

## Regular Article

## Models of estimation on the content of secondary metabolites in some *Hypericum* species

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In the present study, models for estimation of the content of main secondary metabolites, namely hypericin, pseudohypericin and hyperforin were developed for *Hypericum originafolium* Willd, *Hypericum perforiatum* L. and *Hypericum montbretii* Spach. growing in Northern Turkey. Wild growing plants were harvested at vegetative, floral budding, full flowering, fresh fruiting, mature fruiting stages and dissected into stem, leaf and reproductive tissues. Actual secondary metabolite contents of plant materials were measured by High Performance Liquid Chromatography method. Multiple regression analysis with Excel 2003 computer package program was performed for each species and chemicals separately to develop multiple regression models. The produced equation for predicting the content of secondary metabolites in different tissues of the species was formulized as:  $SMC = [a + (b_1 \times S) + (b_2 \times L) + (b_3 \times RP) + (b_4 \times S^2) + (b_5 \times (1/RP))]$  where SMC is whole plant secondary metabolite content, S is stem secondary metabolite content, L is leaf secondary metabolite content, RP is secondary metabolite content of reproductive parts and a, b<sub>1</sub>, b<sub>2</sub>, b<sub>3</sub>, b<sub>4</sub>, and b<sub>5</sub> are coefficients?. The R<sup>2</sup> coefficient values between predicted and observed contents of secondary metabolites were determined as 0.99 for *H. originafolium*, 0.95–0.98 for *H. perforiatum* and 0.90–0.99 for *H. montbretii*. All R<sup>2</sup> values and standard errors were found to be significant at the P<0.05 level.

**Keywords:** *Hypericum originafolium*; *Hypericum perforiatum*; *Hypericum montbretii*; Modeling; Hypericins, Hyperforin; Plant growth stages.

### Introduction

*Hypericum* is a genus of about 400 species of flowering plants in the family Guttiferae. The genus has a nearly world-wide distribution, missing only from tropical lowlands, deserts and Polar Regions (Robson, 1981). Turkey is an important center for the genus *Hypericum* and 43 of the present 89 species are endemic (Davis, 1988). These plants are used as sedatives, antiseptics, and antispasmodics in Turkish folk medicine under the names: "kantaron, peygamber çiçeği, kılıçotu, kanotu, kuzukıran and binbirdelik otu" (Baytop, 1999).

Recently, *Hypericum* species have received considerable interest due to the increasing market demand for crude material of *Hyperici herba*. The plants contain a broad range of structurally diverse natural compounds, namely the phloroglucinol derivatives hyperforin and adhyperforin, the naphthodianthrones hypericin and pseudohypericin and the flavonoids: hyperoside, rutin, quercitrin, quercetin and biapigenin, which possess a wide array of biological properties (Greeson *et al.*, 2001; Patocka, 2003; Radusiene *et al.*, 2004; Tanaka and Takaishi, 2006).

Many pharmacological activities of *Hypericum* extracts appear to be attributable to their hypericins and hyperforin content (Barnes *et al.*, 2001). The naturally occurring red pigments hypericin and pseudohypericin have been reported to exhibit important biological activities, namely photodynamic, antiviral, antiretroviral, antibacterial, antipsoriatic, antidepressant and antitumoral activities (Guedes and Eriksson, 2005). Hypericins have been found only in *Hypericum* species, thus, are chemotaxonomically important for the infrageneric classification of *Hypericum* genus (Kitanov, 2001). Hyperforin is a prenylated phloroglucinol derivative that consists of a phloroglucinol skeleton with lipophilic isoprene chains (Medina *et al.*, 2006). Results from recent studies have indicated hyperforin as the main chemical, responsible for antidepressant effects of *Hypericum* extracts (Roz and Rehavi, 2004). It also exhibits anti-inflammatory (Feisst and Werz, 2004), antitumoral (Schwarz *et al.*, 2003) and antiangiogenic (Dona *et al.*, 2004) effects.

Developmental models have been utilized by using computational or simulation techniques (Uzun, 1996; Odabas, 2003). 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. Standard numerical outputs (i.e. numbers or plots) may be complemented by computer-generated images and animations (Prusinkiewicz, 2004).

Many of the recent studies have focused on investigation of plant developmental periods. Because several important physiological processes e.g. secondary metabolite accumulations in special plant tissues have occurred in different stages of plant phenology (Ellis *et al.*, 1990). Results from our previous studies revealed the presence of significant variations in the content of main chemicals of *Hypericum* namely, hypericin, pseudohypericin and hyperforin in *Hypericum perforatum* L., *Hypericum montbretii* Spach and *Hypericum organifolium* Willd (Cirak *et al.*, 2007a, b; Cirak and Radusiene, 2007; Cirak *et al.*, 2008a, b). We also described the close relationships among phenolic contents of different plant tissues by developing mathematical models in those species of *Hypericum* (Odabas *et al.*, 2008).

Thus in the present study, we aimed to develop models for estimation of the contents of hypericin, pseudohypericin and hyperforin in the aforesaid species of *Hypericum*.

## Materials and Method

### Plant Material

The plant materials were described in our previous studies (Cirak *et al.*, 2007a, b; Cirak and Radusiene, 2007). The plant species were identified by Dr. Hasan Korkmaz, Faculty of Science and Art, Department of Biology, University of 19 Mayıs, Samsun-Turkey. Voucher specimens were deposited in the herbarium of Ondokuz Mayıs University Agricultural Faculty (OMUZF # 101 for *H. perforatum*, OMUZF # 109 for *H. organifolium* and OMUZF # 100 for *H. montbretii*).

### Experimental Procedures

The plant material of the species examined was collected in dry grassland within the Çakallı district of Samsun province, Turkey (41° 04' N; 36° 01' E; 470 m above sea level) from April till September 2005. The mean temperature during the sampling period was 18.5°C, and the precipitation sum 450 mm. The sampling site was not grazed or mown during the plant gathering period. The material represented 20 randomly gathered plants in five phenological stages: vegetative, floral budding, full flowering, fresh fruiting and mature fruiting. Newly emerged shoots (4-6 weeks old-age) with leaves were harvested at the vegetative stage (27<sup>th</sup> of April, 2005 for all species). For the floral budding stage, only shoots with floral buds were selected (20<sup>th</sup> of May for *H. organifolium* and *H. montbretii*; 10<sup>th</sup> of June for *H. perforatum*). At the full flowering stage, only shoots with full opened flowers were harvested (14<sup>th</sup> of June for *H. organifolium* and *H. montbretii*; 24<sup>th</sup> of June for *H. perforatum*).

At the fresh fruiting stage, the shoots which had green capsules were harvested (5<sup>th</sup> of July for *H. origanifolium* and *H. montbretii*; 25<sup>th</sup> of July for *H. perfoliatum*). At the mature fruiting stage, the shoots which had dark brown capsules were harvested (10<sup>th</sup> of August for *H. origanifolium* and *H. montbretii*; 10<sup>th</sup> of September for *H. perfoliatum*). The top of 2/3 plant, was harvested between 12:00 am and 13:00 pm. After collecting 10 shoots were kept as whole plants and the rest were dissected into floral, leaf and stem tissues, then dried at room temperature ( $20 \pm 2$  °C) and assayed for the content of hypericin, pseudohypericin and hyperforin by HPLC.

#### Model Construction

Multiple regression analysis was performed for quantitative data of hypericin, pseudohypericin and hyperforin for each species separately. A search for the best model for predicting secondary metabolite contents was conducted with various subsets of the independent variables, namely secondary metabolite contents of stem, leaf, reproductive parts and whole plant at different stages of plant phenology (Cirak *et al.*, 2007a, b; Cirak and Radusiene, 2007; Cirak *et al.*, 2008a, b). The best estimating equation for the content of secondary metabolite tested were determined with the Excel 2003 and formulized as:  $SMC = [a + (b_1 \times S) + (b_2 \times L) + (b_3 \times RP) + (b_4 \times S^2) + (b_5 \times (1/RP))]$ , where SMC estimate the content of secondary metabolite of whole plant, S indicate secondary metabolite content of stem, L – secondary metabolite content of leaf, RP –secondary metabolite content of reproductive parts and a, b<sub>1</sub>, b<sub>2</sub>, b<sub>3</sub>, b<sub>4</sub>, and b<sub>5</sub> are coefficients of the produced equation. Multiple regression analysis was carried out until the least sum of square was obtained (Odabas, 2007a, b).

#### Results and Discussion

In the present study, prediction equations were developed for hypericin, pseudohypericin and hyperforin contents in three *Hypericum* species: *H. perfoliatum*, *H. origanifolium* and *H. montbretii*. Multiple regression analysis used for determination of the best fitting mathematical equations for estimation of secondary metabolite contents in evaluated *Hypericum* species showed that observed variability was explained by the selected variables: content of secondary metabolite in stem, leaf, reproductive parts and whole shoot during plant growth. Summary statistics of the new produced equations predicting secondary metabolite contents in *H. origanifolium*, *H. perfoliatum* and *H. montbreti* are shown in Table 1. Actual contents of hypericin, pseudohypericin and hyperforin in different tissues of the examined species of *Hypericum* are also shown in Table 2, 3 and 4.

The comparison of observed and predicted by the regression equation values of secondary metabolites in *Hypericum* species are shown in Figure 1, where R<sup>2</sup> values varied between 0.9-0.99.

The variability between actual and predicted secondary metabolite contents in plant parts of *H. origanifolium* was explained by 99% of the observed cases. Secondary metabolite contents for this species were estimated by following equations:  $H = (-0.153) + (0.84 \times S) + (0.77 \times L) + (0.26 \times RP)$ ;  $HY = (0.29) + (0.14 \times L) + (0.22 \times RP) + (11.40 \times S^2)$ ;  $PS = (-0.150) + (0.76 \times S) + (0.75 \times L) + (0.45 \times RP)$ , where H: whole plant hypericin content, HY: whole plant hyperforin content, PS: whole plant pseudohypericin content, L: leaf secondary metabolite content, S: stem secondary metabolite content; RP: secondary metabolite content of reproductive parts (Fig. 2)

The variability explained by the parameters of *H. perfoliatum* was 96% for hypericin, 95% for pseudohypericin and 98% for hyperforin. The produced equations for estimation of secondary metabolite contents for this species can be expressed as:  $H = (0.04) + (0.38 \times S) + (0.23 \times RP)$ ;  $HY = (0.04) + (0.50 \times L) + (1.61 \times S^2) + (0.11 \times (1/RP))$ ;  $PS = (-14.12) + (6.11 \times L) + (170.39 \times S^2) + (1.13 \times (1/RP))$  (Fig. 3)

In *H. montbretii* the variability stand for hypericin was 99%, for pseduhypericin - 90% and for hyperforin - 92%. The produced equations for estimation of secondary metabolite contents for the last species were: H = (-0.34) + (1.22 x L) + (4.83 x S<sup>2</sup>) + (0.02 x (1/RP)); HY= (1.63) + (-1.56 x L) + (-10 x RP) + (-3.78 x S<sup>2</sup>); PS = (0.59) + (0.18 x L) + (-3.02 x S<sup>2</sup>) + (-0.07 x (1/RP)) (Fig. 4).

Table 1. The coefficients, their standard errors and R<sup>2</sup> values of the new produced equations predicting secondary metabolite contents in plant parts of *Hypericum organifolium*, *H. perfoliatum* and *H. montbretii*

<i>Hypericum organifolium</i>								
Secondary Metabolites,	Standard Errors (SE)	Coefficient?	Stem	Leaf	Reproductive part	s <sup>2</sup> ?	1/rp?	R <sup>2</sup>
Hypericin		-0.153±0.10*	0.84±0.14*	0.77±0.13*	0.26±0.02*			0.99
Hyperforin		0.290±0.06*		0.14±0.1*	0.22±0.02*	11.40±1.4*		0.99
Pseudohypericin		-0.150±0.05*	0.76±0.21*	0.75±0.07*	0.45±0.02*			0.99
<i>Hypericum perfoliatum</i>								
Hypericin		0,04±0,12*	0,38±0,25*		0,23±0,04*			0,96
Hyperforin		0,04±0,14*		0,50±0,20*		1,61±0,49*	0,11±0,03*	0,98
Pseudohypericin		-14,12±4,10*		6,11±1,65*		170,39±4,1*	1,13±0,33*	0,95
<i>Hypericum montbretii</i>								
Hypericin		-0.34±0.03*		1.22±0.02*		4.83±0.86*	0.02±0.005*	0.99
Hyperforin		1.63±0.42*		-1.56±1.01*	-0.10±0.03*	-3.78±9.98*		0.92
Pseudohypericin		0.59±0.79*		0.18±0.71*		-3.02±3.08*	-0.07±0.12*	0.90

R<sup>2</sup>: regression coefficient, s<sup>2</sup>? \*, \*\*, \*\*\*: Significant level at p < 0.05, 0.01 and 0.001, respectively.

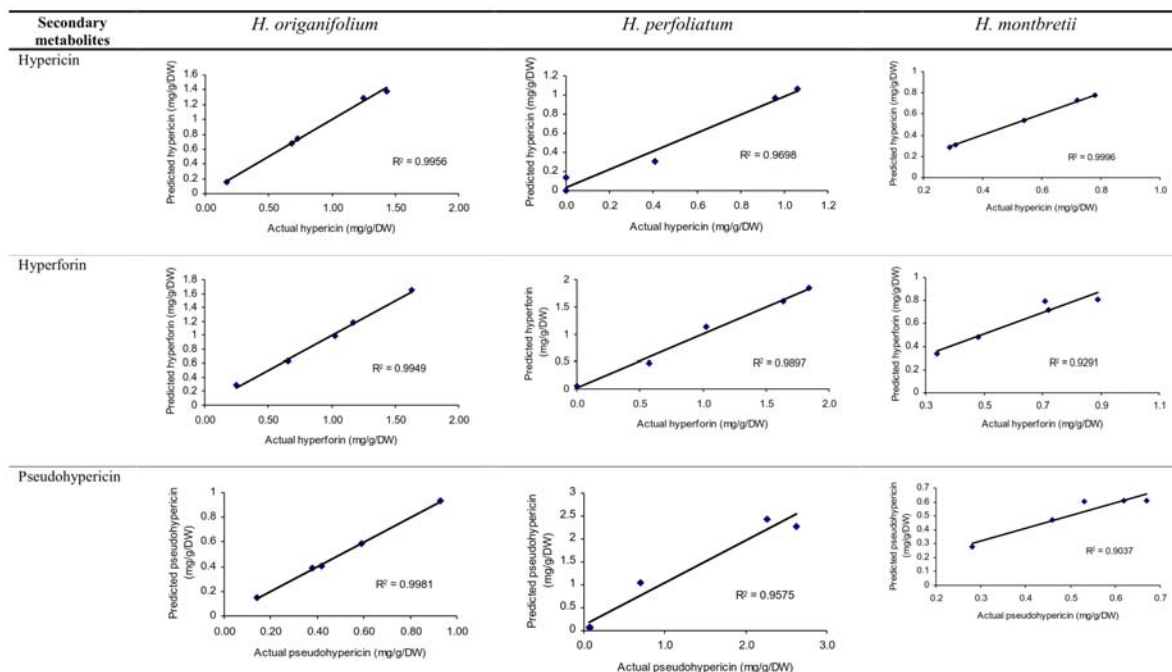


Figure 1. Relationships between actual and predicted contents of secondary metabolites in *Hypericum organifolium*, *H. perfoliatum* and *H. montbretii*.

Table 2. Hypericin content in stem, leaf, reproductive parts and whole shoot of *Hypericum originifolium*, *H. perforiatum* and *H. montbretii* examined at different stages of plant development (mg/g DW) (Cirak et al., 2007a, b; Cirak et al., 2008a).

<i>Hypericum</i> sp.	Plant Growth Stage	Stem	Leaf	Reproductive part	Whole shoot
<i>H. originifolium</i>	Vegetative	0.248	0.754	0	0.68
	Floral budding	0.147	0.742	2.773	1.25
	Full flowering	0.141	0.911	2.64	1.43
	Fresh fruiting	0.129	0.94	0.122	0.73
	Mature fruiting	0.092	0.262	0.225	0.17
<i>H. perforiatum</i>	Vegetative	0	0.48	0	0
	Floral budding	0	0.55	3.82	1.06
	Full flowering	0	0.73	3.09	0.96
	Fresh fruiting	0	0.68	0.35	0.41
	Mature fruiting	0	0	0	0
<i>H. montbretii</i>	Vegetative	0.17	0.42	0	0.31
	Floral budding	0.12	0.65	1.39	0.54
	Full flowering	0.17	0.79	1.8	0.78
	Fresh fruiting	0.15	0.77	1.12	0.72
	Mature fruiting	0.09	0.41	0.24	0.29

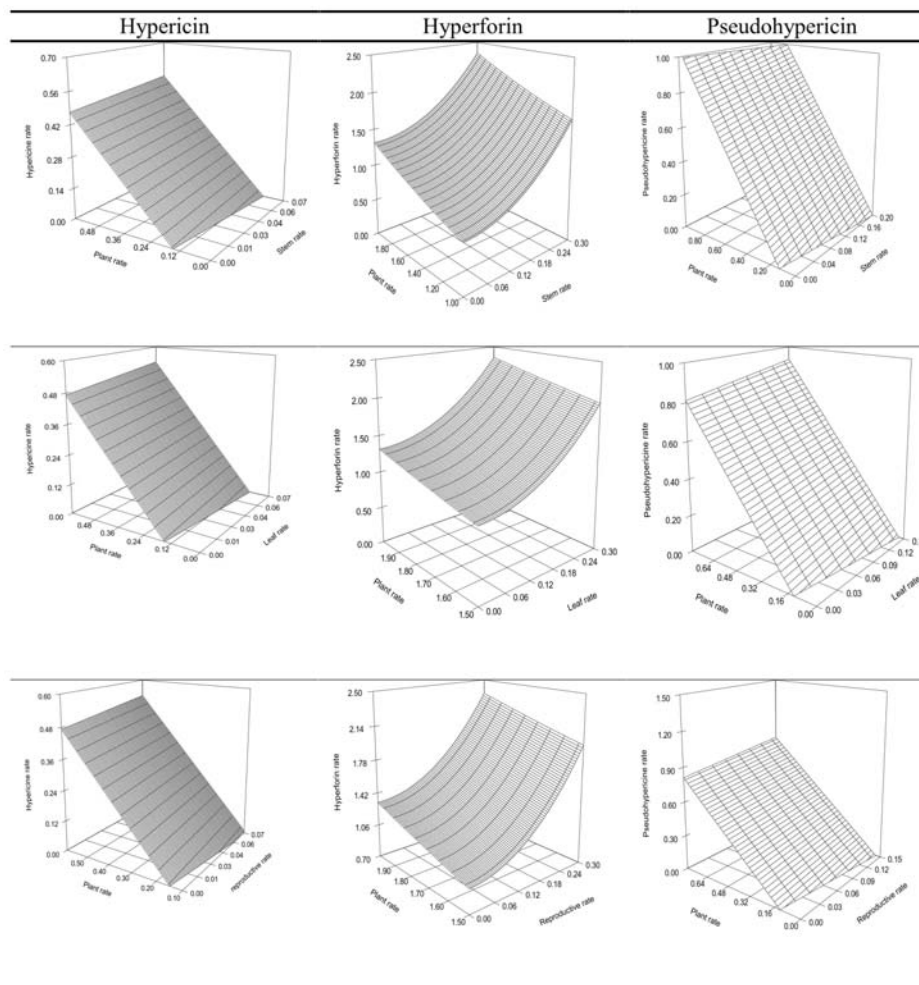


Figure 2. The relationship between the ratio of special plant tissue and content of secondary metabolites in whole plant of *Hypericum originifolium*

Table 3. Hyperforin content in stem, leaf, reproductive part and whole shoot of *Hypericum originafolium*, *H. perforiatum* and *H. montbretii* examined at different stages of plant development (mg/g DW) (Cirak and Radusiene, 2007; Cirak et al., 2008b).

<i>Hypericum</i> sp.	Plant Growth Stage	Stem	Leaf	Reproductive part	Whole shoot
<i>H. originafolium</i>	Vegetative	0.27	0.62	0	1.17
	Floral budding	0.13	0.28	2.18	1.03
	Full flowering	0.15	0.96	4.36	1.63
	Fresh fruiting	0.12	0.88	0.28	0.66
	Mature fruiting	0	0.42	0.35	0.25
<i>H. perforiatum</i>	Vegetative	0.17	0.73	0	0.57
	Floral budding	0.13	1.22	7.8	1.64
	Full flowering	0.65	0.84	5.95	1.84
	Fresh fruiting	0.13	1.33	3.22	1.03
	Mature fruiting	0	0	0	0
<i>H. montbretii</i>	Vegetative	0.18	0.66	0	0.48
	Floral budding	0.12	0.51	0.23	0.34
	Full flowering	0.13	0.46	1.88	0.71
	Fresh fruiting	0.13	0.45	1.92	0.89
	Mature fruiting	0.09	0.52	1.39	0.72

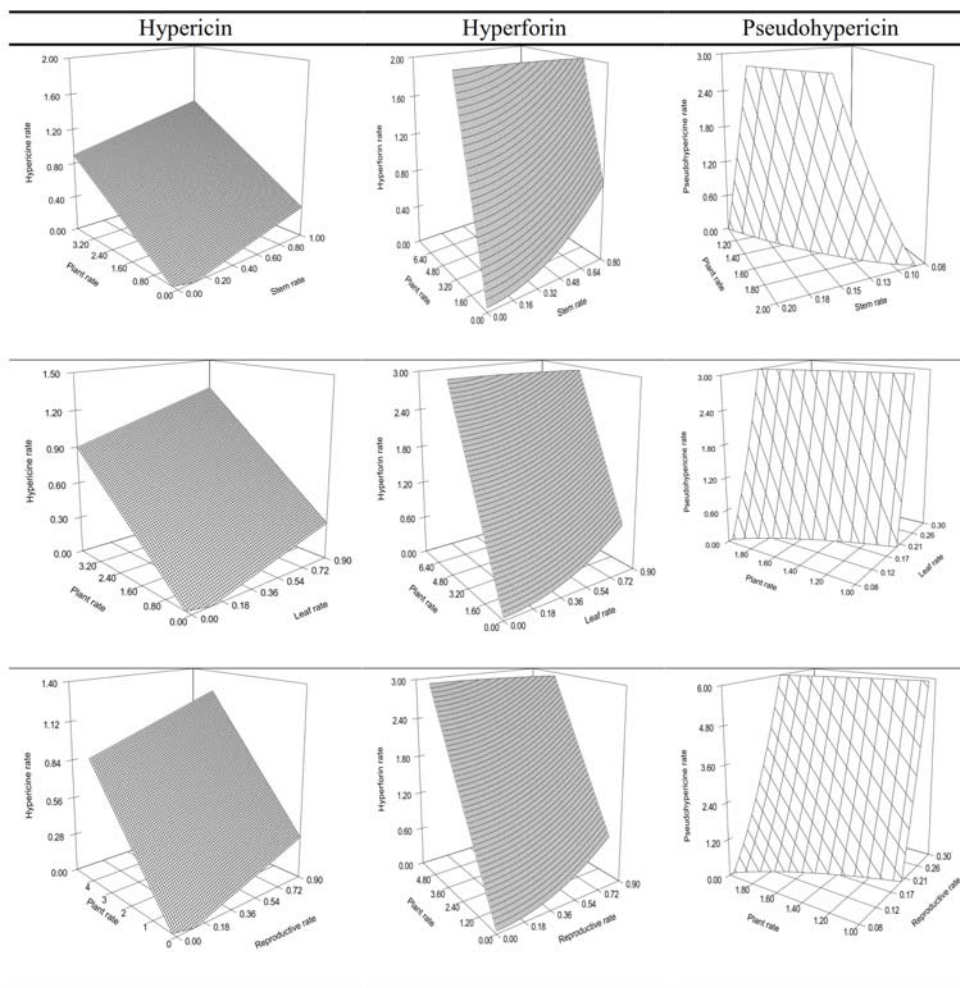


Figure 3. The relationship between the ratio of special plant tissue and content of secondary metabolites in whole plant of *Hypericum perforiatum*

Table 4. Pseudohypericin content in stem, leaf, reproductive part and whole shoot of *Hypericum originafolium*, *H. perforiatum* and *H. montbretii* species examined at different stages of plant development (mg/g DW) (Cirak et al., 2008a, b).

<i>Hypericum</i> sp.	Plant Growth Stage	Stem	Leaf	Reproductive Part	Whole shoot
<i>H. originafolium</i>	Vegetative	0.24	0.47	0	0.38
	Floral budding	0.15	0.23	0.99	0.59
	Full flowering	0.09	0.65	1.18	0.93
	Fresh fruiting	0.12	0.54	0.12	0.42
	Mature fruiting	0.09	0.31	0	0.14
<i>H. perforiatum</i>	Vegetative	0.09	2.07	0	0.08
	Floral budding	0.09	2.45	6.04	2.26
	Full flowering	0.14	2.11	7.41	2.62
	Fresh fruiting	0.14	1.88	3.52	0.70
	Mature fruiting	0	0	0.08	0.07
<i>H. montbretii</i>	Vegetative	0.3	0.79	0	0.46
	Floral budding	0.15	0.78	1.18	0.62
	Full flowering	0.19	0.94	1.5	0.67
	Fresh fruiting	0.16	0.88	0.95	0.53
	Mature fruiting	0.1	0.27	0.22	0.28

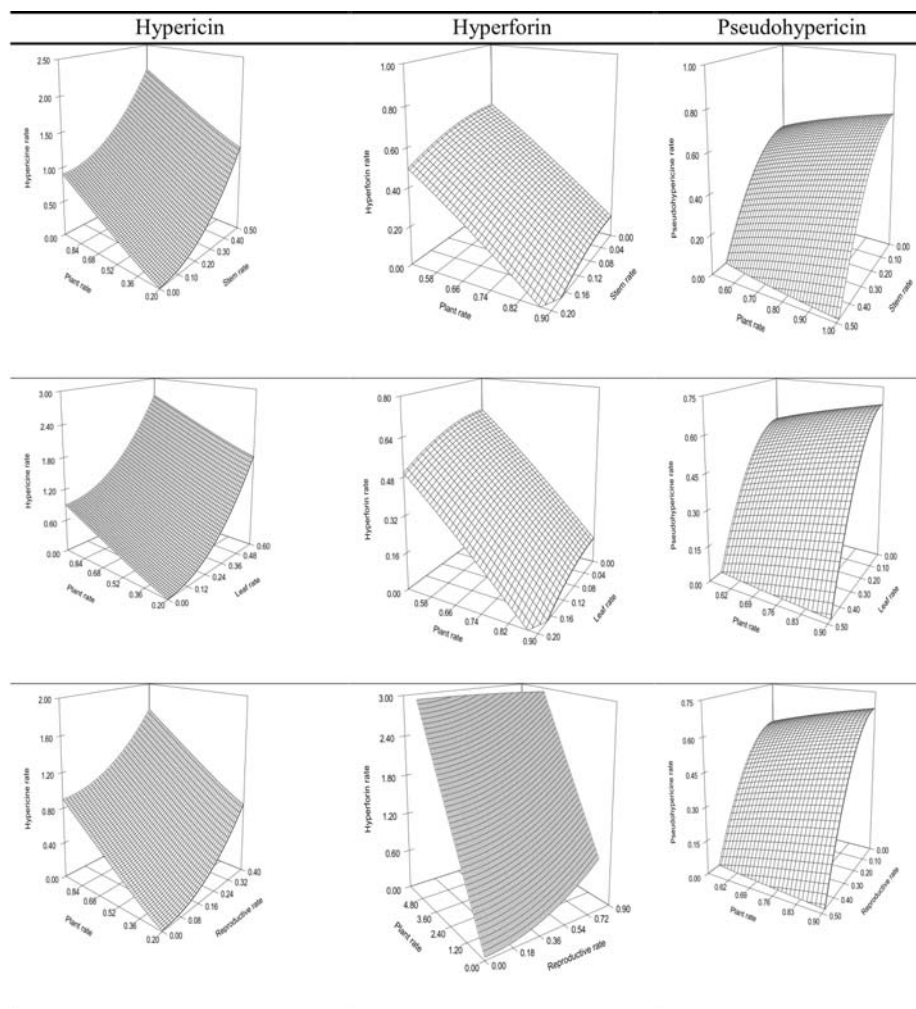


Figure 4. The relationship between the ratio of special plant tissue and content of secondary metabolites in whole plant of *Hypericum montbretii*



## Conclusions

Prediction models for the content of medicinally important secondary metabolites, as hypericin, pseudohypericin and hyperforin were developed for three *Hypericum* species.. The present topic is an important for phytochemical and taxonomical studies on *Hypericum* species because of prediction of secondary metabolite contents by using simple equations models instead of expensive and time-consuming devices during the course of an experiment. The models produced in the present study can be useful only for prediction of corresponding compounds of *Hypericum* species evaluated in following research. On the other hand, different models can be developed for other *Hypericum* species and phytochemicals of other biosynthesis pathway.

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