Cover page with official title: Gut Microbiome and Blood Markers After Habitual Herbal Tea Consumption (SRTT) (ID, INSERT)

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1) Introduction

TeTrimTeas intends is to establish a long-term cooperative with local growers and producers who will become partners in the business, with profit share to local growers and producers. The overall aim of the company is to produce quality, science-based botanical/herbal teas to improve health and wellbeing, growing as many of the ingredients locally and organically, to reduce food-to-fork miles within the decarbonisation and sustainability agendas in Wales.

TeTrimTeas have created herbal green tea blends, improving on existing Chinese formulation, and would like to test it as 'health tea'. The recruited cohort will be randomised into one of three intervention teas (green tea control, senna herbal mix and rhubarb root herbal mix). They would like to explore if consumption of the teas for 21 days has an impact on digestion and potentially help control weight gain.

Aberystwyth University will use high resolution metabolomics to investigate the chemical composition of capillary blood samples, in particular the short chain fatty acids. They will also assess lipid composition in capillary bloods and the microbiome of stools. Diet data, stool consistency and anthropometric measurements will be collected pre and post intervention. Results will advance product development and data would be used in grant applications into the health benefits of the herbal teas.

2) Statement of Purpose

A randomised, placebo controlled parallel human clinical trial of two intervention teas (herbal green tea with rhubarb root and herbal green tea with senna) in healthy adults is proposed, to assess the potential for clinically relevant benefits in terms of weight control and gut health. Researchers are aiming to recruit a cohort (n = 55) of healthy adults (>18 years) who will be randomised into herbal green tea with rhubarb root, herbal green tea with senna) or control green tea (daily hot infusion of tea for 21 days. Subjects will be asked to take the tea at the end of the day and not to consume food afterwards.

3) Investigational Product

3.1) Description

The two investigational products (2.5g tea bags) will contain green tea, honeysuckle flower, Cassia seed, lotus leaf, five-leaf ginseng, and hawthorn fruit and finally senna leaf or rhubarb root. The green tea control will contain 0,75g of green tea (Dartmoor Estate green tea). The format will be a box containing 21 tea bags. In a cup/mug, the researchers would ask the participant to place a 2.5g tea bag and add 190ml of hot water (80-100◦C) and stir clockwise 10 consecutive times to allow for optimal infusion and then allow to brew for 3 minutes, before removal.

3.2) Quality

The teas were blended using food grade ingredients and prepared and packed in a food grade kitchen.

3.3) Dose

In a cup/mug, the researchers would ask the participant to place a 2.5g tea bag and add 190ml of hot water (80-100◦C) and stir clockwise 10 consecutive times to allow for optimal infusion and then allow to brew for 5 minutes, before removal. The researcher would ask the participants to consume a cup of tea after their last meal/snack of the day (post 6pm) daily, for 21 days. Participants will not consume anything after the tea.

4) Investigational Product Safety

The teas contain green tea, which contain low amount of caffeine. In addition, the intervention teas contain honeysuckle flower, Cassia seed, lotus leaf, five-leaf ginseng and hawthorn fruit, and senna leaf or rhubarb root. If large quantities of the herbal teas are consumed in one go, a laxative effect may occur. This product is not hazardous. This product is free of the following components and their products thereof: cereals containing gluten, crustaceans, eggs, fish, peanuts, soybeans, milk (including lactose), nuts, celery, mustard, sesame seeds, sulphur dioxide and sulphite, lupin and molluscs, in compliance with Regulation (EC) No. 1169/2011. This product does not contain added colorants. The teas are vegan, and they are produced and stored in a food grade facility.

5) Study Design

5.1) Objectives of the Study

The **primary objective** of this proposal is to test our hypothesis that rhubarb root tea consumption over 21 days in a healthy human cohort will decrease total Cholesterol, Low-Density Lipoprotein (LDL) cholesterol, Triglycerides (Trig) and non-HDL in capillary bloods (plasma) when compared with baseline, and when compared with the senna and green tea arms after 21 days.

A **secondary objective** of this proposal is to test our hypothesis that rhubarb root tea consumption over 21 days will improve or maintain High-Density Lipoprotein (HDL) cholesterol in capillary bloods (plasma) when compared with baseline, and when compared with the senna and green tea arms after 21 days.

Researchers will measure diet quality using the Prime Diet Quality Score (PDQS), a food-based diet quality index (Gicevic, Mou, Bromage, Fung, & Willett, 2021). It is hypothesized that the score will increase from baseline, indicating an improved diet after consumption of the intervention teas, particularly the Rhubarb root tea when compared with the senna and green tea.

Researchers will explore changes in short chain fatty acids concentrations in plasma measured using Gas Chromatography-Flame Ionization Detection

Researchers will measure stool consistency using the Bristol Stool Form Scale, to monitor laxative effects.

Researchers will employ 16S rRNA gene amplicon sequencing (via Illumina) to characterise the bacterial composition of the faecal samples. From this, alpha and beta indices representing community structure will be compared between the pre and post intervention samples (and between treatments) using classical statistical approaches such as permutational multivariate analysis of variance and linear regression analysis. Researchers will monitor the microbiota to define if there are any key responding microbes that correlate with health measurements (weight, BMI other biomed measurements etc) that are boosted or depleted in response to the tea.

5.2) Subject Selection

55 participants, over 18 years, mixed gender, mixed ethnicity.

5.3) Inclusion Criteria:

- Consenting adults >18 y Age
- Commit to fasting capillary blood collection.
- Commit to stool sampling collection.
- Able to refrain from taking any over-the-counter medication or herbal supplements during the diet monitoring and experimental periods.
- Able to prepare and consume the tea during the experimental days, after the last meal/snack of the day and not to consume anything afterwards.
- Fill in diet questionnaires and stool ranking.
- Able to inform the researcher if any antibiotics or heavy alcohol is consumed over the intervention period.

5.4) Exclusion Criteria

- Serious health conditions that require daily long-term medications (including immunosuppressants)
- A history or current diabetes, lung issues, gut inflammation (Crohn's, IBD), digestive disorders
- Diagnosed with a serious health condition within the last 12 months.
- Pregnant
- Play sports at a high level (more than 7h/week or 1h/day)
- Smoking
- consume high dose of alcohol > 21 unit per week for men and > 14 units per week for women.
- Food allergy /food intolerance/ eating disorder or are on a specially prescribed diet.

5.5) Covid Exclusion criteria

- Showing (or anyone within the household) any COVID-19 symptoms (see COVID-19 basic health screen) *
- Higher risk or vulnerable from coronavirus or live with someone at a higher risk of a severe illness from COVID-19 (over 70, undergoing cancer treatment, high risk of getting infections).
- Had a letter from the NHS advising you to shield (isolate).
- Had been at risk of exposure to COVID-19 such as travel, contact with someone with COVID-19, been exposed to the virus, or has been asked to self-isolate by the track and trace system.
- Serious health conditions that require daily long-term medication.

*If the potential participant has had COVID-19 previously (and are fully recovered and not within isolation) then they are eligible to join the study.

5.6) Study Design

After pre-induction over the phone, if the participant is eligible and still interested, the researcher will firstly run through an induction session. This can be done by phone or Teams (whatever suits the

participant best). The study is randomised so the participant will be allocated into one of three groups. This will be double blinded so neither the participant nor the researcher will know what group the participant have been allocated into. Information can be disclosed at the end of the study if the participant desires. The participant will need to come to WARU or Trimsaran Community Health Centre for a capillary blood sample collected from the finger using a lancet. The participant will need to collect a stool sample using kits and toilet facilities provided.

Induction

The researcher will firstly run though how the researcher are working safely during coronavirus (COVID-19). Then the researcher will introduce the participant to all the study materials the participant will experience during the experimental session.

Materials will include:

- Tea bags for the 21 days
- Stool collection kit and Bristol stool scale
- Blood collection kit
- Prime Diet Quality Score (PDQS) questionnaire

The researcher will run through the logistics of WARU or Trimsaran Community Health Centre visits and organise dates.

The experimental sequence:

'Monitoring' period (two days before the study)

The researcher would ask the participant to refrain from taking any over-the-counter medication (such as ibuprofen, paracetamol, aspirin, cough/ cold remedies) or herbal supplements, and to let the researchers know if the participant find that it is necessary to take any such medication during the trial.

Experimental session- 21 days

Morning: The participant will come to WARU or Trimsaran Community Health Centre at the pre-organised timeslot to allow the researchers to collect a fasted capillary (fingerpick) blood samples and stool sample (which may be brought in with the participant). The participant may consume their breakfast after the visit. The participant will also collect the allocated tea bags. The researcher will also take the participants the weight and height. The researcher will ask the participant to record their stool consistency using the Bristol stool scale and record their diet using the PDQS questionnaire.

Over 21 days: the researchers would like the participant to consume a cup of hot-infused tea after their last meal/snack of the day (post 6pm) daily, for 21 days. The researcher would ask the participant not to consume anything after the tea. In a cup/mug the researcher would ask the participant to place a 2.5g tea bag and add 190ml of hot water (80-100◦C) and stir clockwise 10 consecutive times to allow for optimal infusion and then allow to brew for 5 minutes, before removal.

Whilst undergoing the experimental sessions, if necessary, the researcher will be easily contacted by email, Teams or phone.

After the experimental period:

Morning of the final visit. Please come to WARU or Trimsaran Community Health Centre at the pre-organised timeslot to allow the researchers to collect a fasted capillary (fingerpick) blood samples and stool sample (this may be brought in with the participant). The researcher will also take the he participants the weight and height. The researcher will ask the participant to record their stool consistency using the Bristol stool scale and record their diet using the PDQS questionnaire. The participant may consume their breakfast after their visit. Afterwards, the participant can go back to their 'normal' eating pattern.

Post study
The researcher would like the participant to fill in an additional PDQS questionnaire at least 21 days after the experimental period and provide their weight.

6) Participant Risks

For the capillary blood samples, some people may feel nervous and on rare occasions dizziness may occur. Alongside green tea, the intervention teas may contain honeysuckle flower, Cassia seed, lotus leaf, five-leaf ginseng, hawthorn fruit, senna leaf and rhubarb root. The herbal teas contain low amount of caffeine and, if large quantities of the herbal teas are consumed in one go, a laxative effect may occur. The tea ingredients have already been tested for any adverse effects in a human cohort, however if any negative effects occur, participants are asked to refrain from continuing in the study.

7) Benefits to participant

There is no financial gain for participants if they decide to join this study. They will allow the researchers to gain important insight into the action of health teas using a combination of techniques including metabolomics, gut microbiome analysis and lipid analysis. This will be the first time this type of research will have been conducted and will be a valuable pilot study before they can investigate any human health benefits in the future. Researchers will provide participants with tea for the experimental days.

8) Privacy/confidentiality

Participants are informed that only the researchers involved with the study will be able to look at the information they provide. Specific details and personal identifiers will only be available to the researchers. At the end of the study, any information relating to participants will be made pseudonymous (coded without their name associated). Participants will not be identifiable in any publication that may arise from this research. Electronic files will be kept in a logical manner and will always be kept grouped within specific folders and password protected. All data storage is using the AU network and backed up. Once the raw data has been extracted from a paper version onto a computer, the paper will be destroyed via a paper shredder or in confidential waste bags to ensure the participant's confidentiality. There may be times when keeping paper forms are necessary (consent forms), but in this case the paper versions will be kept in a locked filing cabinet. All files that are stored on the WARU share drive will always be protected with a secure password. Setting passwords will automatically encrypt the document and will not allow any unauthorised access to the data. The Gatekeeper of the passwords delivers the passwords by encrypted emails. When sharing

data, the researchers will be using the WARU shared drive, which will be kept organised, and all documents will be in relevant folders and password protected if containing confidential information. The only people that will have access to the share drive are WARU staff, directly involved with the project. When sharing data between researchers, this will be done by uploading the document to the WARU share drive. Documents will not be sent directly via email. All biofluids are stored in a locked freezer. Key is kept by the gatekeeper.

9) Safety Monitoring

9.1) Participant:

If a participant, or a member of their family/household become unwell during the study, then they are pre-warned to alert a member of the research team immediately using the contact information they have been provided. Participation in the study will be suspended immediately until further discussion with the research team has taken place. If they become unwell at any point and need medical assistance, they are advised to contact 111 and seek advice from the NHS health sector or their doctor's surgery. The researchers have a duty of care towards them and can help monitor their health remotely over 14 days and will help in any way they can.

9.2) Data:

Each computer that belongs to the WARU team is programmed with the university network. Therefore, to gain access to the computer each member of staff will have a unique password to gain access to their own desktop account. Staff members are always expected to lock their computers when not attended. The WARU email account is only accessed by authorised individuals. The Gatekeeper regularly changes the password to enable access. Email addresses of study participants will be recorded, and study specific emails will be diverted to a secure named folder, only accessed by the immediate WARU Team. Each Aberystwyth University computer is scheduled to perform daily updates for anti-virus and firewall protection, this aims at keeping the university network, bug, and virus free. In the event of a power surge the university's network is backed up every 15 minutes therefore content is regularly saved automatically on the universities back up drive. Confidential data will not be stored on mobile devices (phones, laptops, tablets). If for any reason confidential data needs to be stored on a mobile device then permission must be gained from the line manager first, and if granted, all documents will be encrypted. For participant confidentiality, all personal data such as names, addresses, DOB and phone numbers, will be stored and password protected and there will be a gatekeeper of the password. Personal details will be removed or replaced with an ID code when data is being analysed. Only the gatekeeper can link names to codes. Sensitive materials (participant's details e.g., address) will be electronically stored away and access will be restricted. This data will be password protected and there will be a gatekeeper of the password. ID codes are completely randomised. The researchers will select 4 digits to create random codes which can then be assigned to participants, alongside the study start code. All rooms where data is stored will only be accessible via a card key. This will prevent those who are not authorised gaining access into a room where sensitive data (e.g., consent forms) may be. In exceptional circumstances, confidentiality may have to be breached in cases where persons are considered to be at risk or if required by law.

9.3) Data analysis and statistics

Data analysis will be conducted at Aberystwyth University. Chemical composition using metabolomics will be conducted at AberInnovation and Aberystwyth University, and the quantification of short chain

fatty acids as well as clinical biochemistry (Chem21 to include glucose and lipid panel tests) will be analysed at Bronglais hospital.