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Effects of a Food Supplement with a Wild Thyme (*Thymus serpyllum* L.) Extract on Gut Health and the Microbiome in Humans: A Randomized, Double-Blinded, Placebo-Controlled Clinical Trial

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Abstract

Background: Based on the scientific and traditional knowledge, benefits for gut and digestive health were expected from Wild Thyme (*Thymus serpyllum* L.) extract (WThE) consumption, but no controlled human studies were performed yet to prove the proposed health benefits. **Method:** The aim of this human randomized, double-blind parallel arm pilot study was to explore the impact of aqueous Wild Thyme extract, a food supplement, in a healthy but overweight study collective (N = 40). In detail, the impact on digestion, gastrointestinal symptoms, gut microbiome, and quality of life by employing an essential oil-free WThE preparation or a matching placebo was investigated. **Results:** The study results indicate that WThE has the potential to improve gastrointestinal symptoms and increase stool frequency, thus an improved quality of life was observed. The stool microbiome of study collective was characterized by a high *Firmicutes* to *Bacteroidetes* ratio. A decrease in the mean *Firmicutes*/*Bacteroidetes* ratio was seen in WThE group. **Conclusion:** The data support the potential applications of WThE as a food supplement with benefits on gut health.

Keywords

Wild Thyme (*Thymus serpyllum* L.) Extract, Gut Health, Gut Microbiome, Abdominal Comfort, Bowel Movement, Mental Well-Being, Quality of Life

1. Introduction

General gut health problems, such as lower abdominal pain or belly aches accompanied by a bloated abdomen or flatulence, are common health problems in Western societies with different causes and variable outcomes. Moreover, symptoms are highly individual and underlying causes are often difficult to determine. A recent global survey revealed the prevalence of over 40% of their populations in 33 countries on 6 continents to be affected by functional gastrointestinal disorders (FGIDs), now called disorders of gut-brain interaction [1].

Thymus serpyllum L. (wild thyme) is a perennial shrub, native to areas of northern and central Europe. Its aerial parts are most frequently used in ethnomedicine, mainly for treating illnesses and problems related to the respiratory and gastrointestinal systems [2] [3] [4], particularly for symptomatic treatment of digestive disorders such as epigastric distension, sluggish digestion, eructation and flatulence. Due to their non-volatile phenolic components, *Thymus serpyllum* preparations demonstrate spasmolytic effects, stimulate gastric juice secretion, and help digestion.

In addition, wild thyme can be a source of natural antioxidants, nutritional supplements, or components of functional foods in the food industry [3]. Wild thyme herb, Serpylli herba (Lamiaceae), consists of dried and flowering aerial parts of *Thymus serpyllum* L. and mainly contains substance groups, such as flavonoids, polyphenol acids and triterpenes [5].

To our knowledge, there are no controlled studies investigating the described effects of *Thymus serpyllum* herb extract (WThE) in humans.

Therefore, the current human pilot study aimed to investigate the impact of WThE on gut health, especially gastrointestinal symptoms and stool frequency and consistency, and gut microbiome in a healthy but overweight study collective.

2. Material and Methods

2.1. Study Design and Subjects

The study was performed as a prospective, randomized, double-blind, placebo-controlled study in a parallel dose-escalation design. The study included a screening investigation, followed by a 2-week run-in phase for prospective assessment of gastrointestinal parameters and 3 study visits. The intervention phase started at visit 1 with a 4-week supplementation of dosage I (600 mg; between visit 1 and visit 2) and continued with further 4-weeks intervention with dosage II (900 mg; between visit 2 and visit 3).

Schematic overview of study design is showing as **Figure 1**.

The clinical study was approved by the Institutional Review Board (IRB) of Landesärztekammer Baden-Württemberg, Stuttgart. The study was registered at German Clinical Trials Register and received the identification number DRKS00026074. The conduction of the study was in accordance with the guidelines for Good Clinical Practice (GCP) set forth by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

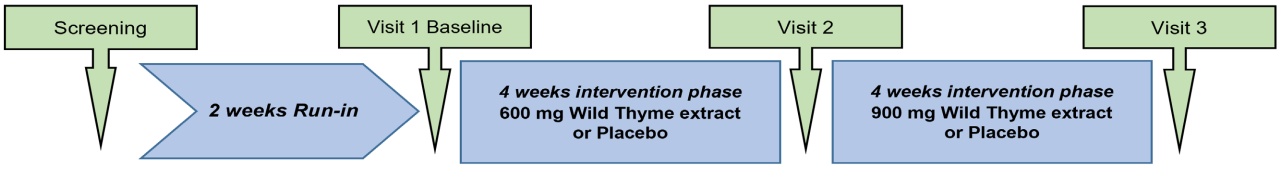

Figure 1. Overview study design.

(ICH), and in accordance with the Declaration of Helsinki regarding the treatment of human subjects in a study.

The clinical part of the study was performed at the study site of BioTeSys GmbH (Esslingen, Germany) between August and December 2021. All study subjects provided a written informed consent before they were screened for their eligibility to take part in the study. According to the inclusion criteria, only non-smoking male and female subjects of age between 30 and 70 years with a Body Mass Index (BMI) between 25 and 35 kg/m^2 and without any diagnosed gastrointestinal diseases were included in the study. Furthermore, the frequency of bowel movement had to be between 2 and 5 times per week. Subjects with a vegan and vegetarian nutrition style or regular consumption of pro- and prebiotics more than once weekly were excluded, as well as subjects consuming fiber rich foods (including fruit and vegetables) more than 3 times per week. Further exclusion criteria were bowel movements characterized by a regular constipation (less than 2 per week) or diarrhea (at least once per week) according to the Bristol Stool Chart classification. A medical examination including clinical laboratory, anamnesis, check of vital signs (blood pressure) and 12-lead resting electrocardiogram (ECG) was performed at screening to ensure that only healthy subjects without any clinically relevant abnormal findings were included in the study. Additionally, subjects were asked about their gastrointestinal symptoms and only subjects with a prespecified cut-off level were enrolled to the study (for details see the section "Assessment of gastrointestinal symptoms" below).

At each visit, subjects were provided with a diary to complete daily documentation. During the run-in phase subjects documented their stool frequency and consistency as well as the intensity of the gastrointestinal symptoms. Based on the diary, the inclusion criteria were re-checked at visit 1. If all criteria were fulfilled, subjects were randomly assigned to placebo or verum group (group size 1:1). At each study visit, subjects handed in a fecal sample collected at home for the analysis of the gut microbiota and a fasting blood sampling was performed for the analysis of routine parameters.

Altogether, 49 subjects underwent the screening, wherefrom 42 were finally included in the study. Two subjects dropped out during the first intervention phase prior to any measurements under treatment and due to reasons not connected to the study procedure or investigational product. For more details on the disposition of subjects see **Figure 2**.

2.2. Investigational Products

The investigational product was a wild thyme preparation consisting of an

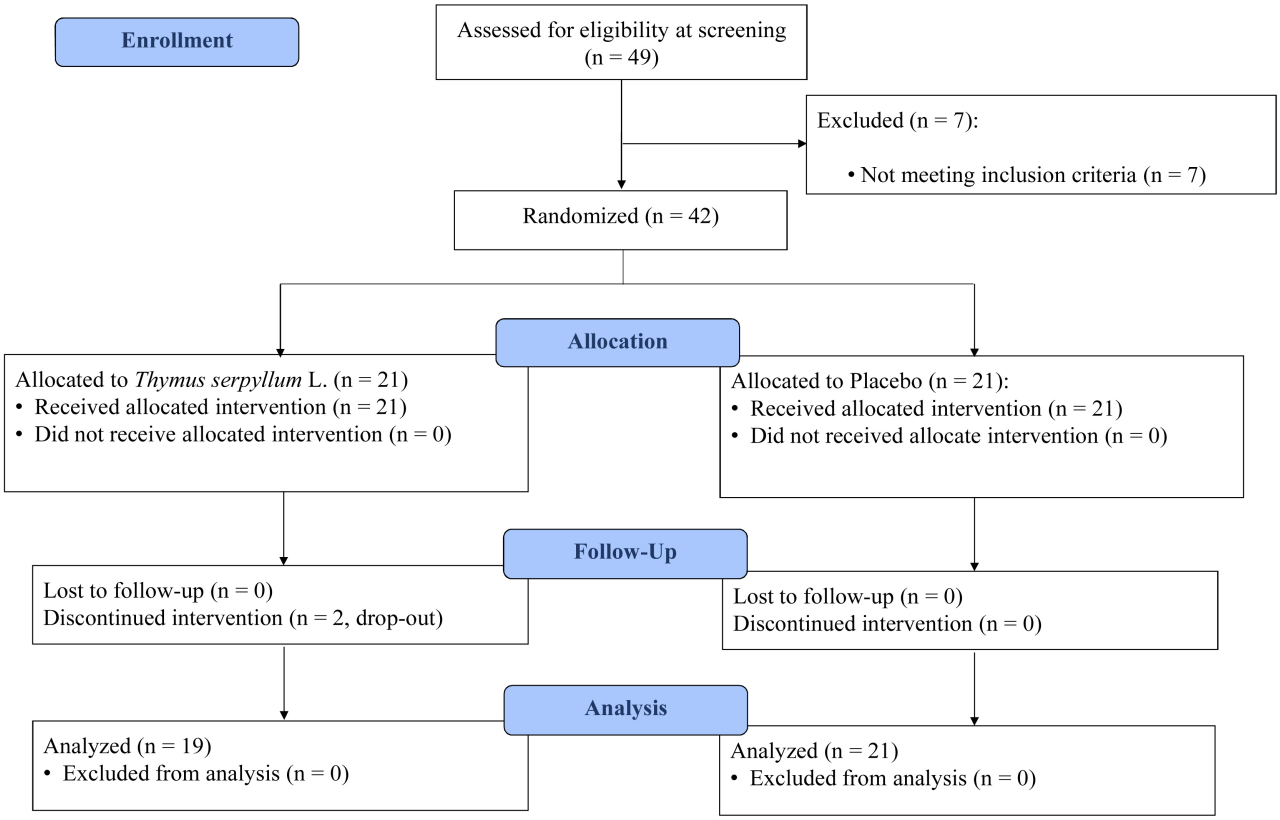

Figure 2. Volunteer disposition.

essential oil-free (less than 0.1%) aqueous extract of Serpylli herba (70%) and Nutriose®, a soluble dietary fiber of Roquette (30%). The wild thyme preparation was produced under high quality GMP-certified conditions, in compliance with Hazard Analysis Critical Control Points (HACCP)-conditions and the ISO standard 22,000 by Finzelberg GmbH & Co KG, 56626 Andernach, Germany. The investigational product is patent protected (EP2858655B1).

The study product and the matching placebo (Maltodextrin) were provided in titanium dioxide-free vegetarian capsules at the following daily regimen: during the first 4 weeks of intervention 600 mg and during the next 4 week-intervention phase 900 mg were administered orally in the morning (before breakfast).

Compliance to the intervention was calculated based on the dispensed and returned study products.

2.3. Evaluation of Stool Frequency and Consistency

Stool frequency was assessed as the average number of bowel movements occurred in a two-week period (week 1 and 2 as well as week 3 and 4 of each intervention phase). To achieve this, study subjects were instructed to document each bowel movement in the subject's diary during the whole study period including the date and time. The two-week run-in phase was assessed as baseline. Additionally, the consistency of each bowel movement was classified by subjects into one from, the seven categories using the Bristol Stool Chart. From this data, the

average consistency was calculated for the two-week intervals, similarly to the stool frequency.

A short symptom questionnaire was filled in by the subjects at screening, to obtain a retrospective assessment of the following gastrointestinal symptoms: 1) stagnant gas/bloated abdomen, 2) gas leakage/flatulence, 3) feeling of fullness/tightness due to constipation, 4) grumbling in the gastrointestinal tract and 5) abdominal discomfort/pain. The symptoms were rated on a 5-point Likert scale (0 = no, 1 = very light/weak, 2 = a little/somewhat, 3 = medium, 4 = strong). Summarizing the single symptoms, a cut-off level of at least 5 points when evaluating the symptoms retrospective for the last month was required for inclusion at screening. These symptoms were additionally rated daily by the subjects via their diary during week 2 in the run-in phase (baseline), as well every second week of the intervention to evaluate the changes during the intervention. Mean values of each symptom were built from each assessed week.

In addition, subjects rated the symptom "feeling of incomplete bowel emptying" for each bowel movement and documented the intensity of the symptom in the diary using the same 5-point Likert scale as described above. The mean values were calculated for the whole run-in phase (baseline) as well as for week 1 and 2 and week 3 and 4 of each intervention phase.

Furthermore, the validated gastrointestinal questionnaire Gastrointestinal Symptom Rating Scale (GSRS) was used for the retrospective evaluation of the five symptom clusters Reflux, Abdominal pain, Indigestion, Diarrhea and Constipation within the last 7 days. To assess the changes in gastrointestinal symptoms, the GSRS questionnaire was filled in by the subjects at each study visit.

2.4. Evaluation of Quality of Life and Global Assessment

To investigate the impact of subjects' general mental and physical health on the quality of life, the self-administered Short Form 12 health scale (SF-12) questionnaire was filled at study visits 1 - 3. Two aggregate summary measures of the questionnaire—the Physical Component Score (PCS) and the Mental Component Score (MCS)—were evaluated separately.

As part of the global assessment, the satisfaction with the stool frequency was rated by subjects on a 5-point Likert scale (very unsatisfied, unsatisfied, neutral, satisfied or very satisfied) at each study visit.

2.5. Gut Microbiome Composition Analysis

Stool samples were collected by subjects at home within 48 hours prior to the start of the intervention (baseline), and prior to the follow-up visits 2 and 3 to assess the changes of fecal microbiome composition. Subjects were instructed to freeze the stool sample at −20˚C within 10 min after sampling. After maximum 48 hours after collection, the samples were stored at study site at −70˚C until shipping to the analysis lab.

To characterize the microbial community of the stool samples 16S-rRNA

technology was used. The analysis was performed at Microsynth AG (Balgach, Switzerland). For the DNA isolation, the QIAamp Fast DNA Stool Mini Kit (51604) was used with a prior bead beating step of the stool samples in InhibitEX® Buffer (Qiagen, Venlo, Netherlands). 16S rRNA gene amplicon sequencing was based on the Illumina protocol for the construction of 16S metagenomic sequencing libraries (Illumina Inc., 2014, San Diego, CA, USA). For the amplification of the third and fourth hypervariable regions of the 16S rRNA gene a two-step PCR with the bacterial primers 341F_ill and 802R_ill was used. The amplicon libraries were constructed with the Nextera DNA Library Prep kit. The sequencing was performed on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) using the Illumina paired-end protocol (250 bp paired end read, V2 chemistry). After sequencing, libraries for each sequencing lane and sample were demultiplexed and the raw reads were sorted by the inline barcodes. Next, adapters and primer sequences were trimmed.

For read assignment to taxonomic units the RDP reference database was used. Based on the output data, the changes of alpha diversity (Shannon index) as well as the distribution of selected bacterial genera were evaluated.

2.6. Safety Assessment

Blood routine parameters were determined at each study visit as described above. Those included differentiated haemogram, liver enzymes (GPT, GOT, γ-GT, AP), creatinine and uric acid. Furthermore, subjects were instructed to document any adverse events as well as new medication in the diary during the whole study period. These entries were monitored and judged by the authorized study staff. The tolerability of the study product was further assessed. Vital signs (blood pressure and heart rate) were monitored at rest at all study visits.

2.7. Statistical Analysis

Data of this pilot study were evaluated exploratory. All subjects who completed all study visits (n = 40) according to the protocol were included in the analysis. In the publication the results after intake of 600 mg are presented. Results are confirmed in general after intake of 900 mg Wild Thyme, but no dose dependency was observed.

For the analysis of differences between treatments (placebo versus verum), delta values between baseline and end of the 4-weeks intervention were compared by means of the unpaired t-test. In case of non-normal distribution of data, the Wilcoxon rank sum test was applied.

To investigate the impact by gender and stool frequency on the outcome of the study, the described statistical approach was additionally applied for the subgroup analysis. For this purpose, the data were separated into a male and female subgroup as well as into the subgroup with lower and higher stool frequency, whereby the median level of 3.5 stools/week during run-in period was defined as the cut-off value for the subgroup definition.

Changes within groups were evaluated with paired t-test statistic or Wilcoxon signed rank test, if appropriate. ANOVA with repeated measurements or appropriate non-parametric tests were applied for parameters with interim assessments after 14 days of intervention.

The results of the global assessment were evaluated with a Chi-Square.

All statistical tests were performed two-sided. A significance level of $p < 0.05$ was used. The analysis was performed with IBM SPSS Statistics Software Version 24.0 (Armonk, NY, USA). Summary tables and graphs were generated using GraphPad Prism software (San Diego, CA, USA).

3. Results

3.1. Demographics and General Health of the Study Population

Demographic data of the subjects and baseline characteristics are specified in Table 1. No significant differences were observed for demographic and baseline data in both groups. Overall, the study collective can be described as a general healthy, moderately active, overweight collective with nutrition habits of moderate quality. Only subjects with a stool frequency of 2 - 5 stools/week and with low to moderate GI discomfort as assessed with a gastrointestinal symptom score (range 0 - 20) were allowed to participate in the study. Food intake, physical activities and body weight were not changed over the study period in both groups.

3.2. Stool Frequency and Consistency

The results of the analysis of stool frequency and consistency are summarized in Table 2.

After 4 weeks of intervention an increase of stool frequency was observed in both study groups when considering overall data, whereby the delta changes were comparable between the groups ($p = 0.5907$). Considering growing evidence that indicates that age and gender can affect the stool frequency and the

Table 1. Demographic and baseline characteristics.

Parameter	WThE (n = 19)		Placebo (n = 21)	
	Mean	95% CI	Mean	95% CI
Age (years)	51.5	46.2 - 56.7	53.8	48.7 - 59.0
BMI (kg/m^2)	27.9	26.6 - 29.2	28.3	27.3 - 29.4
Systolic BP (mmHg)	122.6	115.8 - 129.4	129.9	123.4 - 136.4
Diastolic BP (mmHg)	80.3	76.14 - 84.38	82.0	77.99 - 85.91
Total cholesterol (mg/dL)	210.7	188.8 - 232.5	218.7	193.1 - 244.3
GI symptome score	10.3	8.5 - 12.1	11.1	9.4 - 12.7
Stool frequency	3.6	3.2 - 4.1	3.4	2.9 - 3.9

Values are means (95% CI). WThE: Wild Thyme extract, BMI: body mass index, BP: blood pressure, GI: gastrointestinal.

Table 2. Stool frequency and consistency.

Parameter	WThE				Placebo				Delta change				*p-value*[1]
	Baseline		4 *weeks*		*Baseline*		4 *weeks*		*WThE*		*Placebo*		
	Mean	95% *CI*	*Mean*	95% *CI*	*Mean*	95% *CI*	*Mean*	95% *CI*	*Mean*	95% *CI*	*Mean*	95% *CI*	
Stool frequency													
Overall	3.64	3.21 - 4.07	4.61	3.91 - 5.30	3.43	2.92 - 3.93	4.24	3.77 - 4.71	0.96	0.53 - 1.40	0.81	0.41 - 1.21	0.5907
Males	3.70	2.02 - 5.38	5.20	3.02 - 7.38	4.00	2.61 - 5.39	4.00	2.84 - 5.16	1.50	0.74 - 2.26	0.00	−0.62 - 0.62	0.0141*
Females	3.62	3.20 - 4.05	4.39	3.62 - 5.16	3.25	2.67 - 3.83	4.31	3.74 - 4.89	0.77	0.24 - 1.31	1.06	0.62 - 1.51	0.3734
Baseline stool frequency > median	4.53	4.20 - 4.85	5.88	4.91 - 6.84	4.69	4.31 - 5.07	4.94	4.59 - 5.29	1.35	0.61 - 2.09	0.25	−0.14 - 0.64	0.0126*
Baseline stool frequency ≤ median	3.00	2.66 - 3.34	3.68	3.16 - 4.21	2.65	2.37 - 2.94	3.81	3.16 - 4.46	0.68	0.11 - 1.25	1.15	0.60 - 1.71	0.2076
Stool consistency													
Overall	3.52	3.12 - 3.93	3.44	3.09 - 3.78	2.88	2.58 - 3.17	3.03	2.69 - 3.38	−0.09	−0.47 - 0.29	0.16	−0.22 - 0.53	0.3456

Values are means (95% CI); WThE Wild Thyme extract, [1]p-value for comparison between the groups (unpaired t-test or Wilcoxon rank sum test or ANCOVA in case of baseline differences (consistency)); *p < 0.05.

composition of gut microbiota [6], a subgroup analysis by gender was performed. In the subgroup of men, a significant increase of stool frequency was seen after 4 weeks of intervention with wild thyme, whereas there were no changes after placebo intake. As shown in the **Figure 3**, the delta changes from baseline were significantly different between the groups (p = 0.0141).

In female subjects, a significant increase of stool frequency was observed in both wild thyme and placebo group with no differences between the groups regarding the delta changes (p = 0.3734).

Furthermore, wild thyme intervention showed an effect in subjects with stool frequency >3.5 stools/week at baseline with significantly higher delta changes compared to placebo (p = 0.0126) (see **Figure 4**).

Additionally, subjects were asked about satisfaction with their frequency of bowel movements throughout the study. The results showed a higher rate of subjects being "satisfied" or "very satisfied" in wild thyme group compared to placebo with 63% vs. 43% after the 4-week intervention period (see **Table 3**).

On average stool consistency of study group was characterized in the category "type 3" of normal range with a sausage shape with cracks in the surface. Stool consistency slightly increased over the study period in the placebo group but not in the wild thyme group. The change in stool consistency did not differ significantly between groups (p = 0.5346). In summary, data indicate that there is no impact of WThE on stool consistency that indicates that the long-term intake of WThE does not lead to undesired softening of the stool towards diarrhoea.

3.3. Gastrointestinal Symptoms and GSRS

Table 4 shows an overview of the analysed gastrointestinal symptoms. After 4 weeks of intervention, there was a reduction of the symptom severity observed

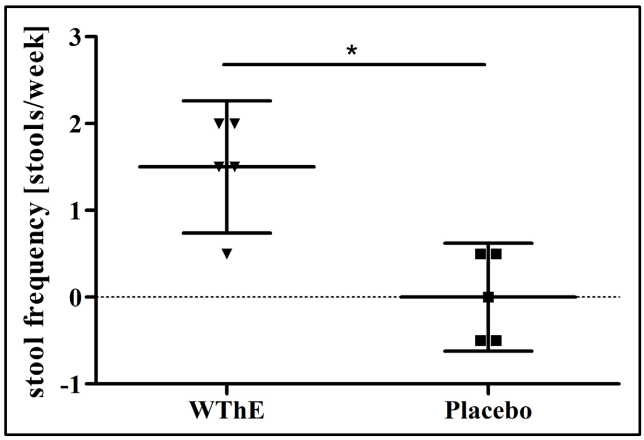

Figure 3. Distribution of delta change of stool frequency in subgroup men [stools/week] (Scatter diagram with mean ± 95% CI); *p < 0.05; WThE: Wild Thyme extract.

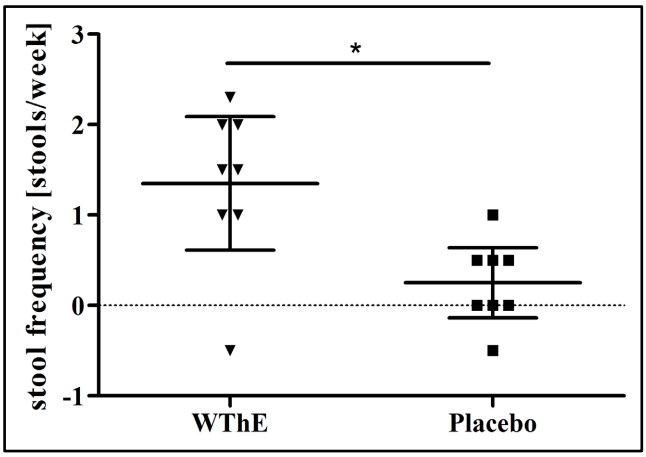

Figure 4. Distribution of delta change of stool frequency in subgroup stool frequency at baseline > median [stools/week] (Scatter diagram with mean ± 95% CI); *p < 0.05; WThE: Wild Thyme extract.

for all evaluated symptoms, which was seen not only after wild thyme intake but also in the placebo group. Although these improvements were more pronounced in the wild thyme group for almost all symptoms, only a trend was found for gas leakage / flatulence with a greater reduction in WThE group. Further, in women a decrease of feeling of fullness by trend was seen in WThE group in comparison to placebo (WThE*:* −1.01 (95% CI: −1.50; −0.51), Placebo: −0.43 (95% CI: −0.86 - 0.00), p = 0.0699).

The analysis of the GSRS questionnaire confirmed the observation of the GI symptoms. An improvement of the subscores Indigestion, Constipation, Abdominal pain, and Reflux as well as of the total GSRS score was observed. Like the gastrointestinal symptoms, this improvement was more pronounced in the verum group (see Table 4) resulting in an higher improvement of total GSRS score by trend in comparison to placebo group (WThE: −3.15 (95% CI: −4.43; −1.83),

Table 3. Global assessment.

Satisfaction with the frequency of bowel movements	Baseline				4 weeks			
	WThE		*Placebo*		*WThE*		*Placebo*	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
very unsatisfied	0	0	0	0	0	0	0	0
unsatisfied	7	37	8	38	3	16	3	14
neutral	9	47	12	57	4	21	9	43
satisfied	3	16	1	5	9	47	8	38
very satisfied	0	0	0	0	3	16	1	5
Total	19	100	21	100	19	100	21	100
p-value[1]	0.4969				0.4090			

WThE: Wild Thyme extract, [1]p-value for comparison between the groups (Chi-Square test).

Table 4. Gastrointestinal symptoms.

Parameter	WThE				Placebo				Delta change				p-value[1]
	Baseline		4 weeks		Baseline		4 weeks		WThE		Placebo		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	
Gastrointestinal symptoms													
Stagnant gas/bloated abdomen	1.84	1.31 - 2.36	0.99	0.60 - 1.38	1.81	1.41 - 2.21	1.10	0.85 - 1.36	−0.84	−1.30; −0.39	−0.71	−1.05; −0.36	0.6091
Gas leakage/flatulence	1.78	1.26 - 2.30	1.13	0.81 - 1.46	1.79	1.36 - 2.23	1.27	0.87 - 1.66	−0.65	−1.03; −0.26	−0.53	−0.86; −0.19	0.0946[t]
Feeling of fullness/tightness due to constipation	1.56	0.98 - 2.14	0.78	0.41 - 1.14	1.76	1.37 - 2.15	1.28	0.91 - 1.65	−0.79	−1.20; −0.37	−0.48	−0.84; −0.13	0.2503
Grumbling in the gastrointestinal tract	1.43	0.92 - 1.94	0.69	0.33 - 1.04	1.10	0.66 - 1.53	0.72	0.42 - 1.03	−0.75	−1.20; −0.29	−0.37	−0.74; 0.00	0.1851
Abdominal discomfort/pain	0.98	0.58 - 1.39	0.39	0.09 - 0.69	1.12	0.78 - 1.46	0.69	0.34 - 1.05	−0.60	−0.98; −0.21	−0.43	−0.75; −0.10	0.4849
Mean gastrointestinal score	1.52	1.07 - 1.97	0.80	0.52 - 1.07	1.52	1.18 - 1.85	1.01	0.75 - 1.28	−0.72	−1.09; −0.36	−0.50	−0.76; −0.25	0.2972
Feeling of incomplete emptying	1.61	1.05 - 2.17	1.12	0.67 - 1.58	1.59	1.18 - 1.99	1.17	0.85 - 1.50	−0.48	−0.87; −0.10	−0.41	−0.79; −0.04	0.8816
GSRS questionnaire													
Diarrhea	1.54	1.18 - 1.91	1.41	1.06 - 1.76	1.51	1.22 - 1.80	1.68	1.33 - 2.03	−0.14	−0.51; −0.24	0.18	−0.16; −0.52	0.1199
Indigestion	3.34	2.74 - 3.94	2.56	2.23 - 2.90	3.43	2.93 - 3.93	2.98	2.59 - 3.36	−0.77	−1.28; 0.27	−0.45	−0.92; −0.01	0.3246
Constipation	3.04	2.38 - 3.70	2.13	1.76 - 2.49	3.56	3.08 - 4.04	2.86	2.35 - 3.37	−0.91	−1.37; −0.45	−0.70	−1.15; −0.25	0.4917
Abdominal pain	2.53	2.10 - 2.96	1.74	1.47 - 2.00	2.47	2.08 - 2.87	2.10	1.76 - 2.44	−0.79	−1.16; −0.43	−0.37	−0.71; −0.03	0.0834[t]
Reflux	1.87	1.37 - 2.36	1.34	1.10 - 1.58	1.76	1.32 - 2.20	1.64	1.23 - 2.06	−0.53	−0.96; −0.10	−0.12	−0.44; −0.20	0.1591
Total score	12.29	10.54 - 14.04	9.15	8.38 - 9.92	12.71	11.08 - 14.35	11.24	9.83 - 12.65	−3.15	−4.43; −1.87	−1.48	−2.77; −0.18	0.0607[t]

Values are means (95% CI); WThE: Wild Thyme extract. [1]p-value for comparison between the groups (unpaired t-test or Wilcoxon rank sum test); t $p < 0.1$.

Placebo: −1.48 (95% CI: −2.77; −0.18) p = 0.0607).

3.4. Quality of Life

Gastrointestinal discomfort can have a great impact on quality of life and psy-

chological well-being. To measure the impact of WThE on an individual's everyday life health a SF-12 questionnaire was used. As shown in Table 5, an improvement was observed in the Mental Component Summary (MCS) score of the SF-12 questionnaire after 4 weeks of intervention with wild thyme, whereas after placebo intake, a slight worsening of the score was seen. The delta changes between baseline and end of intervention with WThE were significantly different in comparison to placebo (p = 0.0323).

Regarding the Physical Component Summary score (PCS), a slight improvement was observed in the placebo group, whereas there were almost no changes in the verum group. Between the groups, the delta values were different by trend towards the placebo group (p = 0.0544).

3.5. Microbiome Analysis

The Shannon diversity index is a sum of the proportion of each species relative to the total number of species in the community under analysis and therefore accounts for both abundance and evenness [7]. In the current investigation, no changes of Shannon diversity index were observed within and between the groups over the study period (WThE: 3.85 (95% CI: 3.73 - 3.99) at baseline and 3.71 (95% CI: 3.55 - 3.90) after 4 weeks; placebo: 3.80 (95% CI: 3.68 - 3.93) at baseline and 3.73 (95% CI: 3.57 - 3.89) after 4 weeks).

In the following, some selected parameters of microbiome analysis are presented on the different bacterial hierarchy levels. Exemplarily the Krona charts for baseline and after 4 weeks of intervention are shown for both study groups (Figure 5).

Firmicutes and *Bacteroidetes* are the two most abundant bacterial phyla. Overall, data indicated a high variability in relative abundances of these phyla-between subjects. As shown in Table 6, data point to a decrease in *Firmicutes* and increase in *Bacteroidetes* predominantly after intake of WThE. In the placebo group there was on average a small decrease in *Bacteroidetes* and increase in *Firmicutes.* This observation is also reflected by the Krona charts. Based on the mean values of *Firmicutes* and *Bacteroidetes* a decrease of the *Firmicutes*/*Bacteroidetes* ratio was observed in the WThE group (WThE: from 7.06 to 5.15, placebo: from 5.15 to 6.14).

By the analysis of microbiome on genus level some differences between wild

Table 5. SF-12 questionnaire.

SF-12 questionnaire	WThE				Placebo				Delta change				p-value[1]
	Baseline		4 weeks		Baseline		4 weeks		Wild Thyme		Placebo		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	
SF-12 PCS	50.93	47.57 - 54.28	50.96	47.90 - 54.01	52.18	49.75 - 54.60	52.81	49.90 - 55.72	0.03	−3.06 - 3.13	0.64	−1.77 - 3.04	0.0544[t]
SF-12 MCS	47.67	43.23 - 52.11	51.25	47.85 - 54.64	52.25	49.75 - 54.74	51.43	48.51 - 54.36	3.58	0.28 - 6.88	−0.81	−3.97 - 2.34	0.0323*

Values are means (95% CI); WThE: Wild Thyme extract; [1]p-value for comparison between the groups (Wilcoxon rank sum test); t: p < 0.1, *p < 0.05.

Table 6. Bacterial phyla *Firmicutes* and *Bacteroidetes*.

Distribution of selected phyla	WThE				Placebo				Delta change				p-value[1]
	Baseline		4 weeks		Baseline		4 weeks		Wild Thyme		Placebo		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	
Firmicutes	80.31	76.49 - 84.12	73.61	66.03 - 81.18	76.28	69.64 - 82.92	78.04	72.95 - 83.13	−6.7	−14.32 - 0.92	1.76	−4.97 - 8.50	0.1714
Bacteroidetes	11.38	7.66 - 15.11	14.30	7.48 - 21.12	14.80	9.62 - 19.97	12.72	7.61 - 17.82	2.92	−3.25 - 9.07	−2.08	−8.34 - 4.18	0.2553

Values are means (95% CI); WThE: Wild Thyme extract. [1]p-value for comparison between the groups (Wilcoxon rank sum test).

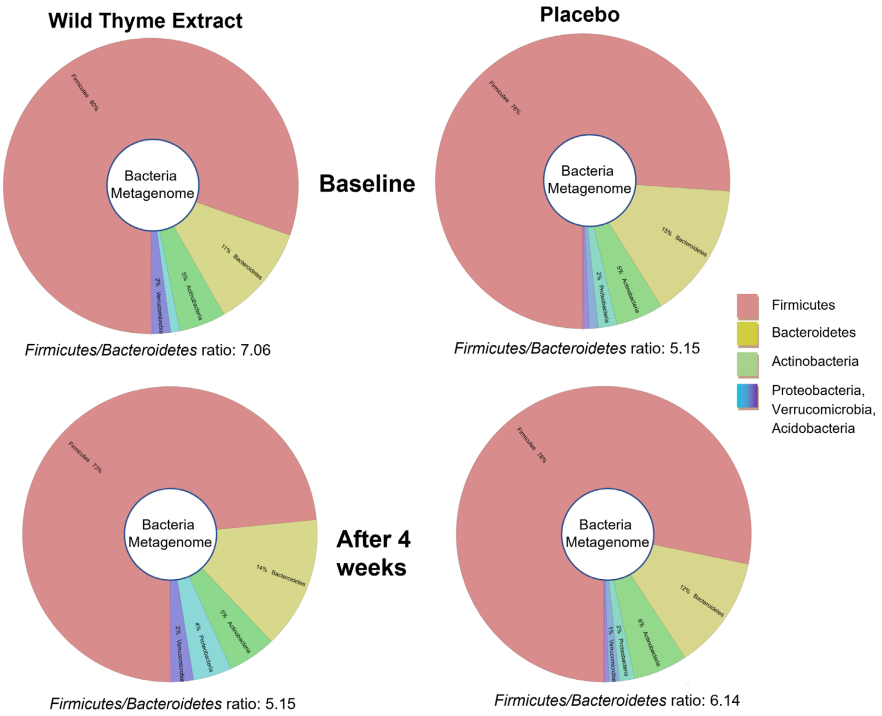

Figure 5. Krona charts, Bacteria at baseline and after 4 weeks of intervention.

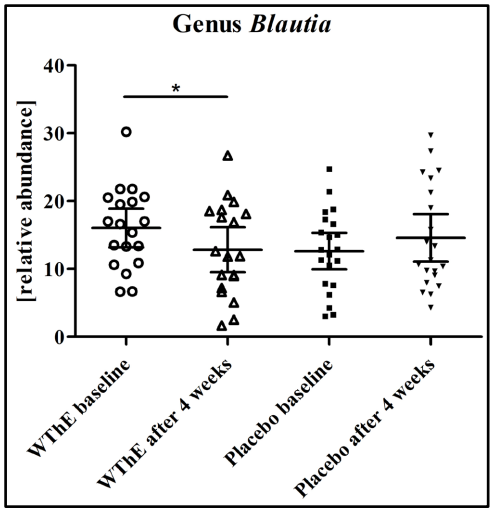

Figure 6. Distribution of *Blautia* in stool samples [relative abundance] (Scatter diagram with mean ± 95% CI); WThE: Wild Thyme extract.

thyme and placebo group were found. For example, in *Blautia*, a significant decrease ($p = 0.0444$) was observed in WThE group after the 4-week intervention period (**Figure 6**). In contrast, in placebo group, the relative abundance rather increased.

3.6. Safety Assessment

All subjects rated the study product with WThE as "well" tolerated after the 4-week intervention period. Furthermore, there were no AEs related to the investigational products.

Regarding the blood routine parameters or vital signs, there were no changes that might indicate a relation with administration of the interventional products. Taken together, the study confirms overall good tolerability and safety of the WThE.

4. Discussion

Thyme species are traditionally used mainly for respiratory and gastrointestinal ailments [8], although for centuries this knowledge was based on folk medicinal experiences. In the last decades the respiratory effects were underpinned with pre-clinical and some clinical data, but the gastrointestinal activity of Thyme was not investigated until recently. Early pre-clinical and only observational human studies are described by Greek gastroenterologists [9]. Based on this evidence an EFSA claim ID4491: "it has an anti-inflammatory effect releasing the gastric digestion; it improves the digestive function (stomach, intestine)" was submitted and is still pending "on hold" by this authority (ESFA claim ID4491). The results of the studies with the proprietary extract of Wild Thyme may support this claim.

Several systematic pre-clinical data have shown the benefits of Thyme species for gastrointestinal ailments, whereby especially *Thymus serpyllum* L., the wild thyme was found to be the most active Thymus species. This was elucidated with comparative antioxidant and anti-inflammatory test batteries (internal data, not published). Further the proprietary *Thymus serpyllum* extract preparation was investigated in different pre-clinical studies. In an experimental colitis model the native WThE showed anti-inflammatory and anti-oxidative effects [10] [11]. Irritable Bowel Syndrome (IBS) is discussed to be triggered at least partially on minor/low-grade inflammation processes in the gut, too. Thus, wild thyme extract was also successfully investigated in an IBS model [12]. Additionally, recently obtained data in an experimental model of high-fat diet in mice showed beneficial effects of WThE on metabolic syndrome [13]. These effects were evidenced by a reduction in body weight gain in obese mice, which was associated with an improvement in the glycemic and lipid metabolic profile, most probably related to the amelioration of insulin resistance process that occurs in obesity. Moreover, the extract was able to modulate the altered gut microbiota, restoring microbial richness and diversity, and augmenting the counts of short-chain fatty

acid producing bacteria, which have been associated with the maintenance of gut permeability and weight regulation [13].

The current human study is the first clinical study investigating *Thymus serpyllum* in humans aiming to investigate the impact of *Thymus serpyllum* extract (WThE) on gut health, especially gastrointestinal symptoms and stool frequency, consistency, and gut microbiome, in a healthy, overweight study collective.

The results of the current study indicate that supplementation with WThE may have a beneficial effect on the digestion and gut health. We observed an improvement of stool frequency in Wild Thyme group. This was especially pronounced in men, whereas in women higher placebo effects were observed. Furthermore, the results of the subgroup analysis by stool frequency at baseline may indicate that especially subjects with higher but still irregular stool frequency (>3.5 and ≤5 stools per week) may benefit from the intake of the investigational product. Possibly in subjects with stool frequency <3.5 stools/week other physical factors might dominate the rather low stool frequency and together with the normal physiologic variability complicates to grasp the effects against placebo.

No changes were observed in stool consistency after intake of WThE. Stool consistency only slightly increased over the study period in the placebo group but not in the wild thyme group. However, at baseline, subjects in the placebo group showed significantly harder stool consistency than subject in the verum group.

Regarding the gastrointestinal symptoms, we observed an improvement not only in the WThE, but also in the placebo group. This may probably be explained due to a large placebo effect. Placebo effects are observed in many clinical trials, across medical diagnoses and treatments. Previous studies on gastrointestinal disorders showed that the placebo effect may reach 35% in IBD and even 40% in IBS. The reasons for such a high response are diverse [14]. It was reported that a high number of study visits by patients with IBD is associated with higher placebo response rates. That would mean that the frequency of investigator-patient interactions is one of the main factors for occurrence of placebo response in patients with this disease. Further, the duration of the study may influence the placebo response rates and studies of short duration may lead to a higher placebo effect rates in IBS patients. To transfer those observations on the current study would mean that alone the fact that the study subjects had to deal with their gastrointestinal problems and come to regular study visits may have affected the study outcome. One also needs to keep in mind, that the occurrence of the placebo effect does not automatically mean that the investigational product does not have any beneficial effects. Again, a within group analysis clearly showed an improvement of stool frequency and gastrointestinal symptoms in the verum group. For total GSRS score, a trend towards WThE regarding the delta change was observed. With respect to single symptoms/complaints the symptom relief was more pronounced in WThE group with an improvement by trend especially for gas leakage/flatulence and abdominal pain (GSRS questionnaire).

Overall, the observed positive impact from Wild Thyme extract on gastrointestinal symptoms could be a result of the differences in stool frequency and consistency and/or modulation of gut microbiota. Other causes of impairment of gut health may be e.g., psychological stress which induces a local inflammatory response in the gastrointestinal tract, increases intestinal permeability and modifies visceral hypersensitivity and motility via cytokine secretion [15] [16]. Furthermore, studies have shown the gut-brain axis, a bidirectional communication system between the gastrointestinal tract with the microbiota and the brain, to be involved in neuropathological changes and a variety of conditions such as irritable bowel syndrome, neurodegeneration and even depression [15] [17]. Very likely related to this, pre-clinical studies showed that cytokine production by macrophages was decreased by WThE [11] [12] [13], which may be associated to a decrease of stress, a better mental state and an improvement of quality of life. The described mechanism may explain the observation of the current clinical study where subjects of WThE group reported an improved quality of life and psychological well-being, whereas with placebo there was no improvement.

In human obesity, abnormal gut microbiota and increased gut permeability (leaky gut) are common risk factors, which can contribute to increasing chronic low-grade inflammation [18], often accompanied with overweight or metabolic syndrome [19]. Moreover, both quantitative and qualitative modifications of gut microbiota are observed in obese patients, which are related to several pathophysiological mechanisms, possibly explaining the relationship between obesity and gastrointestinal disease and disorders [20]. In the current study, we observed a rather low diversity levels of gut microbiome measured with Shannon index (median 3.8 25^{th} - 75^{th} percentile: 3.7 - 4.1 in WThE group and 3.8 25^{th} - 75^{th} percentile: 3.6 - 4.0), when comparing with our experience and other clinical studies (Median in other own clinical studies: 4.1 with 25^{th} - 75^{th} percentile of 3.9 - 4.4) (internal data). Despite no gastrointestinal disease was diagnosed by subjects, the data point to some dysbiosis possibly responsible for some of the gastrointestinal symptoms.

Evidence is growing about changes in gut microbiota that may be linked to energy homeo-stasis, thus predicting that obese and lean individuals have distinct microbiota composition, with measurable difference in the ability to extract energy from the food and to store those energy as fat. According to this point of view, they demonstrate that the *Firmicutes*/*Bacteroidetes* proportion is increased in obese people compared to lean people, and tend to decrease with weight loss [21]. Based on the mean values of *Firmicutes* and *Bacteroidetes* a decrease of the ratio was observed in comparison to placebo in the current study. The data indicate, that WThE could possibly support balancing *Firmicutes*/*Bacteroidetes* ratio back to a healthier ratio.

It was also shown that gut microbiota is involved in the occurrence of obesity by affecting appetite and satiety via vagus nerve activation or immune-neuroendocrine mechanisms [22]. This study revealed that some bacteria implicated in these

mechanisms have been modulated with WThE; for example, the bacteria *Blautia* has been associated with obesity and in our study a significant decrease of relative abundance of *Blautia* was observed in the first 4 weeks of the study. These differences could be explained by the ability of certain bacteria, especially from the *Firmicutes* phylum (like *Blautia*), to produce more enzymes that are responsible for carbohydrate degradation and fermentation. *Blautia* is a common acetic acid producer in the intestine. In literature it was investigated that the abundance of *Blautia* is negatively correlated with some diseases, especially painful GI-related issues and bowel symptoms, like bloating [23]. However, higher abundance of *Blautia* was found in the fecal microbiota of irritable bowel syndrome and ulcerative colitis patients compared with healthy individuals. These conflicting conclusions are focused on the genus level and did not conduct in-depth studies at the species or even strain levels. We must avoid drawing general conclusions at the genus level. There may be differences in the composition of *Blautia* at the species level, and different species of *Blautia* may exert beneficial or adverse effects on human health [24].

The mechanism on how WThE may affect the microbiome composition is not known. One possible explanation may be its high polyphenol content. In general, in both in vitro and in vivo studies, polyphenols or polyphenol-rich sources have shown to influence the relative abundance of different bacterial groups within the gut microbiota, reducing numbers of potential pathogens and enhancing mainly beneficial microbiota [25].

5. Conclusion

The current pilot human study aimed to investigate the impact of *Thymus serpyllum* herbal extract (WThE) on gut health.

The results of the study indicate that Wild Thyme has the potential to improve gastrointestinal symptoms and increase stool frequency. This also resulted in an improved quality of life. The stool microbiome of study collective was characterized by a high *Firmicutes* to *Bacteroidetes* ratio which could positively be affected by the intake of WThE.

However, also significant placebo effects were observed. Together with the high inter-individual differences between subjects, clear statements are limited.

Taking together, the current study confirmed overall good tolerability and safety and showed that WThE may have a beneficial effect on the gut health. The data support the potential applications of WThE as a food supplement addressing gut health.

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Disclosure of Interest

The entire study, including methodology, statistical analysis, and conclusion, was independently conducted by BioTeSys GmbH.

Katharina Knaub and Christiane Schön are employees of BioTeSys GmbH, an independent Nutritional CRO performing the RCT.

Cynthia G. Suarez is an employee of Finzelberg.

Ivo Pischel is an independent scientific consultant to Finzelberg.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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