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1 # The authenticity and quality of *Rhodiola rosea* products

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10 *Background: Rhodiola rosea* L. root (Golden Root, Arctic Root) is a high-value herbal medicinal product,
11 registered in the UK for the treatment of stress-induced fatigue, exhaustion and anxiety and used throughout
12 Europe as a herbal medicinal product for similar indications. There are several Chinese species used in
13 traditional Chinese medicine (TCM), including *Rhodiola crenulata* (Hook.f. & Thomoson) that is believed to be a
14 common adulterant in the R. rosea value chain. We investigate the phytochemistry of the different species
15 and assess the potential of *R. crenulata* as an adulterant in the *Rhodiola rosea* value chain.

16 Aims: The project is embedded in a larger study aiming to investigate the diverse value chains that lead to the
17 production of *Rhodiola rosea* as a herbal medicinal product. Here we focus on a comparison of the quality of
18 the finished products and assess any phytochemical variation between products registered under the
19 Traditional Herbal Medicine Products Directive (THMPD) and products obtained from the market without any
20 registration (i.e. generally unlicensed supplements).

21 There are different species of Rhodiola on the market and the principal aim is to establish how these different
22 species vary in their metabolite profile, how products are commercialised and whether there is potential for
23 adulteration at the product manufacture stage.

24 Methods: Approximately 40 commercial products have been sourced from different suppliers. We analysed
25 these samples using high performance thin layer chromatography (HPTLC), mass spectrometry (MS) and ^{1}H-
26 NMR spectroscopy coupled with multi-variate analysis software following a method previously developed by
27 our group for the analysis of turmeric products.

28 Results: The consistency of the products varies significantly. Approximately one fifth of commercial products
29 that claimed to be *Rhodiola rosea* did not contain rosavin (one of the reference markers used to distinguish *R.*
30 *rosea* from related species). Moreover some products appeared not to contain salidroside, another marker
31 compound found in medicinal Rhodiola species. Approximately 80 % of the remaining commercial products
32 were lower in rosavin content than the registered products and appeared to be adulterated with other
33 *Rhodiola* species.

34 Conclusions: The variation in phytochemical constituents present in Rhodiola products available to European
35 buyers via the internet and other sources is a major cause for concern. Adulteration with different species, and
36 other unknown adulterants, appears to be commonplace. Good quality systems and manufacturing practices,
37 including those required under the THMPD, enable consumers to have confidence that products are authentic
38 and meet a high specification for quality and safety.

39 Key words: Rhodiola, quality, adulteration, metabolomics

40

41

Abbreviations

AMIX	Analysis of mixtures
DMSO	Dimethylsulphoxide
GACP	Good agricultural and collection practice
GMP	Good manufacturing practice
HMP	Herbal medicinal product
HPTLC	High performance thin layer chromatography
HTP	Hydroxytryptophan
LC	Liquid chromatography
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
PCA	Principal component analysis
PL	Product licence
TCM	Traditional Chinese medicine
THR	Traditional herbal registration
THMPD	Traditional Herbal Medicinal Products Directive
TMS	Tetramethylsilane

70 ## Introduction

71 *Sedum roseum* (L.) Scop., Crassulaceae , mainly known within the medicinal plant industry under its synonym
72 *Rhodiola rosea* L., is a perennial flowering plant distributed throughout the northern hemisphere, particularly
73 in circumpolar and high altitude regions of Europe and Asia, and to a lesser extent in North America (Brown,
74 2002).

75 Over 200 Rhodiola species have been documented, and many of these are used as medicine in Asia, including
76 *Rhodiola heterodonta* (Hook.f. & Thomson) Boriss, *Rhodiola quadrifida* (Pall.) Fisch & Mey, *Rhodiola semenovii*
77 (Regel & Herder) Boriss, Rhodiola kirilowii (Regel) Maxim. and *Rhodiola crenulata* (Hook.f. & Thomson)
78 H.Ohba. Among, all of these species, *R. rosea* has been the predominant subject of phytochemical, animal and
79 human studies (Shikov, 2014).

80 The root of *Rhodiola rosea is* mentioned in Carl Linne's Materia Medica, which recommends it as a treatment
81 for headaches, hysteria, and as an astringent. The traditional medicinal uses of *R. rosea* have been well
82 established, and numerous studies have been carried out *in-vitro* and *in-vivo* concerning cardio-, neuro- and
83 hepatoprotective effects, antiviral, anti-inflammatory and antibacterial activities of *R. rosea* extracts
84 (Panossian et al., 2010).

85 *R. rosea* has a long history of use as a medicinal plant in several traditional systems. Between 1748 and 1961 a
86 catalogue of medicinal applications of *R. rosea* appeared in the scientific literature of Sweden, Norway, France,
87 Germany, Iceland, and the Soviet Union, principally as an adaptogen with various health-promoting effects
88 (Panossian et al., 2010).

89 The root of *R. rosea* is mainly wild-harvested, the main region being the Altai Mountains in southern Siberia
90 (Galambosi, 2005). Although some attempts have been made to cultivate (e.g. Canada, Sweden, Bulgaria),
91 there appears to be little interest for investment in large-scale cultivation. This may be due to the long-term
92 nature of any investment (the root needs five years of growth before it can be harvested) or it may be that the
93 costs involved make it difficult for farmers to compete on price with the wild-collected material.

94 However, our initial investigations suggest that due to its popularity, there is a scarcity of authentic raw
95 material available and not enough to satisfy demand. Because of this disparity between supply (at an
96 acceptable price) and demand, there is potential for adulteration with different species, especially *Rhodiola*
97 *crenulata* (Hook.f. & Thomson) H.Ohba and other Chinese species.

98 With a traditional history of medicinal use within Eastern Europe, Scandinavia, arctic countries, Asia and North
99 America – Rhodiola products have become high value commodities, traded internationally. One factor
100 contributing towards this economic growth is its use by sports men and women to help prevent fatigue and
101 improve performance (Parisi et al., 2010).

102 Use of *R. rosea* and other Rhodiola species is allowed by sports regulators but this use highlights a potential
103 danger that if adulterated products are unknowingly used, the reputation of sports competitors could be
104 adversely affected.

105 Herbal products, advertised as containing the root of *R. rosea,* are widely available from retails outlets and the
106 internet. They are generally sold as 'food supplements' and so providing they do not make any medicinal claim
107 they can legally be placed on the market without the need of either a medicines product licence (PL) or a
108 traditional herbal medicinal product registration (THR). However, there are several *R. rosea* products available
109 that are registered herbal medicinal products for use in the treatment of stress-induced fatigue, exhaustion
110 and anxiety.

111 Products registered under the THR scheme must demonstrate that they are safe and of acceptable quality,
112 including adherence to good agricultural and collection practices (GACP) and good manufacturing practice
113 (GMP).

114 Aims and Objectives

115 To select a sample of Rhodiola products, available from the internet and from retail outlets and assess their
116 phytochemical composition.

117 To determine whether there are phytochemical differences between registered products and unregistered
118 products and to assess how this impacts on the products' quality (and thus on safety as well as consumer
119 confidence).

120 Materials and methods

121 **Test samples**

122 Approximately 40 products were obtained from different suppliers including retail outlets and products that
123 are readily available over the Internet. The samples claimed to be consisted of crude drug material (2), bulk
124 powder (3), hard capsule extracts (21), soft gel capsules (1), tablets (9), of Rhodiola rosea and aqueous extracts
125 of *Rhodiola crenulata* (3). 37 were mono-preparations claiming to contain only *Rhodiola rosea* root and
126 rhizome extracts (and excipients), and two were combined with other constituents such as vitamins, and
127 herbal extracts. Two of the products held a traditional herbal registration (THR), but the majority were not
128 registered products and were readily available over the Internet and from retail outlets (as food supplements).

129 A detailed description of all investigated products is provided in the supplementary data.

130 **Solvents, reagents and chemicals**

131 Deuterated dimethyl sulfoxide-D6 lot no. 14F-145 and tetramethylsilane (99.9%) lot no S47541 32108B02
132 were purchased from Cambridge Isotope Laboratories, Inc., Andover, MA, USA. Ethanol absolute (99.8%), lot
133 no 950-0090 purchased from Merck, Germany, Methanol, lot no 982801 purchased from CarlRoth GmbH,
134 Karlsruhe, Germany, Ethylacetate (99.5%), lot no A0343909 purchased from Acros, New Jersey, USA, Formic
135 acid (98+ %) pure, lot no A0333265 purchased from Acros, New Jersey, USA.

136

137

138 **Standards**

139 Reference standard: Salidroside, lot no BCBH4124V and rosavin, lot no 083M4725V were purchased from
140 Sigma-Aldrich Chemicals, St Luis, MO, USA.

141 **^{1}H-NMR spectroscopy**

142 *Preparations of standard solutions and samples*

143 Approximately 50 mg of solid samples was accurately weighed and transferred to a 1.5 ml Eppendorf reaction
144 tube, 1 ml of deuterated DMSO containing 0.05% tetramethylsilane were added. The mixture was mixed on a
145 rotary mixer for 60 s, sonicated for 10 minutes at room temperature and centrifuged for 10 minutes at room
146 temperature (speed; 14,000 rpm). The reference standard solutions of salidroside, and rosavin were prepared
147 at a concentration of 1.0 mg/ml in deuterated DMSO. Seven hundred microliters of supernatant was
148 transferred to a 5 mm diameter NMR tube, and the samples were submitted on the same day for ^{1}H-NMR
149 analysis. Sample 39 was extracted twice (S40) to act as a control for the statistical analysis.

150 *Apparatus and instrumentation*

151 NMR spectra were recorded on Bruker Avance 500MHz spectrometer (^{1}H, 500MHz) equipped with a QNP
152 multi-nuclear probe head with z-gradient . The topspin software version 1.3 was used for spectra acquisition
153 and processing. The AMIX Bruker Biospin multivariate analysis software version 3.0 was used for converting
154 spectra to an ASCII file. The numbers of scans chosen was 64 for optimum resolution of peaks, and locked at
155 zero on the TMS peak. Principal Component Analysis (PCA) carried out using SIMCA software version 13.0.

156 **LC-MS/MS Analysis**

157 *Preparations of standard solutions and samples*

158 One mg of substance was suspended in 1 mL of solvent A (2.5 % (v/v) MeCN, 0.5 % (v/v) HCOOH in water),
159 sonicated for 10 minutes and centrifuged at room temperature for 10 minutes at 5000 *g*.

160 *Apparatus and instrumentation*

161 The LC-MS/MS data was acquired with an Agilent 1100 HPLC system which was coupled to a Bruker Daltonics
162 Esquire HCT ion-trap mass detector. 10 µL of the supernatant were used as injection volume for the HPLC. The
163 chromatographic step was performed with a constant flow rate of 0.2 ml/min and a 45 minutes linear gradient
164 from solvent A to solvent B (95% MeCN (v/v), 0.5% (v/v) HCOOH in water). For separation, a Waters Atlantis T3
165 column (2.1 x 150 mm, Silica 3µm) was used which was heated to 50°C.

166 **Data reduction and multivariate statistics methods**

167 The ^{1}H-NMR spectra were phase-corrected, baseline-corrected, and zeroed to the TMS peak. The spectra were
168 converted to an ASCII file using AMIX software for multivariate analysis. AMIX was used to generate a number
169 of integrated regions (buckets) of the data set. The size of buckets was 0.04 ppm. The signals of deuterated
170 DMSO, residual water, and TMS internal standard were removed before performing the statistical analysis. The

171 data set was imported to Microsoft EXCEL, and the samples were labelled 1 to 40. The Principal Component
172 Analysis (PCA) was carried out using SIMCA software version 13.0.

173 **High performance thin layer chromatography (HPTLC)**

174 *Preparations of standard solutions and samples*

175 The extraction of plant sample was performed based on a method described by the HPTLC association for the
176 identification of dried Rhodiola root. The reference standard solutions of salidroside and rosarin were
177 prepared at concentration of 1.0 mg/ml in methanol. Approximately 500 mg of solid samples were weighed
178 individually into 10 ml reaction tubes and 5 ml of ethanol (99.8 %) was added. The resultant solution was
179 shaken at 300 rpm for 10 minutes and centrifuged for 5 minutes at 5000 rpm. The supernatant solution was
180 transferred into individual vials, and then submitted for HPTLC analysis.

181 *Chromatography*

182 HPTLC analysis was performed on 200.0 * 100.0 mm silica gel 60 F 254 HPTLC glass plates (Merck, Germany).
183 Standard solutions and samples (2 µl) were applied on the plate as bands 8.0 mm wide using CAMAG spray-on
184 technique with Automatic TLC sampler (ATS 4) or CAMAG Linomat 5.

185 Bands were applied to a distance of 8.0 mm from the lower edge of plate and 20 mm from the left and right
186 edges. The space between bands was 11 mm, and the number of tracks per-plate was 15. The development
187 distance was 70.0 mm from the lower edge of the plate using CAMAG Automatic developing chamber (ADC2).
188 The temperature and the relative humidity within the developing chamber (ADC2) were 23 ^{o} C and 33 %
189 respectively.

190 The derivatisation of plates was performed through dipping (Speed: 3, time:0) with 10 % sulphuric acid in
191 methanol reagent using CAMAG Chromatogram Immersion Device and heated to 100 ^{o}C for 5 minutes on the
192 TLC plate heater. The plates were documented using CAMAG Visualizer under white light, UV 254 nm, and UV
193 366 nm with visionCATS software.

194 **Other Apparatus and instrumentation**

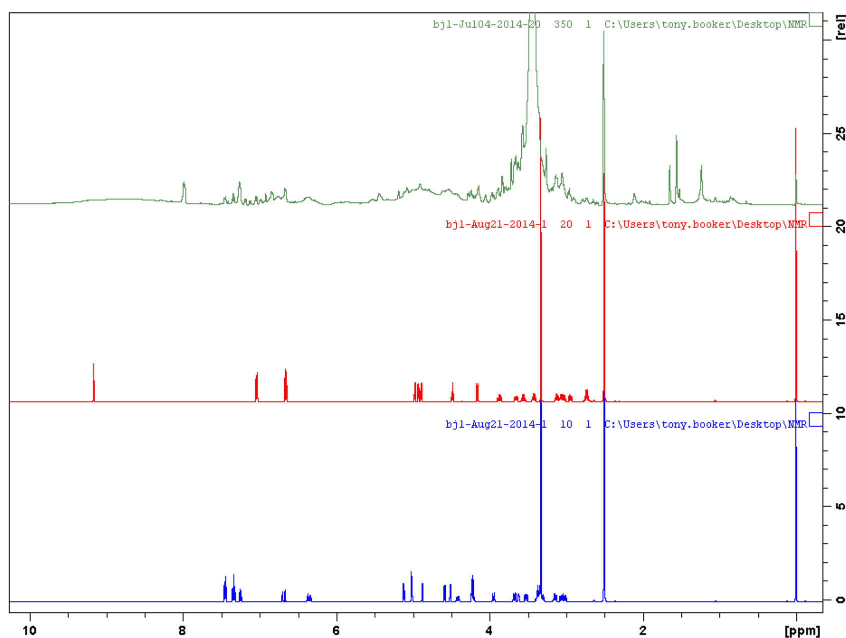
195 POS-3000, Grantbio, Serial no b090250014, Cambridgeshire, England.

196 Centrifuge EBA21, Serial no 0000799-01-00, Hettich (Zentrifugen), Faust Laborbedarf AG, Germany. Balance
197 AG245, Serial no 1114402254, Mettler-Toledo.

198 Results and Discussion

199 The samples were analysed by ^{1}H-NMR spectroscopy coupled with SIMCA multivariate analysis software, mass
200 spectrometry and HPTLC.

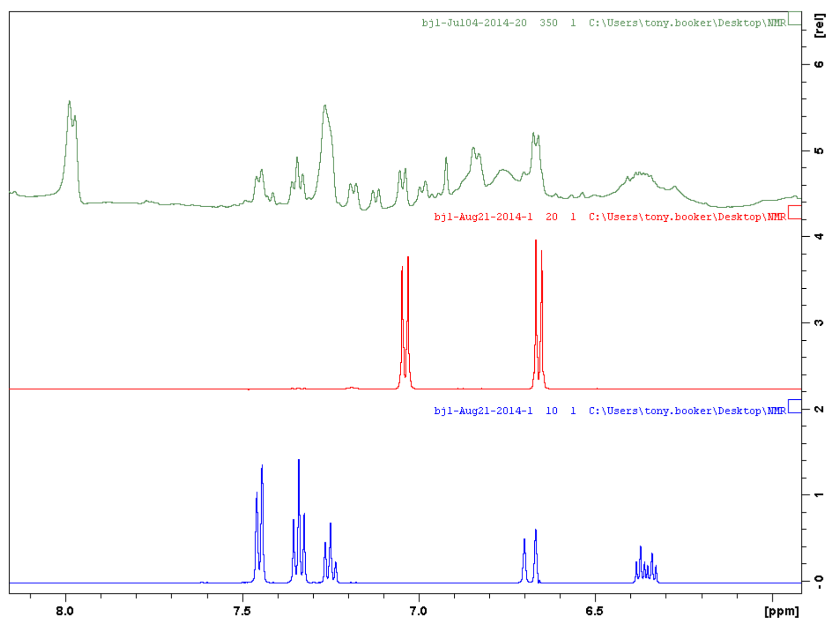
201 In the ^{1}H-NMR spectroscopy analysis, the two main constituents of *R. rosea* root, salidroside and rosavin were
202 assigned and compared with a *Rhodiola rosea* crude dried plant root supplied by a company that manufactures
203 THR products (S35).



204

205 *Figure 1 ^{1}H-NMR spectra of Rhodiola rosea crude dried plant (top) together with spectra of reference standards*
206 *of salidroside (middle) and rosavin (bottom) respectively.*

207 Because of the high concentration of glycosides, including polysaccharides and the presence of excipients in
208 many of the Rhodiola products, it was difficult to identify peaks in the carbohydrate range (3 - 5 ppm). The
209 resonances observed at lower chemical shifts (0.2-3.0 ppm) are most probably attributable to the methyl
210 groups of terpenoids (Fig. 1). However, peaks corresponding to salidroside and rosavin could be observed in
211 the aromatic region of the spectrum (6.2-8.5 ppm) (Fig. 2). Using this method we were able to confirm the
212 presence of both saldroside and rosavin in our samples.



213

214 *Figure 2 ^{1}H-NMR spectra of R. rosea extract (top) together with spectra of reference standards of salidroside*
215 *(middle) and rosavin (bottom), respectively, focusing on aromatic region.*

The PCA model for the ^{1}H-NMR spectroscopy (Fig. 3), utilises data obtained for the entire spectral region (0 – 10 ppm). The Rhodiola products PCA appeared to indicate that although there was much variation between samples, the variation did not distinguish between the different species. The principal variation between these two species is rosavin content.

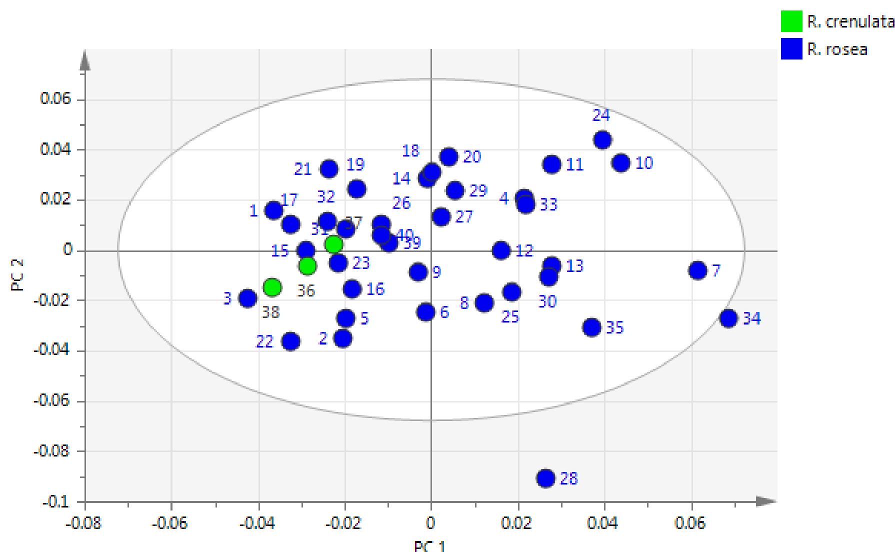


Figure 3 Scores plot showing the whole spectral regions of investigated Rhodiola samples

The PCA grouping in Fig. 3 may be attributable to the similarities of major metabolites, e.g. glycosidic constituents within Rhodiola products or to ingredients added (mainly excipients) to the commercial extracts. This PCA model appeared to poorly discriminate between products containing *R. rosea* extracts and those extracts containing *Rhodiola crenulata* or other Rhodiola species. Our assumption was that this observation was possibly due to adulteration of products by incorrect species, i.e. that a proportion of products labelled *R. rosea* actually contained an amount of *R. crenulata* or other species. In order to investigate this hypothesis, subsequent analysis was performed utilising HPTLC.

We developed an HPTLC method in order to simultaneously detect the presence of rosavin and salidroside. Importantly, *R. rosea* contains both salidroside (R_F 0.35) and rosavin (R_F 0.22), whereas although salidroside is present in many other Rhodiola species, rosavin is absent or in very low concentrations (Panossian 2010). The characteristic marker compound rosavin was not found in seven of the products claiming to be *R. rosea* and the overall fingerprint looked very different to that of *R. rosea* (Fig 4).

Sample (S) 11 was a registered product and S20 was an expired *R. rosea* product that we included to see if the marker compounds were still visible past the expiry date.

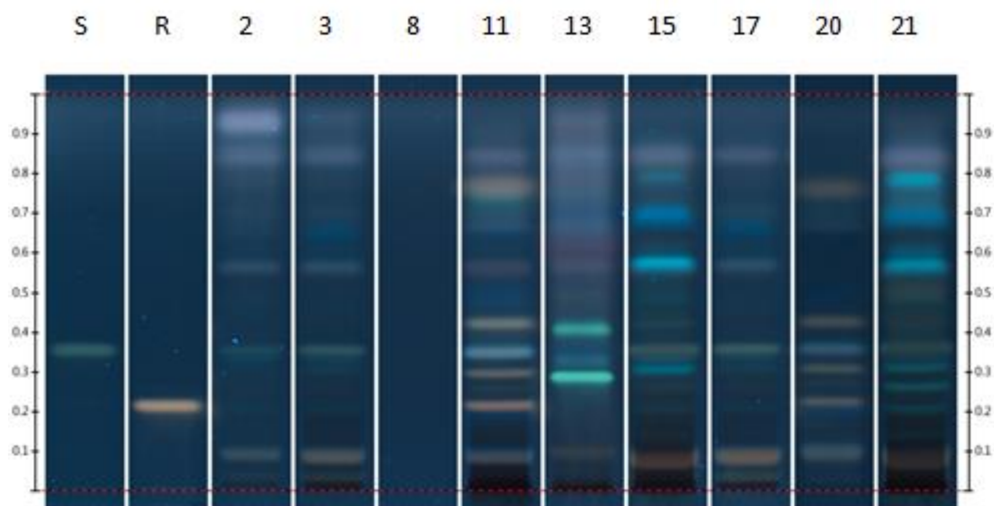


Figure 4 Comparing different Rhodiola products, Mobile phase: Ethylacetate, methanol, water, formic acid (77:13:10:2). D: Sulphuric acid reagent, UV 366 nm.

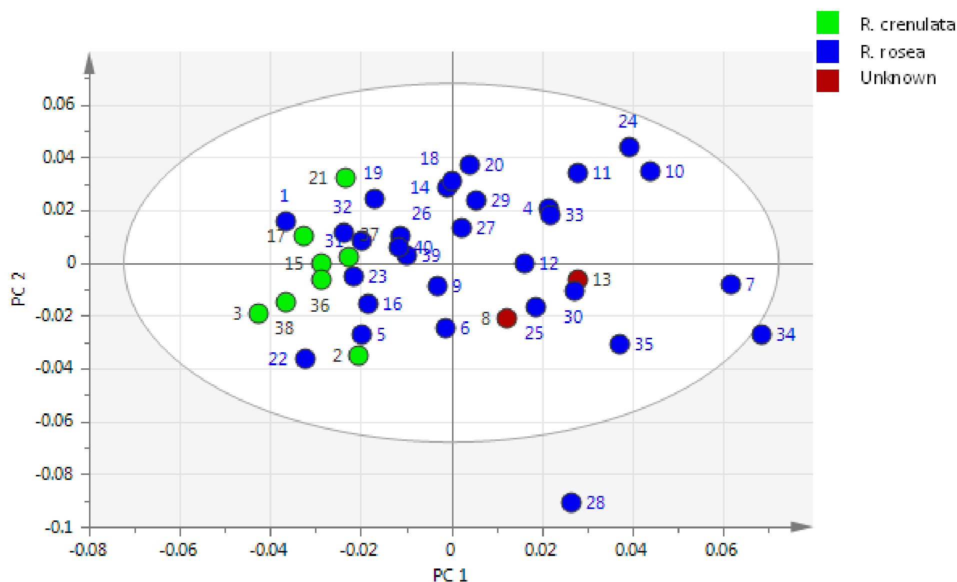
The HPTLC analysis revealed that S2, S3, S15, S17, and S21 samples show a fingerprint not consistent with that of Rhodiola rosea and were probably adulterated with other Rhodiola species that did not contain rosavin (R_F 0.2) but contained salidroside (R_F 0.35) e.g. *Rhodiola crenulata, Rhodiola quadrifida*. Moreover, two of the samples exhibited the presence of unspecified components, not related to *R. rosea or* Rhodiola *species* (S8 and S13).

S2 displayed a strong zone at R_F 0.92, S13 displayed brightly coloured greenish zones (R_F 0.3 and 0.4) S8 appeared not to contain any compounds detectable under this system.

After discovering that these seven samples were adulterated either with other Rhodiola species or unknown species, it was possible for us to re-label the assigned samples in the NMR - PCA.

Once re-labelled, the PCA displayed an improved differentiation between the *R. rosea* and *Rhodiola crenulata* samples (Fig. 5). Although we have re-labelled five of the products that did not contain rosavins as *R. crenulata* it should be recognised that these may be adulterated with other Rhodiola species and we are currently developing methods that will identify adulterant species within mixtures.

256



257 *Figure 5 Scores plot showing the whole spectral regions of investigated Rhodiola samples after re-labelling*

258 *based on HPTLC identification of the possible source species*

259 Analysis of the raw NMR spectroscopy data for S8 in the aromatic region lead to the identification of signals

260 which are characteristic for derivatives of the amino acid tryptophan. A downfield shifted signal at 10.6ppm

261 was characteristic for an indole ring-NH. Closer analysis of the coupling pattern for resonances between

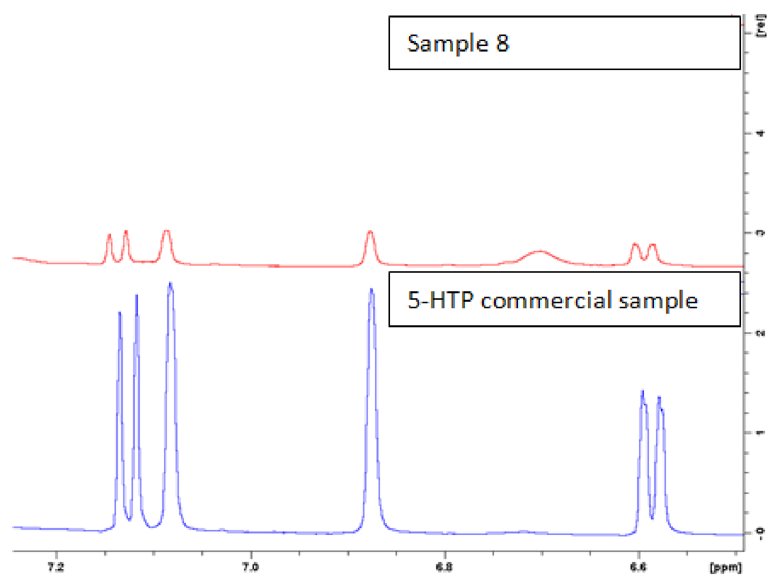
262 6.5ppm and 7.1ppm identified a lack of a carbon bound proton at position 5 of the indole moiety. This led to

263 the assumption, that the substance is 5-hydroxytryptophan (5-HTP). This assumption was proved by comparing

264 NMR (Fig. 6) and LC-MS/MS spectra (Fig. 7) of a commercial sample of 5-HTP with S8. Both, the NMR and the

265 LC-MS/MS spectra confirmed a presence of 5-HTP in S8 with the same resonances, elution times and

266 molecular masses, respectively.



267

268 *Figure 6 NMR spectrum in 6.5ppm to 7.2 region for sample 8 and 5-HTP commercial sample*

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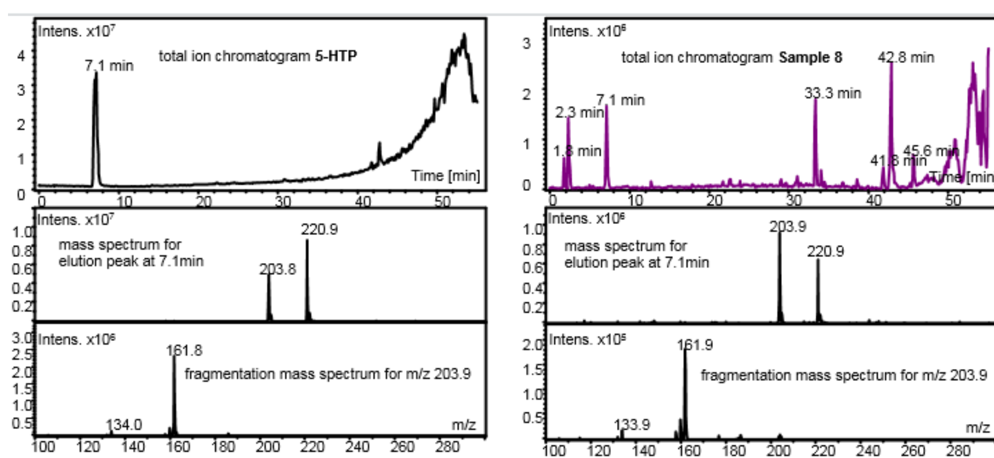
270 

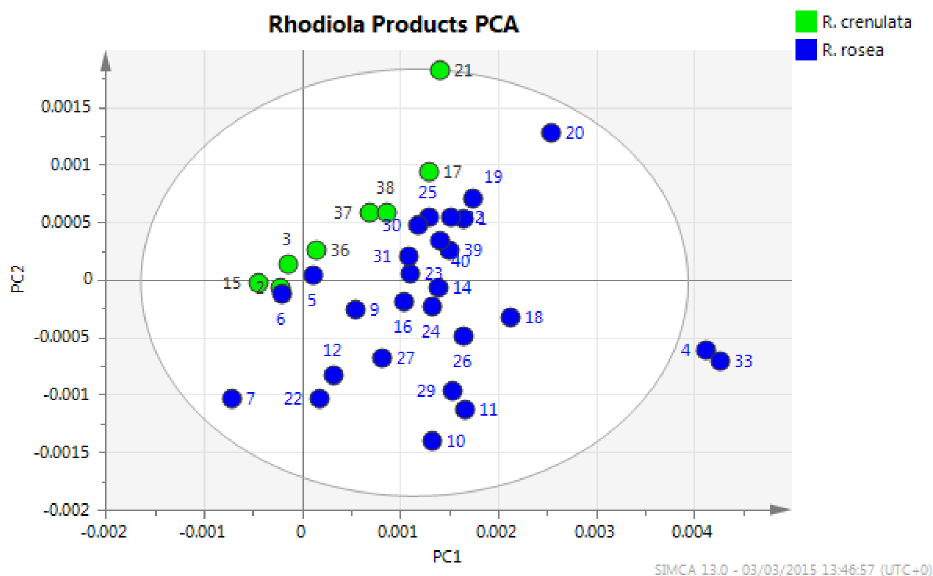
271 *Figure 7 LC-MS/MS chromatogram with the mass spectra and fragmentations in positive and negative ion*
272 *mode for 5-HTP reference standard and S8. The elution fraction at 7.1 min for 5-HTP with its characteristic*
273 *fragmentation is also detectable for S8.*

274

275 One of the problematic factors in the metabolomic analysis of commercial products is the presence of
276 excipients. These tend to occur within the carbohydrate region and, in order to minimise the effect of any
277 excipient, we performed a PCA exclusively on the aromatic region (7 – 10ppm).

278 In order to compare only the aqueous and hydro-ethanolic extract products, the crude raw drugs (samples 1,
279 34 and 35) were removed from the data set, together with the unknown products (samples 8 and 13) and a
280 soft-gel extract (sample 28). The resulting PCA showed a better differentiation between *R. rosea* and the non-
281 rosea species. Further investigations will determine whether these are *R. crenulata or other adulterant*
282 *Rhodiola* species (Fig. 8).

283 It is observed that the products that are highest in rosavin are in the bottom right quadrant. There is a group of
284 seven products that are higher in rosavin content than other products, including the 2 THR products (11 and
285 33). However, there are some *R. rosea* products that appear to have a very low rosavin content; in fact the PCA
286 places these more closely to the group we have labelled *R. crenulata* products (samples 5 and 6). The likely
287 reason for this is that these products contain a mixture of *R. rosea* and *R. crenulata* or other adulterant
288 species. Through closer examination of this PCA we can infer that over 80% of products contain less rosavin
289 than the THR products.



290

291 *Figure 8 Scores plot showing PCA of Rhodiola products (7 – 10 ppm chemical shift).The lower ellipse indicates*
292 *products that are higher in rosavin content.*

293 Table 1 Label claims versus actual findings for Rhodiola products

Sample no.		**Findings**
2	*Rhodiola rosea* sourced in China	Not *R. rosea*, probably other rhodiola species e.g. *R. crenulata*
3	Whole dried root of *Rhodiola rosea*	Not *R. rosea*, probably other rhodiola species e.g. *R. crenulata*
8	*Rhodiola rosea* extract 2000 mg, wild-sourced from Siberia	Not *R. rosea* or any other R. species. Determined as 5-HTP and excipients
13	*Rhodiola rosea* root 1000 mg	Probably not rhodiola species, appears adulterated
15	*Rhodiola rosea* plus multivitamins standardised to contain 3% rosavins and 1% salidroside	Not *R. rosea*, probably other rhodiola species e.g. *R. crenulata*
17	*Rhodiola rosea* standardised to contain 1% salidroside	Not *R. rosea*, probably other rhodiola species e.g. *R. crenulata*
21	*Rhodiola rosea* standardised to contain 1% salidroside	Not *R. rosea*, probably other rhodiopla species e.g. *R. crenulata.* Probable high sugar content

294 **General Discussion**

295 The safety and quality of herbal medicines available to European consumers has been a key issue for
296 medicines regulators. The introduction of the Traditional Herbal Medicinal Products Directive (THMPD) and
297 herbal registration has provided a means whereby consumers can access a wide variety of popular herbal
298 medicines of assured quality and with a well-researched safety profile.

299 However, there are still a large number of medicines left un-regulated and widely available, particularly from
300 internet sources. Economically lucrative products, including *R. rosea* products, are likely candidates for
301 adulteration, especially when the raw plant material is in short supply. Moreover, because *R. rosea* products
302 are popular with sports persons, there is an added danger that they may be adulterated with performance
303 enhancing stimulants.

304 Using a methodology previously developed by our group (Booker et al., 2014), we investigated the utilisation
305 of ^{1}H-NMR spectroscopy coupled with multivariate analysis software and HPTLC to ascertain the identity and
306 composition of *R. rosea* value chain products. Both techniques provide different and complementary data,
307 which we used to discriminate between the wide-variety of sampled finished products. Further investigations
308 will focus on the crude drug material and aim to establish a robust method for identifying mixtures of different
309 species within products.

310 ^{1}H-NMR spectroscopy coupled with multivariate analysis software enabled us to group the Rhodiola products
311 based on similarities between the products. Although chemometric data analysis can be undertaken
312 automatically with the requisite software packages, it requires an in-depth understanding as to what each set
313 of signals in the given spectra represents phytochemically, so that the identification of key constituents within
314 a product can be assigned logically. In this set of samples (mainly extracts) there were a lot of overlapping
315 peaks present, particularly in the carbohydrate region, probably produced by a combination of intrinsic
316 glycosidic material and excipients. This made interpretation of the data difficult for individual compounds and
317 suggested that a more separation technique, e.g. HPTLC should be used.

318 The HPTLC analysis provided detailed qualitative data for the determination of the marker compounds, and
319 allowed to make visual comparisons between different products relatively easily. Because HPTLC allows us to
320 be selective regarding the groups of compounds we want to analyse, we can make comparisons between
321 samples based on the composition of the main marker compounds (salidroside and rosavin).

322 *Rhodiola crenulata* is the main species found in Chinese medicine and it is the only species listed in the Chinese
323 Pharmacopoeia. However, *R. rosea* is the species of main economic interest. The difference in price between
324 the two species, with *R. crenulata* being cheaper for the foreign buyer, and the relative ease of availability in
325 China of *R. crenulata*, may be a reason behind the adulteration of *R. rosea* products with *R. crenulata* and
326 possibly other Rhodiola species. While there is – at this stage – no evidence for potential risks in using *Rhodiola*
327 *crenulata,* apart from a possible lack of effectiveness*,* other examples of phytomedicines highlight the risk of
328 adulteration with a species of the same genus (Li and Yu, 2006).

329 In this study, the characteristic marker compound (rosavin) was not detected in twenty three percent of
330 unregistered products that claimed to contain *R. rose* . Two of these products were adulterated with material
331 not from the genus Rhodiola i.e. that did not contain either rosavin or salidroside and one of these was
332 positively identified as 5-hydroxytryptophan, an amino acid commonly used as an anti-depressant or an aid
333 for weight loss. It is unclear whether this is a deliberate or accidental adulteration since attempts to clarify this
334 with the companies involved have been unsuccessful. However, whether deliberate or not, these findings
335 show that there is a monumental failing in the quality systems of the companies involved.

336 Moreover, there were variations in the amount of marker compounds contained in *Rhodiola* products. This
337 was supported by the PCA data which indicated that approximately 80% of products contained lower amounts
338 of rosavin than the THR products. Thus, this may indicate that there are common but qualitative different
339 species of *Rhodiola* substituted or used as admixtures in *R. rosea* labelled products.

340 While adulteration by other Rhodiola species, including *Rhodiola crenulata* presents one particular problem,
341 adulteration with unknown material is potentially even more worrying. Future investigations will focus on the
342 isolation and identification of these compounds to determine whether there are other plant species or
343 synthetic compounds (including stimulants) and how different value chains are affected.

344 Conclusions

345 Approximately one quarter of unregistered *Rhodiola* products were adulterated and did not conform to their
346 label specification. Approximately 80% of products were of poorer quality than the THR products as regards
347 the rosavin content. This indicates that there are major breakdowns in the quality systems employed along the
348 various stages of Rhodiola value chains.

349 Buying unregistered products, particularly from the internet, presents a clear risk. There is no practical way for
350 the general public to differentiate un-registered genuine products from adulterated products. Products
351 registered under the THMPD were confirmed to contain authentic RR.

352 Based on this analysis we plan to investigate the value chains of *R. rosea* and other Rhodiola species and to
353 investigate how and why such adulterations can happen. This research also calls for more training and for
354 raising awareness of the relevant stakeholders.

355

356 Conflicts of interest

357 We wish to draw the attention of the Editor to the following facts which may be considered as
358 potential conflicts of interest and to significant financial contributions to this work.

359 Anthony Booker's research position is funded through a charitable donation by Dr. Willmar Schwabe GmbH &
360 Co. KG, Germany.

361 Zarko Kulic is an employee of Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany. Based on NMR data,
362 he identified 5-hydroxytryptophan in one of the samples and conducted the LC-MS/MS–experiments for the
363 sample for verification.

364

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