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Valepotriates and valerenic acids in commercial preparations of valerian available in Australia

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Thirty-one commercial valerian preparations available in Australia, including teas, tablets, capsules and liquids, were analysed by HPLC for valepotriates, valerenic acid and valerenic acid derivatives. The concentration of valerenic acid and its derivatives ranged from <0.01 to 6.32 mg/g of product. Powder capsules, on average, contained the highest concentration of valerenic acids (2.46 mg/g) and liquids the lowest concentration (0.47 mg/ml). The mean concentration of valerenic acid in the five products standardized against valerenic acid (3.56 mg/g) was significantly higher than in the 26 non-standardized products (0.89 mg/g). There was a significant relationship between valerenic acids content and added valerian root for the standardized products but not for the non-standardised products. Valepotriates were found at low levels (<1.0 mg/g) in some teas but were not detected in any of the finished products.

1. Introduction

The drug, *Valerianae radix*, consists of the roots, rhizomes and stolons of *Valeriana officinalis* L., and is used as a sedative for nervous tension, sleeplessness, anxiety and stress [1]. It is a respected medicinal plant with entries in the Pharmacopoeia of many countries [2]. Valerian is available on the market in the form of a tea or tincture and incorporated into tablets and capsules as either powdered root or root extract [3].

The plant constituents responsible for beneficial health effects have not been fully identified although the essential oil, valepotriates and valerenic acid and its derivatives are believed to provide biological activity [4]. Valerenic acid and its derivatives are not present in two other species used for phytotherapy, Mexican (*V. edulis* Nutt. ssp. *procera*) and Indian valerian (*V. wallichii* DC.) [5], but are found in the roots of *V. officinalis* and several related species [5, 6]. The valerenic acids are relatively stable compounds [7] and can be used as an identity check and to ensure that extracts are not adulterated [8].

The use of medicinal herbs is becoming more popular in Australia with over 60% of the population using one form of alternative medicine and retail sales over $Aust. 900 million [10]. Consumers expect to purchase high quality products that have efficacy. A recent survey conducted on retail *Echinacea* products in Australia revealed a high variability in the level of active constituents and led to the recommendation of more stringent quality control by manufacturers and more definitive and uniform labelling based on nominated active compounds [11].

Standardization of phytomedicines against one or more leading compounds is considered important due to differences in raw material quality and extraction processes [9]. When products are not standardized, the only guide a consumer can use to determine the chemical concentration of the product is by the stated amount of plant extract or raw material used.

This study was undertaken to determine the levels of valerenic acid and its derivatives and valepotriates in valerian products available in retail outlets in Australia. The aim was to determine the quality and consistency of a range of products and the adequacy of labelling as an indication of quality.

2. Investigations, results and discussion

2.1. Analysis of finished products

A total of 28 solid and three liquid products containing only valerian were purchased during November 1999 and March 2000. Where possible, two samples with different batch numbers were obtained and resulted in the purchase of a total of 55 samples for analysis. The level of valerenic acid and its derivatives and valepotriates were determined by HPLC using a modified method of Bos et al. [7]. The peaks of valerenic, acetoxyvalerenic and hydroxyvalerenic acids were summed to give total valerenic acids and those of valtrate and isovaltrate summed to give total valepotriates. Linear regression analysis and one-way ANOVA's were accomplished using Excel (Microsoft Corporation, Redmond, WA).

2.2. Total valerenic acids and valepotriates by product weight

The level of total valerenic acids in the 55 analytical samples ranged from <0.01 to 6.32 mg/g or ml of product (Table 1). A substantial proportion of samples (16%) contained less than 0.1 mg and this included three tea samples and two liquid samples at non-detectable levels. Samples with higher levels comprised 31% with 0.1–1 mg, 33% with 1–2 mg and 20% >2 mg/g or ml. All standardized samples (that is, those with a stated valerenic acid level on the label) contained valerenic acids at >2 mg/g whereas only one non-standardized sample fell in this range. The mean concentration of valerenic acids in the five standardized products (3.56 mg/g) was significantly higher ($P < 0.001$) than that present in the 26 non-standardised products (0.89 mg/g).

The range and average concentration of valerenic acids in each product class are given in Table 2. Powder capsules, on average, contained the highest concentration of valerenic acids on a product weight basis (2.46 mg/g). The tablets, teas and soft gel capsules had an average valerenic acid content of about 1 mg/g while liquids had the lowest average concentration (0.47 mg/ml) of all product classes.

In general, products were consistent between batches except for one tea which returned a non-detectable amount followed by a concentration of 1.36 mg/g in the repeat purchase (Table 1). The difference between batches was smaller for standardized products (0.2% to 14.8%, average 6.5%) than for non-standardized products (0 to 59%, average 22.9%).

The linear regression of the tested level of valerenic acids against the stated level in the standardized samples was highly significant ($y = 0.87x - 0.09$; $R^2 = 0.99$, $P < 0.001$). This indicates that while the actual valerenic

Table 1: Valerian products tested, their valerian content and level of total valerenic acids in mg/g or ml product, mg/g root and mg/dose

Product	Manufacturer/Distributor	Country of Production	Valerian Content (g/g)	Stated Valerenic Acid (mg/g)	Recommended Dose (g of root)	Total Valerenic Acids				
						m/g product		m/g root		mg/dose
						Sample 1	Sample 2	Sample 1	Sample 2	
Teas										
Healthy Life Valerian Rootlets	Healthy Life	France			nr	1.25	1.47	1.25	1.47	
Blooms Valerian Root	Blooms Health Products	Australia			6.00	nd	1.36	nd	1.36	8.16
Hilde Hemmes' Herbals Valerian Root	Herbal Supplies	Australia			2.00	1.19	1.09	1.19	1.09	2.78
Colonial Farms Valerian Rootlets	Select Foods	Australia			2.00–4.00	nd	nd	nd	nd	nd
Russell's Valerian Tea	Russell's Natural Foods	France			nr	1.64	1.73	1.64	1.73	
Tablets										
Earth's Own Valerian 2500	Allied Master Chemists	Australia	1.82		2.50–5.00	2.35	–	1.29	–	3.22–6.44
Valerian Forte	Blackmores	Australia	1.73	3.46	1.80	3.25	2.78	1.88	1.61	3.15
Valerian Herb-Relax 2000	Blooms Health Products	Australia	1.42		2.00	0.52	–	0.37	–	0.73
Fingerprint Botanicals Valerian 1000	Bullivant's Natural Health	Australia	1.08		1.00–2.00	1.34	1.25	1.24	1.16	1.21–2.42
Chemworld Valerian 500mg	Chemworld Chemist	Australia	0.90		0.50–1.00	0.34	0.32	0.38	0.36	0.19–0.38
Healtheries Valerian 500mg	Health Minders	Australia	0.87		0.50	0.42	0.29	0.48	0.33	0.20
Ethical Nutrients Valerian 1000	Health World	Australia	1.24	2.47	1.00–2.00	2.03	2.05	1.64	1.66	1.65–3.30
Herbal Valerian 500 mg	Herb Valley	Australia	1.14		0.50	0.49	–	0.43	–	0.22
Valerian	Herron	Australia	0.91		0.50–1.00	0.49	0.40	0.54	0.44	0.25–0.50
Valerian	Natures Way Health	Australia	0.82		0.50–1.50	1.29	0.96	1.57	1.17	0.69–1.38
Cirkulin Valerian Tablets	Polcopharma	Germany	1.19		0.56–1.12	0.07	–	0.06	–	0.03–0.06
Valerian 2000	Soul Pattinson	Australia	1.39	2.78	2.00	2.44	2.08	1.76	1.50	3.22
Valerian	VitaGlow	Australia	0.64		0.50–1.00	0.32	–	0.50		0.25–0.50
Powder Capsules										
Bio-organics Valerian 2250	Bullivant's Natural Health	Australia	3.70	7.32	2.25	6.19	6.32	1.67	1.71	3.62
Nature's Own Valerian 500 mg	Bullivant's Natural Health	Australia	1.52		0.50–1.00	1.79	1.95	1.18	1.29	0.62–1.24
Hilde Hemmes' Herbals Valerian	Herbal Supplies	Australia	2.50	4.93	1.50–4.50	4.31	4.30	1.72	1.72	2.59–7.77
Kordel's Valerian 1000	Kordel	Australia	1.82		1.00	0.59	0.69	0.32	0.38	0.35
Valerian Root	Nature's Sunshine	USA	0.84		0.82	1.68	1.32	2.00	1.57	1.47
Nature's Path Valerian Root	Planet Health	USA	1.00		1.06–2.12	1.66	–	1.67	–	1.76–3.52
Valerian 1000	Vitaplex Products	Australia	1.82		1.00	0.67	0.47	0.37	0.26	0.31
Soft Gel Capsules										
Earth's Own Valerian 1000	Allied Master Chemists	Australia	2.25		1.00	1.20	0.93	0.53	0.41	0.47
Valerian 100 mg	Herb Valley	Australia	2.21		1.00	0.95	1.51	0.43	0.68	0.56
Valerian 500	Soul Pattinson	Australia	1.38		0.50–1.00	0.51	0.26	0.37	0.19	0.16–0.32
Liquids										
Valerian	Greenridge Botanicals	Australia	1.00		0.90–3.00	0.94	1.31	0.94	1.31	1.02–3.39
Hilde Hemmes' Valerian Root	Herbal Supplies	Australia	0.20		1.00–3.00	0.09	–	0.45	–	0.45–1.35
Valerian	Thursday Plantation	Australia	0.50		1.50	nd	nd	nd	nd	nd

Blank result when second sample of product could not be obtained, nd = not detected (< 0.01mg/g), nr = not recommended.

Table 2: Concentration of total valerenic acids in each product clas

Product Type	No.	Total Valerenic Acids			
		mg/g Product		mg/g Root	
		Mean	Range	Mean	Range
Tea	5	0.98	<0.01–1.64	0.98	<0.01–1.64
Tablet	13	1.21	0.07–3.25	0.97	0.06–1.88
Powder Capsule	7	2.46	0.47–6.32	1.22	0.26–2.00
Soft Gel Capsule	3	0.89	0.26–1.20	0.44	0.19–0.68
Liquid	3	0.47	<0.01–1.31	0.54	0.01–0.94

acid content was related to the labelled amount, the actual amount was, on average, 13% lower. This discrepancy may have arisen from different analytical methods of determination or from losses during processing and/or storage.

Valepotriates were found at low levels (<1.0 mg/g) in some valerian teas, but were not detected in any of the finished products. The lack of valepotriates in the finished medicines was not unusual and has been reported in other studies [7, 12, 13]. Bos et al. [7] have shown that valepotriates are not extracted by solutions containing less than 70% ethanol. They are also thermolabile and unstable, decomposing under acidic or alkaline conditions and in alcoholic solutions [6].

Hydroxyvalerenic acid was detected in all the samples containing valerenic acid, except in one tablet and three soft gel capsules, at levels of 0.01 to 0.24 mg/g. This agrees with earlier studies [8, 14], in contrast to two recent surveys of valerian products that failed to find hydroxyvalerenic acid in any of the preparations tested [7, 9]. It is believed that hydroxyvalerenic acid is a hydrolysis breakdown product of acetoxyvalerenic acid [8, 14] and that this hydrolysis can occur when roots are stored in high humidity conditions [6].

2.3. Total valerenic acids on an added valerian basis

All products were labelled with the amount of valerian root added, expressed as equivalent dried root per unit. The added valerian ranged from 0.2 to 3.46 g/g or ml. In Table 1 a calculated value of the concentration of valerenic acids in the extract or raw material (mg/g root) was derived from the product label claim of the amount of added valerian**.** The concentrations ranged from <0.01 mg to 2.0 mg which is similar to that reported in European studies [7, 8]. Hänsel and Schulz [8] recommended that valerian extracts contain a minimum of 1.2 mg/g of valerenic acids for alcoholic extracts and 0.6 mg/g for aqueous extracts. This study found 49% of extracts contained <0.6 mg/g and that all standardized samples were manufactured from extracts containing >1.2 mg/g. In Table 2 the average valerenic acids concentration for the tea, tablet and powder capsule products was about 1.0 mg/g root whereas the liquid and soft gel products contained about 0.5 mg/g root.

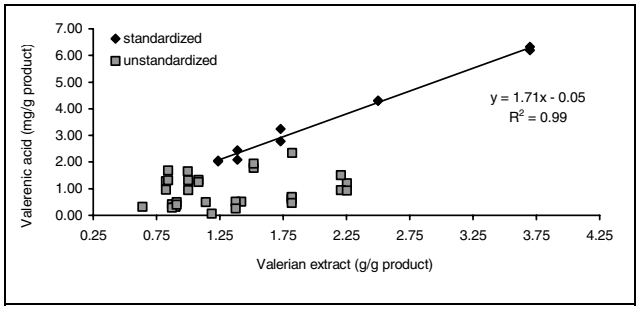

Fig. 1: Regression of total valerenic acids (mg/g or ml of product) and added valerian root (g/g or ml of product) for standardised and non-standardized products

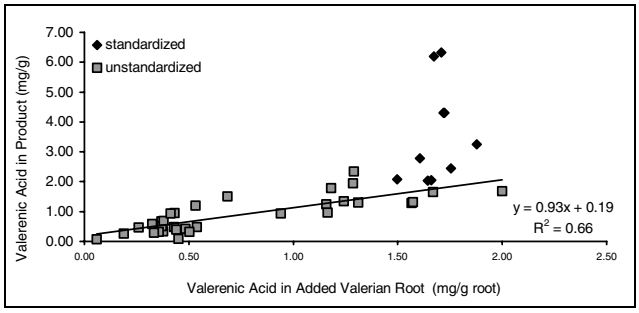

Fig. 2: Regression of total valerenic acids in the product (mg/g or ml) and a calculated value of the total valerenic acids in the added root (mg/g root)

The relationship between total valerenic acids and the amount of added valerian extract is shown in Fig. 1. A linear regression for the standardized samples was highly significant ($y = 1.71x - 0.05$; $P < 0.001$) indicating that the valerenic acid content in these samples was directly related to the amount of extract added. There was, however, no significant relationship for the non-standardized samples indicating that the level of valerenic acids was only poorly related to the amount of added extract.

To examine if the valerenic acids level of a sample was related to the quality of the extract or raw herb used in manufacture, the linear regression of valerenic acids in each sample against the calculated value in the extracts or raw material was determined (Fig. 2). The significant regression equation for non-standardized samples ($y = 0.93x + 0.19$; $P < 0.05$) indicated that much of the variation in these samples was due to the quality of the added valerian extract or raw material which is known to be variable [8, 14]. For the standardized samples, no significant regression was obtained indicating that the quality of the sample did not depend on the quality of the added extract. In addition, the calculated concentrations of extracts of all standardised samples were in a narrow range of 1.50 to 1.88 mg/g of root suggesting that the amount of extract/g of product was increased to bring the valerenic acid content to the claim on the label.

2.4. Recommended daily dose of products

Information on recommended daily doses was found on the labels of all products except for two teas (Table 1). Large differences were found in terms of both the recommended amount of valerian root (0.5 to 6.0 g/day) and total valerenic acids (<0.01 to 8.16 mg). This reflects the large variation of 2 to 9 g/day that has been proposed as a recommended daily dose [15]. In 16 products, the recommended dose was <2 g/day while 12 products were within the recommended range and 3 products were above.

2.5. Conclusions

There was a wide range in concentrations of total valerenic acids in the analysed samples with a substantial pro-

portion (16%) having less than 0.1 mg/g or ml. Significant concentration differences were seen in the various product classes indicating substantial effects of processing on valerenic acid content. Standardised products had higher and more consistent levels of total valerenic acid that were related to the amount of valerian root material used per unit of product. The concentrations of valerenic acids in non-standardised products were directly related to the concentration in the raw material which was found to be highly variable. Therefore, the labelling of products with a total valerenic acid content provides consumers with a better indication of the product quality than the amount of added valerian root alone.

3. Experimental

3.1. Calibration of working standards

Caffeine (HPLC grade, Fluka, Steinheim) was calibrated to isovaltrate (Crop and Food Research, Dunedin, New Zealand) at 255 nm and biphenyl (99.5%, Aldrich, Milwaukee, WIS) to valerenic acid and hydroxyvalerenic acid (Indofine, Belle Mead, NJ) at 225 nm. Standard curves were prepared for all compounds in the range of 1 to 10 µg. The slope of the working standard curve was divided by the slope of the compound curve to obtain a correction factor. All data generated using working standards were multiplied by the correction factor.

3.2. Sample preparation

3.2.1. Teas and tablets

Tea (10 g) and 10 weighed tablets were crushed to a powder. Weighed aliquots (2–4 g) were extracted three times in 30 ml methanol (AR grade, Merck, Kilsyth, Vic) with 5 min sonication. The solution was filtered through No. 1 filter paper (Whatman, Maidstone, Kent), diluted to 100 ml with methanol and two 20 µl aliquots were analysed by HPLC. This procedure was repeated twice for each sample. The residue after filtration was re-extracted in methanol, analysed and added to the total.

3.2.2. Capsules

The contents of 10 powder filled capsules were weighed and extracted as above. Ten soft gel capsules were weighed, cut in half and the capsules and contents extracted as above. The weight of the gel capsule contents was determined by separating the contents from another set of 10 capsules and weighing.

3.2.3. Liquids

Aliquots (2 ml) of liquid preparations were diluted to 100 ml in methanol, filtered through filter paper and analysed directly.

3.3. Chromatography

The Shimadzu HPLC system consisted of 10AT pump, SIL 10AXL autosampler, SPD 10A UV-Vis dual wavelength detector and CTO-10A column oven. The operating conditions were: Column: reversed phase Alltima C18, 250 × 4. 6 mm (Alltech, Sydney); Guard column: Alltima C18 5 µm (Alltech, Sydney); Mobile Phase: solution A of 200 ml CH_3CN (Burdick and Jackson, Muskegon, MISS) +800 ml H_2O (Milli-Q) + 1.2 ml 85% H_3PO_4 (BDH, Poole, England), solution B of 800ml CH_3CN + 200 ml H_2O + 1.2 ml 85% H_3PO_4; Gradient: 45% B for 5 min, linear gradient to 100% B in 24 min, isocratic 100% B for 5 min, linear gradient to 45% B in 2 min, isocratic 45% B 5 min.; Flow rate: 1 ml/min; Detector wavelengths: 225 and 255 nm.

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