



**PPP Annual Report 2019**

PPP projects which are under supervision of the "Topsectoren" must report annually on the scientific content and financial progress. This form is used to report the progress of the content of the project. PPP projects that finish in 2019 should make use of a different form: "PPP-final report."

**The annual report will be published on the TKI / topsector website. Therefore, please ensure that there is no confidential information in the annual report.**

*Please, submit the report before 15 February 2020 to Hans van der Kolk*

| General information                             |                                                                           |
|-------------------------------------------------|---------------------------------------------------------------------------|
| PPP-number                                      | <b>AF18124</b>                                                            |
| Title                                           | Valorization of licorice root by-product for safe, natural antimicrobials |
| Theme                                           | Gezond & Veilig                                                           |
| Implementing institute                          | Laboratory of Food Chemistry, WUR                                         |
| Project leader research (name + e-mail address) | Prof. Dr. Ir. Jean-Paul Vincken (jean-paul.vincken@wur.nl)                |
| Coordinator (on behalf of private partners)     | Dr. Marian Verbruggen (marianverbruggen@ruitenberg.com)                   |
| Project-website address                         | -                                                                         |
| Start date                                      | 01-06-2019                                                                |
| Final date                                      | 01-06-2023                                                                |

| Approval by the coordinator of the consortium                                                                                                        |                                       |
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| The annual report must be discussed with the coordinator of the consortium. The "TKI's" appreciate additional comments concerning the annual report. |                                       |
| Assessment of the report by the coordinator on behalf of the consortium:                                                                             | X Approved<br>Not approved            |
| Additional comments concerning the annual report:                                                                                                    | 11/2/2020<br><i>Marian Verbruggen</i> |

| Summary of the project |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Problem definition     | <p>Consumers are demanding more naturally preserved (food) products. Plant antimicrobials offer high effectiveness, low toxicity and low incidence of resistance development. Therefore, industrial interest in developing products containing such compounds to combat (persistent) microorganisms is growing.</p> <p>Licorice roots have a longstanding safe use in traditional medicine, and their prenylated phenolic compounds (e.g. glabridin) have shown antimicrobial activity with potency similar to commonly used antimicrobials. In current industrial applications of licorice roots, these antimicrobial phenolics are left untouched, as part of the licorice root by-product (<i>spent</i>). Based on the Dutch sales of licorice root (water) extract in 2016, it is estimated that 51,000 tonnes of licorice root spent were produced and discarded. Considering the phenolic content of licorice roots, it is estimated that ~ 700 tonnes of prenylated phenolics are present in this spent and can be utilised as natural antimicrobial ingredients in different markets (e.g. food, feed and oral care products).</p> |

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|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|               | In this project, prenylated phenolic compounds from licorice root spent will be valorised to obtain clean label and safe antimicrobial compounds.                                                                                                                                                                                                                                                                                                                                                                                                   |
| Project goals | This project seeks the development of broad spectrum antimicrobial extracts. This will be done by (1) identifying the most potent antimicrobials from licorice root spent and their spectrum of activity against problematic spoilage and pathogenic microorganisms relevant to the food, feed and oral care; (2) assessing the safety and health aspects of licorice root spent antimicrobials by using a 2D-organoid model system and (3) defining the routes of valorization of licorice root spent from commercially relevant licorice species. |

| <b>Results</b>        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|-----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Planned results 2019  | Chemical characterization of prenylated phenolic compounds in roots and spent extracts from the three most relevant licorice species.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Achieved results 2019 | <p>Optimization of extraction of licorice root and licorice root-spent to obtain extracts rich in prenylated phenolic compounds.</p> <p>Chemical characterization of prenylated phenolic compounds in three species of licorice root and root-spent extracts, as established by RP-UHPLC-PDA-MS. The species characterized are <i>Glycyrrhiza glabra</i> (three different batches, originating from Iran, Turkey and Afghanistan), <i>G. inflata</i> (wild and cultivated, origin China) and <i>G. uralensis</i> (origin China).</p> <p>First screening of antibacterial activity of licorice crude extracts against the food pathogen <i>Listeria monocytogenes</i>.</p> |
| Planned results 2020  | <p>Preparation of fractions of prenylated phenolic compounds from licorice root/spent extracts via FLASH chromatography, based on their polarity.</p> <p>Purification of fractions to obtain single prenylated compounds via preparative HPLC-MS.</p> <p>Antimicrobial susceptibility assays using broth-microdilution assay against different food, feed and oral care related bacteria. Crude extracts, enriched extracts (fractions) and pure compounds will be tested.</p> <p>Safety profiling of top 5 candidate extracts or fractions will be performed using "simple cell lines" (e.g. Caco2, STC-1 cells).</p>                                                    |

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| <b>Deliverables/products in 2019</b> (provide the titles and /or a brief description of the products/deliverables or a link to a website.) |
| <u>Scientific articles:</u><br><br>none                                                                                                    |
| <u>External reports:</u><br><br>VLAG project proposal<br><br>Annual report for TKI                                                         |
| <u>Articles in professional journals/magazines:</u><br><br>none                                                                            |

(Poster) presentations at workshops, seminars, or symposia.

-Oral presentation on project outline and planned activities to consortium (project meeting at WUR June, 2019)

-Oral presentation on progress to consortium (project meeting at Ruitenberg in November, 2019)

TV/ radio / social media / newspaper:

none

Remaining deliverables (techniques, devices, methods, etc.):

- Extracts from roots and spent from three different licorice species

- Optimized UHPL-UV-MS method for analysis and quantification of licorice phenolic compounds

- Database of licorice root phenolic compounds

