

## DETERMINATION OF QUALITY PARAMETERS IN *Hypericum perforatum* GROWN IN SASKATCHEWAN

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### ABSTRACT

*Hypericum perforatum*, a medicinal plant known as Saint John's Wort, has been used extensively for its antidepressant activity in North America over the past three years. The objective of this study was to establish the influence of plant part and time of harvest on phytochemical quality of Saint John's Wort grown in Saskatoon. Varietal influence on quality was also investigated. Flowering tops, upper leaves and stems, and lower leaves and stems of two and three years old plants, variety Standard, harvested at seven different times from budding to post-blooming from June to September, 1998, were used for quality assessment. Extraction protocol for optimal recovery and an high performance liquid chromatography (HPLC) method for quantification of 7 marker compounds for Saint John's Wort, hypericin and pseudohypericin, and 5 selected flavonoids (quercitrin, quercetin, rutin, hyperoside and biapigenin) were developed. The flowering tops followed by the upper most leaves contained the highest concentration of hypericins and flavonoids when harvested in late June, 0.35 % and 4.0%, respectively. The hypericins content declined by more than 90% between late June and end of August. The content of flavonoids showed a similar declining trend from early July onward. A correlation between date of harvest and quality, and plant part and quality was apparent. Two varieties, Anthos and Elixir™, were found superior in both plant yield and plant quality.

The St. John's Wort was recently documented to possess mild antidepressant activity, which caused an explosive increase in demand for this herb in North America. It was an opportune time for herb growers in Saskatchewan to test the feasibility of large scale cultivation of St. John's Wort in the province. Given that St. John's Wort is known as a noxious weed in pastures throughout North America, Europe, and Asia, growing it on a large scale was not considered a challenge. However, the competitiveness of commercial cultivation depends greatly on critical factors such as selection of best suited cultivars and harvesting of proper plant parts at optimal time (when content of active compounds is at its maximum), which have not been well established and reported in the

literature. With intention to assist herb industry in Saskatchewan in producing high quality St. John's Wort, we conducted the study in 1998 and 1999.

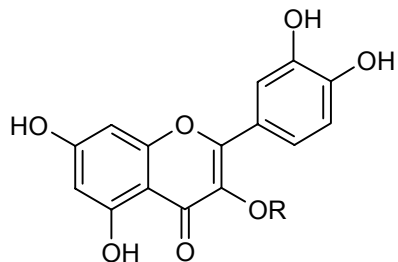
Saint John's Wort has been grown in the Herb Demonstration and Resource Garden at the Horticulture Field Headquarters, University of Saskatchewan campus, since 1996. In 1998, two and three years old plants, variety Standard, grown from seed purchased at Richters Herbs (Goodwood, Ontario) were harvested two times a day at seven different times from budding to post-blooming over three months period (Table 1).

**Table 1.** Effect of Harvest Time on Quality of St. John's Wort<sup>1,2</sup> - Study Design

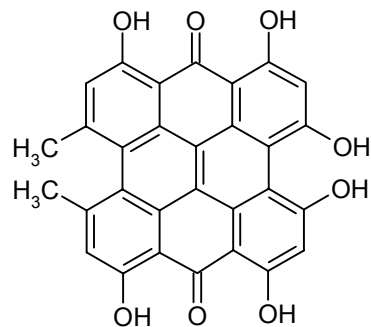
Parameter	Description
<b>Plant age:</b>	2 and 3 year old
<b>Plant part:</b>	Flowers, upper leaves and stems (top 10 cm), lower leaves and stems, flowering tops (upper 30 cm, parts not separated)
<b>Date of harvest:</b>	June 29, July 6, July 15, July 23, July 30, Aug. 11, Aug. 20, Sept. 4, Oct. 7, Oct. 23
<b>Time of day:</b>	9 a.m., 4 p.m.
<b>Date of second harvest:</b>	Sept. 4, Oct. 7, Oct. 23 (row was cut back to 15 cm on July 30 and flowering tops reharvested)

Content of hypericins and 5 selected flavonoids in flowering tops (the top 25 cm of the plant), upper leaves and stems (next 20 cm below the top), and lower leaves and stems (20 cm further below) of Saint John's Wort, were used as quality parameters. Analysis of St. John's Wort is a real challenge for an analyst, since this herb synthesizes a great number of physiologically active compounds, some of them at very low levels and with similar chemical structures. An HPLC assay developed in our laboratory included seven markers: hypericin and pseudohypericin, unique compounds for St. John's Wort, and quercitrin, quercetin, rutin, hyperoside and biapigenin, more widely distributed compounds in plant kingdom. Their chemical structures are presented in Figure 1. Due to high difference in polarities of hypericin on one hand and other flavonoid compounds on the other, it was challenging to establish one set of conditions that would allow for baseline separation of all marker compounds.

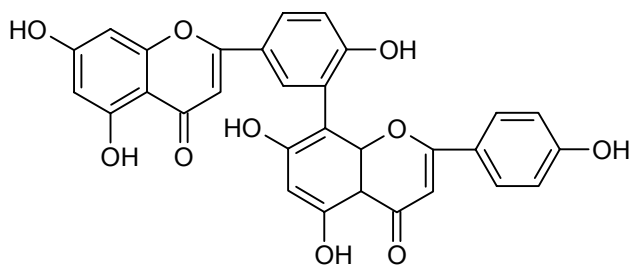
**Figure 1.** Chemical Structures of Marker Compounds in St. John's Wort



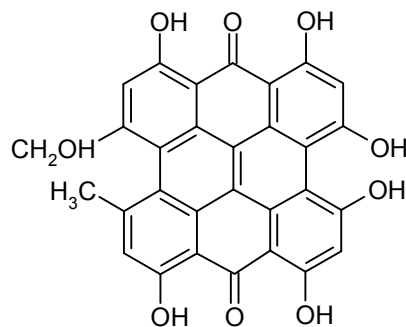
Quercetin R = H  
 Hyperoside R = Galactose  
 Quercitrin R = Rhamnose  
 Isoquercitrin R = Glucose  
 Rutin R = Rhamnose-Glucose



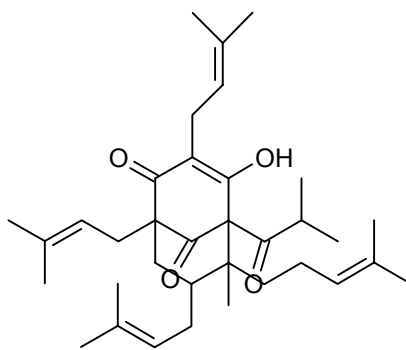
Hypericin



Amentoflavone



Pseudohypericin



Hyperforin

The results of analysis are presented in Table 2. The flowering tops followed by the upper most leaves contained the highest concentration of hypericins and flavonoids when harvested in late June, 0.48 % and 3.8%, respectively. The content of both groups of compounds was slightly higher in 2 years than in 3 years old plants. The hypericins content declined by more than 90% between late June and the end of August. The content of selected flavonoids, particularly rutin and hyperoside, showed a similar declining trend with increasing plant maturity for both 2 and 3 year old plants. The time of day (morning vs. afternoon harvest) did not seem to have a significant impact on phytomedicinal quality of plants. Second harvest of flowering tops in late August appeared possible, however, it caused higher susceptibility to winter kill and negatively impacted growth the following year. Plant quality was also compromised.

In 1999 we investigated an effect of harvest time on quality characteristics of three St. John's wort cultivars that are available on the market: Topas (advertised as improved strain for commercial production, up to 0.5% hypericin, yields 2.5 tonnes/ha), Anthos (advertised as highly uniform strain, suited for mechanical harvest, disease resistant) and Elixir™ (advertised as having higher active constituents, hypericin and related compounds, Richters exclusive ). The plant material of these cultivars was kindly provided by Alberta Agriculture Crop Diversification Centre-South in Brooks, which also provided yield data. The yields (dry matter in kg/ha) were the following: about 1600 for Anthos, 900 for Topas and 2800 for Elixir™ (Liz Russel, personal communications). The results of testing for hypericins and flavonoid contents in our laboratory are presented in Tables 3 and 4. Considering both yield and quality, it appears that varieties Anthos and Elixir™ should preferably be considered for cultivation. No comparative data of these three varieties and Standard variety are available at present.

**Table 2.** Analysis of St. John's Wort Flowers (1998 Harvest)

Sample ID	Date of Harvest	Moisture (%)	Hypericin <sup>1</sup> (%)	Pseudohypericin <sup>1,2</sup> (%)	Total Hypericins <sup>1,3</sup> (%)	Quercitrin <sup>1</sup> (%)	Quercetin <sup>1</sup> (%)	Rutin <sup>1</sup> (%)	Hyperoside <sup>1</sup> (%)	Biapigenin <sup>1</sup> (%)
<i>Flowers 2 yr.</i>										
#5 HRP	29 Jun 98	11.21	0.139	0.179	0.318	0.407	0.106	1.439	2.161	0.026
#12 HRP	6 Jul 98	8.75	0.262	0.215	0.477	0.495	0.188	1.087	1.994	0.020
#20 HRP	15 Jul 98	8.91	0.133	0.125	0.258	0.366	0.237	0.842	1.499	0.015
#29 HRP	23 Jul 98	8.03	0.098	0.083	0.181	0.317	0.178	0.684	1.439	0.010
#48 HRP	30 Jul 98	12.80	0.132	0.147	0.279	0.235	0.198	0.453	1.188	0.011
#65 HRP	11 Aug 98	12.41	0.075	0.070	0.145	0.195	0.137	0.433	1.089	0.010
#81 HRP	20 Aug 98	10.91	0.021	0.019	0.040	0.075	0.143	0.210	0.457	0.012
<i>Flowers 3 yr.</i>										
#8 HRP	6 Jul 98	8.63	0.120	0.145	0.265	0.427	0.230	0.857	2.081	0.021
#16 HRP	15 Jul 98	8.26	0.064	0.078	0.142	0.313	0.234	0.656	1.613	0.014
#26 HRP	23 Jul 98	8.19	0.055	0.062	0.117	0.263	0.212	0.623	1.566	0.011
#45 HRP	30 Jul 98	12.75	0.023	0.038	0.061	0.096	0.156	0.387	0.927	0.006
#61 HRP	11 Aug 98	11.32	0.024	0.036	0.060	0.078	0.135	0.329	0.786	0.007
#77 HRP	20 Aug 98	11.62	0.009	0.012	0.021	0.044	0.096	0.228	0.621	0.007

<sup>1</sup> Content of hypericin, pseudohypericin, total hypericins, quercitrin, quercetin, rutin, hyperoside and biapigenin are reported on dry weight basis.

<sup>2</sup> Pseudohypericin was quantitated using the calibration curve for hypericin, assuming a similar  $\epsilon$  value.

<sup>3</sup> Total hypericins were expressed as the sum of hypericin and pseudohypericin.

**Table 3.** Effect of Harvest Time and Variety on Hypericins Content in St. John's Wort (*Hypericum perforatum*)

Variety	Date of Harvest	Hypericin (%)	Pseudohypericin (%)	Total Hypericins (%)
2 year				
Anthos	6-Jul-99	0.026	0.071	0.097
Topas	6-Jul-99	0.015	0.040	0.055
Elixir™	6-Jul-99	0.025	0.049	0.074
Anthos	17-Sep-99	0.014	0.030	0.044
Topas	17-Sep-99	0.009	0.018	0.026
Elixir™	17-Sep-99	0.019	0.029	0.048

**Table 4.** Effect of Harvest Time and Variety on Flavonoids Content in St. John's Wort (*Hypericum perforatum*)

Variety	Date of Harvest	Quercitrin (%)	Quercetin (%)	Rutin (%)	Hyperoside (%)
2 year					
Anthos	6-Jul-99	0.102	0.025	0.737	0.913
Topas	6-Jul-99	0.078	0.018	0.921	0.775
Elixir™	6-Jul-99	0.097	0.031	0.662	1.086
Anthos	17-Sep-99	0.043	0.021	0.738	0.641
Topas	17-Sep-99	0.042	0.011	0.832	0.601
Elixir™	17-Sep-99	0.082	0.067	0.719	0.909

#### ACKNOWLEDGEMENT

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