# Rhodiolife™ THE FINGERPRINT MATTERS

When it comes to *Rhodiola rosea*, identity – the fingerprint - matters... to your customers!

Manufactured by: **nektium** 



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## RAW MATERIAL



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ABOUT THE ORIGIN

Rhodiolife<sup>®</sup> is different from the very beginning. Starting with the siberian origin, wildcrafting, roots selection, programmes for sustainability and social responsibility.

### 1.About the origin

#### **1.1 Botanical Identity**

Botanical identity is essential. Because of significant speciesdependent variation in phytochemistry and pharmacology, the use of "Rhodiola" as a general term is inaccurate and misleading.

The correct identification of all Rhodiola species according to precise and generally accepted botanical, phytochemical, and genetic taxonomic criteria is not merely an abstract intellectual exercise. It is critical for both scientific and phytopharmacological accuracy, as well as for product labelling for the public. The pharmacological and medicinal properties of Rhodiola are species dependent phenomena. Of all the Rhodiola species, *Rhodiola rosea* has been the predominant subject of phytochemical, animal, and human studies.

Botanical Identity	
Class	Magnoliopsida
Superorder	Saxifraganae
Order	Saxifragales
Family	Crassulaceae – stonecrops, orpins
Genus	Rhodiola L. – stonecrop
Species	Rhodiola rosea L. – roseroot stonecrop



ABOUT THE ORIGIN

### 1.2 The Region

While Rhodiola as a genus may have originated in the mountainous regions of Southwest China and the Himalayas, botanists have established that Rhodiola rosea naturally display a circumpolar distribution in mountainous regions in the higher latitudes and elevations of the Northern Hemisphere. In Central and Northern Asia, the genus is distributed from the Altái Mountains across Mongolia into many parts of Siberia.

Rhodiola rosea used for the production of Rhodiolife® is wildcraft collected, under the Russian Government License, in the Altái State. Altái Mountains represents a pristine area free from contamination in of the most well preserved and remote natural environments.



ABOUT THE ORIGIN

### Altái Republic [Russia]



#### Key facts about Altái

One of the meanings of the word Altái is "Golden Mountains" (from the Mongolian Word "altan")

Forests cover more than half of the Mountain territory.

There are more than 2000 species of supreme vascular plants and approximately 200 kinds of vegetable plants

A calculated total area of 728 km<sup>2</sup> of lakes is distributed amongst more than 7000 lakes in the region. The calculated amount of glacial water in the region is approximately 52 km<sup>2</sup> and there are more than 60000 km worth of waterways.

Altái has a temperate continental climate with short hot summers and long cold, frosty winters. The average yearly temperature is approximately 0°C with 50-700 mm annual rainfall.

The area is regulated by elaborated and established norms of recreational exploitation to ensure preservation of rare plants. In fact more than 60% of the surface is composed of strictly protected areas.

ABOUT THE ORIGIN



Figure 1 / Pictures from Altái (Siberia)

THE PROCESS

### 2.The process

#### 2.1 Collection

Nektium closely collaborates with our supplier implementing operating procedures that provide general technical guidance for the sustainable collection and processing of *Rhodiola rosea* roots, following the overall context of quality assurance and the WHO guidelines on Good Agricultural and Collection Practices (GACP).

GACP provides a basis for a quality assurance system for the starting *Rhodiola rosea* roots used in the production of Rhodiolife<sup>®</sup>. This includes the collection of the plants in the wild, as well as primary processing of the plant material, such as drying, packaging in bulk, storage and transport of the raw materials until it arrives to the Nektium factory. The impact of collection on the environment and ecological processes, and the welfare of local communities are also considered.

GACP apply to the first part of the production, for which Good Manufacturing Practice (GMP) does not apply yet. Applying for GACP eliminates or reduces the risks of microbiological or chemical contamination, mistaken identity, and deterioration during primary processing and storage. Eliminating and reducing these risks enhance the reliability of the starting *Rhodiola rosea* roots used in the production of Rhodiolife<sup>®</sup>.

Nektium works with a local company, which is responsible for the Collection Management Plan. This Plan integrates all the aspects related not only to the GACP but also to the regulatory and permission necessary for the legal export of *Rhodiola rosea* raw materials from Russia.

#### **Good Collection Practices**

Application of GACP is primarily intended to provide a general technical guidance on obtaining *Rhodiola rosea* roots of high quality for the sustainable production of Rhodiolife<sup>®</sup>. Applying for Good Collection Practices pretends to ensure the long-term survival of *Rhodiola rosea* wild populations and their associated habitats.

#### Permision to Collect

Permission for collecting plants is given by the management of the Wood Industry of Altái Region after demand coordination in the Ministry of Natural Resources of the Russian Federation, which is also responsible for issuing the Export Licenses. Collecting permissions are strictly connected to export licences to prevent over collecting of Rhodiola roots. Nektium Pharma, SL (Nektium) is one of the few companies granted with an Export License by the Russian Government.

Collection areas and crafting activities are supervised by the Federal Office for Natural Resource Exploitation (FONRE).

Nektium audits regularly our supplier facilities and the whole process.

THE PROCESS

#### **Collection Management Plan**

Collection activities are supervised by the FONRE. The collection management Plan includes the following requisites



THE PROCESS

#### 2.2 Personell

Collectors are organized in brigades (3 – 10 workers) and are transported to the collection fields up the mountains, where they live from mid-May till the end of September (about 4 months). They are familiar with good collecting techniques, transport, and handling of equipment and plant materials, including cleaning, drying and storage. Training of personnel is conducted regularly.

There are local experts (brigade leaders) responsible for the field collection with practical education and training in Rhodiola rosea biology and plant identification, with practical experience in fieldwork. They are also responsible for the supervision of workers and the full documentation of the work performed. Field personnel should have adequate botanical training, and be able to recognize medicinal plants by their common names and, ideally, by their scientific (Latin) names. Collectors should also receive instructions on all issues relevant to the protection of the environment and the conservation of plant species, as well as the social benefits of sustainable collection of medicinal plants.

Collectors are instructed for addressing three personnel important issues: training, safety and hygiene. This is relevant to all phases of collecting and post-harvest handling.





#### Training Safety In collection and post-harvest activities. Clothing. Ensure that personnel wear clothing and shoes that provide In the positive identification of Rhodiola protection that is appropriate to the work rosea plants. environment. In proper hygienic practices with specific Environmental factors. Consider and attention to preventing microbial establish procedures to protect personnel contamination of handled Rhodiola. from environmental factors that are relevant to worker safety.

Tools and equipment. Maintain all tools, equipment and vehicles used by personnel to ensure that these will be reasonably expected to be reliable and safe.

#### Hygiene

Prevention of contamination.

Provide toilets, hot running water and soap at postharvest handling facilities.

Establish minimum hand washing requirements.

Sick personnel or those with open wounds/skin infections are not allowed to work.

THE PROCESS

#### 2.3 Processing

*Rhodiola rosea* extract processing follows the indications given by the Russian Pharmacopoeia. After collection, the *Rhodiola rosea* is subjected to appropriate preliminary processing, including elimination of undesirable materials, the excess of soil, sorting and cutting. The collected *Rhodiola rosea* roots are protected from insects, rodents, birds and other pests, and from livestock and domestic animals.

Collecting tools, such as machetes, shears, saws and mechanical tools, are kept clean and maintained in proper condition. Those parts that come into direct contact with the collected medicinal plant materials should be free from excess oil and other contamination.

As the collection sites are located at large distances from the consolidation/processing facilities, the process is divided in two main steps.

### PRIMARY PROCESSING



THE PROCESS



THE PROCESS

#### 2.4 Environmental Practices & Sustainability Programmes

Collection practices applied by the collectors to the harvest of *Rhodiola rosea* address not only their need to gain economic benefits from the sale of the harvested plants, but also to make sure that *Rhodiola rosea* survives. In addition to preserving plant populations, harvest practices are oriented to minimize the damage to the local habitat.

Re-harvesting in the same location is restricted to every 5 years to allow sufficient reestablisment of the plant population. Furthermore, only 50% of the population from a specific location can be collected so that enough plants able to produce an adequate amount of seeds to sustain the population are kept.

Since Rhodiola can regenerate through vegetative growth, a portion of the root is left in the ground for plant regeneration.

The use of basic standardized procedures contributes to the batch-to-batch conformity and thus, to the reliability and high quality of *Rhodiola rosea* starting raw material. The application of GACP significantly contributes to improving the quality of starting raw materials before Nektium's GMP system takes the lead in the production of Rhodiolife<sup>®</sup>. Nektium´s ID testing assessment program

Raw material & final product



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### I. INTRODUCTION

We understand the concern and importance of a proper botanical identification of raw materials used in extracts manufacturing processes. Each company has proposed its own identification program, ranging from traditional methods (macroscopic or sensorial) to very novel methods, as genetic identification of DNA barcode. All these methods provide useful information for the identification process through the analysis of genetic identification, what brings unquestionable proof of the biological material identity.

Unfortunately, the experience has shown that due to the production processes, the final products do not contain genetic material that allows applying the genetic analysis. Similarly, such processes often alter the final products phytochemical profiles with respect to its raw materials, especially in highly purified extracts materials. Therefore, there is no single analytical method to trace the identity of the product from raw materials to finished products.

This identification process is performed through the application of different complementary methodologies that allow assessing what methodology is appropriate to apply at every step and what should be used as reference markers on the positive evaluation of the product identity. With regard to this, Nektium has proposed an Identity Assessment Program that permits, combining different methodologies, performing the traceability of product identity from raw materials to finished product, guaranteeing our customers the correct identity and quality of supplied products.

The ID methods used in the identification of raw materials and final products are:

Raw material	Methodology	Principle	
•	Macroscopic identification	Reference description according to standard sources	
	DNA barcoding	Molecular taxonomy (DNA barcode) Phytochemical fingerprint. Identification of specific markers	
	UHPLC fingerprint		
Final product	Methodology	Principle	
	UHPLC fingerprint	Identification of specific markers	

### II. ID TESTING ASSESSMENT PROGRAM



\* All of the extraction, concentration, purification and drying steps could modify the phytochemical profiles of the final extracts with respect to the raw materials.

## Nektium's opinion of the different methodologies suggested for identification of species is:

### 1. Raw material

Raw materials DNA barcode results obtained are unequivocal. The Ultra-High Performance Liquid Chromatography (UHPLC) and HPTLC techniques provide the supplementary data only. DNA barcode determines characteristic sequences of particular specie, what is easily comparable with public databases. Also, when possible, UHPLC profiles are used, where one or more characteristic markers are employed as a genus or species evidence. Commercially available, analytical standards supplied by well recognized companies are employed by Nektium in our UHPLC methodologies.

On the other hand, a macroscopic evaluation is also used as a part of methodology identification.

### 2. Final product

UHPLC profiles in the final products identification are only used when their active compounds are determined by liquid chromatography. With our point of view, the gualitative information supplied by UHPLC determination is more reliable than data obtained by HPTLC. Positive identification of active compounds and other markers by UHPLC is made on basis of retention times and absorbance spectra obtained from analytical standards. In addition, absorbance spectra obtained can be compared with libraries created by Nektium with confirmatory purposes. On the other hand, UHPLC and HPLC are fully automated instrumental techniques with a high reproducibility, which does not allow any discrepancies in the chromatogram interpretation. In contrast, HPTLC profile interpretation is more subjective and the technique is not fully automated, so it is more susceptible to human bias. Finally, UHPLC as well as HPLC techniques are commonly employed and less scientific information can be found on HPTLC. To conclude. Nektium selected UHPLC as the most suitable technique for its purposes.

## 1. Rhodiola rosea

We attach the following results obtained for Rhodiola rosea ID testing:

- 1. Raw material DNA barcode report; includes a photo of raw material used by Nektium. (Document 1)
- Raw material and final product phytochemical profile comparison (UHPLC chromatograms). *Rhodiola rosea* extract is a clear example of the final product that keeps all identified markers in the raw material. Unfortunately, not all extracts keep the characteristic profile of raw material; during manufacturing processes some markers (not active compounds) are lost. <u>(Document 2)</u>



### IV. EXAMPLES OF ID TESTING PERFORMED BY NEKTIUM

#### 1. Rhodiola rosea

Document 1 | Rhodiola rosea raw material DNA barcode report



Report Date: 03/07/2017 Client Sample Description: rhizome fragments (1 bag): RM-RRR17-1003 (Fig. 1) Internal code CSIC: RJB 0601021701061 Sample Internal number: RF08 Type of Analysis: DNA identification of plant samples

#### Results

DNA isolation from 1 sample in the batch was performed and sequences from diagnostic nuclear ribosomal DNA *ITS* obtained. The ITS region is used for DNA identification of *Rhodiola* and other Crassulaceae species identification. The analyzed sequence was compared to related sequences in GenBank by BLAST search algorithm. BLAST search retrieved accessions from the following species: *Rhodiola rosea, Rhodiola quadrifida, Rhodiola pamiroalaica, Rhodiola kirilowii, Hylotelephium ewersii, Rhodiola linearifolia* and *Rhodiola recticaulis* (Table 2. Figure 2)

The resulting analyses conclude that sequences from samples RM-RRR17-1003 has an identity of 99.7% with the species *Rhodiola rosea* (L.) Scop., and therefore identified as such species.

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Signed and reviewed Javier Fuertes Aguilar Científico Titular/Senior Scientist Real Jardín Botánico, CSIC

### IV. EXAMPLES OF ID TESTING PERFORMED BY NEKTIUM

#### 1. Rhodiola rosea

Document 1 | Rhodiola rosea raw material DNA barcode report





#### **CERTIFICATE OF ANALYSIS**

**Sample Description**: rhizome fragments (1 bag): RM-RRR17-1003 (Fig1)



#### Table1. DNA isolation results

Table I.		ouno		
Sample	[DNA] (ng/µl)	Ratio 260nm/230nm	Ratio 260nm/280nm	PCR result
RF08	16.44	1.7	1.9	++

Table 2. Average amount (%) of nucleotide identity between rbcL aligned sequence (PI) and				<ol> <li>and the closest speci</li> </ol>	es		
	Taxon	Length	Rhodiola rosea	Rhodiola linearifolia	Rhodiola kirilowii	Rhodiola quadrifida	
	Sample	No. nucleotides					
	RF08	740	99.7	93	93	90	

#### 1. Rhodiola rosea

Document 1 | Rhodiola rosea raw material DNA barcode report



Figure 2. Maximum-likelihood tree based on a GTR+G substitution model. Numbers above branches represent bootstrap values.

### Methodology

#### **Genomic DNA isolation**

Tissue from rhizome fragments was homogenized in a Tisssue Lyser using iron beads prior to isolation. The DNA isolation protocol using the Qiagen DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) was followed with modifications in incubation time (14 h). DNA quality was assessed by 260/280nm and 260/230 nm absorbance ratio in a Nanodrop UV spectrometer (Thermo Fisher Scientific). gDNA integrity was visualized on an agarose gel by electrophoresis using SybrSafe (Invitrogen) as a stain.

#### PCR and sequencing

Polymerase Chain Reaction (PCR) was performed by using illustra<sup>™</sup> PuReTaq<sup>™</sup> Ready-To-Go<sup>™</sup> PCR Beads, which are premixed, predispensed, single-dose reactions optimized for hot-start. Reactions were done in provided predispensed 0.5-ml PCR tubes. When a bead is reconstituted to a final volume of 25 ml, the concentration of each dNTP is 200 mM in 10 mM Tris-HCl (pH 9.0), 50 mM KCl, and 1.5 mM MgCl2. PCR temperature profile 25-ml reactions was: 95 °C for 5 min followed by 40 cycles of 95 °C for 30 s, 47 °C for 1 mins, and 72 °C for 2 min. Reactions were analyzed by gel electrophoresis with a 3 µl loading volume. Primers (Fuertes Aguilar et al. 1999)

#### ITS P1A TCCGTAGGTGAACGTGCGG

#### ITS P4R TCCTCCGCTTATTGATATGC

PCR results were visualized by agarose gel electrophoresis.and purified for sequencing using ExoSAP-IT For PCR Product Cleanup (Affymetrix). Sequencing reactions (Sanger method) was performed in Secugen (CIB, Madrid). Sequencing reactions were carried out using the TaqDyeDeoxy Terminator Cycle Sequencing Kit (ABI Applied Biosystems, Darmstadt, Germany), and products were analyzed on an ABI 3730XL DNA Analyser automated sequencer.

### IV. EXAMPLES OF ID TESTING PERFORMED BY NEKTIUM

#### 1. Rhodiola rosea

Document 1 | Rhodiola rosea raw material DNA barcode report





#### Sequence analyses and taxon assignment

Sequence data sets were subject to 3 analyses: 1)a heuristic maximum likelihood phylogenetic analysis (ML), where models of nucleotide substitutions and the gamma distribution shape parameter were selected using JMODELTEST 3.6 (Posada and Crandall, 1998) in conjunction with PAUP\*, version 4.0b10 (Swofford, 2001). 2) a Neighbor-Joining tree based on the genetic distance between sequence pairs (GD) after a global multiple sequence alignment 3) the percentage identity (PI) following a basic local alignment search tool (BLAST). The PI method is similar to the GD method, but the value in the matrix is the minimum dissimilarity between samples, based on the percentage identity, as provided by a BLAST method implemented in Blastclust.

#### References

Blastclust (version 2.2.23, ftp://ftp.ncbi.nih.gov/blast/executables/release).

RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies". In Bioinformatics, 2014, http://bioinformatics.oxfordjournals.org/content/early/2014/01/21/bioinformatics.btu033.abstract? keytype=ref&ijkey=VTEqgUJYCDcf0kP

Posada, D. & K. A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817–818. Swofford, D. L. 2001. PAUP\*: phylogenetic analysis using parsimony (\* and other methods). Sinauer, Sunderland, Massachusetts, USA.

#### 1. Rhodiola rosea

Document 2 | Rhodiola rosea chromatographic comparison: raw material vs final product





### V. TRACEABILITY

We keep the traceability from the incoming material to the manufactured extract for each batch.

We state the raw material used into each CoA showing all the implemented step from the ID program. Therefore, we also keep the traceability for each Report of Macroscopic Identification, DNA Barcode for each lot of raw material, and Phytochemical identification by UHPLC fingerprint.

### V. TRACEABILITY

#### Rhodiola rosea

Document 5 | CoA - Rhodiola rosea extract 3%



## WHEN IT COMES TO *RHODIOLA ROSEA*, IDENTITY – THE FINGERPRINT - MATTERS... TO YOUR CUSTOMERS!

Growing your business today means creating transparency and building trust with your customers. If you're including *Rhodiola rosea* in your formulations, why not take a look at RhodioLife<sup>™</sup>, marketed in the United States exclusively by PLT Health Solutions.

THESE STATEMENTS HAVE NOT BEEN EVALUATED BY THE FOOD AND DRUG ADMINISTRATION. THIS PRODUCT IS NOT INTENDED TO DIAGNOSE, TREAT, CURE, OR PREVENT ANY DISEASE.

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