



## The new intelligent ingredient for gut health Scientific Summary

### What is Benegut®?

Benegut® is a high quality, sustainable, natural food ingredient, which is able to reduce gastrointestinal discomfort.

It is a proprietary *Perilla frutescens* (L.) leaf extract, an annual edible plant native to Asia. Perilla leaves are used traditionally as tea or spice as well as in traditional medicinal preparation to support the respiratory tract and the immune system.

In vitro, ex vivo studies and human studies demonstrated that Benegut® combines prokinetic and antispasmodic as well as anti-inflammatory effects leading to an immediate, perceptible relief of gastrointestinal discomfort.

Benegut® is scientifically proven, IP protected and approved to be used in dietary supplements in Europe and the U.S.



### Benegut® – Science

Several scientific studies have been carried out using the proprietary Perilla leaf extract to investigate the effects on digestive health:

1. **Nutritional human study to investigate effects on GI discomfort**
2. **Ex vivo human study to test anti-inflammatory and immune effects**
3. **Ex vivo animal study to explore anti-spasmodic effects**
4. **In vitro study to screen for brain-gut axes activities**

Study facts and results are summarized within the leaflet.

### Benegut® helps to support...

- digestive health.
- the improvement of gastrointestinal discomfort.
- the reduction of bloating.
- the reduction of abdominal discomfort like pain, cramps and inflammations.
- the freedom to wear the clothes of choice like a skinny jeans.
- the motivation to have social activities even when suffering from GI symptoms.
- quality of life.

## 1. Nutritional human study to investigate effects on GI discomfort

Results of a first nutritional human study showed that daily supplementation with Benegut® supports gastrointestinal health.

### Study facts:

- double blind, randomized, placebo-controlled, parallel design human
- 50 healthy people, ages 30-70, BMI 19-30 kg/m<sup>2</sup>, 41 women and 9 men
- intake 150 mg Benegut® twice a day for 4 weeks
- volunteers with GI discomfort & bowel movements between 1.5-3x/week

The study population represented the distribution of GI discomfort within the general population. Particularly woman suffer from GI discomfort and reduced bowel movements and they are generally more willing to participate in trials. The study was conducted by Biotesy, Esslingen, Germany.

### Challenges:

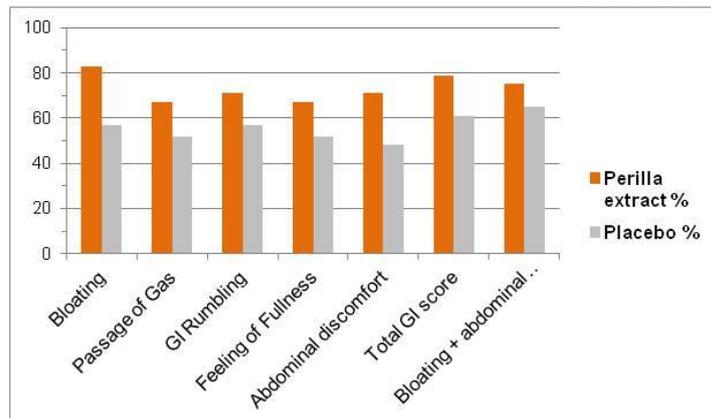
Nutritional human studies investigate effects within healthy people, with the aim to demonstrate an improvement of a physiological status which is still defined as healthy, but affects quality of life – improvements range only from slightly to very slightly. In addition GI symptoms show a high fluctuation and gut health studies report high placebo effects.

### Study flow chart:

|   | Screening | Whole transit time     | Run-In Phase | Treatment Period   |         | Whole transit time | Follow up          |
|---|-----------|------------------------|--------------|--------------------|---------|--------------------|--------------------|
|   |           |                        |              | Visit 1            | Visit 2 |                    |                    |
| Time  |           | 1 week prior to run-in | 2 weeks      | Day 1              | Day 29  | Day 30 - 38        | Day 39             |
| Informed consent  | x         |                        |              |                    |         |                    |                    |
| In- and exclusion criteria  | x         |                        |              | x                  | x       |                    |                    |
| Adverse events  |           |                        |              | x                  | x       |                    | x                  |
| Anamnesis / Checkup / ECG   | x         |                        |              |                    |         |                    |                    |
| Blood routine parameters  | x         |                        |              |                    |         |                    |                    |
| Gastrointestinal constipation module based on ROME III criteria       | x         |                        |              |                    |         |                    |                    |
| Questionnaire of bowel movements                                      |           |                        | x            | daily for 28 days  |         |                    |                    |
| Questionnaire of stool consistency                                    |           |                        | x            | daily for 28 days  |         |                    |                    |
| Questionnaire on daily gastrointestinal characteristics               |           |                        | x            | daily for 28 days  |         |                    |                    |
| Questionnaire of intestinal symptoms (PAC-SYM) + additional questions |           |                        |              | x                  | x       |                    |                    |
| PAC-QoL   |           |                        |              | x                  | x       |                    |                    |
| Perceived stress questionnaire (PSQ)                                  |           |                        |              | x                  | x       |                    |                    |
| Determination of whole transit time                                   |           | x                      |              | Hand in of results |         | x                  | Hand in of results |
| Hand out of study products  |           |                        |              | x                  |         |                    |                    |
| Intake of study products  |           |                        |              |                    | x       |                    |                    |
| Global assessment   |           |                        |              |                    | x       |                    |                    |

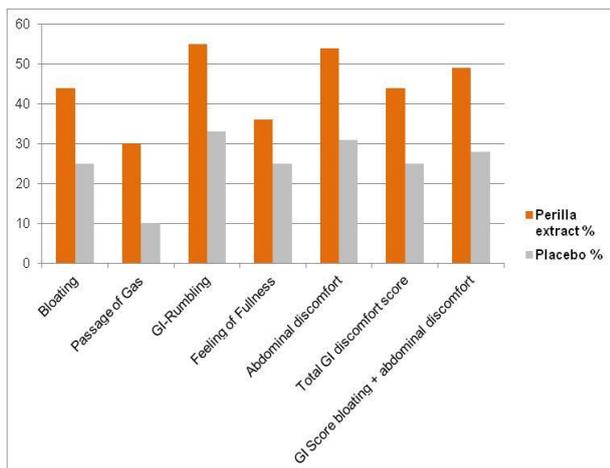
### ➔ **Bene/gut® is able to reduce gastrointestinal discomfort**

- All GI symptoms (bloating, passage of gas, GI-rumbling, feeling of fullness and abdominal discomfort) were significantly improved in healthy people.
- 80% of all volunteers taken Perilla extract reported substantial improvement of gastrointestinal discomfort.

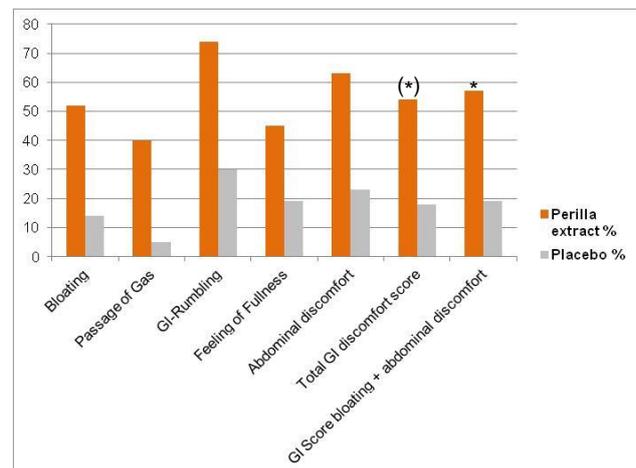


Responder (%)

- GI discomfort was on average improved by over 40%.
- Perilla was most effective to reduce bloating and abdominal discomfort particularly in women. Effects were statistically significant against placebo ( $p=0.048^*$ ).



GI discomfort Improvement (%) All

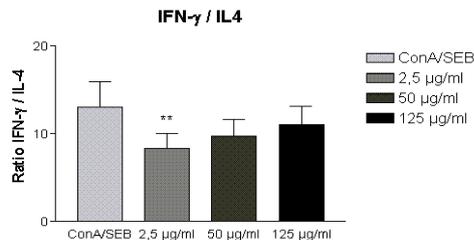
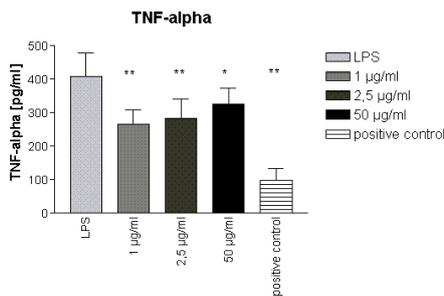


Subgroup women

- Study results confirmed in vitro and ex vivo results, which identified that the mode of actions of Perilla extract are prokinetic and anti-spasmodic effects, efficacy leading to an immediate, perceptible relief from GI discomfort.
- The study results are very promising taking into consideration the challenging set-up of a nutritional human study with healthy volunteers.

### 2. Ex vivo human study on anti-inflammatory & immune effects

Ex vivo whole blood stimulation assays with human blood leucocytes from 10 volunteers specifically stimulated with lipopolysaccharide (LPS) to determine TNF $\alpha$  and with concanavalin A (ConA) and staphylococcal enterotoxin B (SEB) to determine interleukins.



➔ Inhibition of TNF $\alpha$  release

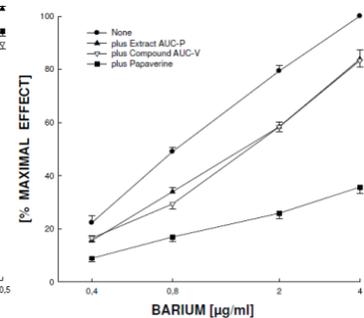
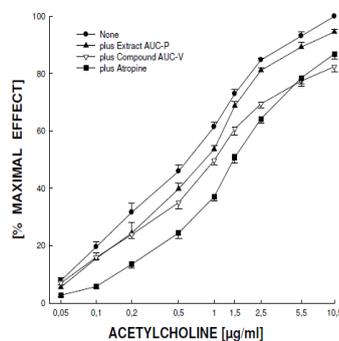
➔ Th1 to Th2 shift

Fujii H., et al., Investigation of a Perilla frutescens special extract. Anti-inflammatory and immune-modulatory properties. Agrofood Industry hi-tech (2012)

### 3. Ex vivo animal study to explore antispasmodic effects

The antispasmodic effect of Bene<sup>g</sup>ut<sup>®</sup> and its key active constituent was investigated on isolated adult male Wistar rats' ileum contraction.

| Mode of action                                      | Agonist       | Positive control |
|---|---------------|------------------|
| Neurotropic effect induced by e.g. stress           | Acetylcholine | Atropine         |
| Muscolotropic effect induced by e.g. food allergens | Barium        | Papaverine       |



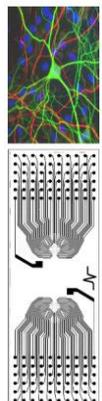
➔ Bene<sup>g</sup>ut<sup>®</sup> and isolated Vicenin 2 demonstrated antispasmodic effects inhibiting musculotropic activity and neurotropic, cholinergic activity.

Verspohl E.J., et al., Testing of Perilla frutescens extract and Vicenin 2 for their antispasmodic effect. Phytomedicine (2013)

### 4. In vitro study to screen for brain-gut axes activities

An acute neuroactive effect of Bene<sup>g</sup>ut<sup>®</sup> and isolated Vicenin 2 on the neuronal activity of murine frontal cortex networks was tested by means of electrophysiological multi-channel recording in comparison with well-known neuroactive substances.

➔ Bene<sup>g</sup>ut<sup>®</sup> and isolated Vicenin 2 act as a reversible cholinesterase inhibitor having prokinetic effect to balance the rhythm and support regularity of the small intestine.



Buchwald-Werner S., Fujii H., Prokinetic and antispasmodic constituent discovered in Perilla Frutescens – Development of a gut health ingredient. Planta medica (2012)