



In-vitro Antibacterial Activity of Sri Lankan Traditional Rice (*Oryza sativa* L.) Extracts against Bacteria Causing Skin and Soft Tissue Infections

Pahan Indika Godakumbura*¹, Thiloka Ishani Kariyawasam¹, Prashantha Malavi Arachchi¹,
Neluka Fernando², Sirimal Premakumara³

¹Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda 10250, Sri Lanka

²Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda 10250, Sri Lanka

³Industrial Technology Institute, Colombo 7, 00700, Sri Lanka

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ABSTRACT

The aim of this study was to evaluate the potential antibacterial activity of the extracts of selected parboiled and un-parboiled Sri Lankan traditional rice against bacteria causing skin and soft tissue infections. Methanolic extracts of five Sri Lankan traditional rice including Kalu Heenati, Pokkali, Rathdal, Kahawanu and Sudu Murunga were used for *in vitro* antibacterial analysis. Antibacterial activity was evaluated in both the parboiled and un-parboiled rice samples. Concentrations of rice extracts used for the assays were 1000 µg/mL and 2000 µg/mL from the each extract. The antibacterial activity was evaluated against common bacteria causing skin and soft tissue infections (*Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and three clinical isolates of Methicillin resistant *staphylococcus aureus* (MRSA)) by well diffusion method and viable colony count technique. According to the results, methanolic extracts of all the selected Sri Lankan traditional rice varieties exhibited a potent antibacterial activity against *Staphylococcus aureus* with minimum bactericidal concentrations (MBC) of 200 µg/mL (minimum incubation time (MIT); 30 min) for Rathdal, 200 µg/mL (MIT; 60 min) for Kalu Heenati, Pokkali and Kahawanu, and 2000 µg/mL (MIT; 60 min) for Sudu Murunga. The largest inhibition zones were observed in the extracts of Kalu Heenati and Rathdal. Kalu Heenati, Pokkali and Rathdal showed an efficacious inhibitory effect against MRSA (MBC; 200 µg/mL, MIT; 60 min), whereas the highest inhibitory activity was observed for Rathdal. Only the extract of Kalu Heenati was slightly active against *Pseudomonas aeruginosa*. None of the rice extracts studied showed an antibacterial activity against *Escherichia coli*. Reduction and loss of antibacterial activity was detected in rice after subjected to parboiling. In conclusion, Sri Lankan traditional rice varieties with red pericarp are good sources of antibacterial compounds mainly against Gram positive bacteria. Methanolic extract of Rathdal and Kalu Heenati showed a high efficacious inhibitory effect against skin and wound pathogens of *Staphylococcus aureus* and MRSA.

KEY WORDS: Traditional rice; Parboiled; Antibacterial activity; Skin and soft tissue infections

1. INTRODUCTION

Bacterial skin and soft tissue infections (SSTIs) are some of the most common infections which can occur from infants to older adults [1,2,3]. Clinical manifestations of SSTIs encompass a wide spectrum of clinical presentations ranging from mild superficial epidermal infections to life threatening rapidly progressive infections [3,4]. This includes cellulitis, erysipelas, impetigo, ecthyma, erythrasma, bacterial

folliculitis, furuncles, carbuncles, and abscesses, hidradenitis suppurativa, surgical site infection, pressure ulcers and venous and arterial ulcers, necrotizing skin and soft tissue infections, fournier gangrene and clostridialmyonecrosis. Bacterial SSTIs involves bacterial invasion of the layers of the skin and underlying soft tissues. Gram positive *Staphylococcus aureus* and *Streptococcus pyogenes* and Gram negative *Pseudomonas aeruginosa* and *Escherichia coli* are the most commonly identified causes of SSTIs [5,6]. Multidrug resistant Methicillin resistant *staphylococcus aureus* (MRSA) strains are being identified more frequently as the causative agents for SSTIs [7]. In recent years, these infections have become more difficult to treat, as pathogens have developed resistance to many different types of antibiotics [8,9]. Despite drugs being

***Corresponding author.**

Dr. Pahan Indika Godakumbura

Department of Chemistry,

Faculty of Applied Sciences,

University of Sri Jayewardenepura,

Nugegoda 10250, Sri Lanka

continuously developed for bacterial SSTIs and tested, many reported cases document the drawbacks and adverse side effects [10,11]. This signifies the urgent need of development of novel drugs and treatment to combat bacterial SSTIs.

Medicinal plants play a vital role in traditional medicine for many centuries worldwide. The compounds extracted from these plants have yielded invaluable drugs against many diseases. However, with drug resistance and burgeoning population, vulnerability to novel diseases have been escalated significantly compared to the past. The lifelong battle against pathogens and humans has reached the climax stage where we witness various disease modifications giving rise to novelty, severity and broadening the spectrum of diseases today. The virulence of pathogens evolves rapidly and our best drugs against them become out dated by due to acquired resistance developed by the pathogens. Therefore scientists have been constantly challenged to come up with remedial drugs against the newly evolving strains of pathogens. Majority of these remedial drug introduced have been derived from chemical compounds extracted from plants and microbial origins. Therefore the search for new bioactive compounds with pharmaceutically valuable properties with natural origin has become a novel approach in drug discovery. Rice can be considered as a treasure trove of bioactive compounds. In addition rice is the major cereal crop as well as the main staple food in Sri Lanka and other parts of the Asia. There are over 2000 different varieties of rice grown all over Sri Lanka since ancient days. Unlike improved rice varieties, Sri Lankan traditional rice varieties (STRV) consist of a unique texture, appearance, aroma and taste and high medicinal value according to traditional medicinal texts [12,13,14].

Rice subjected to two types of processing conditions is available in the Sri Lankan market namely raw rice and parboiled rice. Paddy rice parboiling process is an old practice which dates back more than 1000 years ago as a post-harvest processing method. According to Bhattacharya et al, (1985) about one-fifth of the world's rice is parboiled [15]. Parboiling consists of soaking paddy, either cold or hot water method, steaming and drying. Parboiling makes rice easier to mill, reduce the deterioration of raw rice during storage, improves its nutritional profile and changes its texture which helps to meet the demands of certain consumer preferences [16]. Moreover, gelatinization of rice starch during the steaming process makes the grain translucent, hard, uniform and resistant to breakage during milling. This enables the dehulling process easier giving better quality rice with hard and uniform color and higher yield [16].

It has been demonstrated that STRV are rich source of bio active compounds against various diseases. Recent studies done for STRV

showed bio activities including antioxidant anti-amylase, anti-glycation and anti-inflammatory properties and higher nutritional composition compared to improved varieties [17,18]. The advantage of using STRV in medicine is that these are natural products grown under organic farming conditions and therefore safer than synthetic or chemical compounds.

According to the folk medicinal evidences obtained from Ola leaf manuscripts and traditional medicinal texts ("Sararthasangrahaya" and "Thalpathe piliyam"), pastes prepared using traditional rice flour had been used to cure microbe borne skin infections. Therefore the present study focuses on the potent bacterial activity of the selected STRV against common bacteria that cause skin and soft tissue infections and the effect of parboiling on the antibacterial activity.

2. MATERIALS AND METHODS

2.1 Plant materials

Five STRV (*Oryza sativa* L.) of long grain rice consisting of Kalu Heenati, Pokkali, Rathdal and Sudu Murunga and one short grain rice variety, Kahawanu were selected for *in vitro* studies of the antibacterial activity present in rice grain. Fresh paddy samples of the STRV obtained from a traditional farmer were grown under organic farming conditions. The criteria for selecting the rice varieties were based on the traditional medicinal evidences regarding the effective utilization for skin and soft tissue infections. The respective voucher specimens have been deposited at the Department of Chemistry of the University of Sri Jayewardenepura, Nugegoda, Sri Lanka. Paddy samples were packed in polyethylene bags and stored in a refrigerator at -10° C.

2.2 Parboiling process

Each paddy variety was parboiled by soaking the paddy at 60 °C for 3 ½ hours for short grain rice and 4 hours for long grain rice and steaming at 100 °C for 20 minutes followed by air drying at 50 °C for 24 hours. It was kept open for 48 hour to reach moisture equilibration. Parboiled paddy samples were packaged in polyethylene bags and stored in a refrigerator at -10 °C until the time of analysis. The parboiling procedure was triplicated for each paddy variety.

2.3 Preparation of rice extracts

Both parboiled (PB) and un-parboiled (UPB) paddies were de-hulled (THU 35B, Satake, Hiroshima, Japan), ground and passed through a 60-mesh sieve to obtain a homogeneous fine powder. Ten grams of rice flour of each of the rice varieties were extracted with 10 times the sample weight of 70% methanol/water (v/v) for 24 h at room temperature (28±2°C) and filtered. Filtered extracts were evaporated

under reduced pressure in a rotary evaporator and freeze dried (Christ-Alpha 1-4 Freeze dryer, Biotech International, Germany). The extracts were then used to prepare known concentrations of rice extracts using dimethyl sulfoxide (DMSO, Merck, Germany) and the solutions were passed through 0.2 µm Millipore filters. Filtered extracts were used for antibacterial assays.

2.4 Determination of antimicrobial activity

The antimicrobial activities of extracts were determined by well diffusion assay^[19] and viable colony count technique^[20].

2.4.1 Test organisms

The test microorganisms used in this study include *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and three Clinical isolates of MRSA. Bacterial strains were obtained from the laboratory collection of Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka. The strains were sub-cultured in fresh nutrient agar plates and incubated at 37 °C for 24 h prior to any antimicrobial test.

2.4.2 Culture media

The media used for well diffusion assay and viable colony count technique were Muller Hinton agar (Mast, UK) and Blood agar (Oxoid, UK) respectively. Nutrient agar (Mast, UK) was used as the media to culture bacterial strains.

2.4.3 Standard antibiotics used for antimicrobial assay

Gentamycin (Neon Laboratories, India) was used as a positive control against *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). Cefoxitin (Oxoid, UK) and Vancomycin (Swiss Export Pvt Ltd, India) were used as positive controls against *Staphylococcus aureus* (ATCC 25923) and MRSA respectively.

2.4.4 Well diffusion assay

Bacterial suspensions were prepared for each strain in sterile normal saline and turbidity was adjusted to 0.5 McFarland standard. The suspension of organisms was 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 rice extracts of 1000 µg/mL and 2000 µg/mL concentrations were separately loaded into the wells. At the same time, 200 µL of Gentamycin (50 µg/mL), Vancomycin (50 µg/mL) and Cefoxitin (30 µg/mL) were used as positive controls whereas DMSO 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 and it was measured (Diameter of the inhibition zone – Diameter of the well) in millimeters. Tests were performed in triplicate.

2.4.5 Viable colony count technique

Bactericidal activities of the extracts were determined by viable colony counts. Ten-fold dilution series (concentration ranges from 0.2 - 2000 µg/mL) from each of the rice extracts was made. A volume of 100 µL of bacterial suspensions (1×10^8 bacteria/mL) were added to 900 µL of each of the rice 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 µL of each of the diluted extracts were inoculated onto Blood agar plates and incubated at 37°C for overnight. Rice extracts that killed 100% of bacterial cells during 60 min (i.e. no colonies grew) were further tested at different incubation periods of 0, 15, 30, and 60 min. Three independent trials were conducted for each concentration. Concentration of an extract that killed 100% of bacterial cells was considered as the Minimum Bactericidal Concentration (MBC) of that rice extract.

3. RESULTS AND DISCUSSION

This study evaluated the *in vitro* antibacterial activity of crude rice extracts from five different types of STRV, against pathogenic bacteria causing SSTI. Their potential activities were accessed by the diameter of the inhibition zones and the MBC values. Since the well diffusion assay is a qualitative method used for preliminary screening of antibacterial activity^[21], activities discovered with well diffusion assay were further confirmed by expressing the antibacterial activity as MBC.

Methanol extract of the rice were used for the investigation of antibacterial activity. Methanol can be considered as the best solvent used for extraction of antimicrobial substances compared to other solvents such as water, ethanol, ethyl acetate and hexane^[22-26]. This is associated with the polarity, concentration or nature of methanol, which facilitates the solubility and enhances the extraction of large quantities of secondary metabolites from plants including tannins, polyphenols, terpenoids, saponins, xanthoxylines, totarol, quassinoids, lactones, flavones, and phenones^[27].

It was observed that all the rice extracts produced significant zone of inhibition only against *S. aureus* (Table 1) and with no zones of inhibition against *E. coli*. Only the crude extract of Kalu Heenati displayed an inhibition against *P. aeruginosa*. According to the results shown in Table 1, the highest activities were reported for crude extract of Rathdal against *S. aureus* and MRSA with inhibition

Table 1. Antibacterial activity of the methanolic extracts of STRV by well diffusion assay

Rice variety	Concentration (µg/mL)	Diameter of the inhibition zone around the well (mm)				
		I	II	III	IV	V
Kalu Heenati	1000	5.3 ± 0.6	-	4.0 ± 0.0	-	4.0 ± 1.0
	2000	8.3 ± 1.2	3.3 ± 0.6	5.7 ± 0.6	7.3 ± 0.6	5.6 ± 0.6
Rathdal	1000	8.7 ± 0.6	-	-	5.3 ± 1.2	6.0 ± 0.0
	2000	10.7 ± 1.2	-	7.3 ± 0.6	8.3 ± 0.6	9.7 ± 0.6
Pokkali	1000	2.3 ± 0.6*	-	-	-	-
	2000	5.3 ± 0.6*	-	5.4 ± 0.3	6.4 ± 0.5	5.7 ± 0.3
Kahawanu	1000	3.3 ± 0.3	-	-	-	-
	2000	4.5 ± 0.5	-	-	-	-
Sudu Murunga	1000	-	-	-	-	-
	2000	2.0 ± 0.0	-	-	-	-
Gentamycin ^a	50	n.a.	14.6 ± 0.4	n.a.	n.a.	n.a.
Vancomycin ^a	50	n.a.	n.a.	17.5 ± 0.5	18.8 ± 0.6	17.9 ± 1.0
Cefoxitin ^a	30	17.0 ± 0.2	n.a.	n.a.	n.a.	n.a.

I: *Staphylococcus aureus* (ATCC 25923), II: *Pseudomonas aeruginosa* (ATCC 27853), III, IV, V: Clinical isolates of MRSA,

^a Standard antibiotic, n.a.: Not applicable, *: Partial inhibition, -: No activity

Table 2. Antibacterial activity of the methanolic extracts of PB-STRV by well diffusion assay

Rice variety	Concentration (µg/mL)	Diameter of the inhibition zone around the well (mm)
Kalu Heenati-PB	1000	-
	2000	2.8 ± 0.4
Rathdal-PB	1000	-
	2000	3.5 ± 0.7

I: *Staphylococcus aureus* (ATCC 25923), -: No activity

zone diameters range from 5.3 to 10.7 mm, whereas least activities were shown by Sudu Murunga. Crude extract of Pokkali was partially active against *S. aureus*. This can be explained by the nature and the diffusing ability of antibacterial compounds present in the extract of Pokkali. Rice extracts were further investigated against clinically isolated skin and wound bacteria, MRSA. Results show the sensitivity of MRSA clinical isolates to three rice extracts (Kalu Heenati, Rathdal and Pokkali) with inhibition zones ranging from 4.0-6.0 mm for lower concentrations and 5.4- 9.7 mm for higher concentrations of the extracts. Conversely clinical isolates of MRSA were resistant to both the crude extracts of Kahawanu and Sudu Murunga.

MBC values of the crude extracts of UPB and PB STRV are shown in Table 3. The data indicates that the MBC for all the UPB rice extracts was 200 µg/mL against *S. aureus* and *P. aeruginosa* except for Sudu Murunga, which showed the MBC of 2000 µg/mL. Among the selected STRV Rathdal showed the fastest bactericidal activity

Table 3. Bactericidal activity of STRV determined using viable colony count

Rice variety	Minimum bactericidal concentration (µg/mL)				
	I	II	III	IV	V
Kalu Heenati	200 ± 0 ^b	200 ± 0 ^b	2000 ± 0 ^b	200 ± 0 ^b	200 ± 0 ^b
Kalu Heenati-PB	2000 ± 0 ^c	n.a.	n.a.	n.a.	n.a.
Rathdal	200 ± 0 ^a	n.a.	200 ± 0 ^b	200 ± 0 ^b	200 ± 0 ^b
Rathdal-PB	200 ± 0 ^b	n.a.	n.a.	n.a.	n.a.
Pokkali	200 ± 0 ^b	n.a.	200 ± 0 ^b	200 ± 0 ^b	200 ± 0 ^b
Kahawanu	200 ± 0 ^b	n.a.	n.a.	n.a.	n.a.
Sudu Murunga	2000 ± 0.0 ^b	n.a.	n.a.	n.a.	n.a.

I: *Staphylococcus aureus* (ATCC 25923), II: *Pseudomonas aeruginosa* (ATCC 27853), III, IV, V: Clinical isolates of MRSA, n.a. - Not applicable due to no activity in well diffusion assay

with 30 min of incubation against *S. aureus*, whereas for the rest of the bacterial strains minimum incubation time was 60 min. Kalu Heenati, Pokkali, Kahawanu and Sudu Murunga were able to kill the bacterial strains within 60 min at their corresponding MBC. Moreover all the above rice extracts showed MBC value of 200 µg/mL against MRSA after 60 min incubation period.

The nature and the level of antimicrobial agents present in the extracts and their mode of action on the different test microorganisms explain the above differences in the antibacterial activity. Reduction in the antibacterial activity at lower concentrations may be attributed to the less diffusibility of active compounds, which may improve with stringent extraction procedure. Also the pH of bioactive constituents mainly consisting of phenolic and carboxylic compounds can be altered during the dilution process, which also modifies the results in lower concentrations [28,29].

Data reveals that the parboiling treatment has resulted to reduce or loose the antibacterial activity of the extracts of the STRV. According to the results (Table 2) Kalu Heenati and Rathdal showed a reduced antibacterial activity only against *S. aureus* after parboiling. MBCs of parboiled Kalu Heenati and Rathdal against *S. aureus* indicate a reduction and slowdown of bactericidal activity after parboiling. This is in an agreement with the previous reports of Chen et al., 1985 and Sah et al., 2012, which reveals the reduction or loss of antibacterial properties of the plant extracts subjected to heat treatment [30,31].

It was observed that the Gram negative bacteria (*P. aeruginosa* and *E. coli*) show greater resistant to rice extracts compared to Gram positive bacteria (*S. aureus* and MRSA). This is supported by the previous studies done by Paz et al., 1995 and Kudi et al., 1999 [32,33]. According to Tortora et al., 2001, the above observation can be explained by differences in the cell wall substances in Gram positive and Gram negative bacteria and the ability of Gram negative bacterial outer membrane to act as a barrier for many environmental constituents including antibiotics [34].

This study reveals a relationship between antibacterial activity of rice extracts and the pericarp colour of the rice grain. Rice varieties having red pericarps display higher antibacterial activity compared to the rice varieties having white pericarps. This can be explained by the presence of various bioactive compounds including flavones, anthocyanins, tannin, phenolics, sterols, tocopherols, γ -oryzanol, amino acids, and essential oils in rice varieties having mainly black, red and purple pericarps [35,36].

4. CONCLUSION

The present study highlights the importance of STRV as a potential source of antibacterial compounds associated with SSTIs. Selected STRV show potent antibacterial activity mainly against Gram positive bacteria. Methanolic extract of Rathdal and Kalu Heenati showed a high efficacious inhibitory effect against *S. aureus* and MRSA. Accordingly the results of this study support with the folk medicinal applications of STRV to cure skin diseases. Parboiling rice cannot be recommended for medicinal applications associated with bacterial infections. Furthermore, bioassay guided purification and identification of bioactive compounds responsible for the antibacterial activity are needed.

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