



Antimicrobial Properties of Ethanolic and Methanolic Extracts of Finger millet (*Eleusine coracana* (L.) Gaertn.) Varieties Cultivated in Sri Lanka

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ABSTRACT

*The emerging significance of natural antimicrobial agents creates an imperative need to identify novel plant sources with antimicrobial activities. The objective of the present study was to evaluate antibacterial, antifungal and β -lactamase enzyme inhibitory activities of ethanolic and methanolic extracts of Ravi, Rawana and Oshadha finger millet varieties. Flours of whole grains of the finger millet varieties were extracted with absolute ethanol and methanol separately. Antibacterial activities against six antibiotic-sensitive and four antibiotic-resistant pathogenic bacterial strains were evaluated using the resazurin reduction assay. Antifungal activities against six antimicrobial-sensitive pathogenic fungal strains were evaluated using the agar tube dilution method. β -Lactamase enzyme inhibitory activity was evaluated using a standard method. Both ethanolic and methanolic extracts of the three finger millet varieties showed dose-dependent inhibitory activities against the tested antibiotic-sensitive and antibiotic-resistant bacterial strains while exhibiting high inhibitions against Gram-positive antibiotic-sensitive bacterial strains when compared to Gram-negative antibiotic-sensitive bacterial strains. The findings revealed the antibacterial potential of both ethanolic and methanolic extracts of the three finger millet varieties against antibiotic-sensitive *Staphylococcus aureus* (ATCC[®] 6538[™]) and *Bacillus subtilis* (ATCC[®] 23857[™]) strains and the minimum inhibitory concentrations of the extracts against *S. aureus* and *B. subtilis* were found to be 2.1 and 1.8 mg/ml, respectively. However, none of the extracts can be considered as significantly active against the tested antibiotic-sensitive and antibiotic-resistant bacterial strains when compared to the standard drugs. In addition, none of the extracts can be considered as active against the tested fungal strains at the tested concentrations. Nevertheless, all extracts showed more activities against the tested bacterial strains when compared to the tested fungal strains. Since all extracts showed less than 40% β -lactamase inhibitory activities even at 2 mg/ml concentration, they do not qualify as promising sources of β -lactamase inhibitors at the tested concentration.*

KEYWORDS: *Antibacterial activity, Antifungal activity, Finger millet, β -Lactamase enzyme inhibitory activity*

1 INTRODUCTION

The incidences of resistance of pathogenic microorganisms to antimicrobial agents are increasing at an alarming rate (Khan *et al.*, 2009; Saravanan and Parimelazhagan, 2014; Khan *et al.*, 2015). Indiscriminate use of antimicrobial agents has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms. Consequently, the efficiencies of the currently available antimicrobial agents are declining (Khan *et al.*, 2009; Silva *et al.*, 2015). This has become a great threat in the effective management of infectious diseases and has become a global concern to public health (Khan *et al.*, 2015). In addition, many of the currently available antimicrobial agents are expensive and sometimes induce serious side effects (Silva *et al.*, 2015). The antimicrobial agents commonly used for food preservation are mostly synthetic chemicals and now there is an emerging demand for natural products with antimicrobial activities for preserving foods (Banerjee *et al.*, 2012). Therefore, identifying novel therapeutically active antimicrobial drugs and edible antimicrobial agents, preferably from natural sources, is receiving attention worldwide (Khan *et al.*, 2009; Banerjee *et al.*, 2012; Khan *et al.*, 2015; Silva *et al.*, 2015).

Kingdom Plantae has been regarded as an important source of natural products with antimicrobial activities (Su *et al.*, 2014). Antimicrobial activities of plants are

attributed to the presence of bioactive components such as polyphenols including flavonoids (Khan *et al.*, 2015). Several authors have reported the antimicrobial effects of plant polyphenols and have recognized plant polyphenols as potential inhibitors of the growth of a wide spectrum of microorganisms (Viswanath *et al.*, 2009; Banerjee *et al.*, 2012; Saravanan and Parimelazhagan, 2014; Su *et al.*, 2014; Singh *et al.*, 2015).

Several studies have confirmed the presence of phenolic compounds in cereal grains. Polyphenols are the most important phytochemicals of millets due to their nutraceutical potentials (Singh *et al.*, 2015). Finger millet (*Eleusine coracana* (L.) Gaertn.) grains are rich in polyphenols when compared to other cereals such as rice, wheat, maize and barley (Viswanath *et al.*, 2009; Chandra *et al.*, 2016). Several therapeutic properties of finger millet including antibacterial and antifungal activities have been reported previously (Mathanghi and Sudha, 2012; Srivastava and Sharma, 2012; Singh *et al.*, 2015). Finger millet is commonly cultivated and consumed in Sri Lanka since ancient times (Kumari *et al.*, 2015). It is considered as a medicinal plant and used in the treatment and management of several diseases in the traditional system of medicine (Hettiarachchige and Edirisuriya, 2013). However, there is a scarcity in scientific information on antibacterial and antifungal activities of the finger millet varieties which are commonly cultivated in Sri Lanka. Since there is a genetic diversity in finger millet varieties worldwide and there are varietal differences in composition with respect to

every constituent (Kaluthanthri and Dasanayaka, 2016), it is important to study the antimicrobial potential of the finger millet varieties which are commonly cultivated in Sri Lanka. Therefore, the objective of the present study was to evaluate ethanolic and methanolic extracts of Sri Lankan finger millet varieties for antibacterial activities against six antibiotic-sensitive and four antibiotic-resistant pathogenic bacterial strains, antifungal activities against six antimicrobial-sensitive pathogenic fungal strains and β -lactamase enzyme inhibitory activities.

2 MATERIALS AND METHODS

2.1 Sample collection and preparation

Finger millet varieties which are recommended for cultivation by the Department of Agriculture, Sri Lanka namely Ravi, Rawana and Oshadha were collected from Field Crop Research and Development Institute (FCRDI), Mahailuppallama, Sri Lanka. These varieties were cultivated in experimental plots in the Low Country Dry Zone, at FCRDI, Mahailuppallama and the seeds were certified by the Seed Certification Service of Department of Agriculture, Sri Lanka. Finger millet seeds were dehulled (TM 05C, Satake Corporation, Japan) and flours from whole grains were obtained by milling (Pulverisette 14, Fritsch, Germany) and passing through a 0.5 mm sieve. Flours of whole grains were extracted with absolute ethanol and methanol separately. Whole grain flour (100 g) was extracted with the solvent

(400 ml) by soaking overnight at room temperature (28 ± 2 °C) while stirring continuously using a magnetic stirrer and centrifuging (Sigma 318 K, Sigma Laborzentrifugen GmbH, Germany) at 4000 rpm for 20 min. The supernatant was collected separately and the residue was re-extracted twice under the same conditions. The supernatants were combined and evaporated to dryness under reduced pressure at 40 °C using a rotary evaporator (R-114, Büchi Labortechnik AG, Switzerland). The solvent-free extracts were stored in airtight glass containers at -20 °C until use for the analysis.

2.2 Chemicals, enzymes and reagents

Dimethyl sulfoxide (DMSO), penicillinase, potassium clavulanate, resazurin sodium salt, ampicillin and miconazole were purchased from Sigma Aldrich, MO, USA. Oxoid Mueller-Hinton broth and nitrocefin were purchased from Thermo Fisher Scientific Inc., Massachusetts, USA. Ofloxacin were purchased from Bio Basic Inc., Markham, Canada. Amphotericin B was purchased from ICN Biomedicals, Inc., Ohio, USA. Sabouraud dextrose agar was purchased from Merck Specialities Private Limited, Goa, India. All other chemicals and reagents used in the experiments were of ACS, HPLC and analytical grades.

2.3 Test microorganisms

Six bacterial strains having sensitivity to antibiotics, namely *Shigella flexneri* (ATCC[®] 12022[™]), *Pseudomonas aeruginosa* (ATCC[®] 10145[™]), *Escherichia coli* (ATCC[®] 25922[™]),

Salmonella typhi (ATCC® 14028™), *Bacillus subtilis* (ATCC® 23857™) and *Staphylococcus aureus* (ATCC® 6538™), four bacterial strains with recorded antibiotic resistivity, namely *Klebsiella pneumoniae* (ATCC® 700603™), *Escherichia coli* (ATCC® 35218™), *Salmonella enterica* (ATCC® 700408™) and *Staphylococcus aureus* (ATCC® BAA-1720™) and six fungal strains, namely *Candida albicans* (ATCC® 14053™), *Candida glabrata* (ATCC® 2001™), *Aspergillus niger* (ATCC® 1015™), *Fusarium lini* (NRRL 2204), *Microsporium canis* (ATCC® 10214™) and *Trichophyton rubrum* (ATCC® MYA-4438™) were obtained from the Microbial Bank of International Center for Chemical and Biological Sciences, University of Karachi, Pakistan.

2.4 Determination of antibacterial activity

Antibacterial activity was evaluated using the resazurin reduction assay as described by Osaka and Hefty (2013) with slight modifications. Using aseptic techniques, a glass bottle containing sterilized Mueller-Hinton broth (10 ml) was inoculated with one colony of the bacterial strain,

incubated at 37 °C for 24 hrs and adjusted to the 0.5 McFarland turbidity index by diluting with Mueller-Hinton broth. A known amount of finger millet extract was dissolved in DMSO. Dissolved finger millet extract (10 µl), Mueller-Hinton broth (183 µl) and bacterial suspension (7 µl) were added to a tissue culture treated 96-well microplate, covered with a parafilm and incubated at 37 °C for 20 hrs. Parafilm was removed, the microplate was covered with a sealing film and pre-plate reading was recorded at 570 nm and 600 nm using a microplate reader (SpectraMax M5e, Molecular Devices Inc., USA). Resazurin sodium salt (20 mg) was dissolved in 100 ml of sterile distilled water and filtered. In dark, the prepared dye (20 µl) was added to the wells, the microplate was covered with aluminium foil and incubated at 37 °C in a shaking incubator (80 rpm) for 2 hrs. Aluminium foil was removed, the microplate was covered with a sealing film and absorbances were recorded at 570 nm and 600 nm. Ampicillin and ofloxacin were used as standard drugs. DMSO was used as the control. Inhibition percentage was calculated using the following equations.

$$\text{Reduction \%} = \frac{(\epsilon_{OX\lambda 2} \times A_S\lambda 1) - (\epsilon_{OX\lambda 1} \times A_S\lambda 2)}{(\epsilon_{OX\lambda 2} \times A_C\lambda 1) - (\epsilon_{OX\lambda 1} \times A_C\lambda 2)} \times 100$$

$$\text{Inhibition \%} = 100 - \text{Reduction \%}$$

Where,

$\epsilon_{OX\lambda 1}$ is the molar extinction coefficient of oxidized form of resazurin at 570 nm (80,586), $\epsilon_{OX\lambda 2}$ is the molar extinction coefficient of oxidized form of resazurin at 600 nm (117,216), $A_S\lambda 1$ is the absorbance of sample at 570 nm, $A_S\lambda 2$ is the absorbance of sample at 600 nm, $A_C\lambda 1$ is the absorbance of control at 570 nm and $A_C\lambda 2$ is the absorbance of control at 600 nm.

Antibacterial activities of the extracts were evaluated at three different concentrations (150, 600 and 2400 µg/ml) and the extracts which showed no color change from blue to pink or colorless were subjected to the determination of minimum inhibitory concentration (MIC) values as described by Coronado-Aceves *et al.* (2016). To determine the MIC value, the resazurin reduction assay was performed using seven different concentrations (600, 900, 1200, 1500, 1800, 2100 and 2400 µg/ml) of finger millet extract and the lowest concentration at which the blue color of the dye remained unchanged was recorded as the MIC value.

2.5 Determination of antifungal activity

Antifungal activity was evaluated using the agar tube dilution method as described by Atta-ur-Rahman *et al.* (2001), Uddin *et al.* (2011) and Thadhani *et al.* (2012). A

known amount of finger millet extract was dissolved in DMSO. Using aseptic techniques, dissolved finger millet extract (66.6 µl) was added to a glass tube containing sterilized Sabouraud dextrose agar (4 ml) and the tube was allowed to solidify in a slanting position at room temperature to prepare an agar slant. From each extract, four agar slants were prepared having four different concentrations (400, 800, 1600 and 3200 µg/ml). Each tube was inoculated with a piece of fungus having a diameter of 4 mm taken from a seven-day old culture of fungus and incubated at 27 °C for 7 days. For *A. niger*, amphotericin B was used as the standard drug and miconazole was used for the other fungal strains. DMSO was used as the control. Growth of the fungus was evaluated by measuring the linear growth (in mm) and growth inhibition percentage was calculated with reference to the control using the following equation.

$$\text{Inhibition \%} = \left\{ \frac{100 - \text{Linear growth in test (mm)}}{\text{Linear growth in control (mm)}} \right\} \times 100$$

2.6 Determination of β-lactamase enzyme inhibitory activity

β-Lactamase enzyme inhibitory activity was determined according to the method described by Atta-ur-Rahman *et al.* (2001) with slight modifications. A known amount of finger millet extract was initially dissolved in DMSO and diluted with 100 mM phosphate buffer (pH 7.4). Diluted finger millet extract (50 µl) was added to a 96-well microplate, mixed with 40 µl of penicillinase enzyme

(1000 U/ml) and incubated at 25 °C for 20 min. Preplate reading was taken at 482 nm using a microplate reader (SpectraMax Plus³⁸⁴, Molecular Devices Inc., USA). The reaction was initiated with the addition of 2 mM nitrocefin (10 µl) and the change of absorbances was monitored at 482 nm for a period of 10 min at 30 s intervals and the maximum velocity (V_{\max}) value was recorded. Reaction mixture without the sample was used as the control. Potassium clavulanate

was used as the standard. β -Lactamase enzyme inhibitory activity as percentage

inhibition was calculated using the following equation.

$$\beta\text{-Lactamase enzyme inhibitory activity (\%)} = [(V_{\max C} - V_{\max S}) / V_{\max C}] \times 100$$

Where,

$V_{\max C}$ is the V_{\max} of control and $V_{\max S}$ is the V_{\max} of sample.

2.7 Data analysis

Data of all experiments were statistically analyzed by one-way analysis of variance (ANOVA) and Tukey's test using the IBM SPSS Statistics (Version 20) software. Statistical significance was set at 95% confidence level.

3 RESULTS AND DISCUSSION

3.1 Antibacterial activity

Antibacterial activities of the extracts were evaluated using the resazurin reduction assay. Resazurin is an oxidation-reduction based cell viability indicator widely used to evaluate cell growth in various assays (Sarker *et al.*, 2007; Osaka and Hefty, 2013). It is a blue color non-fluorescent and non-toxic dye, which becomes pink and fluorescent when reduced to resorufin by oxidoreductases produced in viable cells. Resorufin is further reduced to hydroresorufin which is colorless and non-fluorescent (O'Brien *et al.*, 2000; Sarker *et al.*, 2007). The oxidized and reduced forms of resazurin can be measured separately and used to evaluate the reduction capabilities of the cells, which reflect the cell viability (Osaka and Hefty, 2013). There is a direct correlation between the reduction of resazurin and the proliferation of living organisms, ranging from bacteria to mammalian cells (O'Brien *et al.*, 2000).

Antibacterial activities of plant extracts vary with the solvents use for the extraction (Sunder *et al.*, 2011). Silva *et al.* (2015) and Khan *et al.* (2015) reported that methanol is the best solvent to investigate antibacterial activities of plant extracts. Sunder *et al.* (2011) have stated that both ethanol and methanol are commonly used to extract antibacterial compounds. In the present study, ethanolic and methanolic extracts of Ravi, Rawana and Oshadha finger millet varieties were used. Antibacterial activities of the extracts against Gram-negative antibiotic-sensitive bacteria at 150, 600 and 2400 $\mu\text{g/ml}$ concentrations are shown in Figure 1. All extracts showed dose-dependent inhibitory activities against the tested antibiotic-sensitive bacteria. However, none of the extracts showed more than 40% inhibition against *S. flexneri* and *S. typhi* at the tested concentrations. Both extracts of the three varieties showed more than 50% inhibitory activities against *E. coli* and more than 60% inhibitory activities against *P. aeruginosa* at 2400 $\mu\text{g/ml}$ concentration. However, all extracts showed significantly ($P < 0.05$) low inhibitory activities, against the tested Gram-negative antibiotic-sensitive bacteria when compared to the standard drugs.

Both ethanolic and methanolic extracts of the three finger millet varieties showed dose-dependent inhibitory activities against the tested Gram-positive antibiotic-sensitive bacterial strains (Figure 2). Both extracts of the three varieties showed more than 50% inhibitions against *S. aureus* and *B. subtilis* at 600 µg/ml concentration. Furthermore, they showed more than 70% inhibitions against *S. aureus* and *B. subtilis* at 2400 µg/ml concentration. Nevertheless, both extracts of the three varieties cannot be considered as significantly active against the tested Gram-positive antibiotic-sensitive bacterial strains when compared to the standard drugs.

According to the findings of Banerjee *et al.* (2012), different extracts of an Indian variety of finger millet showed considerable proliferation inhibitory activities against *E. coli*, *S. aureus*, *P. aeruginosa*, *Bacillus cereus*, *Listeria monocytogenes*, *Streptococcus pyogenes*, *Proteus mirabilis*, *Serratia marcescens*, *K. pneumonia* and *Yersinia enterocolitica*. Singh *et al.* (2015) have reported antibacterial activities of ethyl acetate extract of an Indian variety of finger millet against *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *Shigella dysenteriae*, *Enterococcus* species and *Salmonella* species. In addition, Shukla *et al.* (2015) have reported antibacterial activities of hexane extracts of three Indian finger millet varieties against *E. coli*, *S. aureus*, *P. aeruginosa*, *Enterococcus* species and *Salmonella* species. However, those results cannot be compared with the results of the present

study due to the differences in the extracts and the methods used to determine antibacterial activities. Phenolic compounds including flavonoids have been shown to act as antibacterial agents by interacting with the proteins on bacterial cell wall and thereby disrupting the functional and structural integrity (Silva *et al.*, 2015). Therefore, the phenolic compounds, which are present in finger millet extracts, may be responsible for the antibacterial activities observed in the present study as well.

Generally, Gram-negative bacterial strains are more resistant to inhibition than Gram-positive bacterial strains (Nair and Chanda, 2007). Gram-negative bacteria have a complex cell wall structure when compared to Gram-positive bacteria. Due to the thick murein layer and periplasmic space, the outer membrane of the cell wall of Gram-negative bacteria restricts the diffusion of antibiotics and plant extracts. Multidrug efflux pumps also contribute to create a high intrinsic resistance by pumping out the antibacterial agents through the active efflux processes (Khan *et al.*, 2015). Banerjee *et al.* (2012) have reported that finger millet extracts are more active against Gram-positive bacteria when compared to Gram-negative bacteria. In agreement with aforementioned studies, ethanolic and methanolic extracts of Sri Lankan finger millet varieties also exhibited high inhibitions against Gram-positive antibiotic-sensitive bacterial strains when compared to Gram-negative antibiotic-sensitive bacterial strains.

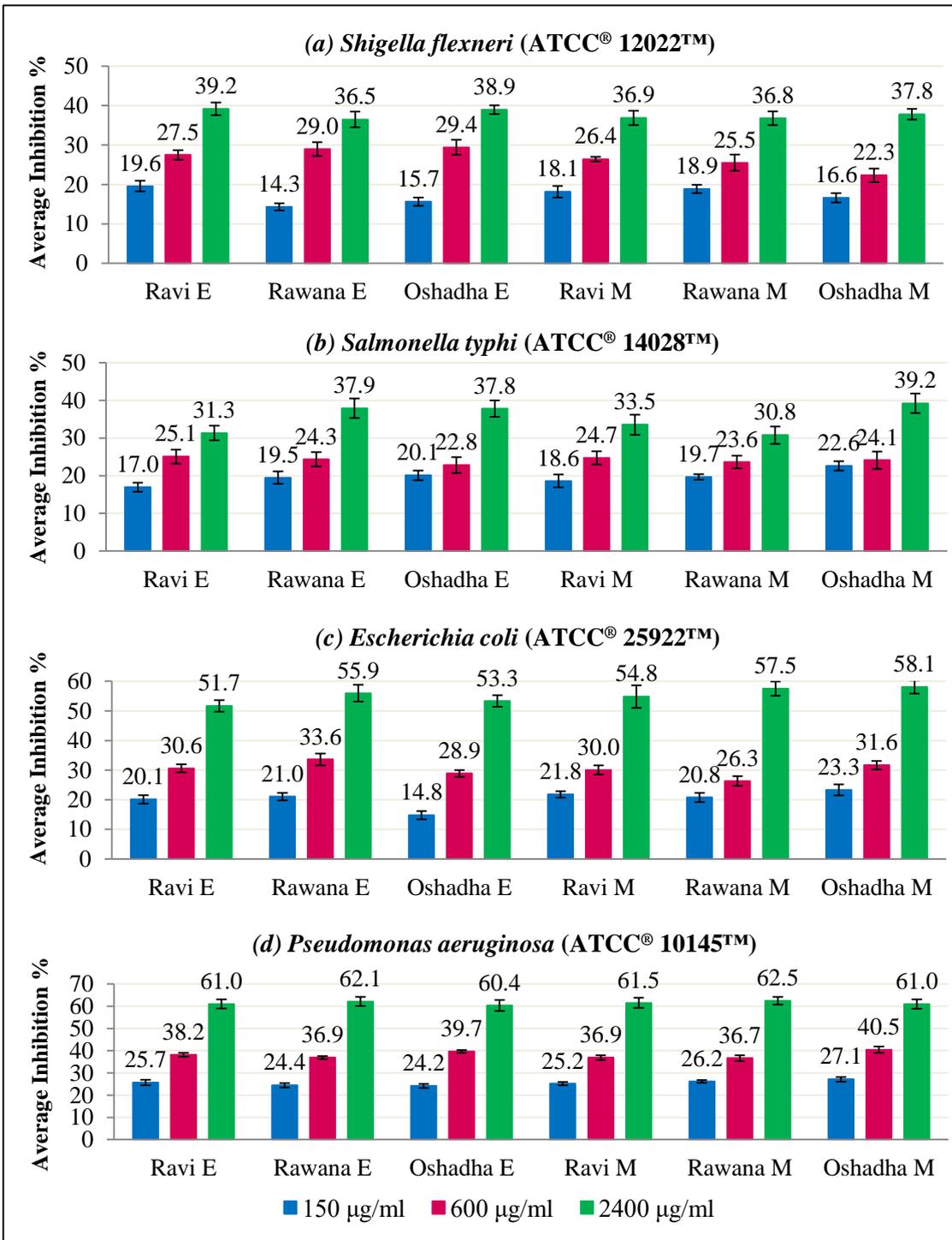


Figure 1: Antibacterial activities of the finger millet extracts against Gram-negative antibiotic-sensitive bacterial strains at 150, 600 and 2400 µg/ml concentrations. Results are presented as mean values (n = 3). Error bars indicate the standard error. E: Ethanol extract, M: Methanol extract. Ofloxacin and ampicillin showed 100% inhibitions even at 150 µg/ml concentration.

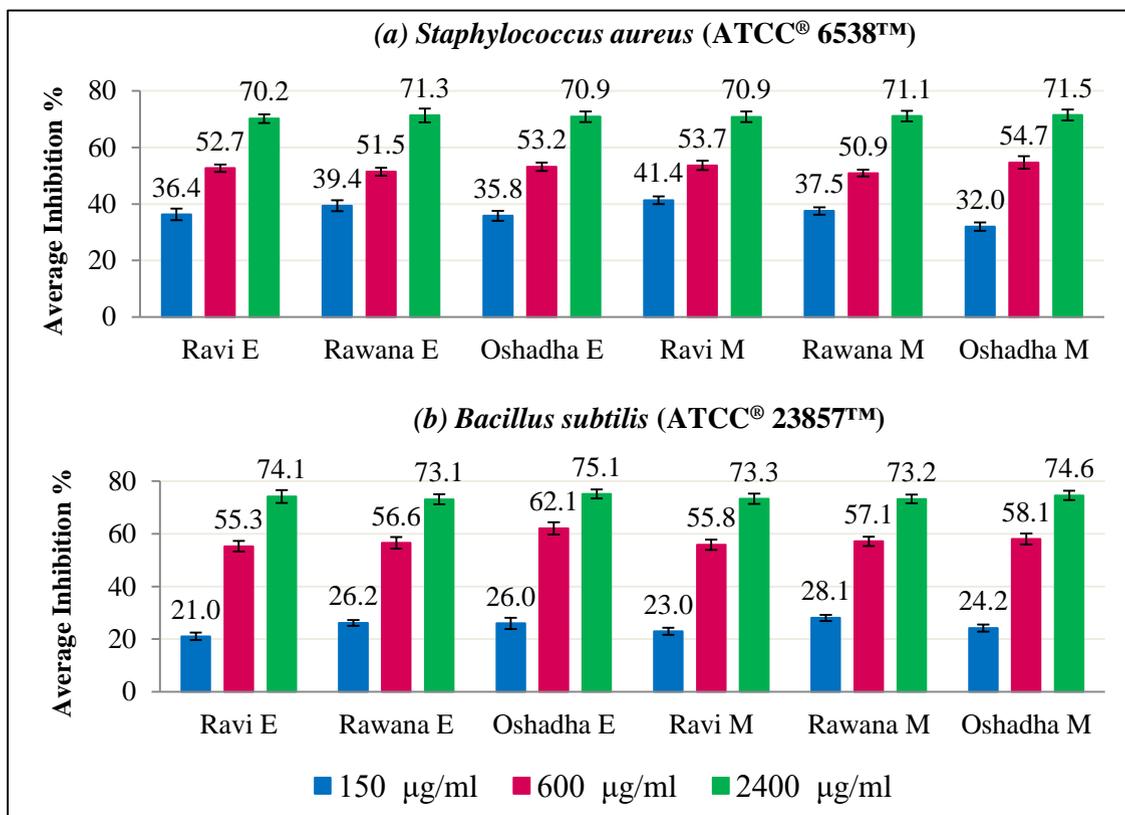


Figure 2: Antibacterial activities of the finger millet extracts against Gram-positive antibiotic-sensitive bacterial strains at 150, 600 and 2400 µg/ml concentrations. Results are presented as mean values (n = 3). Error bars indicate the standard error. E: Ethanolic extract, M: Methanolic extract. Ofloxacin and ampicillin showed 100% inhibitions even at 150 µg/ml concentration.

MIC value is the lowest concentration of an antimicrobial agent that completely inhibits the growth of a microorganism as detected by the unaided eye (Balouiri *et al.*, 2016). When both extracts of the three finger millet varieties were tested against the antibiotic-sensitive strains of *S. aureus* and *B. subtilis* at 2400 µg/ml concentration, no color changes from blue to pink or colorless were observed. Therefore, MIC values of the extracts against *S. aureus* and *B. subtilis* were determined using seven different concentrations (600, 900, 1200, 1500,

1800, 2100 and 2400 µg/ml) and the MIC values of the extracts against *S. aureus* and *B. subtilis* were found to be 2100 and 1800 µg/ml, respectively (Table 1). However, the MIC values of the extracts were significantly higher than the MIC values of the standard drugs. Since color changes from blue to pink were observed when both extracts of the three varieties were tested against the antibiotic-sensitive strains of *S. flexneri*, *P. aeruginosa*, *E. coli* and *S. typhi* at 2400 µg/ml concentration, MIC values of the extracts against these four bacterial strains should

be higher than 2400 µg/ml. Crude plant extracts are generally a mixture of active and non-active compounds. Therefore,

higher MIC values were expected when compared to the standard drugs.

Table 1: Minimum inhibitory concentration values of the finger millet extracts against *Staphylococcus aureus* (ATCC® 6538™) and *Bacillus subtilis* (ATCC® 23857™)

Extract / Drug	Minimum inhibitory concentration value (µg/ml)	
	<i>Staphylococcus aureus</i> (ATCC® 6538™)	<i>Bacillus subtilis</i> (ATCC® 23857™)
Ravi E	2100	1800
Rawana E	2100	1800
Oshadha E	2100	1800
Ravi M	2100	1800
Rawana M	2100	1800
Oshadha M	2100	1800
Ampicillin	6.25	0.19
Ofloxacin	100	0.19

Results are presented as mean values (n = 3). E: Ethanolic extract, M: Methanolic extract.

Antibacterial activities of the ethanolic and methanolic extracts of the three finger millet varieties against antibiotic-resistant bacterial strains are shown in Figure 3. Both extracts showed dose-dependent inhibitory activities against the tested antibiotic-resistant bacterial strains. However, none of the extracts of the three varieties showed more than 45% inhibition against the antibiotic-resistant

bacterial strains even at 2400 µg/ml concentration. The inherent resistance mechanisms of these antibiotic-resistant bacterial strains which function through a restrictive outer membrane barrier and transenvelope multidrug resistance pumps (Girish and Satish, 2008) may be responsible for withstanding the inhibitory activities of the finger millet extracts.

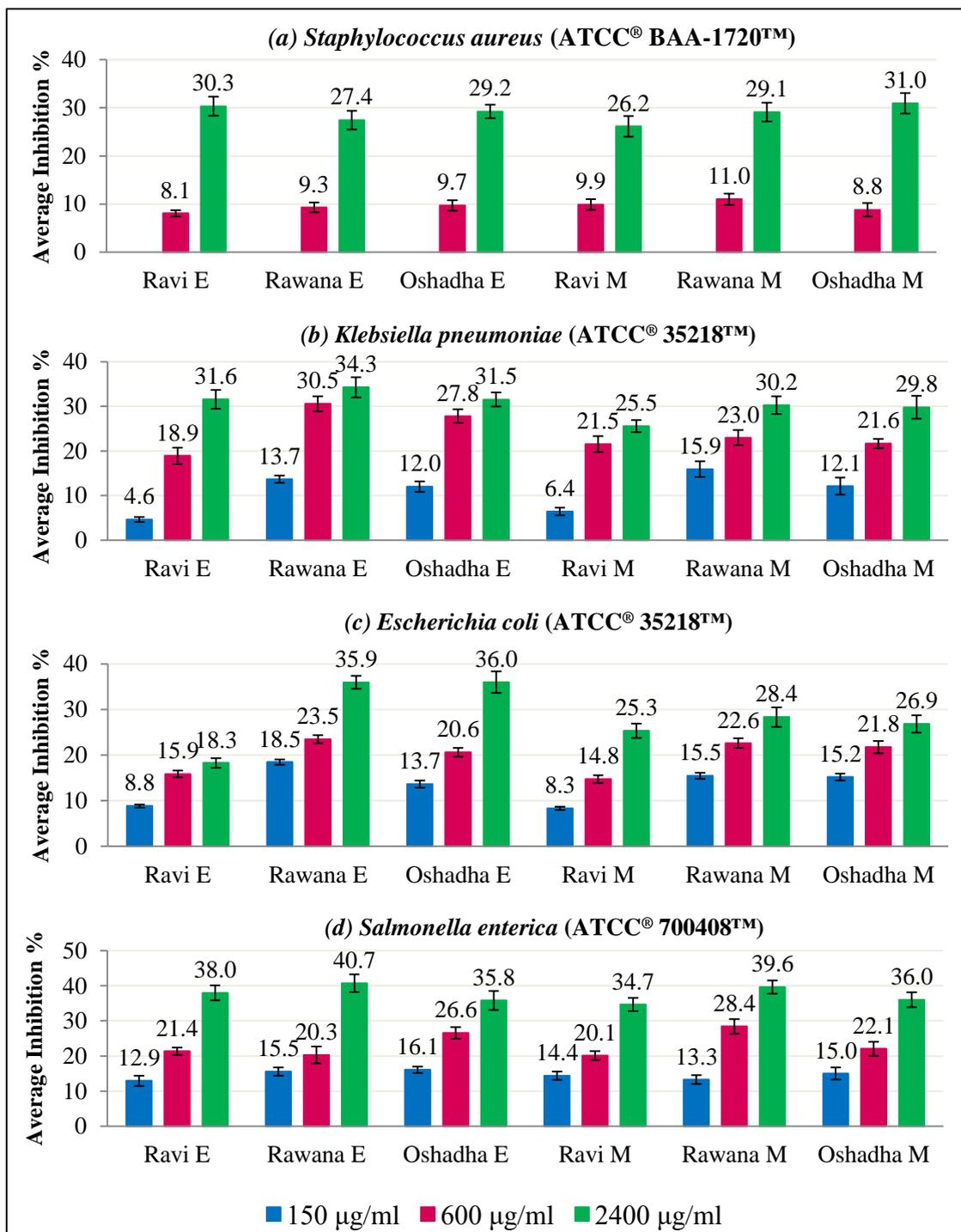


Figure 3: Antibacterial activities of the finger millet extracts against antibiotic-resistant bacterial strains at 150, 600 and 2400 µg/ml concentrations

Results are presented as mean values (n = 3). Error bars indicate the standard error. E: Ethanolic extract, M: Methanolic extract. Ofloxacin and ampicillin showed more 100% inhibitions even at 150 µg/ml concentration.

3.2 Antifungal activity

Most of the antifungal research conducted to date has assessed ethanol or methanol extracts of plants while few studies have utilized aqueous extracts. Furthermore, alcohols facilitate a complete extraction of polar compounds and many of alcoholic plant extracts possess antifungal activities (Webster *et al.*, 2008). In the present study, ethanolic and methanolic extracts of Ravi, Rawana and Oshadha finger millet varieties were evaluated for

antifungal activities. Although none of the extracts of the three finger millet varieties was active against the tested fungal strains at 400, 800 and 1600 µg/ml concentrations, the extracts showed less than 10% and 30% inhibitions against *F. lini* and *A. niger*, respectively at 3200 µg/ml concentration (Table 2). However, none of the extracts can be considered as active against the tested fungal strains at the tested concentrations.

Table 2: Antifungal activities of the finger millet extracts at 3200 µg/ml concentration

Extract / Solvent / Drug	% Inhibition					
	CA	CG	MC	TR	AN	FL
Ravi E	NI	NI	NI	NI	17.3 ± 0.7 ^d	4.3 ± 0.7 ^c
Rawana E	NI	NI	NI	NI	24.3 ± 0.7 ^b	6.7 ± 0.9 ^{bc}
Oshada E	NI	NI	NI	NI	20.7 ± 0.7 ^c	5.7 ± 0.7 ^c
Ravi M	NI	NI	NI	NI	15.3 ± 0.7 ^d	4.7 ± 0.9 ^c
Rawana M	NI	NI	NI	NI	25.7 ± 0.3 ^b	9.7 ± 0.9 ^b
Oshada M	NI	NI	NI	NI	20.7 ± 0.7 ^c	5.7 ± 0.7 ^c
DMSO	NI	NI	NI	NI	NI	NI
Amphotericin B	NA	NA	NA	NA	100.0 ± 0.0 ^a	NA
Miconazole	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	NA	100.0 ± 0.0 ^a

Results are presented as mean ± SE (n = 3). Mean values in a column superscripted by different letters are significantly different at $P < 0.05$. E: Ethanolic extract; M: Methanolic extract; NI: No inhibition; NA: Not applicable; CA: *Candida albicans*; CG: *Candida glabrata*; MC: *Microsporium canis*; TR: *Trichophyton rubrum*; AN: *Aspergillus niger*; FL: *Fusarium lini*.

3.3 β -Lactamase enzyme inhibitory activity

The common antibiotics which are extensively used for the treatment of many infectious diseases such as penicillins, cephalosporins, carbapenems and monobactams are β -lactam antibiotics which contain a β -lactam ring in the molecular structure (Atta-ur-Rahman *et al.*, 2001; Drawz and Bonomo, 2010). β -Lactamases such as penicillinases, cephalosporinases and carbapenemases are plasmid or chromosomally encoded

bacterial enzymes, which can inactivate β -lactam antibiotics by efficiently hydrolyzing the amide bond of the β -lactam ring. These enzymes are produced by bacteria as a part of the resistance mechanism against β -lactam antibiotics (Atta-ur-Rahman *et al.*, 2001). Since life-saving antibiotic drugs are losing their efficacy due to β -lactamase-mediated bacterial resistance, there is an increasing demand to identify novel β -lactamase inhibitors.

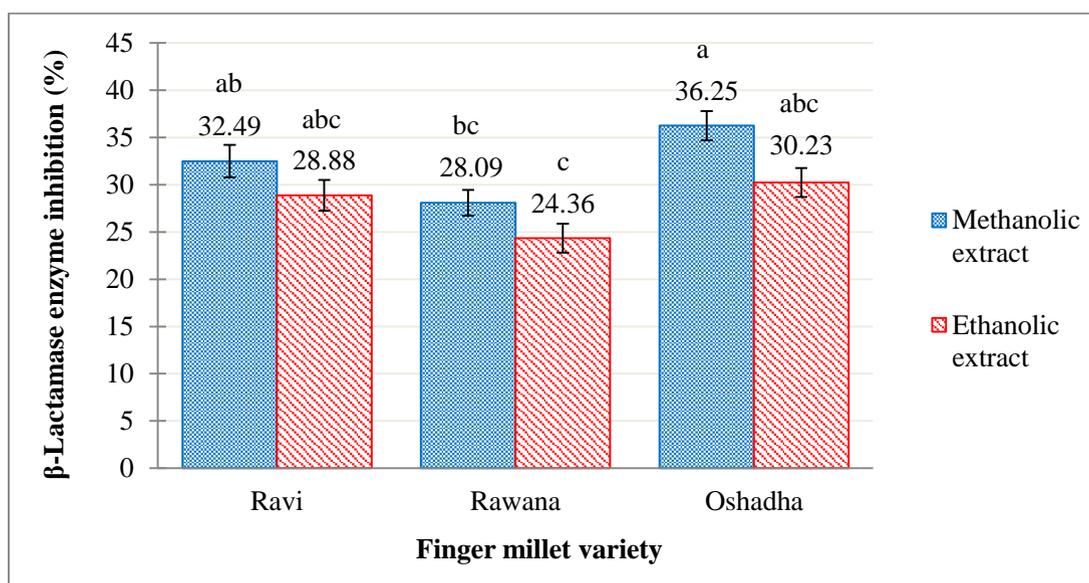


Figure 4: β -Lactamase enzyme inhibitory activities of the finger millet extracts at 2000 $\mu\text{g/ml}$ concentration

Results are presented as mean values ($n = 3$). The values followed by different letters are significantly different at $P < 0.05$. IC_{50} value of the standard, potassium clavulanate is 2.33 $\mu\text{g/ml}$.

In the present study penicillinase was used as a β -lactamase enzyme and nitrocefin, a chromogenic cephalosporin, was used as the substrate. Nitrocefin is sensitive to hydrolysis by all known β -lactamase enzymes produced by both Gram-positive and Gram-negative bacteria and exhibits a

distinctive color change from light yellow to red with the hydrolysis of the amide bond in the β -lactam ring by a β -lactamase (Bidya and Suman, 2014). There were no significant differences ($P > 0.05$) between the ethanolic and methanolic extracts of Ravi, Rawana and

Oshadha finger millet varieties in inhibiting the β -lactamase enzyme. However, all extracts showed less than 40% inhibitions even at 2000 $\mu\text{g/ml}$ concentration (Figure 4). Therefore, they do not qualify as promising sources of β -lactamase inhibitors at the tested concentration.

4 CONCLUSIONS

To the best of our knowledge, this is the first report revealing antibacterial, antifungal and β -lactamase enzyme inhibitory activities of any extract of any finger millet variety cultivated in Sri Lanka. Ethanolic and methanolic extracts of Ravi, Rawana and Oshadha finger millet varieties showed dose-dependent inhibitory activities against the tested antibiotic-sensitive and antibiotic-resistant bacterial strains. Both extracts exhibited high inhibitions against the tested Gram-positive antibiotic-sensitive bacterial strains when compared to the tested Gram-negative antibiotic-sensitive bacterial strains. The results of the present study provided evidence for the antibacterial potential of both ethanolic and methanolic extracts of the three finger millet varieties against antibiotic-sensitive *S. aureus* (ATCC[®] 6538TM) and *B. subtilis* (ATCC[®] 23857TM) strains. However, none of the extracts of the three varieties can be considered either as active against the tested fungal strains or as promising sources of β -lactamase inhibitors at the tested concentrations.

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