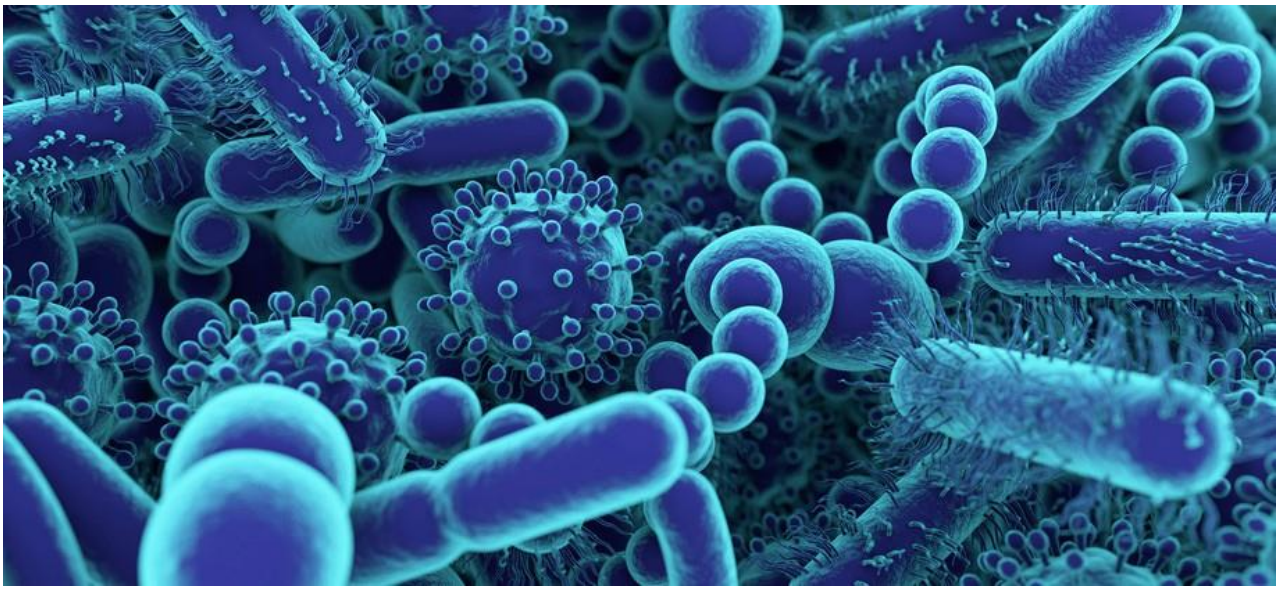
EFFECTS ON GUT HEALTH

2022 double-blind placebo-controlled study



The human microbiome study (2022)

The aim of the study was to measure the effect of prolonged Somarka/Analemma water consumption on the human gut microbiota.

The **gut microbiome**, also known as the gut flora, is the collection of trillions of microorganisms living inside the human digestive tract.

The gut microbiome helps us break down food, absorb nutrients, and produce short-chain fatty acids, which can be used as an energy source for the body. Through complex interplay with immune system pathways, **the gut microbiome protects the body from harmful pathogens and helps prevent infections**.

Studies have also shown that changes in the gut microbiome can **affect our mood and behavior**, and that negative changes of the gut microbiome play a role in conditions such as anxiety and depression.

As part of this study, human participants were instructed to consume Somarka/Anàlemma or Placebo water daily for 3 months.

The change in the state of their microbiome was assessed by profiling different beneficial and potentially pathogenic bacteria before and after the water consumption period.

The results of this preliminary study indicate that **consuming Somarka/Anàlemma water daily has a positive effect on the state of the human gut microbiota**, affecting both the beneficial and the pathogenic bacteria.

RESEARCH REPORT

AUGUST 2023

The human microbiome study (2022)

EXPERIMENTAL DESIGN

OBJECTIVE: To assess the effect of prolonged Somarka/Analemma water consumption on the microbiome of adult humans.

PARTICIPANTS: 16 adult human subjects, aged 18-70.

STUDY DESIGN: The study was designed as a **double blind, placebo-controlled, randomized, parallel group clinical study**. Participants were randomly divided into two groups and given Wands producing either Somarka/Analemma Water (n=8) or non-treated water (Placebo Control, n=8). The setup was double blind, with neither the participants nor the clinical investigators aware of how the subjects were distributed into groups. All participants were instructed to consume at least 1.5 L of the water treated with their assigned Wand for ~100 days, with regular compliance assessment check-ups. No other changes in diet, exercise or lifestyle regimes were made. Participants' stool samples were taken prior to treatment and on the last day of treatment for a comprehensive microbiome analysis.

MICROBIOME ANALYSIS: The microbiome analysis produced two main outputs. The first was the **dysbiosis index**, which is a comprehensive measure of the health of the human gut based on the presence of different bacteria. The second parameter measured the **pathogenic bacteria index**. This parameter was calculated based on the presence of 11 different bacterial species with potentially negative (pathogenic) effects.

For easier reading, both the dysbiosis index and the pathogenic bacteria index are expressed as percentage of change, with a positive score indicating improvement and a negative score indicating worsening.

INSTITUTION

Clinical phase: Den Hoek (De Bilt, The Netherlands)
C. de Gooijer-Kant; **P. Voshol**, Medical Physiologist, PhD; **G. Roozemond**, MSc., neurotherapist; **R. Steinmann**, MD; **T. van Elst**, MD ; **L. Barnhoorn, BA**, neurotherapist

Microbiome analysis: Biovis Diagnostik MVZ GmbH (Limburg an der Lahn, Germany)

The human microbiome study (2022)

RESULTS: Improvement in the **dysbiosis index** and **pathogenic bacteria index** indicates a positive effect of Somarka/Analemma Water!

On average, participants in the Somarka/Analemma group had a **10.7% improvement** in the dysbiosis index, while participants in the Placebo group had **5.3% worsening** the dysbiosis index (**Figure 1, left panel**) amounting to an overall **16% improvement in the Somarka/Analemma group over Placebo**.

Similarly, on average, participants in the Somarka/Analemma group had an **11% improvement** in the pathogenic bacteria index, compared to a striking **35% worsening** in the Placebo group (**Figure 1, right panel**). Although this amounts to a 46% increase in the Somarka/Analemma group over Placebo, this large difference is highly contributed to by the worsening in the Placebo group. Therefore, in this case, it is more informative to look at this data separately to avoid over interpretation.

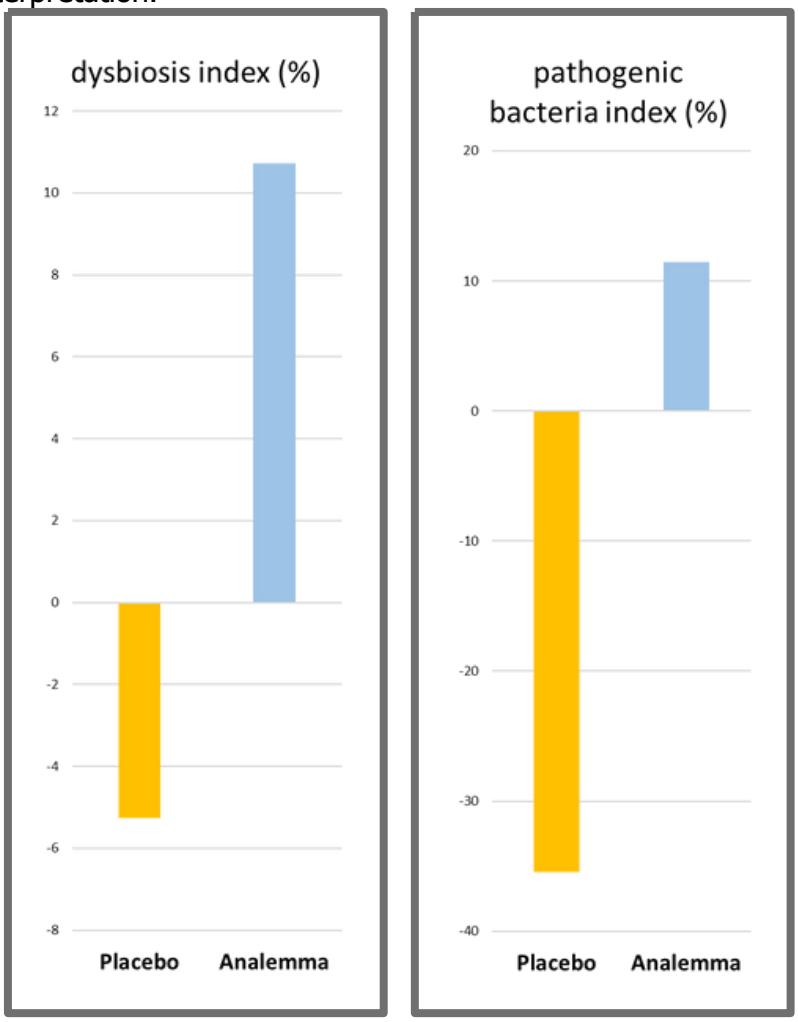


Figure 1. The changes in the dysbiosis index (left panel) and the pathogenic bacteria index (right panel) calculated by comparing the results before and after prolonged consumption of Somarka/Analemma water (blue bars) or Placebo (yellow bars).

The values are expressed as percentage of change, with positive values indicating improvement, and negative values indicating worsening. The values are based on averaged scores of 8 participants per group.

The human microbiome study (2022)

RESULTS: The greater number of participants with improved **dysbiosis index** in the Somarka/Analemma group!

The human microbiome can change in response to a variety of factors, and inter-individual differences play an important role in that process. While one person may experience a significant change in the gut microbiome in response to a certain factor, another person might not be as strongly affected by it, resulting in large differences between individuals.

The following illustrations show the number of participants who experienced **improvement**, **no change** or **worsening** in the dysbiosis index after consuming Somarka/Analemma Water vs. Placebo. This schema shows the general effect of the treatment, without focusing on the actual scores. From this viewpoint, it is evident that in the Somarka/Analemma group, in contrast to Placebo, the vast majority of participants experienced a general improvement in the dysbiosis index.

PLACEBO GROUP

In the Placebo group, **38% of participants** experienced an **improvement** in the dysbiosis index, while **the remaining 62%** experienced either a **worsening or no change at all.**

SOMARKA/AN'ALEMMA GROUP

In the Somarka/Analemma group, **the majority (88%) of participants** experienced **an improvement in the dysbiosis index**.

* average percentage of change

The human microbiome study (2022)

RESULTS: The greater number of participants with improved **pathogenic bacteria index** in the Somarka/Analemma group!

The following illustrations show the number of participants who experienced either **improvement**, **no change** or **worsening** in the pathogenic bacteria index after consuming Somarka/Analemma Water vs. Placebo.

Similar to the dysbiosis index, in the Somarka/Analemma group, the majority of participants experienced either an improvement or stability in this parameter.

PLACEBO GROUP

In the Placebo group, **25% of participants** experienced **an improvement in the pathogenic bacteria index,** while **50%** experienced **a worsening in this index**.

SOMARKA/AN'ALEMMA GROUP

In the Somarka/Analemma group, **50% of participants** experienced **an improvement in the pathogenic bacteria index**.

* average percentage of change

CONCLUSION

Taken together, the results of this preliminary double-blind placebo-controlled study indicate that Somarka/Analemma water has a positive effect on human gut health.

In only 3 months of everyday consumption of Somarka/Analemma water, participants experienced, on average, more improvement in the dysbiosis index compared to Placebo. The dysbiosis index describes the state of the gut by measuring the presence of relevant bacterial species with known beneficial roles in maintaining healthy gut function. While the scores differed between individuals, **the majority of participants in the Somarka/Analemma group experienced an improvement in the dysbiosis index**.

Moreover, in the Somarka/Analemma group, **a greater percentage of participants experienced a decline or stability in the number of pathogenic bacteria** compared to Placebo, indicating a protective effect of Somarka/Analemma water on the human gut.

These exciting early results have been tremendously important for the organization of our upcoming research study on the effects of Somarka/Analemma water on human gut health using the latest advancements in human gut microbiome analysis.

A5. RANDOMIZATION AND BLINDING

A5.1 SUBJECT RANDOMIZATION

As per the study design, (A and B) randomization schedule was generated by a Biostatistician at Raptim Research Pvt. Ltd., India by PROC PLAN procedure (such that the design being balanced over the period and sequence combination) using Statistical Analysis Software SAS® Version 9.4 (SAS Institute Inc., U.S.A.). Subjects were allocated sequential numbers starting from 01 on the day of check-in for period I and were assigned randomization sequence as per their number stated in randomization schedule. The numbers assigned to subjects were as follows:

Water-1 dosed Subjects: 2, 3, 6, 7, 9, 11, 13, 16, 18, 19, 21, 23 and 25.

Water-2 dosed Subjects: 28, 29, 31, 34, 36, 38, 40, 42, 44, 46, 48 and 49.

Placebo dosed Subjects: 1, 4, 5, 8, 10, 12, 14, 15, 17, 20, 22, 24, 26, 27, 30, 32, 33, 35, 37, 39, 41, 43, 45, 47 and 50.

A5.2 BLINDING

The present study was designed as a blinded. The study subjects, the Clinical Investigator, the study staff involved in study activities, bio-statistician, bio-analyst and the sponsor were blinded for the treatment administered to subject. Only Pharmacist, the assistant pharmacist who are responsible for dispensing of investigational products and the QA auditor monitoring the dispensing activity had access to the randomization schedule and they were not be involved in any other study related activities until completion of the analysis.
The pharmacist assigned the treatment code (which is an alphabetic code i.e. X or Y or any other notation) for the randomization notation (i.e. if A/B, T/R or any other notation) given in the Randomization schedule generated by the Bio-statistician for test or placebo treatment. The treatment code assigned for test and placebo was recorded in the 'Assignment of treatment code for blinded study design' format which was kept in a sealed envelope in the pharmacy. The photocopy of this format was provided to biostatistician after completion of clinical and after completion of ATP level analysis of the study for statistical analysis.
For code breaking procedure (in case of occurrence of any SAE or in any emergency condition citing safety concern of the subject) individual envelope containing the label with the details of subject number and the randomization sequence of the subject was prepared by the Pharmacist prior to baseline day visit. These labels were verified with the randomization schedule by the assistant pharmacist and the QA auditor. The sealed envelopes with subject number written on were kept in a secure place in Pharmacy or with Clinical Investigator.

APPENDIX B

The human microbiome study (2022)

B1. INVESTIGATORS AND ADMINISTRATIVE STRUCTURE

The study was designed and performed with the following contributions:

Screening & planning – Performed by R. Steinmann
Clinical phase – conducted by R. Steinmann, G. Roozemond, C. de Gooijer-Kant and T. van Elst
Analysis – performed by R. Steinmann, G. Roozemond and P. Voshol

B2. BASELINE ACTIVITIES

Baseline activities were comprised of: obtaining study-specific written informed consent, completing a questionnaire for collecting demographic data, handout of requirements for stool sample analysis and handout of Water Wands including instructions on how to use the device. The Wands were filled with either Somarka/ Analemma water or Placebo (tap water).

The participants were instructed on how to collect their baseline stool sample. The timeline of stool sample delivery, see **Table B1**.

B3. RANDOMIZATION AND BLINDING

20 adult participants were included in this pilot study.

They were blindly randomized to consume either Somarka/ Analemma water or Placebo in a 1:1 ratio during aperiod of 100 days. The only person who had access to the randomization process and schedule was in no other way involved in the study.

One participant dropped out of the study due to personal reasons. Furthermore, three participants finished the study, but their scores were omitted from the analysis presented here due to use of probiotics and/or antibiotics during the water intake period.

This was the distribution of the remaining 16 participants into groups:

Subjects in the Somarka/ Analemma group: 13, 20, 26, 31, 35, 43, 52, 53

Subjects in the Placebo group: 1, 7, 8, 14, 25, 34, 38, 44

B4. ACTIVITIES DURING THE WATER INTAKE PERIOD

The participants were instructed to consume a minimum of 1.5 L of water daily after treating it with the assigned Water Wand as per the instructions. The participants were discouraged from making any other changes to their lifestyle during the study period.

All participants received a short questionnaire every 3-4 weeks where they could fill in relevant lifestyle changes that had happened over this period of time (e.g. illness, vacation, etc.).

The participants were instructed to collect a stool sample at the end of the study period. For the timeline of stool sample delivery, see **Table B1**.

Table B1. Stool sample collection details.

	Aǹalemma group	Placebo group
Subject number	13, 20, 26, 31, 35, 43, 52, 53	1, 7, 8, 14, 19, 25, 34, 36, 38, 44
Microbiome analysis #1	Participants sent their stool samples to the laboratory during the first week of July 2022, with the exception of participant 52 who sent their stool sample to on August 10th 2022.	Participants sent their stool samples to the laboratory during the first week of July 2022
Microbiome analysis #2	Participants sent their stool samples to the laboratory during the week before October 15th 2022, with the exception of participant 52 who sent their stool sample to on November 22nd 2022.	Participants sent their stool samples to the laboratory during the week before October 15th 2022.

B5. MICROBIOME ANALYSIS

Two main parameters were measured as part of the study.

The dysbiosis index (DI) is an index based on a proprietary formula developed by Biovis Diagnostik GmbH, which describes the degree of deviation in the microbiome, taking into account different bacterial phyla and species and their weighting factor. It does not take into the degree the amount of inflammation. Its value can range from 0 to 30, with lower score indicating a healthier gut microbiome. For simplicity, the change in this parameter between the baseline measurement and end measurement was expressed as percentage of change, in which a positive value indicates a positive change (improvement), and a negative value indicates a negative change (worsening).

The number of potentially pathogenic bacteria is a parameter based on measuring the presence of 11 different potentially pathogenic bacterial species. The score can range from 0 to 11, where 0 indicates "no highly abundant presence of pathogenic/potentially pathogenic bacteria" and any other number indicates "the number of pathogenic bacteria are present in higher abundance than normal". For simplicity, the change in this parameter between the baseline measurement and end measurement was expressed as percentage of change and referred to as the pathogenic bacteria index, in which a positive value indicates a positive change (improvement), and a negative value indicates a negative change (worsening).

B6. REFERENCES

Clapp M, Aurora N, Herrera L et al. (2017) Gut microbiota's effect on mental health: The gut-brain axis. Clin Pract 7:987.
Flint H, Scott K, Louis P et al. (2012) The role of the gut microbiota in nutrition and health. Nat Rev Gastroenterol Hepatol 9, 577–589.
Lazar V, Ditu L, Pircalabioru GG, Gheorghe I et al. (2018) Aspects of gut microbiota and immune system interactions in Infectious diseases, immunopathology, and cancer. Front Immunol 9:1830.