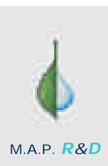

STUDY OF THE PHYTOCHEMICAL VARIABILITY OF THE ESSENTIAL OIL OF HYPERICUM PERFORATUM IN RELATION TO VEGETATIVE STAGE

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Abstract

Aims: The reproducibility of effects of herbal medicinal preparations is strongly affected by changes in the composition of the phytochemical compounds of the herb. Geographic origin, choice of cultivars, and harvesting times are parameters with a distinct impact on raw material quality.

The aim of this study was to demonstrate the variability of the phytochemical composition of essential oils of St. John's wort (SJW, *Hypericum perforatum*), and to determine the quantity and qualitative composition of the essential oil of the SJW cultivar "Topas" of Iranian provenience by GC-MS at three vegetative stages: before flowering, at full flowering, and at the time of fruit formation.

Results: The composition of essential oil from SJW collected by wild crafting in South Africa clearly differs from the composition of the oil from controlled cultivation of the cultivar Topas in Mashad, Iran. Such variability must also be expected from commercial oils without clear identication of the origin of the plant material.

For the SJW cultivar Topas grown under controlled conditions in Iran, fresh and dry yield of biomass per hectare was highest at the time of fruit formation. However, SJW in full flowering gave the highest amounts of essential oil (0.35 ml/100 g of dry matter) as compared to earlier (0.12 ml/100 g) respectively later vegetative stages (0.16 ml/100g). Major constituents of the essential oil of SJW of all vegetative stages are bicyclogermacrene, α-cadinol and spathulenol. Bicyclo-germacrene and α-cadinene are marker compounds of full full flowering stage. β-Caryophyllene and ?-muurolene are markers of early flowering, and high quantities of β-bisabolene and globulol indicate late flowering stages, as do low quantities of γ-Eudesmol.

Longifolene is not present in full flowering, but present in very high amounts (18.71 respectively 21.99%) in early respectively late flowering.

Conclusions: Our results allow us to identify marker compounds for the retrospective control of the vegetative stage of SJW grown in Mashad, Iran, on harvesting. The analytical results would suggest the stage of full flowering as the optimal harvesting time, in accordance to traditional use of SJW.

Introduction

SJW accumulates an essential oil in translucent spheroidal secretory glands (1;2). The composition of the essential oil is primarily determined by genetical, environmental and ontogenetical factors. As was demonstrated for other secondary metabolites of SJW, such as hypericin or flavonoids (3), a variability of the qualitative and quantitative composition of secondary metabolites according to vegetative cycle and harvesting time would have also have to be expected for the essential oil. Our study was aimed on the determination of such differences and, as a practical consequence, the evaluation of the optimal harvesting time of SJW.

We analyzed the composition of the essential oil of willd crafted SJW from South Africa, and the yield and composition of the essential oil of SJW (cultivar Topas) grown in Mashad, Iran, at three vegetative stages: before flowering (BF), in full flowering (FF), and at the stage of fruit formation (FR).

Materials and Methods

Samples of SJW in full flowering were collected from wild populations in the region Pickedberg, North of Capetown (South Africa). Distillation of the essential oil and analytical profiling was carried out as described below.

Pickedberg: 600-700 m above sea level, 32°50' Southern latitude, mediterranian-type climate with approximately 300-350 mm of annual average rainfall with typical winter rains.

Experimental cultivation of the cultivar Topas was carried out in the Mashhad university research station (Iran).

Mashad: 1215 m above sea level, 35°43' Northern latitude, lowest temperature in winter -7.6°C, semi-dry climate with 242.7 mm of annual average rainfall. Substrate: Sandy loam, pH 7.3, CEC 13.8, EC 0.9, organic matter 0.92%, N 0.05%, P 4.2 mg/kg, K 305 mg/kg.

At springtime, seeds of the *Hypericum perforatum* L. cultivar "Topas" were swollen in water over night, air dried, and planted outdoors in a depth of 5 mm. After 6 months, the 25 cm high plantlets were transplanted to the field. All experiments were carried out in triplicate for the three envisaged harvesting times on 3x3 plots of 160×125 cm. Row spacing was 40 cm, planting distance 25 cm. The patches were regularly hoed and irrigated. Weeds were removed mechanically. In the first year after transplanting growth rate was very low, but after a sufficient cold stimulus during winter, stem initiation took place. The plant material harvested at vegetative stages BF, FF, or FR, respectively, was collected from the upper third of the individual plants. The material was then dried under exclusion of light at 30±0.5°C, and subjected to analysis. All extractions respectively analyses were carried out in triplicate.

Extraction of essential oil: The dried plant material was subjected to water steam distillation for 3 hours using a Clevenger apparatus according to method recommended by the European Pharmacopoeia. The resulting oils were of watery consistence and pale yellow in colour.

Identification of compounds: Constituents were identified by comparing their Kovats retention indices to data given in the literature (4-6). GC-MS was used for the confirmation of compound identity and for the identification of further constituents.

GC analysis: GC analysis was carried out on a Shimadzu GC-9A gas chromatograph fitted with a Silicon DB-1 capillary column (60m×0.25mm). Carrier gas was Helium with an inlet pressure of 3 kg/cm² in split mode. A temperature gradient was set to 50-250°C, with an increase of 4°C/min. Injector temperature was 250°C, detection temperature was 265°C. For detection, a dual FID was used. For all samples, a volume of 0.1 µl was injected.

GC-MS analysis: GC-MS analyses were carried out on Varian 3400 gas chromatograph fitted with a Silicon DB-1 capillary column (60 m×0.25mm). The temperature gradient was programmed for an initial temperature of 40°C, a final temperature of 250°C, and an increase of 4°C per minute. Carrier gas was Helium in a flow rate of 3.7 ml/min. Injector temperature was 260°C. The GC was coupled to a Saturn II A mass selective detector (70eV).

Results and Discussion

Essential oil composition: There was a distinct difference in type and quantity of major compounds found in the essential oil of wild crafted SJW from South Africa as compared to the oil from cultivated SWJ from Iran (tables 1 and 3).

Table 1: Major compounds detected in the essential oil of SJW from South Africa (wild crafted) as compared to oil originating from Iran (Mashad, cultivation, cultivar Topas; cf. table 3). Flowers harvested at full flowering stage. Values in %.

Origin	RSA	Iran
a-pinene	25.38	0.72%
2-methyl-octane	20.64	n.d.
a-thujene	0.07	n.d.
camphene	0.03	n.d.
ß-pinene	0.30	1.07
sabinene	1.60	n.d.
myrcene	0.61	n.d.
a-phellandrene	0.03	n.d.
limonene	0.24	n.d.
cis-ocimene	0.67	n.d.
?-terpinene	0.27	n.d.
(E)-ß-ocimene	0.06	0.49
ß-caryophyllene	3.77	2.07
germacrene D	15.12	1.62
a-curcumene	3.47	n.d.

Essential oil quantity: Harvesting time under controlled conditions had a significant effect on essential oil content of SJW. As shown in table 2, harvesting at stage FF yielded 191% more essential oil than collecting the plants at BF, and 50% more than was found for FR. The increased yield of essential oil at full flowering is not reflected in the biomass of fresh respectively dry matter, which was highest at the time of fruit formation. The yields of essential oil would suggest the stage of full flowering as the optimal harvesting time, in accordance to traditional use of SJW.

Table 2: Effects of harvesting time on fresh and dry herb yield and essential oil content of *Hypericum perforatum* L. (Origin:Mashad)

Harvesting time	BF	FF	FR
Fresh herb yield (t/ha)	7.5	15.7	18.0
Dry herb yield (t/ha)	2.0	3.9	4.7
Essential oil content (ml/100 g)	0.12	0.35	0.18
Relative oil content (%)	100	291	150
Essential ol yield (l/ha)	2.4	13.8	8.4

Dependency of essential oil composition from flowering stage: Next to the quantity of the essential oil, the qualitative composition of the essential oil was strongly influenced by the harvesting time (table 3). For BF, 39 different constituents were identified, in contrast to 30 at FF and 37 at FR.

There are striking differences in the composition of the essential oil obtained from material harvested at the three different vegetative stages, especially with respect to the relative amounts of bicyclogermacrene, ß-bisabolene, a-cadinol, ß-caryophyllene, a- and ?-eudesmol, longifolene, ?-muurolene, and spathulenol.

Bicyclogermacrene shows a distinct peak with a quantity of 16.9% at full flowering, whereas at the times BF and FR, the concentration is much lower with 5.5 respectively 4.3%. β-Caryophyllene and ?-muurolene are only present in major amounts in early flowering (4.6 respectively 4.5%), whereas α-cadinene is clearly indicative for full flowering with a concentration of 27.2% in contrast to <<2% at BF and FR. In contrast, β-bisabolene respectively globulol are representative for late flowering stages, and are not detected at full flowering. γ-Eudesmol sharply decreases after full flowering from 6.5% to <1% at FR. Longifolene is not present in full flowering, but present in very high amounts (18.71 respectively 21.99%) in early respectively late flowering. Together with bicyclogermacrene, longifolene can be used as a quality parameter for essential oils from SJW.

A reproducible composition of SJW preparations is of commercial interest, as extracts and the essential oil are used in various pharmaceutical preparations (7). The quality and quantity of secondary metabolites of medicinal plants depends (among others) on the vegetative stage at harvesting time (3). As could be shown by our investigation, this is also true for the essential oil obtained from SJW.

Table 2: Compounds identified in the essential oil of SJW at different vegetative stages (n.d. = not detected; RI = Kovats Retention Index). Origin: Mashad.

Component	BF (%)	FF (%)	FR (%)	RI
Aromadendrone	0.83	0.89	0.95	1462
Bicyclogermacrene	**5.51**	**16.93**	**4.31**	1481
ß-bisabolene	n.d.	n.d.	**9.02**	1478
ß-bisabolol	1.32	n.d.	0.99	1659
?-bourbonene	0.60	0.80	0.58	1386
Cadelene	1.30	1.64	1.10	1642
a-cadinene	1.16	**27.17**	0.89	1427
ß-cadinene	2.59		1.95	1513
?-cadinene	2.17	1.00	1.36	1505
d-cadinene	0.88	1.61	n.d.	1508
a-cadinol	**3.20**	**5.70**	**4.87**	1627
Camphor	0.50	n.d.	n.d.	1096
ß-caryophyllene	**4.62**	2.07	2.04	1458
caryophyllene oxide	1.86	0.99	1.06	1598
ß-cedrene	1.89	1.18	1.91	1414
a-coraene	n.d.	n.d.	1.20	1384
a-cubebene	0.88	n.d.	0.85	1349
ß-cubebene	1.00	0.54	n.d.	1485
Cubebol	0.66	n.d.	n.d.	1529
?-cuprenene	1.38	2.44	1.07	1490
o-cymene	1.13	0.38	n.d.	1010
ß-elemene	0.79	n.d.	n.d.	1377
?-elemene	0.83	0.69	0.72	1543
Elemol	n.d.	n.d.	0.45	1529
?-eudesmol	2.90	**3.81**	2.77	1664
G-eudesmol	**10.75**	**6.52**	0.76	1579
Farnesol	2.98	1.57	2.23	1675
farnesyl acetone	0.77	1.01	0.69	1863
geranyl acetate	0.54	0.39	n.d.	1353
geranyl butyrate	0.84	n.d.	n.d.	1541
germacrene D	1.69	1.62	1.93	1446
Glubulol	n.d.	n.d.	**5.15**	1571
Guaiol	n.d.	n.d.	2.13	1564
a-humulene	1.15	1.67	1.50	1450
Longifolene	**18.71**	n.d.	**21.99**	1421
Linaloal	0.52	n.d.	n.d.	1082
G-muurolene	**4.52**	n.d.	2.14	1471
trans-nerolidol	1.15	1.52	1.22	1549
(E)-ß-ocimene	n.d.	0.49	n.d.	1035
a-pinene	0.57	0.72	1.44	928
ß-pinene	1.12	1.07	1.36	968
Sabinene	n.d.	n.d.	1.83	953
Spathulenol	**6.90**	**6.95**	**8.45**	1575
?-terpinene	n.d.	n.d.	0.96	1054
terpinene-4-ol	n.d.	0.44	n.d.	1166
a-terpineol	0.66	n.d.	1.42	1161
Terpinolene	0.82	0.81	0.58	1061
a-thujene	n.d.	n.d.	2.13	911
Viridiflorol	0.78	0.50	n.d.	1582

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