Asian Journal of Chemistry Vol. 20, No. 6 (2008), 4792-4802

Prediction Models for the Phenolic Contents in Some *Hypericum* Species from Turkey

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In this research, models for prediction of the content of several phenolics namely chlorogenic acid, hyperoside, apigenin-7-O-glucoside, rutin, quercitrin, quercetin and viteksin were developed for *Hypericum originafolium* Willd, *Hypericum perfoliatum* L. and *Hypericum montbreii* 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 phenolic content of plant materials was measured by high performance liquid chromatography method. Multiple regression analysis with Excel 2003 computer package program was performed for each species and phenolic separately to develop the models. The produced equation for predicting of phenolic content in different tissues of the species was formulized as: $PC = [a + (b_1 \times S) + (b_2 \times L) + (b_3 \times R) + (b_4 \times S^2) + (b_5 \times (1/RP))]$ where PC is whole plant content of phenolic compound, S is phenolic content of stem, L is phenolic content of leaf, RP is phenolic content of reproductive parts and a, b_1, b_2, b_3, b_4 and b_5 are coefficients. R^2 values varied between 0.65-0.99 for *H. originafolium*, 0.67-0.99 for *H. perfoliatum* and 0.96-0.99 for *H. montbreii* depending of the phenolics examined. All R^2 values and standard errors were found to be significant at the $p < 0.05$ level.

Key Words: *Hypericum originafolium*, *Hypericum perfoliatum*, *Hypericum montbreii*, Modelling, Phenolic contents, Plant growth stages.

INTRODUCTION

Over many centuries, plants of several *Hypericum* species have been of great interest to mankind for medicinal purposes[1]. Their pharmaceutical

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importance includes well-documented antioxidant[2], antitumor[3], antimutagenic[4] and antibacterial[5] properties. The most common are the extracts from *Hypericum perforatum* L., which are now widely used in Europe for treating depression[6]. Approximately 400 *Hypericum* species grow in the temperate regions of the world, alone in Turkey. This genus includes 89 species, 43 of which are endemic[7].

The methanolic extract from the aerial parts of *Hypericum* species has been reported to contain many bioactive compounds namely the naphthodianthrones hypericin and pseudohypericin[8], the phloroglucinol derivatives hyperforin and adhyperforin[9], several phenolics *e.g.* flavonoids[10], phenyl propanes[11], amino acids, xanthones[12], essential oils, tannins, procyanidins and other water-soluble components[13] which possess a wide array of biological properties.

Phenolic compounds are important for their contribution to the colour, sensory attributes and nutritional and antioxidant properties of plants[14]. Especially flavonoids have attracted considerable interest as dietary constituents and results from clinical studies indicated their possible role in preventing cardiovascular diseases and several kinds of cancer[15]. Although hypericins and hyperforin have been reported to mainly contribute to the pharmacological effects of *Hypericum* extracts, flavonoids have also made an important contribution to the antidepressant activity[16,17]. Due to these reasons, many individual or groups of *Hypericum* species have been investigated for the presence and/or variation of several phenolics to date[18-23].

Developmental models are commonly explored using computational or simulation techniques[24,25]. 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[26].

Most of the researches have focused on investigation of plant developmental periods. Because different physiological processes have occurred in different periods of plant growth stage[27]. In previous studies, we found significant variations in the content of several phenolics *e.g.* chlorogenic acid, hyperoside, apigenin-7-O-glucoside, rutin, viteksin, quercetin and quercitrin in *Hypericum perfoliatum* L., *Hypericum montbretii* Spach and *Hypericum origanifolium* Willd[28-30]. In the present study, models for prediction of the contents of aforesaid phenolics in those three species of *Hypericum* were developed for the first time.

EXPERIMENTAL

The plant materials were described in our previous studies[28-30]. The plant species were identified by Dr. Hasan Korkmaz, Faculty of Science and Art, Department of Biology, University of Ondokuz Mayis, Samsun, Turkey. Voucher specimens were deposited in the herbarium of Ondokuz Mayis University Agricultural Faculty (OMUZF # 101 for *H. perfoliatum*, OMUZF # 109 for *H. origanifolium* and OMUZF # 100 for *H. montbretii*).

The plant material of the species examined was collected in dry grassland within the Çakalli 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 (April 27, 2005 for all species). For the floral budding stage, only shoots with floral buds were selected (May 20 for *H. origanifolium* and *H. montbretii*; June 10 for *H. perfoliatum*). At the full flowering stage, only shoots with full opened flowers were harvested (June 14 for *H. origanifolium* and *H. montbretii*; June 24 for *H. perfoliatum*). At the fresh fruiting stage, the shoots which had green capsules were harvested (July 5 for *H. origanifolium* and *H. montbretii*; July 25 for *H. perfoliatum*). At the mature fruiting stage, the shoots which had dark brown capsules were harvested (August 10 for *H. origanifolium* and *H. montbretii*; September 10 for *H. perfoliatum*). The top of 2/3 plant, was harvested between 12:00 am and 13:00 pm. After collected, 10 individuals were kept as whole plants and the rest were dissected into floral, leaf and stem tissues, dried at room temperature (20 ± 2 °C) and assayed for phenolic contents by HPLC[28-30] (Tables 1-7).

Model construction: Multiple regression analysis of the data was performed for each phenolic in each species separately. A search for the best model for predicting phenolic contents was conducted with various subsets of the independent variables, namely, phenolic contents of stem, leaf, reproductive parts and whole plant at different stages of plant phenology. The best estimating equation for the content of phenolics tested were determined with the Excel 2003 and formulized as $PC= [a + (b_1 \times S) + (b_2 \times L) + (b_3 \times RP) + (b_4 \times S^2) + (b_5 \times (1/RP))]$ where PC is whole plant content of phenolic compound, S is phenolic content of stem, L is phenolic content of leaf, RP is phenolic content of reproductive parts and a, b_1, b_2, b_3, b_4 and b_5 are coefficients of the produced equation. Multiple regression analysis was carried out until the least sum of square was obtained[25].

TABLE-1
CHOLOROGENIC ACID CONTENT IN STEM, LEAVES, REPRODUCTIVE PARTS AND WHOLE SHOOTS OF *Hypericum* SPECIES EXAMINED AT DIFFERENT STAGES OF PLANT DEVELOPMENT (mg/g/DW)

Hypericum sp.	Plant growth stage	Stems	Leaves	Reproductive	Whole plant
H. originafolium	Vegetative	1.11	3.42	0.00	2.88
	Floral budding	0.71	4.41	0.22	2.33
	Full flowering	0.91	3.60	1.31	2.34
	Fresh fruiting	1.01	5.56	1.94	2.65
	Mature fruiting	0.27	2.58	0.00	0.52
H. perfoliatum	Vegetative	2.62	56.63	0.00	18.20
	Floral budding	1.94	70.33	8.80	24.00
	Full flowering	2.39	65.85	9.39	20.40
	Fresh fruiting	2.85	55.07	3.39	7.20
	Mature fruiting	0.94	0.00	2.14	0.80
H. montbretii	Vegetative	0.61	3.92	0.00	3.19
	Floral budding	0.75	3.99	2.59	2.43
	Full flowering	0.84	4.32	1.12	2.48
	Fresh fruiting	0.84	5.13	0.54	1.66
	Mature fruiting	0.28	2.59	0.00	0.81

TABLE-2
RUTIN CONTENT IN STEM, LEAVES, REPRODUCTIVE PARTS AND WHOLE SHOOTS OF *Hypericum perfoliatum* AT DIFFERENT STAGES OF PLANT DEVELOPMENT (mg/g/DW)

Hypericum sp.	Plant growth stage	Stems	Leaves	Reproductive	Whole plant
H. perfoliatum	Vegetative	0.003	0.000	0.000	0.001
	Floral budding	0.006	0.000	0.009	0.007
	Full flowering	0.011	0.000	0.024	0.020
	Fresh fruiting	0.034	0.000	0.036	0.035
	Mature fruiting	0.008	0.000	0.084	0.046

TABLE-3
HYPEROSIDE CONTENT IN STEM, LEAVES, REPRODUCTIVE PARTS AND WHOLE SHOOTS OF *Hypericum* SPECIES EXAMINED AT DIFFERENT STAGES OF PLANT DEVELOPMENT (mg/g/DW)

Hypericum sp.	Plant growth stage	Stems	Leaves	Reproductive	Whole plant
H. originafolium	Vegetative	1.24	26.29	0.00	19.25
	Floral budding	0.81	25.87	19.16	16.43
	Full flowering	1.35	26.28	12.79	15.88
	Fresh fruiting	1.20	29.55	9.25	10.97
	Mature fruiting	0.36	9.65	0.46	2.39

Hypericum sp.	Plant growth stage	Stems	Leaves	Reproductive	Whole plant
H. perfoliatum	Vegetative	1.26	23.74	0.00	12.30
	Floral budding	1.26	28.67	36.82	14.90
	Full flowering	1.93	30.44	26.96	17.00
	Fresh fruiting	2.14	22.01	14.37	6.30
	Mature fruiting	0.31	0.00	0.49	0.30
H. montbretii	Vegetative	2.13	26.95	0.00	18.28
	Floral budding	1.85	27.35	23.62	15.47
	Full flowering	1.40	28.31	15.54	12.64
	Fresh fruiting	1.39	27.28	8.69	8.57
	Mature fruiting	0.46	11.71	0.96	5.60

TABLE-4
APIGENIN–7-O-GLUCOSIDE CONTENT IN STEM, LEAVES, REPRODUCTIVE PARTS AND WHOLE SHOOTS OF *Hypericum* SPECIES EXAMINED AT DIFFERENT STAGES OF PLANT DEVELOPMENT (mg/g/DW)

Hypericum sp.	Plant growth stage	Stems	Leaves	Reproductive	Whole plant
H. originafolium	Vegetative	0.01	0.00	0.00	0.00
	Floral budding	0.01	0.00	0.02	0.00
	Full flowering	0.02	0.00	0.03	0.00
	Fresh fruiting	0.02	0.02	1.72	0.02
	Mature fruiting	0.01	0.02	0.00	0.01
H. perfoliatum	Vegetative	0.09	0.00	0.00	0.02
	Floral budding	0.02	0.00	0.14	0.02
	Full flowering	0.02	0.00	0.10	0.03
	Fresh fruiting	0.03	0.00	0.67	0.21
	Mature fruiting	0.09	0.00	0.23	0.18
H. montbretii	Vegetative	0.00	0.00	0.00	0.00
	Floral budding	0.00	0.00	0.00	0.00
	Full flowering	0.03	0.00	6.69	2.25
	Fresh fruiting	0.04	0.00	4.32	1.01
	Mature fruiting	0.09	0.00	0.00	0.02

TABLE-5
VITEKSIN CONTENT IN STEM, LEAVES, REPRODUCTIVE PARTS AND WHOLE SHOOTS OF *Hypericum montbretii* AT DIFFERENT STAGES OF PLANT DEVELOPMENT (mg/g/DW)

Hypericum sp.	Plant growth stage	Stems	Leaves	Reproductive	Whole plant
H. montbretii	Vegetative	0.00	0.14	0.00	0.05
	Floral budding	0.00	1.53	0.00	0.58
	Full flowering	0.00	1.85	0.00	0.96
	Fresh fruiting	0.00	0.76	0.00	0.15
	Mature fruiting	0.00	0.00	0.00	0.00

TABLE-6
QUERCITRIN CONTENT IN STEM, LEAVES, REPRODUCTIVE PARTS AND WHOLE SHOOTS OF *Hypericum* SPECIES EXAMINED AT DIFFERENT STAGES OF PLANT DEVELOPMENT (mg/g/DW)

Hypericum sp.	Plant growth stage	Stems	Leaves	Reproductive	Whole plant
H. originafolium	Vegetative	2.58	0.97	0.00	1.41
	Floral budding	2.55	1.06	4.20	2.26
	Full flowering	4.52	0.03	6.47	3.89
	Fresh fruiting	4.41	0.06	0.13	2.39
	Mature fruiting	1.10	0.83	0.91	1.06
H. perfoliatum	Vegetative	1.10	0.63	0.00	0.47
	Floral budding	0.92	0.91	4.09	1.82
	Full flowering	1.09	1.03	4.55	2.11
	Fresh fruiting	1.04	0.00	0.55	0.84
	Mature fruiting	0.09	0.00	0.26	0.15
H. montbretii	Vegetative	1.69	1.32	0.00	1.21
	Floral budding	2.21	1.29	2.30	1.21
	Full flowering	3.32	1.58	2.23	2.72
	Fresh fruiting	3.32	1.32	1.09	1.95
	Mature fruiting	0.88	1.05	1.05	1.01

TABLE-7
QUERCETIN CONTENT IN STEM, LEAVES, REPRODUCTIVE PARTS AND WHOLE SHOOTS OF *Hypericum* SPECIES EXAMINED AT DIFFERENT STAGES OF PLANT DEVELOPMENT (mg/g/DW)

Hypericum sp.	Plant growth stage	Stems	Leaves	Reproductive	Whole plant
H. originafolium	Vegetative	0.02	0.02	0.00	0.04
	Floral budding	0.04	0.05	0.23	0.09
	Full flowering	0.02	0.00	0.16	0.05
	Fresh fruiting	0.01	0.00	0.77	0.02
	Mature fruiting	0.05	0.03	0.04	0.03
H. perfoliatum	Vegetative	0.01	0.00	0.00	0.00
	Floral budding	0.01	0.00	0.09	0.04
	Full flowering	0.01	0.00	0.18	0.05
	Fresh fruiting	0.01	0.00	0.04	0.04
	Mature fruiting	0.02	0.00	0.03	0.02

RESULTS AND DISCUSSION

In the present study, prediction equations of phenolic contents were developed for chlorogenic acid, hyperoside, apigenin-7-O-glucoside, quercitrin and quercetin in *H. origanifolium*; chlorogenic acid, hyperoside,

apigenin-7-O-glucoside, rutin, quercitrin and quercetin in *H. perfoliatum*; chlorogenic acid, hyperoside, apigenin-7-O-glucoside, quercitrin and viteksin in *H. montbretii*. Multiple regression analysis used for determination of the best fitting mathematical equations for estimation of phenolic contents in *Hypericum* plants evaluated here showed that most of the variations in phenolic contents values were explained by the selected parameters (phenolic content of stem, leaf, reproductive parts and whole shoots during plant growth).

The variation explained by the parameters in *H. origanifolium* was 99 % for chlorogenic acid, 82 % for hyperoside, 99 % for apigenin-7-O-glucoside, 65 % for quercetin and 99 % for quercitrin. The produced phenolic content prediction equations for this species were CA= 0.3 + (0.035 × L) + (2.023 × S^2) + [0.191 × (1/RP)]; HP = 56.92 + (2.25 × S) + (-1.58 × L) + [-18.32 × (1/RP)]; AP= 0.009 + (-0.56 × S) + (0.012 × RP); QC = 0.01 + (1.99 × S) + [-0.002 × (1/RP)]; QT = 0.8 + (0.22 × RP) + (0.08 × S^2) where CA: whole plant content of chlorogenic acid, HP: whole plant content of hyperoside, AP: whole plant content of apigenin-7-O-glucoside, QC: whole plant content of quercetin, QT: whole plant content of quercitrin, L: phenolic content of leaf, S: phenolic content of stem; RP: phenolic content of reproductive parts (Table-8 and Fig. 1).

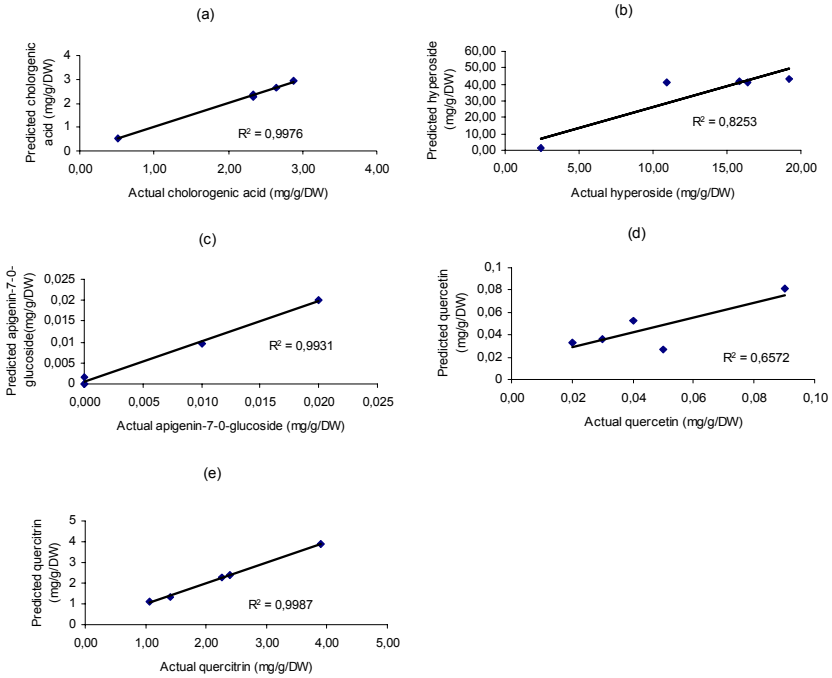

Fig. 1. Relationship between actual and predicted content of chlorogenic acid (a), hyperoside (b), apigenin-7-O-glucoside (c), quercetin (d) and quercitrin (e) content in *H. originafolium*

TABLE-8
THE COEFFICIENTS, THEIR STANDARD ERRORS AND R^2 VALUES OF THE NEW PRODUCED EQUATIONS PREDICTING PHENOLIC CONTENTS IN *H. origanifolium, H. perfoliatum* AND *H. montbretii*

Secondary Metabolites and Standard Errors (SE)	Coefficient	S	L	RP	S^2	1/RP	R^2
Hypericum originafolium							
Hyperoside	56.92 ± 13.01*	2.25 ± 4.13*	-1.58 ± 0.49*	–	–	-18.32 ± 3.93*	0.98
Cholorogenic acid	0.300 ± 0.16*	–	0.035 ± 0.05*	–	2.023 ± 0.13*	0.191 ± 0.03*	0.99
Quercetin	0.010 ± 0.02*	1.99 ± 1.05*	–	–	–	-0.002 ± 0.10*	0.65
Quercitrin	0.800 ± 0.04**	–	–	0.220 ± 0.01**	0.080 ± 0.03**	–	0.99
Apigenin-7-O-glucoside	0.009 ± 0.01**	-0.56 ± 0.09**	–	0.012 ± 0.01**	–	–	0.98
Hypericum perfoliatum							
Rutin	0.0001 ± 0.001*	0.51 ± 0.07*	–	0.50 ± 0.02*	–	–	0.99
Quercetin	0.0200 ± 0.020*	-0.66 ± 1.47*	–	0.20 ± 0.12*	–	–	0.67
Quercitrin	0.0190 ± 0.120*	0.49 ± 0.13*	–	0.33 ± 0.02*	–	–	0.99
Apigenin-7-O-glucoside	-0.0500 ± 0.040*	1.21 ± 0.58*	–	0.36 ± 0.08*	–	–	0.91
Hyperoside	-11.0400 ± 8.960*	–	1.00 ± 0.31*	–	-0.96 ± 0.79*	5.59 ± 4.5*	0.97
Cholorogenic acid	15.6300 ± 1.680*	–	0.26 ± 0.02*	–	-1.78 ± 0.16*	-28.41 ± 3.31*	0.99
Hypericum montbretii							
Quercitrin	-5.630 ± 3.24*	–	5.21 ± 2.60*	–	-0.04 ± 0.09*	1.24 ± 0.79*	0.96
Hyperoside	-594.400 ± 494.60*	–	19.13 ± 15.78*	–	21.31 ± 15.16*	354.8 ± 292.9*	0.96
Cholorogenic acid	-3.180 ± 1.32*	–	1.51 ± 0.35*	–	0.99 ± 1.42*	-1.94 ± 0.48*	0.97
Viteksin	0.009 ± 0.02*	–	–	–	0.26 ± 0.01*	–	0.99
Apigenin-7-O-glucoside	-0.020 ± 0.18*	–	–	0.31 ± 0.04*	-0.26 ± 3.74*	–	0.96

R^2 = Regression coefficiency, SE = Standard error, S = Phenolic contents of stem, L = Phenolic contents of leaf, RP = Phenolic contents of reproductive parts of produced equations; *,**,***: Significant at the level of $p < 0.05$, 0.01 and 0.001, respectively.

As for *H. perfoliatum*, the variation between actual and predicted phenolic contents of plant parts was explained as 99 % for chlorogenic acid, 97 % for hyperoside, 91 % for apigenin-7-O-glucoside, 67 % for quercetin, 99 % for quercitrin and 99 % for rutin. The produced phenolic content prediction equations for this species were CA = 15.63 + (0.26 × L) + (-1.78 × S^2) + [-28.41 × (1/RP)]; HP = -11.04 + (1.00 × L) + (-0.96 × S^2) + [5.59 × (1/RP)]; AP = -0.05 + (1.21 × S) + (0.36 × RP); QC = 0.02 + (-0.66 × S) + (0.02 × RP); QT= 0.19 + (0.49 × S) + (0.33 × RP); RU = 0.0001 + (0.51 × S) + (0.50 × RP) where RU: whole plant content of rutin (Table-8 and Fig. 2).

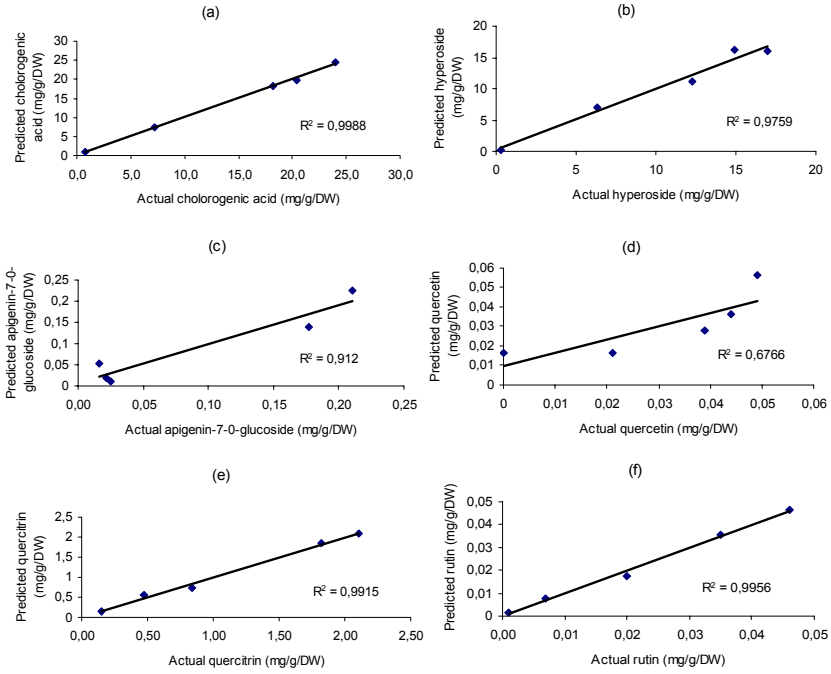

Fig. 2. Relationship between actual and predicted content of chlorogenic acid (a), hyperoside (b), apigenin-7-O-glucoside (c), quercetin (d), quercitrin (e) and rutin (f) content in *H. perfoliatum*

In *H. montbretii*, the variations stand for 97 % for chlorogenic acid, 96 % for hyperoside, 96 % for apigenin-7-O-glucoside, 96 % for quercitrin and 99 % for viteksin according to the parameters. The produced phenolic content prediction equations for this species were CA = -3.18 + (0.99 × S^2) + (1.51 × L) + [-1.94 × (1/RP)]; HP = -594.4 + (21.31 × S^2) + (19.13 × L) + [354.8 × (1/RP)]; AP= -0.02 + (-0.26 × S^2) + (0.31 × RP); QT= -5.63 + (-0.04 × S^2) + (5.21 × L) + [1.24 × (1/RP)]; VT= 0.009 + (0.26 × S^2) where VT: whole plant content of viteksin (Table-8 and Fig. 3).

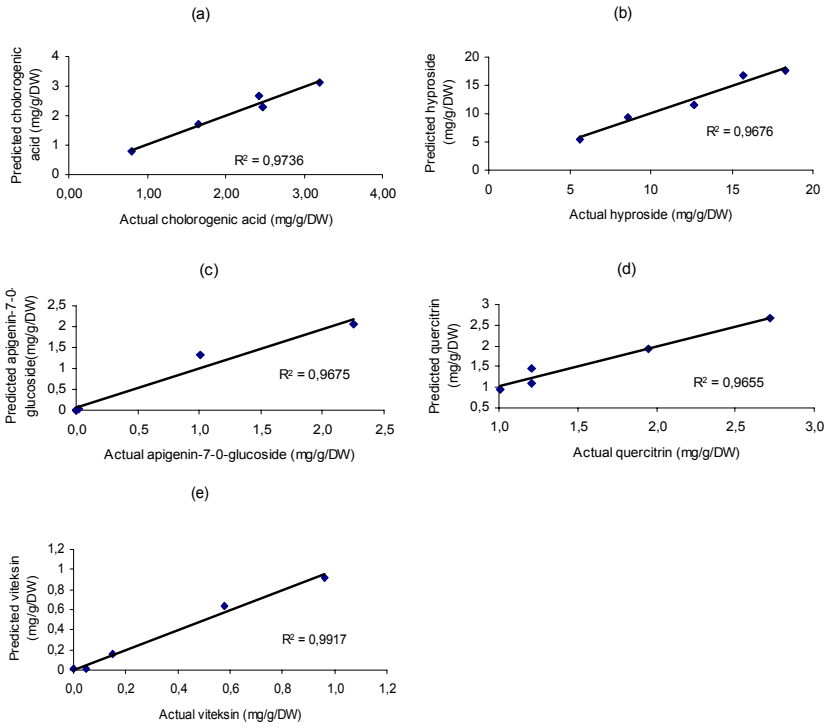

Fig. 3. Relationship between actual and predicted content of chlorogenic acid (a), hyperoside (b), apigenin-7-O-glucoside (c), quercitrin (d) and viteksin (e) content in *H. montbretii*

Conclusion

In the present study, it is the first time we have developed prediction models for the content of phenolics, namely chlorogenic acid, hyperoside, apigenin-7-O-glucoside, rutin, quercitrin, quercetin and viteksin during plant growth in three *Hypericum* species, each has potential using fields in medicinal treatments and botanical industry with their well-documented chemical contents. As the understanding of plant growth and development has been increasing, such mathematical models as shown in Table-8 will be very useful tools for prediction of secondary metabolite contents for many plants without using of expensive analytical devices. Also, considering the importance of determining the chemical contents in *Hypericum* plants, prediction of secondary metabolite contents by using of simple equations instead of using expensive and time-consuming devices during the course of experiment is an also important topic for phyochemical and taxonomical studies on the genus *Hypericum*. Hence, the models produced in the present study can be used safely by *Hypericum* researchers for the species used in this research. On the other hand, different models can be developed for other *Hypericum* species and phytochemicals different from those used in the present study.

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(*Received*: 10 October 2007; *Accepted*: 10 March 2008) AJC-6456