

Comparative phytochemical analysis of wild and cultivated rhizomes of *Hedychium spicatum* Buch. Ham. of north west Himalaya

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Manuscript received online 02 November 2013, revised 29 July 2013, accepted 08 August 2014

Abstract : The pharmaceutical, cosmetic and agricultural demand of medicinal and aromatic plant species causes promotion of their commercial cultivation in order to meet the increasing demand within the domestic and export markets. Rhizomes of *Hedychium spicatum* Buch. Ham. (Family : Zingiberaceae), contain bioactive compounds that are responsible for anti-inflammatory, ant-asthmatic, hypoglycaemic, vasodialator and other antimicrobial properties. The present investigation aimed to evaluate chemical profile of *H. spicatum* collected from north west Himalaya. Processed rhizomes were analyzed for the presence of metals, alkaloids, fatty acids and volatiles by Atomic Absorption Spectrophotometer and GC-MS combined with HS-SPME. Comparison of data was done with the help of Student's t-test and is presented as mean $\pm$ standard deviation at significance level $p \leq 0.05$. Metal/nutrients generally significantly increased in cultivated plant except B (55.50 to 51.60 ppm) and Cl (150.00 to 141.50 ppm). Except linoleic acid (18 : 2 ω 6 50.00 to 57.33), oleic acid (18 : 3 ω 9 10.00 to 14.24) and γ -linolenic acid (18 : 3 ω 6 1.00 to 1.30), all the tested fatty acids significantly decreased in cultivated *H. spicatum*. Only considerable variations were recorded in chemical constituents after three years of experimental cultivation. Acid value ranged from 1.30 to 1.40 while saponification value 108.00 to 111.80 for wild and cultivated rhizomes respectively. There is considerable qualitative variation in the composition of the chemical contents of both the wild and cultivated samples.

Keywords : *Hedychium spicatum*, phytochemical analysis, wild, cultivated, metals.

Introduction

Hedychium spicatum Buch. Ham. (Family : Zingiberaceae), commonly known as spiked ginger lily, is found in the entire Himalayan region¹. It is a perennial rhizomatous and herb erect herb with leafy stem upto 5–150 cm height and is used in traditional system of medicine to treat various diseases. Leaves are broad, ovate-lanceolate, 30–60 $\times$ 10–20 cm acuminate, glabrous above, sparsely and pubescent beneath. Flowers fragrant, white with an orange-yellow or red base, dense, terminal, 15–25 cm long spikes; floral bracts large, green and singled flowered. Calyx membranous, 3-lobed, ovate, obtuse and shorter than bracts. Corolla tube 5–6.5 cm long, much longer than calyx; petals white, linear, spreading; lip white with 2-elliptic lobes and orange or yellow base; filaments of stamen-red. Capsules are globular, 3-valved with an

orange-red lining and seeds black with red aril². Rhizomes are 15–20 cm long, 2.0–2.5 cm in diameter, externally yellowish-brown, but turn dark brown on storage, one edge of each piece is covered by a rough reddish brown layer marked with numerous scars and circular rings and rudiments of rootlets were visible³. The reported main constituents are essential oil components⁴, starch, resins, organic acids, ethyl ester of *p*-methoxy cinnamic acid, *d*-sabinene and sesquiterpenes⁵. Its rhizomes are used in Ayurvedic and Unani medicine. Aromatic rootstock contains essential oil, saccharin, albumin, starch and mucilage. The rhizomes are considered to be useful in stomachic, carminative and stimulant for the treatment of liver complaints, diarrhoea, food poisoning and inflammation. It is useful in the treatment of asthma and bronchitis⁶. Rhizome powder is used as an antiseptic

agent and also used in various aches and pains⁷. Its rhizome has been valued in the Traditional System of Medicine for possessing variety of therapeutic properties like carminative, expectorant, tranquilizer, stomachic, antiseptic, vasodilator, analgesic, anti-inflammatory, antimicrobial, antifungal, antioxidant, pediculicidal and cytotoxic activities^{5,8,9}. Different formulations of this plant are Agastyaharitaki Rasayana and Shatyadi Churn¹⁰. Eranda paka, Shribahushalo guda, Dantodbhedagantaka rasa, Katphaladi churna, Pippalyadi taila, Aravindasava, Ushirasava, Chandanasava, Sarivadyasava, Amritaprashaghrita and Pradarantakalauha are the other important formulations¹¹. The trade value of its rhizome is Rs. 175 per kg in the retail market¹². The literature survey of the plant revealed that not much research work dealing with its comparative chemical analysis has been carried out so far. In this study we aimed to provide the chemical composition of *H. spicatum* for the acclaimed medicinal use and determine the quality of the drug prepared by using this herb.

Results

Phytochemical analysis results for wild and cultivated samples of *Hedychium spicatum* were tabulated in Tables 1–3. The analysis shows that high variety of nutrients/metals, fatty acids and other chemical constituents are present in wild crafted as well as in cultivated drugs which can be used in different compositions of medicines.

Table 1. Metal/nutrients content of wild and cultivated *H. spicatum*

Chemical constituent Metal/nutrients	Wild sample (ppm)	Cultivated sample (ppm)
Cu	10.37 ^a ± 0.54	12.67 ± 1.25
Zn	21.40 ± 1.64	22.89 ± 1.02
Mn	38.00 ^b ± 0.50	41.33 ± 0.63
Fe	285.00 ^b ± 6.08	310.59 ± 0.52
K	18800.00 ± 360.56	19300.00 ± 100.00
Ca	13200.00 ^b ± 264.58	15200.00 ± 100.00
Mg	6700.00 ± 200.00	6700.00 ± 45.83
Al	225.00 ^b ± 3.61	255.00 ± 10.00
Ba	44.62 ^b ± 1.16	51.86 ± 1.02
B	55.50 ^b ± 1.32	51.60 ± 0.53
Mo	0.25 ± 0.06	0.21 ± 0.02
Cl	150.00 ^a ± 5.00	141.50 ± 1.80

^aSignificant at $p \leq 0.05$ and ^bsignificant at $p \leq 0.01$.

Table 2. Fatty acid composition of wild and cultivated *H. spicatum*

Chemical constituent Fatty acid	Wild sample Percent w/w	Cultivated sample Percent w/w
Linoleic acid (18 : 2 ω 6)	50.00 ^a ± 4.58	57.33 ± 0.66
α -Linolenic acid (18 : 3 ω 3)	15.00 ^b ± 1.00	11.45 ± 0.61
Oleic acid (18 : 1 ω 9)	10.00 ^b ± 1.00	14.24 ± 0.26
Palmitic acid (16 : 0)	6.00 ± 1.00	5.50 ± 0.36
Stearic acid (18 : 0)	2.00 ± 0.00	2.20 ± 0.26
γ -Linolenic acid (18 : 3 ω 6)	1.00 ^b ± 0.00	1.30 ± 0.10
Eicosanoic acid (20 : 0)	0.79 ^a ± 0.06	0.65 ± 0.04
Eicosenoic acid (20 : 1)	0.39 ± 0.04	0.43 ± 0.03
Eicosadienoic acid (20 : 2)	0.08 ± 0.04	0.09 ± 0.01

^aSignificant at $p \leq 0.05$ and ^bsignificant at $p \leq 0.01$.

Table 3. Other chemical constituent composition of wild and cultivated *H. spicatum*

Chemical constituent	Wild sample	Cultivated sample
α -Tocopherol (ppm)	7.00 ± 1.00	6.80 ± 0.36
γ -Tocopherol (ppm)	7100.00 ^b ± 100.00	813.50 ± 2.60
Acid value	1.30 ± 0.17	1.40 ± 0.10
Saponification value	108.00 ± 3.61	111.80 ± 2.88
Terpenoid (%)	16.40 ^a ± 0.10	19.00 ± 1.47
Hedychenone (%)	22.30 ^b ± 0.10	26.45 ± 0.43
Ethyl- <i>trans</i> - <i>p</i> -methoxy cinnamate (%)	67.80 ^b ± 0.98	59.50 ± 1.32
Ethyl cinnamate (%)	10.20 ^b ± 0.82	14.50 ± 0.50
d-Sabinene (%)	4.00 ± 1.00	5.43 ± 0.20
1,4-Cineole (%)	6.00 ± 0.50	6.45 ± 0.13
Sesquiterpene-cadinene (%)	5.50 ± 0.50	5.90 ± 0.36
Sesquiterpene alcohol (%)	4.70 ± 0.20	5.10 ± 0.17

^aSignificant at $p \leq 0.05$ and ^bsignificant at $p \leq 0.01$.

Chemical analysis showed that wild sample of *Hedychium spicatum* contained metals/nutrients – 10.37 ppm copper (Cu), 21.40 ppm zinc (Zn), 38.00 ppm manganese (Mn), 285.00 ppm iron (Fe), 18800.00 ppm potassium (K), 13200.00 ppm calcium (Ca), 6700.00 ppm magnesium (Mg), 225.00 ppm aluminium (Al), 44.62 ppm barium (Ba), 55.50 ppm boron (B), 0.25 ppm molybdenum (Mo), 150.00 ppm chlorine (Cl), and fatty acids– linoleic acid (18 : 2 ω 6) 50.00% w/w, α -linolenic acid (18 : 3 ω 3) 15.00% w/w, oleic acid (18 : 1 ω 9) 10.00% w/w, palmitic acid (16 : 0) 6.00% w/w, stearic acid (18 : 0) 2.00% w/w, γ -linolenic acid (18 : 3 ω 6) 1.00% w/w, eicosanoic acid (20 : 0) 0.79% w/w, eicosenoic acid (20 :

1) 0.39% w/w and eicosadienoic acid (20 : 2) 0.08% w/w. Other chemical constituents detected in *Hedychium spicatum* were vitamins α -tocopherol and γ -tocopherol (7.00 and 7100.00 ppm respectively), terpenoid (16.40%), hedychenone (22.30%), ethyl-*trans*-*p*-methoxy cinnamate (67.80%), ethyl cinnamate (10.20%), d-sabinene (4.00%), 1,4-cineole (6.00%), sesquiterpene-cadinene (5.50%) and sesquiterpene alcohol (4.70%). It has an acid value of 1.30 and saponification value of 108.00.

The cultivated sample of *H. spicatum* contains metals/nutrients – 12.67 ppm Cu, 22.89 ppm Zn, 41.33 ppm Mn, 310.59 ppm Fe, 19300.00 ppm K, 15200.00 ppm Ca, 6700.00 ppm Mg, 255.00 ppm Al, 51.86 ppm Ba, 51.60 ppm B, 0.21 ppm Mo, 141.50 ppm Cl and fatty acids-linoleic acid (18 : 2 ω 6) 57.53% w/w, α -linolenic acid (18 : 3 ω 3) 11.45% w/w, oleic acid (18 : 1 ω 9) 14.24% w/w, palmitic acid (16 : 0) 5.50% w/w, stearic acid (18 : 0) 2.20% w/w, γ -linolenic acid (18 : 3 ω 6) 1.30% w/w, eicosanoic acid (20 : 0) 0.65% w/w, eicosenoic acid (20 : 1) 0.43% w/w and eicosadienoic acid (20 : 2) 0.09% w/w. Other chemical constituents isolated from cultivated *H. spicatum* were vitamins including α -tocopherol and γ -tocopherol (6.80 and 813.5 ppm respectively), terpenoids (19.50%), hedychenone (26.45%), ethyl-*trans*-*p*-methoxy cinnamate (59.50%), ethyl cinnamate (14.50%), d-sabinene (5.43%), 1,4-cineole (6.45%), sesquiterpene-cadinene (5.90%) and sesquiterpene alcohol (5.10%). It had an acid value of 1.40 and saponification value of 111.8.

Discussion

The mean contents of Cu, Mn, Fe, Ca and Al in wild sample were significantly lower than in the cultivated sample of *H. spicatum*. Magnesium content was same in wild and cultivated sample. The boron and chlorine content was significantly higher in wild sample as compared to cultivated sample.

The linoleic acid (18 : 2 ω 6), oleic acid (18 : 1 ω 9) and γ -linolenic acid (18 : 3 ω 6) content in wild sample was significantly lower than cultivated sample. The γ -linolenic acid (18 : 3 ω 3) and eicosanoic acid (20 : 0) content in wild sample were significantly higher than cultivated sample. The content of γ -linolenic acid (18 : 3 ω 6) in wild sample was lower than cultivated sample. The α -tocopherol content in wild sample was higher than cultivated sample.

The content of γ -tocopherol in wild sample was much higher than cultivated sample. The acid value, saponification value and terpenoid content in wild sample were lower than cultivated sample.

Since indiscriminate use of synthetic drugs and antibiotics has resulted into serious symptoms all over the world, the demand of plant based raw materials for pharmaceuticals has increased enormously. Moreover, the synthetic drugs and intermediary chemicals are extremely expensive. The World Health Organization has emphasized the utilization of indigenous systems of medicines based on the locally available raw materials, i.e. medicinal plants¹³.

The qualitative analysis of both the samples viz. wild and cultivated showed that all the chemical contents found in the wild samples were also present in cultivated samples. Whereas, when wild and cultivated samples were compared quantitatively, in the cultivated sample, the quantity of most of the metals/nutrients was significantly higher while quantity of some metals/nutrients was slightly lower as compared to wild samples. This increment in the quantity is may be due to the presence of nutrients in the fertilizer by which the cultivation beds were supplemented. While the quantity of fatty acids and other chemical constituents were slightly influenced.

Recently the antioxidant phytochemical study of *H. spicatum* was done by Bhatt *et al.* (2008) and concluded that the phenolics and DL- α -tocopherol content was significantly ($p < 0.01$) higher in one year old planted rhizome as compared to those which are collected from the wild¹⁴. The aromatic volatile oil commercially known as 'Kapur Kachri Oil' had a methoxy cinnamate as major chemical constituent and had been extracted from the plant rhizome¹⁵. The crude extract of the rhizome had been used in the preparation of an anticancerous drug, PDMA 28¹⁶. Dried rhizomes of *H. spicatum* on steam distillation give essential oil (yield 2–4%), which consists mainly of monoterpenes (e.g. myrcene, cineole), sesquiterpenes (e.g. β -bisabolene, β -pactchoulene, elemol, eudesmol) and benzene derivatives (e.g. eugenol, *p*-methoxycinnamic acid). Other compounds isolated from the rhizomes were three labdane diterpenoids which include hedychenone and 7-hydroxyhedychenone¹⁷. Benzyl acetate, benzyl cinnamate, β -caryophyllene, cinnamaldehyde, 1,8-cineole, *p*-cymene, ethylcinnamate, limonene, linalool, linalylacetate, *p*-

methoxyethylcinnamate, pentadecane, β -phellandrene, α - and β -pinenes, d-sabinene, γ -terpinene, β -terpeniol, β -sitosterol and its glucoside, cryptomeridiol, 7-hydroxyhedychenone, hedychenone, 6-oxo-labda-7,11,13-trien-16-oic acid lactone isolated from the rhizomes of *H. spicatum*¹⁸. Ali (1998) reported essential oil (4%), furanoid diterpene, 7-hydroxyhedychinone, organic acids, resinic acids and starch (50%)¹³. The principal constituents of the volatile oil were ethyl *p*-methoxycinnamate (68%); the others being limonene, β -phellandrene, *p*-cymene, linalool, β -terpineol, β -caryophyllene, ethyl cinnamate (10%), d-sabinene (4%), 1,4-cineole (6%), sesquiterpenes (5.5%) and sesquiterpene alcohol (4.7%). Sakhanokho *et al.* (2013) investigated chemical composition, antifungal and insecticidal activities of *Hedychium* essential oils and found that the oil was rich in 1,8-cineole (0.1–42%), linalool (< 0.1%–56%), α -pinene (3%–17%), β -pinene (4%–31%) and (*E*)-nerolidol (0.1%–20%)¹⁹.

Materials and method :

Plant collection :

Rhizomes were collected in the month of August 2010 from nearby wild sources, Tarikhet before the onset of dormancy and some of the rhizomes were chemically analyzed and the remaining rhizomes were experimentally cultivated at SMP Garden, Regional Research Institute of Himalayan Flora, Tarikhet, Ranikhet. After completion of agro trials for three subsequent years, rhizomes were collected from the treatment showing best yield was again chemically analyzed to see the effect of *ex-situ* cultivation on chemical constituents of this herb.

Plant grinding :

Prior grinding, all the samples were examined to determine if they are dry enough to be ground, properly labelling and for anything unusual, such as fungus growth on them etc. Proper drying of each sample was ensured by vacuum drying without heating. Samples were ground with a grinder. Then, utilizing a Tyler Industrial Products model RX24 portable shaker, the ground plant sample was sieved through a series of mesh sizes.

Extract preparation :

Fine powder (100 g) was mixed thoroughly with 500 mL of 50% ethanol (1 : 1 ratio of ethanol and distilled water) and kept at room temperature for overnight. On

2nd day 500 mL of fresh 50% ethanol was added to this mixture and after mixing thoroughly kept at room temperature for overnight. The same process was repeated on 3rd day. On 4th day, mixture was filtered and processed for rotavaporation to concentrate the filtrate. Then the concentrated filtrate was freeze dried (lyophilized) to get a powder. The samples were then processed as per the specific protocols for different analysis.

Metal analysis :

Metal analysis was done using the grounded powder of each sample. One gram of each of the grounded sample was digested by gentle heating, initially with 10 mL concentrated nitric acid (HNO₃) and perchloric acid (HClO₄) [4 : 1 v/v] in Kjeldahl flask until no brown fumes appeared²⁰. The final volume was made up to 10 mL in a volumetric flask with 1% HNO₃. Analysis of the metal was carried out on an Atomic Absorption Spectrophotometer (GBC Avanta Sigma). The measurements were taken using an external standard calibration graph. Each analysis was done in triplicate including blanks.

Analysis of alkaloids, fatty acids and other volatiles :

GC-MS (Perkin-Elmer Turbomass) combined with headspace solid-phase micro extraction (HS-SPME) method was used to analyze the profiling of free volatile compounds in given plant extracts. Prior the analysis, conditions for sample preparation (SPME fiber type, extraction time, extraction temperature and dilution solvent) and GC-MS conditions were standardized to get optimized methodologies and results. Following the standardization, the extract were resuspended thoroughly with water and 8 mL of sample were placed in a 20 mL headspace vial with addition of NaCl; a polydimethylsiloxane SPME fiber was used for extraction at 40 °C for 30 min with continuous stirring. Identification of components was obtained by comparison of their retention time with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of *n*-hydrocarbons (C₉–C₃₃). Further validation and authentication of the product was done using Library of databases available with GC-MS (NIST, Wiley). The calculations and quantification were done using software attached to the equipment.

Statistical analysis :

Comparison of data was done with the help of Student's

t-test and is presented as mean $\pm$ standard deviation. Comparison of the result was done in triplicate and values of $p < 0.05$ were considered to be significant.

Conclusion

The present study shows that in the wild and cultivated samples of *H. spicatum*, all the chemical contents qualitatively remain same. However, there is a considerable variation in the chemical composition of both the samples.

Therefore, it may be concluded that the mass scale cultivation of this herb may help in fulfilment of the increasing raw drug demand of pharmaceutical companies but still more work in its chemistry is required for its proper use in pharmaceutical formulations.

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