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Phytochemical characteristics and antibacterial activity of selected Sri Lankan herbal porridges

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Abstract

Porridges based on rice varieties are common Sri Lankan dietary remedies since early days. This study was conducted to evaluate the biological activity of herbal porridges based on selected Sri Lankan traditional rice varieties. In this study, four rice porridges of Madathawalu (MWP), Kalu Heenati (KHP), Mixed porridge with Sudu heenati, Goda heenati, Masuran, Dik wee (1:1:1:1) (MXP) and Special traditional porridge (STP) were prepared. Methanolic extracts of these four porridges were subjected to phytochemical analysis and determination of antibacterial activity. Some phytochemicals including phenols, flavonoids, alkaloids, steroids, terpenoids, saponins, glycosides, anthocyanins and xanthoproteins were identified in all four porridge extracts. The porridge extracts showed antimicrobial activity against food borne pathogens (both gram positive *Staphylococcus aureus* and gram negative *Pseudomonas aeruginosa* and *Escherichia coli*), which are the most commonly identified causes of bacterial skin and soft tissue infections. In conclusion, the Sri Lankan traditional rice based porridges are rich in phytochemicals and they possess antibacterial activity.

Keywords: antimicrobial activity, phytochemicals, porridge, Sri Lankan traditional rice

1. Introduction

The porridge made of rice varieties consume as breakfast as a practice by most Sri Lankans since ancient times. These meals offered healthy life and believed to be good remedy for non-communicable diseases. Furthermore, a rice porridge is easy to prepare and digest, can be obtained with low cost and consume as a whole food by any age group. Thus, porridges consume by many ethnic groups in Sri Lanka as an ideal food for the sick because nutritional properties of the porridge can be altered by changing the rice variety. Various rice varieties are used to prepare rice-based porridges. Among them, Sri Lankan traditional rice varieties play a vital role due to their high bioactivities^[1]. Millet porridge and drink possess high antimicrobial activity^[2]. Due to their health properties cereal/legume drink and porridge mixtures were developed using locally available raw materials^[3]. Previous studies have shown that methanolic extracts of some Sri Lankan traditional rice varieties exhibited potential antibacterial activity against *Staphylococcus aureus*^[4].

The compounds extracted from plants have yielded invaluable drugs against many diseases. Natural evolution has contributed to tremendous diversity in natural products which has always been an inspiring source for scientists to discover novel compounds beneficial to mankind. Nowadays these traditional rice varieties based porridge packets have been re-emerged in the Sri Lankan market due to the awareness of the nutritional and medicinal properties of traditional rice varieties, of which the consumption is said to be a remedy for non-communicable diseases^[5]. These porridges are recommended by indigenous doctors to cure several non-communicable diseases. According to the previous studies, Sri Lankan traditional rice varieties possess high nutritional value^[6,7].

Sri Lankan traditional rice based porridges are said to be, less studied treasure trove of bioactive compounds with potential medicinal benefits. Although individual rice brans have tested for varies bioactivities, the rice porridges have not or less focused been tested yet. The main objective of the research project was to analyze the phytochemicals and antibacterial activity of herbal porridges based on selected Sri Lankan traditional rice varieties.

2. Materials and Methods**2.1 Selection of herbal porridges**

A total of four Sri Lankan traditional rice based porridges were analyzed for their phytochemicals and antimicrobial activity. Madathawalu rice porridge (MWP), Kalu Heenati

rice porridge (KHP), Mixed rice porridge with Sudu Heenati, Goda Heenati, masuran, Dik Wee (1:1:1:1) (MXP) and Special traditional porridge (STP) porridge recipes were selected based on the recommendations of indigenous doctors for this study.

2.2 Preparation of herbal porridges

The Madathawalu rice porridge was made of Madathawalu rice (100 g). The Kalu Heenati rice porridge was made of Kalu Heenati rice (100 g). For mixed rice porridge processing 25 g of Sudu Heenati, 25 g of Goda Heenati, 25 g of Masuran, 25 g of Dik Wee rice were weighed and used. 100 g of Madathawalu rice, the spices of coriander, cloves, cardamom, cinnamon, fenugreek, ginger (1 teaspoon of each were placed in a separate cloth pocket), onion, pepper, tamarind, pandan leaves and lemongrass (1 teaspoon of each) were used to prepare special traditional porridge. For the general procedure of preparing porridges, an amount of 100 g of rice was weighed, washed five times and soaked in water for 12 hours. The wet rice was poured in to a 2.5 L pot. Then 1.5 L of water, 10g of chopped garlic and 5g of salt were added to the pot. Porridges were cooked about 1 hour at medium high heat with stirring using a wooden spoon to avoid formation of lumps until the final volume was approximately 400ml. Porridge was then blended using a blender. Prepared four porridges were lyophilized in a freeze drier until free of moisture. After the porridge freeze dried, they were stored at -20 °C until analysis for less than 1 month. All the lyophilized porridges were ground and sieved using a 500 µm sieve to obtain a consistent fine residue.

2.3 Preparation of the porridge extracts for bio assays

Ten grams of dehydrated porridge powder of each of the porridges were extracted with 10 times the sample weight of 70% methanol/water (v/v) for 24 h at room temperature (28±2 °C). Then extracts were filtered through Whatman 52 filter paper to obtain filtrate. Filtered extracts were evaporated under reduced pressure in a rotary evaporator to remove methanol. Remaining water was removed by freeze drying. The extracts were then used to prepare known concentrations of porridge extracts and the solutions were passed through 0.2 µm eruginos filters. Filtered extracts were used for the bioassays.

2.4 Phytochemical Analysis

The prepared porridge extracts were used for the phytochemical analysis (phenols, flavonoids, alkaloids, steroids, terpenoids, quinones, tannins, saponins, glycosides, anthocyanins and xanthoproteins).

2.4.1 Test for phenols

Few drops of 1% FeCl₃ was added to the porridge extract. The solution turned into purple color and it indicated the presence of phenols [8].

2.4.2 Test for flavonoids

One milliliter of 10% NaOH was added to the 1ml of porridge extract to get intense yellow color [9].

2.4.3 Test for alkaloids

One milliliter of 1% HCl was added to the 3ml of porridge extract. The solution was heated gently for 20 min. Then it was cooled and filtered. Few drops of Mayer's reagent was added to the filtrate. Turbidity or creamy precipitate indicated the presence of alkaloids [10].

2.4.4 Test for steroids

Steroids were identified according to the Salkowski method. Concentrated H₂SO₄ acid and chloroform were added the porridge extract. The solution turned into red color, indicating the presence of steroids [11].

2.4.5 Test for terpenoids

About 0.8 g of porridge extract was taken and 10 ml methanol was added, shaken and filtered. Then 2 ml of chloroform and 3 ml H₂SO₄ were added to this filtrate. Reddish brown color indicated the presence of terpenoids [11].

2.4.6 Test for quinones: Concentrated HCl acid was added to a small amount of porridge extract. Yellow precipitate indicated the presence of quinones [11].

2.4.7 Test for tannins: Few drops of Ferric chloride solution and 1 ml of water were added to 0.5 ml of porridge extract. Blue or green black color indicated the presence of tannins [12].

2.4.8 Test for saponins: About 2 ml of porridge extract was taken in a test tube and shaken vigorously. The froth formation indicated the presence of saponins [8].

2.4.9 Test for glycosides: Few drops of glacial acetic acid and few drops of ferric chloride were added to the porridge extract. Concentrated H₂SO₄ acid was then added by the side of the tube. Reddish brown color was observed at the interface of the two layers and bluish green color appeared in the upper layer which indicated the presence of glycosides [13].

2.4.10 Test for anthocyanins

About 2 ml of 2N HCl was added to 2ml of porridge extract. The solution was turned in to pink red color. Next, NH₄OH was added and solution turned in to purple – blue color, indicating the presence of anthocyanins [11].

2.4.11 Test for xanthoproteins

Few drops of concentrated HNO₃ acid and NH₃ solution were added to 1 ml of porridge extract. Formation of reddish orange precipitate indicated the presence of xanthoproteins [11].

2.5 Determination of antibacterial activity

2.5.1 Preparation of bacterial cultures, media and standards

The antimicrobial activities of extracts were determined by well diffusion assay [14]. The test microorganisms used in this study include *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). Bacterial strains were obtained from the laboratory collection of the Medical Research Institute, Sri Lanka. The media used for well diffusion assay was Muller Hinton agar (Mast, UK). Nutrient agar (Mast, UK) was used as the media to culture bacterial strains. Gentamycin (Neon Laboratories, India) was used as a positive control against *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). Muller Hinton agar (38 g) was suspended in one liter of distilled water. The mixture was stirred well and autoclaved at 121 °C and 15 lb pressure for 20 minutes. Autoclaved Muller Hinton agar (20 mL) was poured into sterilized petri dishes

(Fisher, USA) under sterile conditions in a laminar air flow and kept undisturbed for solidification.

The bacterial strains were sub-cultured in fresh nutrient agar plates and incubated at 37 °C for 24 h prior to any antimicrobial test. Bacterial suspensions were prepared by transferring several colonies of microorganisms to sterile normal saline (5 mL). The suspensions were mixed for 15 s and subsequently diluted to match the turbidity of at 0.5 McFarland standard.

McFarland standards are suspensions of barium sulphate that allows visual comparison of bacterial density. This was prepared by adding 0.5 mL of 1.175% (v/v) BaCl₂·2H₂O to 99.5 mL 1% (w/v) H₂SO₄. This solution was mixed well and the turbidity was verified by measuring the absorbance of the solution at 625 nm using a spectrophotometer. Absorbance of the 0.5 McFarland standard should be 0.08 – 0.1 at this wavelength.

2.5.2 Well diffusion assay

Bacterial suspensions were used to inoculate Muller Hinton agar plates to obtain a confluent growth. Wells were cut in the agar surface with the help of a cork borer. The diameter of a well was 8 mm and had a height of 4 mm. A volume of 200 µL from each of the porridge extracts of 10 mg/mL, 25 mg/mL, 50 mg/mL and 100 mg/mL concentrations were separately loaded into the wells.

At the same time, 200 µL of Gentamycin (50 µg/mL) was used as positive control whereas water was used as the negative control. All the plates were incubated at 37 °C for overnight to allow bacterial growth. Any zone of inhibition around the extracts containing wells was considered as sensitive (i.e. inhibition activity against the bacterial growth) and it was measured (Diameter of the inhibition zone – Diameter of the well) in millimeters. Tests were performed in triplicate.

3. Result and Discussion

3.1 Phytochemical Analysis

From the phytochemical prospecting, phenols, flavonoids, alkaloids, steroids, terpenoids, saponins, glycosides, anthocyanins and xanthoproteins were identified in methanolic extracts of all four porridges. Quinones were not detected in MWP, KHP and STP whereas tannins were not detected in all four porridge extracts. (Table 1).

Table 1: Phytochemical analysis of porridge extracts

Phytochemicals	MWP	KHP	MXP	STP
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Quinones	-	-	+	-
Tannins	-	-	-	-
Saponins	+	+	+	+
Glycosides	+	+	+	+
Anthocyanins	+	+	+	+
xanthoproteins	+	+	+	+

MWP: Madathawalu Porridge; KHP: Kaluheenati Porridge; MXP: Mixed rice Porridge; STP: Special traditional Porridge; +: phytochemicals present; -: phytochemicals absent.

3.2 Antimicrobial activity of rice based porridge extracts

In vitro antibacterial assays were carried out to investigate the bio activity of four porridges. The figure 1 illustrates the images of inhibition zones of porridge extracts, positive control and negative control. The measured mean value of diameter of selected inhibition zones for all porridge extracts and compounds are shown in Table 2.

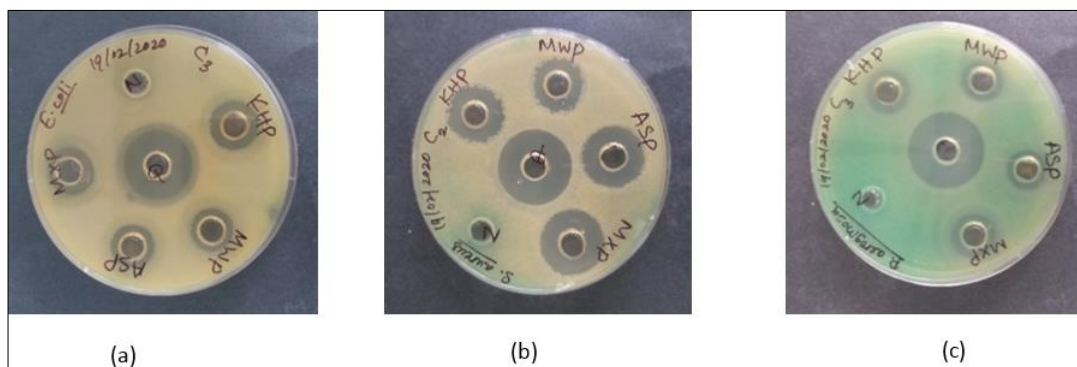


Fig 1: Images of inhibition zones for MWP, KHP, MXP and STP, where P- positive control (Gentamycin), N- negative control (distilled water). (a) Inhibition zones of *Escherichia coli* (ATCC 25922), (b) Inhibition zones of *Staphylococcus aureus* (ATCC 25923) and (c) Inhibition zones of *Pseudomonas aeruginosa* (ATCC 27853).

This study evaluated the *in vitro* anti-bacterial activity of four porridge extracts which were extracted using 70% methanol against food born pathogenic bacteria because food borne diseases are of major concern worldwide. According to the data obtained for the microbial assay, all four porridge extracts showed anti-microbial activity for the higher concentration.

E. coli (*Escherichia coli*), is a gram negative, rod shaped bacteria that lives in human intestines and in the gut of some animals [15]. Even though some *Escherichia coli* strains are harmless and are part of the normal microbiota of the gut

which can provide vitamin K2 for their host, some strains of *Escherichia coli* are associated with food poisoning and pneumonia and thereby cause diarrhea if the food or water are contaminated. It can be infected by ground meat, vegetables and fruit, untreated milk, unpasteurized fruit juices, yogurt and cheese etc [15]. Moreover, *Escherichia coli* is responsible for 75% to 95% of urinary tract infections [16]. Shiga toxin-producing *E. coli* who produce a toxin called Shiga damages the lining of human intestine [16]. In fact, some varieties lead to cause life-threatening symptoms, for instance, adult kidney failure, fever, bleeding and seizures [15].

Table 2: Mean diameter of inhibition zones for porridge extracts and compounds

Porridge extract/compound	Mean diameter of the inhibition zone (mm)		
	ATCC 25922	ATCC 25923	ATCC 27853
MWP (50 mg/ml)	15.1	20.9	15.7
KHP (50 mg/ml)	17.2	17.8	14.8
MXP (50 mg/ml)	9.1	17.1	13.9
STP (50 mg/ml)	10.0	18.8	12.7
Positive (500 µg/ml)	28.1	25.3	24.3
Negative (Distilled water)	-	-	-

MWP: Madathawalu Porridge; KHP: Kaluheenati Porridge; MXP: Mixed rice Porridge; STP: Special traditional Porridge

According to the data obtained for the anti-microbial assay, KHP porridge extract (50 mg/ml) has shown the widest inhibition zone (17.2 mm) while MXP exhibited the least inhibition activity (diameter of 9.1 mm inhibition zone) among the tested four porridges against *Escherichia coli* (ATCC 25922) bacterial species. The positive control gentamycin showed the 28.1 mm diameter of the inhibition zone.

Staphylococcus aureus is a gram positive, round shaped facultative anaerobic bacterium. *S. aureus* is also responsible for food poisoning by producing toxins. *Staphylococcus aureus* is one of the major causes for gastroenteritis due to the consumption of contaminated food [17]. The most common staphylococcal infections are skin infections, bloodstream infections, endocarditis, osteomyelitis and lung infection [18]. MWP porridge extract (50 mg/ml) has shown the largest inhibition zone (diameter of the zone is 20.9 mm) for *Staphylococcus aureus* (ATCC 25923) species. The least inhibition activity was reported for MXP which exhibited the inhibition zone of 17.1 mm diameter whereas the positive control gentamycin had the comparatively higher inhibition activity (diameter of the zone was 25.3 mm). Previous studies have shown that methanolic extract of Rathdal Sri Lankan traditional rice variety (200 µg/mL, minimum incubation time; 30 min) exhibited potential antibacterial activity against *Staphylococcus aureus* [4]. According to the results, Rathdal possesses higher anti-bacterial activity than tested four porridge extracts against *Staphylococcus aureus* because MWP, KHP, MXP and STP showed anti-bacterial activity at higher concentrations (50 mg/ml) than that of Rathdal (200 µg/mL).

Pseudomonas aeruginosa is a gram negative, rod-shaped, asporogenous bacillus bacterium which is classified as an opportunistic pathogen which can cause infections in the blood and lungs. *Pseudomonas aeruginosa* can be spread to human when exposed to water or soil that is contaminated with these germs [19]. For *Pseudomonas aeruginosa* (ATCC 27853) species, the most active porridge extract was MWP showing the 15.7 mm of the inhibition zone diameter while the lowest activity was found in STP extract (12.7 mm diameter of the inhibition zone). The positive control gentamycin (500 µg/ml) demonstrated high inhibition activity against ATCC 27853 showing 24.3 mm diameter of the inhibition zone. In relation to the anti-bacterial activity of Sri Lankan traditional rice varieties, only the extract of Kalu Heenati has shown slight activity (diameter of inhibition zone ; 3.3±0.6, concentration ; 2000 µg/mL) [4]. Research findings have demonstrated that garlic and garlic extracts possess growth inhibition of *Pseudomonas aeruginosa*. More specifically, garlic extracts (50 mg/ml) caused zone of growth inhibition that is equivalent to the disk of gentamycin [20]. Moreover, the obtained anti-bacterial activity against *Pseudomonas aeruginosa* can be further explained as the effect of the used ingredient of garlic. Therefore, by analyzing

these results and considering literature, it implies that garlic which was used as an ingredient to prepare porridges, gives considerable anti-bacterial activity [21].

4. Conclusion

In conclusion, this study reveals the antimicrobial activity and phytochemical analysis of rice based porridge composites. Secondary metabolites; phenols, flavonoids, alkaloids, steroids, terpenoids, saponins, glycosides, anthocyanins and xanthoproteins present in the methanolic extracts of MWP, KHP, MXP and STP porridges. These porridge extracts showed a high efficacious inhibitory effect against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, which associated with food poisoning and causes of bacterial skin and soft tissue infections. Accordingly, the results of this study support with the folk medicinal applications of rice based porridges to cure food borne pathogenic diseases. The data obtained in this study can be utilized for the future approaches of the biological activities and health benefits of these Sri Lankan traditional rice based porridges.

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