissolving in HPLC-grade methanol. Calibration curves were established on six concentrations in following ranges: 6.0–300 µg/mL for chlorogenic acid and rutin, 3.0–300 µg/mL for hyperoside and isoquercetin, 2.0–200 µg/mL for quercitrin and quercetin. All calibration cures showed good linear regression ($r^2 \geq 0.999$) within the test range. Each sample was analyzed twice and the mean value was used for calculation. The concentration of compounds was expressed as mg/g dry mass (DM).

Chemicals

The reference substances chlorogenic acid (purity 98.03%), rutin trihydrate (purity 99.02%), isoquercetin (purity 99.0%), quercitrin (purity 99.0%), were purchased from Karl Roth (Germany). Hyperoside (purity 98.6%) and quercetin (purity 95.4%) reference materials were obtained from ChromaDex (USA). Acetonitrile and methanol of HPLC grade were supplied by Karl Roth (Germany).

Model construction

The general purpose of multiple regressions is to learn more about the relationship between several independent or predictor variables and a dependent or criterion variable. The linear regression model assumes that the relationship between the dependent variable y_i and the p-vector of the regressors x_i is linear. Some remarks on terminology and general use: y_i is called the dependent variable. The decision as to which variable in a data set is modelled as the dependent variable and which is modelled as the independent variable may be based on the presumption that the value of one of the variables is caused by, or directly influenced by the other variables. x_i is called independent variable. Usually a constant is included as one of the regressors. This variable captures all other factors that influence the dependent variable y_i other than the regressors x_i. The relationship between the error term and the regressors, for example, whether they are correlated, is a crucial step in formulating a linear regression model, as it will determine the method to be used for estimation [15].

The best estimating equations for the rooting percentage and root growth were determined with the R-program and formulized as $P_C = (a) + (b \times L) + (c \times L^2)$ where P_C is phenolic content, L is light intensity (µmol m^2 s^{-1}) and a, b and c are coefficients. Multiple regression analysis was carried out until the least sum of squares (R^2) was obtained.

RESULTS AND DISCUSSION

The light intensity is one of the major environmental factors affecting plant physiology, especially photosynthesis and plant development. Main function of plant secondary metabolites is thought to be the adaptation of plants to their environment. The physiological changes in plants in response to different stress factors may stimulate secondary metabolite production for the restoration of the defensive systems. The increase in secondary metabolite concentrations of plants observed in the present study under high light intensities may be attributed to those possible physiological changes. The development of prediction models for the content of phenolics, namely, chlorogenic acid, rutin, hyperoside, isoquercetrin, quercitrin and quercetin in *H. triquetrifolium* have potential using fields in pharmacological treatments. The developed mathematical models could be applied as very useful tools for prediction of phenolic compounds content for *H. triquetrifolium* instead of using expensive and time-consuming analytical devices. The obtained results are also important for plant physiology, agronomy and phyochemical studies on *H. triquetrifolium*.

In the present study, prediction equations were developed for estimation of the contents of chlorogenic acid, rutin, hyperoside, isoquercitrin, quercetin and quercitrin. Results of the statistical analysis revealed that most of variations in phenolic compounds levels in plant material could be explained by differences in light intensity.

The variation in the content of chlorogenic acid was explained by 97% of changing environmental parameters. The equation for chlorogenic acid was as follows: P_{chol} = (27.659)-(0.124 × L)-(1.43E^{-3} × L), where P_{cho}: chlorogenic acid content in the aerial parts (Table 1). Relationship between actual and predicted content of chlorogenic acid is shown in Figure 1.

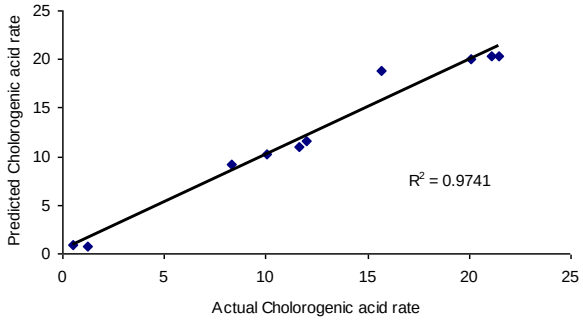

Fig. 1. Relationship between actual and predicted content of chlorogenic acid (mg/g DM) in greenhouse-grown *Hypericum triquetrifolium*.

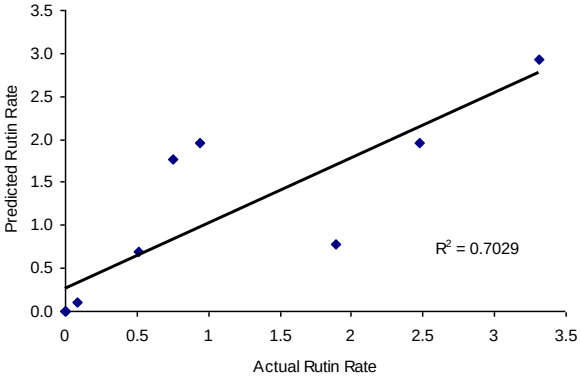

Fig. 2. Relationship between actual and predicted content of rutin (mg/g DM) in greenhouse-grown *Hypericum triquetrifolium.*

The variation between actual and predicted contents for hyperoside was explained by 86%. The equation for hyperoside was as follows: $P_{hyp.}$= (62.518)-(0.245 × L)+(2.27 E^{-4} × L²), where P_{hyp}: hyperoside acid content in the aerial part of the plant (Table 1). Relationship between actual and predicted content of hyperoside is shown in Figure 3.

Table 1. Coefficients, their standard errors and r² values of the new produced equations predicting phenolic compounds contents in greenhouse-grown *Hypericum triquetrifolium.*

Secondary metabolites (mg/g) with standard errors	Coefficient (a)	L (b)	L² (c)	r²
Chlorogenic acid	27.659 ± 10.269***	-0.124 ± 0.0361***	1.43E^{-4} ± 2.99E^{-5}***	0.97***
Rutin	5.715 ± 8.483***	-0.023 ± 0.030***	2.32E^{-5} ± 2.5E^{-5}***	0.70***
Hyperoside	62.518 ± 34.327***	-0.245 ± 0.121***	2.27 E^{-4} ± 9.98E^{-5} ***	0.86***
Isoquercetrin	70.328 ± 51.083***	-0.276 ± 0.179***	2.58 E^{-4} ± 1.49E^{-4} ***	0.86***
Quercitrin	15.588 ± 23.689***	-0.062 ± 0.0833***	6.11E^{-5} ± 6.89E^{-5} ***	0.87***
Quercetin	6.867 ± 5.683***	-0.025 ± 0.020***	2.21E^{-5} ± 1.65E^{-5} ***	0.85***

R²: regression coefficient, SE: standard error, L: light (µmol m^{-2} s^{-1}) of produced equations.
**: Significant at the level of p < 0.001.

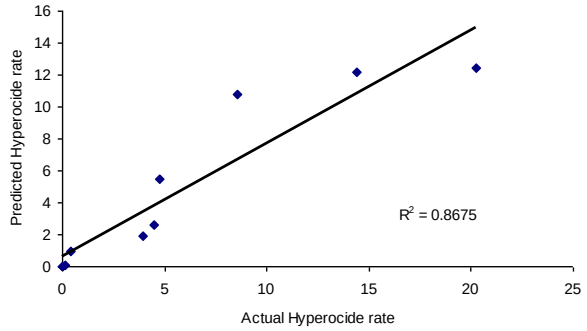

Fig. 3. Relationship between actual and predicted content of hyperoside (mg/g DM) in greenhouse-grown *Hypericum triquetrifolium*.

The variation explained by the parameters for isoquercetrin was 86%. The equation for isoquercetrin was P_{qcetin} = (70.328)-(0.276 × L)+(2.58E^{-4} × L²), where P_{isoq}: isoquercetrin content in the aerial parts of the plant (Table 1). Relationship between actual and predicted content of isoquercetrin in *H. triquetrifolium* is shown in Figure 4.

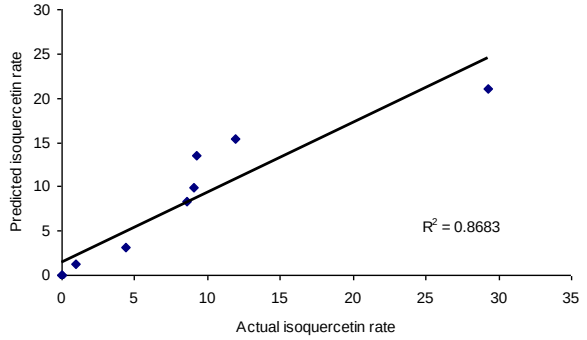

Fig. 4. Relationship between actual and predicted content of isoquercetrin (mg/g DM) in greenhouse-grown *Hypericum triquetrifolium*.

As for quercitrin, the variation explained by the parameters was 87%. The equation for quercitrin was: $P_{qcitrin}$ = (15.588)-(0.062 × L)-(2.76E^{-3} × L+[6.11E^{-5} × L), where $P_{qcitrin}$: quercitrin content in the aerial parts of the plant (Table 1). Relationship between actual and predicted content of quercitrin in *H. triquetrifolium* is shown in Figure 5.

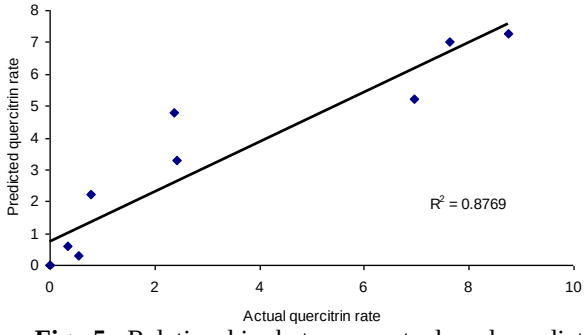

Fig. 5. Relationship between actual and predicted content of quercitrin (mg/g DM) in greenhouse-grown *Hypericum triquetrifolium*.

The variation explained by the parameters for quercetin was 85%. The equation for quercetin was: P_{qcetin} = (6.867)-(0.025 × T-(0.025 × L)+(2.21E^{-5} × L²), where P_{qcetin}: quercetin content in the aerial parts of the plant (Table 1). Relationship between actual and predicted content of quercetin in *H. triquetrifolium* is shown in Figure 6.

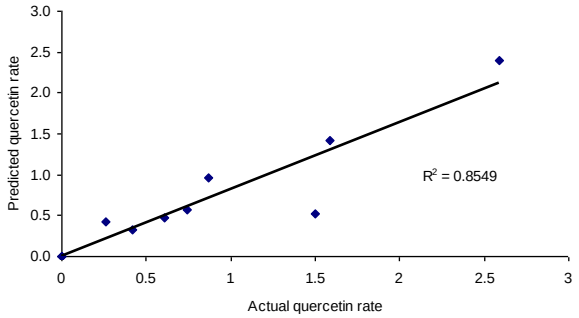

Fig. 6. Relationship between actual and predicted content of quercetin (mg/g DM) in greenhouse-grown *Hypericum triquetrifolium.*

H. triquetrifolium was treated during different phenological phases: vegetative, floral budding, full flowering, fresh fruiting, mature fruiting. In the present study, the contents of chlorogenic acid, rutin, hyperoside, isoquercetrin, quercetin and quercitrin increased in greenhouse-grown plants in response to elevating temperatures from 20 to 30 °C. We observed that increases in light intensities from 789.4 µmol m^{-2} s^{-1} to 1716.6 µ mol m^{-2} s^{-1} resulted in a continuous increase in the contents of chlorogenic acid, rutin, hyperoside, isoquercetrin, quercetin and quercitrin. The obtained results are also important for plant physiology, agronomy and phyochemical studies on *H. triquetrifolium*.

CONCLUSION

Environmental factors have a prominent effect on secondary metabolism resulting in high variability of phytochemical contents of wild/cultivated plants. On the other hand, they determine the quality of the products derived from the corresponding plants. Hence, description of the effect of different environmental factors on secondary metabolism seems to be the first step in optimizing the production technologies of medicinal plant materials in terms of chemical profile. In the present study, we have developed prediction models for the secondary metabolite contents in *H. triquetrifolium*. The phenolic contents determined by the produced models (chlorogenic acid, rutin, hyperoside, isoquercetrin, quercitrin and quercetin) were found statistically acceptable (R²=0.70 – 0.97). Thus, the models produced in the present study can be used safely by researchers for the standardization of *H. triquetrifolium* plant material quality.

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МОДЕЛ, ПРЕДСКАЗВАЩ ЕФЕКТА НА ИНТЕНЗИВНОСТТА НА СВЕТЛИНАТА ВЪРХУ ФЕНОЛНОТО СЪДЪРЖАНИЕ В *HYPERICUM TRIQUETRIFOLIUM* TURRA

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Целта на настоящото изследване е да се разработи модел, предсказващ фенолното съдържанието в Hypericum triquetrifolium Turra с множествена регресия. Най-добрите изчислителни уравнения за съдържанието на фенолните съединения (хлорогенова киселина, рутин, хеперозид, изокверцетин, кверцитрин и кверцетин) са представени като PC = (a) + (б × L) + (в × L^2), където PC е фенолното съдържание, L е интензитетът на светлината (ммол м² $сек^{-1}$) и , B и C са коефициенти. Множествин регресионен анализ се провежда, докато се получи сумата от най-малките квадрати (R^2). R^2 стойност е 0,70 за рутина и 0.97 за хлорогенновата киселина. Стандартните грешки се оказаха значителни при ниво p <0.001.