

Studies on Chemical Compositions, Health Benefits and Bioactivities of Selected Sri Lankan Herbal Porridges

*Research proposal
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1. Title of the proposed project:

Study the chemical compositions, health benefits and bioactivities of selected Sri Lankan herbal porridges.

2. Research background:

Rice is the staple food of the inhabitants of Sri Lanka. Rice in Sri Lanka plays an important, major role in the country's functioning and survival for centuries. Because of its nutritional values, rice consumption is becoming popular around the world with almost 95% of the production reported in Asia.¹ Rice is mainly consumed as cooked intact grains but a small amount of it is used as ingredient for processed foods and as feed. Green leafy porridges made with leaf water extracts, rice and coconut milk are common Sri Lankan dietary remedies. Being a tropical country, green leafy vegetables and fruits with high fibre are abundant. Green leafy vegetables are utilized in many ways in Sri Lankan diets such as salads, curries and porridges to supplement the cereal-based traditional diets. Green leafy vegetables are also a source of minerals (iron, calcium, potassium and magnesium), vitamins (K, C, E and many of the B vitamins) and phytonutrients (beta-carotene, lutein zeaxanthin and phytosterols).² The porridge made of green leaves consume as breakfast as a practice by most Sri Lankans since ancient times. These meals relied to be helpful to have a healthy life style.

According to the studies dietary fibre, tocopherols, tocotrienols, oryzanol, flavonoids and other phenolic compounds are the major constituents found in rice which are responsible for the management of diabetes, cancers, high blood pressure and coronary heart diseases.^{3,4,5} And also some of green leafy extracts are used in folk medicine to treat diabetes, cancers and etc.⁶ By preparing rice-based porridges and using the knowledge of indigenous medicine, health benefits of these porridges such as antioxidant activity, anticancer activity can be studied. Although individual rice brans have tested for cytotoxic effect, the cooked rice has not tested. Hence, the objectives of the project is to study the chemical compositions, health benefits and bioactivities of herbal porridges which are based on selected Sri Lankan traditional rice varieties.

3. Specific objectives:

- Survey to find the demand for herbal porridges from cancer patients, traditional farmers and mothers' of school children from selected primary schools
- To determine the proximate composition and minerals of selected indigenous rice varieties based herbal porridges
- To determine antioxidant activity, antiglycation activity and glycemic index of prepared porridges
- To determine the anticancer activity in human cancer cell lines of selected Sri Lankan rice varieties based herbal porridges
- To determine the antimicrobial activity of selected Sri Lankan rice varieties based herbal porridges

4. Literature review:

Since ancient times, rice is the highest priority crop of Sri Lanka. The rice grain consists of 75-80 % carbohydrates as starch and other nutrients such as protein with a full complement of amino acids, fats, fiber and antioxidants such as α -tocopherol, γ -tocopherol and γ -oryzanol.⁷ According to previous studies phenolic compounds found in rice which are responsible for the management of diabetes, cancers, high blood pressure and coronary heart diseases.⁴ Minerals like calcium, magnesium and phosphorus are present in rice along with some traces of iron, copper, zinc and manganese.⁷ Crude protein content of the selected rice varieties (Kalu heenati (KH), Pokkali (PK), Gurusinghe wee (GW), Kahawanu (KW), Sudu murunga (SM) and Unakola samba (US) were in the range of 9.7 ± 0.3 % - 11.0 ± 0.4 %.⁸ This indicates that above selected rice varieties contain higher amount of proteins compared to that of improved varieties.⁸

Furthermore, rice-based porridges are consumed by the Sri Lankan people since ancient times. Porridge is easy to digest, can be obtained with low cost and consume as a whole food, so it is used traditionally in many cultures as a food for the sick, and often is eaten to have a healthy life. According to the previous studies the proximate compositions and glycaemic index (GI) of some commonly consumed green leafy porridges have been evaluated.² Green leafy porridges of *Murraya koenigii spreng* (Karapincha), *Asparagus racemosus* (Haathawariya),

Hemidesmus indicus (Iramusu), *Aegle marmelos* (Beli), *Cassia auriculata* Linn (Ranawara), *Cardiospermum halicacabum* (Wel Penela), *Aerva lanata* (Polpala), *Clitoria ternatea* Linn. (Ela katarolu), *Scoparia dulcis* (Wal koththamalli), *Atlantia zeylanica* Linn. (Yaki narang), *Osbeckia octandra* (Heen bovitiya) and *Cephalandra indica* (Kowakka) show low GI and have the ability to reduce peak blood glucose.² Porridge made with *Scoparia dulcis* (Wal koththamalli) leaf extract reduces the weight loss in diabetes, hyperglycaemia and hyperlipidaemia and the rate of pancreatic cell damage due to STZ in Wistar rats.⁹ And also literature shows that green leafy porridges have very high antioxidant activity.¹⁰ Findings of the study of Abeysekara 2015 indicate that the Sri Lankan traditional rice bran or its fractions have great promise as supplements/nutraceuticals for management of cancer.¹¹ The results demonstrate that the bran of individual Sri Lankan traditional red rice varieties (Sudu Heeneti, Goda Heeneti, Masuran and Dik Wee) possess growth inhibition and cytotoxicity against human lung cancer and cervical cancer cell lines and GST inhibitory activity *in vitro*.¹¹ Millet porridge and drink possess high antimicrobial activity.¹² Due to their health properties cereal/legume drink and porridge mixtures were developed using locally available raw materials.¹³

Also porridge made out of these rice varieties are commonly used by school children and cancer patients. As a result, there is an increasing interesting studies of health benefits after preparing of porridges. There is no any results reported indicating their effects after preparing porridges with plants.

5. Methodology:

Traditional rice varieties and green herbal leaves will be selected according to the literature survey and the information gathered from cancer patients, farmers and ayurvedhic doctors. The selected traditional rice varieties will be obtained from Rice Research and Development Institute, Bathalegoda and traditional farmer community respectively.

According to the known procedure rice based leaf porridges will be prepared.² Coconut milk will be obtained from 150 g of coconut kernel blended with 400ml of water will be used in porridge preparation. Tender herbal leaves (about 40 g) will be blended with 150ml of coconut milk and 100ml of water. The slurry will be filtered (1 mm), and the filtrate will be taken for porridge preparation. Rice (25 g) will be cooked with 250ml of water. The mentioned leaf

extract (90ml) and water (60ml) were added to cooked rice with salt to taste. All porridges will be cooked until the final volume was approximately 300–400ml.

Proximate composition will be analyzed according to the AOAC methods (2000). Micro nutrients will be analyzed using Atomic absorption Spectrophotometry.¹⁴ FRAP, ABTS assays will be used for determination of antioxidants.¹ The total phenolic content of porridge extracts will be determined using the Folin-Ciocaltea reagent method.

SRB (Sulforhodamine B) assay will be conducted to porridge crudes against human breast, lung, cervical cancer cell lines.¹¹ For this experiment, cancer cell lines were used to perform the SRB assay. Cancer cells were plated on 96 well plates (5000 cells/well) with 200 µl of growth medium. Cells were exposed to test compounds (concentrations ranging from 25 to 400 µg/mL) for 24, 48 and 72 hours. Then the cytotoxicity was assessed by Sulforhodamine B assay. The cell supernatant was completely removed and washed with PBS. TCA (50%, 25 µl) was added on top of FBS free fresh medium (200 µl) to make final concentration of 10% TCA, and was incubated at 4 °C for one hour former to the SRB assay. The plate was then washed with five washing cycles using tap water and dried completely. 0.4% SRB dissolved in 1% TCA, 100 µl was added to each well and allowed to stain for 15 minutes. The plate was again washed with five washing cycles to remove unbound dye using 1% (vol/vol) acetic acid after removing the stain. The protein bound dye was solubilized with tris base (10 mM, pH 7.5, 200 µl), after air drying. The plates were then shaken for 60 minutes to homogenize the dye solution. The absorbance was then measured at 540 nm using Synergy HTBioTek micro plate reader. The percentage viability (IC50) was calculated as given below (Eq. 1)

$$\text{Viable cell (\%)} = \left(\frac{\text{Absorbance of treat cells}}{\text{Absorbance of untreated cells}} \right) \times 100 \dots \dots \dots \text{(Eq. 1)}$$

The antimicrobial activities of extracts will be determined by well diffusion assay and viable colony count technique. The test microorganisms will be used in this study include pathogenic bacteria that produce different food borne diseases.

Ex:-Salmonella (can contract salmonellosis by consuming raw and undercooked eggs, undercooked poultry and meat, contaminated raw fruits and vegetables such as sprouts and melons, as well as unpasteurized milk and other dairy products)

Clostridium perfringens (illness usually occurs by eating foods contaminated with large numbers of this bacteria that produce enough toxin to cause sickness in the form of abdominal cramping and diarrhea. It grows fastest in large portions of food, such as casseroles, stews and gravies that have been sitting at room temperature in the danger zone) *Campylobacter* (Sources include consuming raw and undercooked poultry and other meats, unpasteurized dairy products and untreated water or contaminated produce)

Staphylococcus aureus (the bacteria can be found in unpasteurized dairy products and salty foods such as ham and other sliced meats. Foods that are made or come in contact with hands and require no additional cooking are at highest risk, including: Salads, such as ham, egg, tuna, chicken, potato and macaroni, Bakery products, such as cream-filled pastries, cream pies, Sandwiches)

Escherichia coli (these include eating raw or undercooked ground beef or drinking unpasteurized beverages or dairy products)

Bacterial suspensions will be prepared by transferring several colonies of microorganisms to sterile normal saline (5 mL). The suspensions will be mixed for 15 s and subsequently diluted to match the turbidity of at 0.5 McFarland standard. Bacterial suspensions will be used to inoculate Muller Hinton agar plates to obtain a confluent growth. Wells will be cut in the agar surface with the help of a cork borer. A volume of 200 μ L from each of the porridge extracts of 1000 μ g/mL and 2000 μ g/mL concentrations will be separately loaded into the wells. At the same time, 200 μ L of Gentamycin (50 μ g/mL, Vancomycin (50 μ g/mL) and Cefoxitin (30 μ g/mL) will be used as positive controls whereas DMSO will be used as the negative control. All the plates will be incubated at 37 °C for overnight to allow bacterial growth. Any zone of inhibition around the extracts containing wells was considered as sensitive and it will be measured (Diameter of the inhibition zone – Diameter of the well) in millimeters. Tests will be performed in triplicate.

Bactericidal activities of the extracts will be determined by viable colony counts. Ten-fold dilution series (concentration ranges from 0.2 - 2000 μ g/mL) from each of the porridge extracts will be made. A volume of 100 μ L of bacterial suspensions (1×10^8 bacteria/mL) will be added to 900 μ L of each of the porridge extracts (1 in 5 dilutions) and incubated for 60 min at 37 °C. Control consisted of bacterial suspension incubated with sterile normal saline. At the end of 60 min, 100 μ L of each of the diluted extracts will be inoculated onto Blood agar plates and

incubated at 37 °C for overnight. Three independent trials will be conducted for each concentration. Concentration of an extract that killed 100% of bacterial cells was considered as the Minimum Bactericidal Concentration (MBC) of that porridge extract.

6. Time Scale bar chart for study:

Activity	2017						2018					
	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Literature review and survey to identify the suitable rice varieties and herbal leaves	■	■	■									
Identify the suitable recipes to prepare porridges				■								
Proximate analysis of porridges					■	■						
Analysis of antioxidants Antiglycation activity							■	■	■			
Analysis of phytochemicals							■	■	■			
Antibacterial activity of porridges										■	■	■

Anticancer activity of porridges																		
	2018							2019										
Anticancer activity of porridges																		
Thesis writing																		

7. Expected outcomes:

Porridge is common Sri Lankan breakfast which is made of green leaves and traditional rice varieties. These porridges are given to cancer patients and school children to improve their health states. Additionally, these porridges are consumed by pregnant mothers to have healthy babies. However, traditional herbal porridges have been consuming by Sri Lankans from ages, but there is no any scientific report other than the indigenous medicinal knowledge. The purpose of this study is to explore the health benefits of these porridges such as nutritional values, anticancer activity and antioxidant activity. After determining the nutritional and medicinal values of these porridges, we can get the recognition for our Sri Lankan meals.

8. Source of funding:

University of Sri Jayewardenepura Funding: Grant

9. Principal investigator/supervisor:

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10. Other supervisors:

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