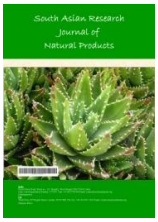

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Glutathione S-Transferase and β-Glucuronidase Enzymes Inhibitory and Cytotoxic Activities of Ethanolic and Methanolic Extracts of Sri Lankan Finger Millet (*Eleusine coracana* (L.) Gaertn.) Varieties

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Authors' contributions

This work was carried out in collaboration among all authors. Author SASJ conducted the experiments, managed the literature searches, performed the statistical analysis and wrote the first draft of the manuscript. Authors RI and AN assisted in conducting cytotoxicity studies. Authors JKRRS, GHCMH, MJG and MIC supervised the research and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: The adverse side effects of cancer management and the increasing rate of mortality associated with cancers create a necessity to discover alternative strategies in cancer management and new anticancer agents especially derived from plant materials.

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Aims: This study was focused on evaluating glutathione S-transferase and β-glucuronidase enzymes inhibitory activities and cytotoxicities against human lung and breast cancer cell lines of ethanolic and methanolic extracts of Ravi, Rawana and Oshadha finger millet varieties in view of the anticancer potential.

Methodology: Flours of whole grains of the finger millet varieties were extracted with ethanol and methanol separately. Glutathione S-transferase and β-glucuronidase enzymes inhibitory activities of the extracts were evaluated using standard methods. Cytotoxic effects on brine shrimps were evaluated as a preliminary assessment of toxicity. Growth inhibitory properties were studied on human lung and breast cancer cell lines and mouse embryo normal cell line using the MTT assay.

Results: The findings revealed dose-dependent glutathione S-transferase and β-glucuronidase enzymes inhibitory activities of ethanolic and methanolic extracts of Ravi, Rawana and Oshadha finger millet varieties. All extracts were non-cytotoxic upon normal cells and showed growth inhibitory properties upon human breast cancer cells. Methanolic extract of Oshadha showed the highest glutathione S-transferase (IC_{50} value: 236.18 µg/mL) and β-glucuronidase (IC_{50} value: 125.31 µg/mL) inhibitory activities and highest cytotoxicity upon human breast cancer cells. However, all extracts did not qualify as promising sources of cytotoxic agents against human lung cancer cells at the tested concentrations.

Conclusion: The findings of the present study suggested the potential of ethanolic and methanolic extracts of Ravi, Rawana and Oshadha finger millet varieties to enhance the efficacy of chemotherapy and to prevent and manage liver, colon and breast cancers. The specific compounds which are responsible for each activity will be studied in future.

Keywords: Brine shrimp lethality assay; cancer cell lines; cytotoxicity; enzyme inhibition; finger millet; β-glucuronidase; glutathione S-transferase

1. INTRODUCTION

Cancer is one of the most severe health problems in both developing and developed countries worldwide [1]. It is one of the most life-threatening diseases, which occurs due to complex molecular changes within the cells [2,3]. Cancer is a population of abnormal cells which grow and spread through uncontrolled cell division. These cells have the ability to invade other tissues and spread to more distant parts of the body [1,4]. Pathogenesis mechanisms of cancer include DNA damage, oxidative stress and chronic inflammation. More than 200 different types of human cancers are known up to date and lung cancer is the most common cancer diagnosed in males, while breast cancer is the most common cancer diagnosed in females worldwide [1,5].

Radiotherapy, chemotherapy and surgery are among the conventional treatments of cancer. However, the adverse or toxic side effects of cancer radiotherapy and chemotherapy and the increasing rate of mortality associated with cancer create a necessity to discover alternative strategies in cancer management and new anticancer agents derived from nature, especially from plant materials [1,3]. Plants have been used as important sources of anticancer constituents and the application of plant based compounds as anticancer agents can be traced back to 1950s [5,6]. Plant based phytochemicals which possess anticancer and chemo protective effects are safer for long-term use in cancer patients and they reduce the side effects of conventional cancer therapy due to effective antioxidant activities [7].

Glutathione (GSH) is a tripeptide which involves in the synthesis of proteins and nucleic acids and in protecting cells by detoxifying free radicals and peroxides. GSH involves in the detoxification of electrophiles. Glutathione S-transferase (EC 2.5.1.18) is a detoxification enzyme which catalyze the reaction between GSH and cytotoxic agents, containing electrophilic centers, to produce a conjugate that is chemically less reactive, less toxic, water-soluble and can be easily excreted from the body. Consequently glutathione S-transferase involves in inactivation of various electrophile-producing anticancer agents via conjugation to GSH. Electrophilic centers present in current anticancer agents react with GSH to easily form conjugates, which are then excreted from the body. In this case, glutathione S-transferase utilizes its detoxification mechanisms in the biotransformation of these anticancer agents into harmless metabolites leading to the failure of chemotherapy [8,9]. The involvement of glutathione S-transferase in anticancer drug

resistance leads to discover novel agents that can inhibit glutathione S-transferase, in order to enhance the efficacy of chemotherapy.

Glucuronidation is a major detoxification process in mammals and a major pathway of the phase II xenobiotic biotransformation. During glucuronidation, toxic compounds including metabolic wastes and xenobiotics are conjugated with glucuronic acid. This conjugation deactivates toxic compounds and subsequently eliminates them from the body. β-Glucuronidase (EC 3.2.1.31) is a typical lysosomal enzyme which interrupts this process by catalyzing the hydrolysis of glucuronide conjugates. Therefore, over expression of this enzyme is related to liver injuries and certain cancers including liver and colon cancers [10-12]. β-Glucuronidase inhibitors are capable of reducing the carcinogenic potential of toxic compounds normally excreted in bile after glucuronidation. Using silymarin, a commercially available plant derived β-glucuronidase inhibitor, in the treatment of liver disorders and certain cancers confirms the therapeutic significance of inhibiting the catalytic action of β-glucuronidase. However, silymarin has a poor bioavailability and certain other limitations related to the gastrointestinal tract like bloating, dyspepsia, nausea, irregular stool and diarrhea. It also causes pruritus, headache, exanthema, malaise, asthenia and vertigo. Therefore, there is a need to identify effective β-glucuronidase inhibitors with clinical efficacy and safety [12,13].

During the past few years, dietary plant polyphenols have received a remarkable attention for their health benefits [14]. Plant polyphenols are extensively used in cancer research due to their potent antioxidant properties [15]. Among the common cereals such as rice, wheat, maize and barley, finger millet (*Eleusine coracana* (L.) Gaertn.) is a rich source of polyphenols [15,16]. Finger millet grains are well known for the health benefits and have the ability of reducing the risk of cancers [17]. Sen et al. [18] and Sen and Dutta [19] have reported the anticancer properties of finger millet against human chronic myeloid leukemia cells (K562). Singh et al. [15] reported the anticancer activity of finger millet against human hepatic cancer cells (HepG2).

Finger millet is the third important cereal cultivated in Sri Lanka after rice and maize [20] and commonly cultivated and consumed in Sri Lanka since ancient times [21]. However, there is a scarcity in scientific evidences on anticancer properties of locally grown finger millet varieties. Since there is a genetic diversity in finger millet worldwide and there are varietal differences in composition with respect to every constituent [22], it is important to study the anticancer potential of the finger millet varieties which are commonly cultivated and consumed in Sri Lanka. The present study was focused on evaluating Sri Lankan finger millet varieties for glutathione S-transferase and β-glucuronidase inhibitory activities and cytotoxicities against brine shrimps, human lung and breast cancer cell lines and normal cell lines in search of their anticancer potential.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The finger millet varieties which are currently recommended for cultivation by the Department of Agriculture, Sri Lanka namely Ravi, Rawana and Oshadha were selected for the study and the samples were collected from the Field Crops Research and Development Institute (FCRDI), Mahailuppallama, Sri Lanka. Finger millet grains were certified by the Seed Certification Service of the Department of Agriculture, Sri Lanka. The grains were dehusked (TM05C, 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 ethanol and methanol separately. Whole grain flour (100 g) was extracted with the solvent (400 mL) overnight at the room temperature (28 ± 2°C) using a magnetic stirrer and centrifuged at 4000 rpm for 20 min. The supernatant was collected separately and the residue was re-extracted twice using the same conditions. The supernatants were combined and evaporated to dryness under reduced pressure at 40°C using a rotary evaporator. The solvent free extracts were stored in airtight glass containers at -20°C until use for the analysis.

2.2 Chemicals, Reagents, Enzymes and Cell Lines

Dimethyl sulfoxide (DMSO), glutathione S-transferase, GSH, 1-chloro-2,4-dinitrobenzene, tannic acid, β-glucuronidase, 4-nitrophenly-β-D-glucuronide, D-saccharic acid 1,4-lactone and doxorubicin were purchased from Sigma Aldrich, MO, USA. Roswell Park Memorial Institute

(RPMI) 1640 medium was purchased from Thermo Fisher Scientific Inc., Massachusetts, USA. 3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Bio Basic Inc., Markham, Canada. Etoposide was purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan. Sea salt was purchased from Instant Ocean, Inc., USA. Shrimp eggs were purchased from San Francisco Bay Brand, Inc., USA. Human lung carcinoma (NCI-H460), human breast adenocarcinoma (MCF-7) and mouse embryo (3T3) cell lines were purchased from American Type Culture Collection (ATCC), Manassas, USA. All other chemicals and reagents used in the experiments were of ACS, HPLC and analytical grades.

2.3 Determination of Glutathione S-Transferase Enzyme Inhibitory Activity

Glutathione S-transferase enzyme inhibitory activity was determined according to the method described by Samaradivakara et al. [23]. Finger millet extract (50 µL) was mixed with potassium phosphate buffer (170 µL) and 10 µL of glutathione S-transferase enzyme (6.5 U/mL) and incubated at 37°C for 10 min. Then, 30 mM GSH (10 µL) and 30 mM 1-chloro-2,4-dinitrobenzene (10 µL) were added and the change of absorbance was monitored at wavelength of 340 nm for a period of 15 min at 20 s intervals using a microplate reader (SpectraMax Plus^{384}, Molecular Devices Inc., USA). The maximum velocity (V_{max}) values of the sample (V_{maxS}) and control (V_{maxC}) were recorded. Tannic acid was used as the standard. Glutathione S-transferase enzyme inhibitory activity as percentage inhibition was calculated using the following equation. IC_{50} value was calculated using dose-response graphs.

$$\text{Inhibition \%} = [(V_{maxC} - V_{maxS}) / V_{maxC}] \times 100$$

2.4 Determination of β-Glucuronidase Enzyme Inhibitory Activity

β-Glucuronidase enzyme inhibitory activity was determined according to the method described by Acharya and De [11]. Finger millet extract (20 µL) was mixed with 120 µL of 0.1 M sodium phosphate buffer (pH 7.0) and 10 µL of β-glucuronidase enzyme (5000 U/mL) and incubated at 37°C for 30 min. The reaction was initiated with the addition of 0.4 mM 4-nitrophenyl β-D-glucuronide (50 µL) and the reaction mixture was incubated at 37°C for 20 min. Absorbance values of the sample (A_S) and control (Ac) were measured at wavelength of 540 nm using a microplate reader (SpectraMax Plus^{384}, Molecular Devices Inc., USA). D-Saccharic acid 1,4-lactone was used as the standard. β-Glucuronidase enzyme inhibitory activity as percentage inhibition was calculated using the following equation. IC_{50} value was calculated using dose-response graphs.

$$\text{Inhibition \%} = [(A_C - A_S) / A_C] \times 100$$

2.5 Determination of Cytotoxic Effects Using the Brine Shrimp Lethality Assay

Cytotoxicities of the ethanolic and methanolic extracts of the three finger millet varieties were evaluated using the brine shrimp lethality assay as described by Hussain et al. [24] and Olowa and Nuñeza [25]. Finger millet extract (20 mg) was dissolved in 2 mL of the respective solvent and 5, 50 and 500 µL of the solution were added to 3 glass vials (to obtain final concentrations in the vials as 10, 100 and 1000 µg/mL, respectively). The solvents were allowed to evaporate overnight at the room temperature. Artificial sea water was prepared by dissolving 38 g of sea salt in 1 L of deionized water and added to a hatching chamber. Shrimp eggs were added into the dark area of the hatching chamber and kept at the room temperature for the shrimps to hatch and mature as nauplii. After 48 hrs, phototropic nauplii, who were attracted to the illuminated area of the hatching chamber, were used for the assay. Using pasteur pipettes, 10 freshly hatched free-swimming nauplii were introduced into each glass vial, total volume in the vial was adjusted to 5 mL with artificial sea water and incubated at the room temperature under illumination. After 24 hrs, the number of surviving nauplii were counted and recorded. Etoposide was used as the standard reference cytotoxic drug. Data were analyzed by the probit analysis method using the Finney statistical software and LC_{50} value was determined as the measure of cytotoxicity of the extracts. Mortality percentage was calculated using the following equation.

$$\text{Mortality \%} = \frac{\text{Number of dead nauplii}}{\text{Initial number of nauplii}} \times 100$$

2.6 Determination of Cytotoxic Effects on Cancer and Normal Cell Lines

Cytotoxicities of the ethanolic and methanolic extracts of the three finger millet varieties against

human lung cancer (NCI-H460), human breast cancer (MCF-7) and mouse embryo (3T3) cell lines were evaluated using the MTT assay as described by Atta-ur-Rahman et al. [26]. Cell lines were cultured in RPMI 1640 medium and incubated in a humidified 5% CO_2 incubator (IR Water-Jacketed Incubator NU-8700 E, NuAire, USA) at 37°C for 24 hrs. Cells were harvested, counted using a haemocytometer and diluted with media. Lung cancer (5000 cells/well), breast cancer (7000 cells/well) and mouse embryo (5000 cells/well) cells (100 µL) were plated in 96-well tissue culture treated flat bottom microplates and incubated at 37°C for 24 hrs in a humidified 5% CO_2 incubator. After the incubation, media were discarded, finger millet extract (20 µL) and fresh media (180 µL) were added to the wells and incubated at 37°C for 48 hrs in a humidified 5% CO_2 incubator. After discarding the extracts and media, 200 µL of MTT reagent (0.5 µg/mL) was added to each well and incubated at 37°C for 3 hrs in a humidified 5% CO_2 incubator. Then, MTT reagent was discarded and the formazan crystals, formed by reduction of MTT were dissolved in DMSO (100 µL). Absorbance values of the sample (A_S), control (Ac) and blank (A_B) were measured at wavelength of 570 nm using a microplate reader (SpectraMax 340, Molecular Devices Inc., USA). Doxorubicin was used as the standard drug. Percentage growth inhibition of the cells were calculated using the following equation.

$$\text{Growth inhibition \%} = 100 - \left\{ \frac{(A_S - A_B)}{(A_C - A_B)} \times 100 \right\}$$

2.7 Data Analysis

In brine shrimp lethality assay, data were analyzed by the probit analysis method using the Finney statistical software and LC_{50} value was determined with a 95% confidence level. Data of other experiments were statistically analyzed using the IBM SPSS Statistics (version 20) software and results were expressed as mean ± standard error (SE). Statistical significance was set at 95% confidence level. One way analysis of variance (ANOVA) and Tukey's test were used to determine the differences among the varieties and the extracts.

3. RESULTS AND DISCUSSION

3.1 Glutathione S-Transferase Enzyme Inhibitory Activities

The abilities of the ethanolic and methanolic extracts of Ravi, Rawana and Oshadha finger millet varieties to inhibit glutathione S-transferase mediated conjugation of 1-chloro-2, 4-dinitrobenzene to GSH was evaluated and the results revealed that both extracts of the three finger millet varieties were capable of inhibiting the catalytic action of the glutathione S-transferase enzyme in a dose-dependent manner (Table 1). There were significant differences ($P < 0.05$) between the ethanolic and methanolic extracts in glutathione S-transferase enzyme inhibition and methanolic extracts of the three varieties exhibited significantly high ($P < 0.05$) inhibitory activities when compared to ethanolic extracts. Among the three varieties, Oshadha showed the highest glutathione S-transferase enzyme inhibitory activity while Rawana showing the lowest activity. Among the six finger millet extracts, methanolic extract of Oshadha had the lowest IC_{50} value indicating the highest glutathione S-transferase enzyme inhibitory activity. Since, glutathione S-transferase enzyme involves in the biotransformation of anticancer agents into harmless metabolites leading to failure of chemotherapy, glutathione S-transferase enzyme inhibitors are capable of enhancing the efficacy of chemotherapy [8,9]. Therefore, the abilities to inhibit the glutathione S-transferase enzyme indicated the anticancer potential of Ravi, Rawana and Oshadha varieties.

3.2 β-Glucuronidase Enzyme Inhibitory Activities

The abilities of the ethanolic and methanolic extracts of Ravi, Rawana and Oshadha finger millet varieties to inhibit β-glucuronidase mediated deconjugation of 4-nitrophenyl-β-D-glucuronide were evaluated and the results indicated that both extracts of the three finger millet varieties were capable of inhibiting the catalytic action of the β-glucuronidase enzyme in a dose-dependent manner (Table 2). There were no significant differences ($P > 0.05$) between the ethanolic and methanolic extracts in β-glucuronidase enzyme inhibition. Among the three varieties, Oshadha showed the highest β-glucuronidase enzyme inhibitory activity while Rawana showing the lowest activity. Among the six extracts, methanolic extract of Oshadha had the lowest IC_{50} value indicating the highest β-glucuronidase enzyme inhibitory activity. Since β-glucuronidase enzyme is capable of interrupting the glucuronidation process, the catalytic action of β-glucuronidase enzyme is related to liver injuries and certain cancers including liver and colon cancers. Therefore, β-

glucuronidase enzyme inhibitors are capable of preventing and managing liver and colon cancers [10,11]. Consequently, the abilities to inhibit the β-glucuronidase enzyme indicated the anticancer potential of Ravi, Rawana and Oshadha varieties.

3.3 Cytotoxic Effects on Brine Shrimps

Various assays are used to evaluate potential toxicity of plant extracts based on different biological models. Due to the ethical issues in toxicological tests, substituting animals with alternative models is very important. Brine shrimp lethality assay can be considered as an appropriate solution for this, especially since it is classified as an *in vivo* assay [27]. In the present study, ethanolic and methanolic extracts of Ravi, Rawana and Oshadha finger millet varieties were evaluated for their cytotoxic effects on brine shrimp nauplii as a preliminary assessment of toxicity. All extracts were evaluated at three different concentrations (10, 100 and 1000 µg/mL) and observed for their toxic effects on brine shrimp nauplii after 24 hrs of treatment and the results are presented in Table 3. According to Meyer's toxicity index, extracts with LC_{50} values lower than 1000 µg/mL are considered as toxic, while extracts with LC_{50} values higher than 1000 µg/mL are considered as non-toxic. According to Clarkson's toxicity criterion for the toxicity assessment of plant extracts, extracts with LC_{50} values higher than 1000 µg/mL are non-toxic, extracts with LC_{50} values between 500 and 1000 µg/mL are low toxic, extracts with LC_{50} values between 100 and 500 µg/mL are medium toxic while extracts with LC_{50} values between 0 and 100 µg/mL are highly toxic [28]. According to the results of the present study, both ethanolic and methanolic extracts of the three finger millet varieties showed no brine shrimp lethality at the tested concentrations indicating the extracts are non-cytotoxic.

3.4 Cytotoxic Effects on Cancer and Normal Cell Lines

MTT *in vitro* cell proliferation assay is one of the most widely used assays to evaluate preliminary anticancer activity. It is based on the capacity of mitochondrial dehydrogenase enzymes in metabolically active cells to reduce the yellow colour water-soluble MTT and to convert into purple colour water-insoluble formazan which precipitates in the cellular cytosol and can be dissolved after cell lysis.

Table 1. Glutathione S-transferase enzyme inhibitory activities of ethanolic and methanolic extracts of the finger millet varieties

Extract / Standard	Glutathione S-transferase enzyme inhibitory activity	
	Inhibition % at 800 µg/mL assay concentration	IC_{50} values (µg/mL)
Ravi E	74.19 ± 0.79^{c}	305.53 ± 2.33^{bc}
Rawana E	73.79 ± 1.09^{c}	348.13 ± 4.29^{a}
Oshadha E	78.10 ± 0.34^{b}	283.50 ± 3.09^{cd}
Ravi M	76.49 ± 0.59^{bc}	273.27 ± 9.91^{d}
Rawana M	74.53 ± 0.27^{c}	307.80 ± 2.00^{b}
Oshadha M	83.34 ± 0.31^{a}	236.18 ± 1.17^{e}
Tannic acid	NA	11.76 ± 0.15^{f}

Results are presented as mean ± SE (n = 3). Within a column, mean values superscripted by different letters are significantly different at P < 0.05. E: Ethanolic extract; M: Methanolic extract; NA: Not applicable.

Table 2. β-Glucuronidase enzyme inhibitory activities of ethanolic and methanolic extracts of the finger millet varieties

Extract / Standard	β-Glucuronidase enzyme inhibitory activity	
	Inhibition % at 500 µg/mL assay concentration	IC_{50} (µg/mL)
Ravi E	80.96 ± 1.37^{a}	144.43 ± 5.60^{abc}
Rawana E	83.69 ± 0.47^{a}	162.02 ± 6.14^{a}
Oshadha E	85.46 ± 2.12^{a}	134.87 ± 1.86^{bc}
Ravi M	81.79 ± 1.28^{a}	155.42 ± 3.55^{ab}
Rawana M	83.31 ± 1.42^{a}	163.91 ± 4.43^{a}
Oshadha M	85.42 ± 0.47^{a}	125.31 ± 3.36^{c}
D-Saccharic acid 1,4-lactone	NA	12.40 ± 0.45^{d}

Results are presented as mean ± SE (n = 3). Within a column, mean values superscripted by different letters are significantly different at P < 0.05. E: Ethanolic extract; M: Methanolic extract; NA: Not applicable.

Table 3. Cytotoxic effects of ethanolic and methanolic extracts of the finger millet varieties on brine shrimps

Extract / Solvent / Standard	Mortality % at different concentrations			LC_{50} values (µg/mL)
	10 µg/mL	100 µg/mL	1000 µg/mL	
Ravi E	0.0 ± 0.0	3.3 ± 3.3	6.7 ± 3.3	>1000
Rawana E	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 3.3	>1000
Oshada E	0.0 ± 0.0	0.0 ± 0.0	6.7 ± 3.3	>1000
Ravi M	0.0 ± 0.0	0.0 ± 0.0	6.7 ± 3.3	>1000
Rawana M	0.0 ± 0.0	3.3 ± 3.3	10.0 ± 5.8	>1000
Oshada M	0.0 ± 0.0	3.3 ± 3.3	0.0 ± 0.0	>1000
Methanol	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 3.3	>1000
Ethanol	0.0 ± 0.0	3.3 ± 3.3	0.0 ± 0.0	>1000
Etoposide	6.7 ± 3.3	23.3 ± 3.3	93.3 ± 3.3	477.6 ± 16.8

Results are presented as mean ± SE (n = 3). E: Ethanolic extract; M: Methanolic extract.

Table 4. Cytotoxic effects of ethanolic and methanolic extracts of the finger millet varieties on human breast cancer (MCF-7) cells at 100 and 500 µg/mL assay concentrations

Finger millet variety	Growth inhibition (%)			
	100 µg/mL		500 µg/mL	
	Ethanolic extract	Methanolic extract	Ethanolic extract	Methanolic extract
Ravi	34.55 ± 1.47^{b}	47.24 ± 0.74^{a}	49.68 ± 1.50^{b}	58.46 ± 1.39^{b}
Rawana	27.52 ± 0.77^{c}	32.53 ± 1.08^{b}	40.02 ± 1.16^{c}	48.57 ± 1.30^{c}
Oshadha	42.60 ± 0.93^{a}	47.83 ± 0.70^{a}	55.45 ± 1.08^{a}	63.93 ± 1.00^{a}

Results are presented as mean ± SE (n = 3). Within a column, mean values superscripted by different letters are significantly different at $P < 0.05$.

Cells which are dead following a toxic damage cannot reduce MTT into formazan and production of formazan is proportionate to the number of viable cells [29,30]. Since lung cancer and breast cancer are the most prevalent cancers among males and females, respectively [1,5], human lung cancer and breast cancer cell lines were selected for the study. Cytotoxic effects of ethanolic and methanolic extracts of finger millet varieties on human breast cancer cell line are presented in Table 4. The results showed significant differences ($P < 0.05$) in growth inhibitions among three varieties and between the extracts. Methanolic extracts of the finger millet varieties showed significantly high ($P < 0.05$) cytotoxic effects when compared to ethanolic extracts. Ethanolic and methanolic extracts of Oshadha and methanolic extract of Ravi at 500 µg/mL assay concentration showed more than 50% growth inhibition in human breast cancer cells. At 500 µg/mL assay concentration, methanolic extract of Oshadha demonstrated the highest cytotoxicity upon human breast cancer cells.

4. CONCLUSION

The findings of the present study revealed glutathione S-transferase inhibitory activities of ethanolic and methanolic extracts of Ravi, Rawana and Oshadha finger millet varieties while indicating the potential of enhancing the efficacy of chemotherapy. β-Glucuronidase inhibitory activities of the extracts indicated the potential application in the treatment of liver disorders and liver and colon cancers. In addition, the findings revealed growth inhibitory activities of ethanolic and methanolic extracts of Ravi, Rawana and Oshadha finger millet varieties upon human breast cancer cells and indicated none of the extracts of the three varieties are cytotoxic upon normal cells. Collectively, these findings indicated the anticancer potential of ethanolic and methanolic extracts of Ravi, Rawana and Oshadha finger millet varieties. Among the six finger millet extracts, methanolic extract of Oshadha showed the highest glutathione S-transferase and β-glucuronidase enzymes inhibitory activities and highest cytotoxicity upon human breast cancer cells. Crude plant extracts are generally a mixture of active and non-active compounds and the ethanolic and methanolic extracts of the three finger millet varieties used in this study may also contain a mixture of active and non-active compounds. Therefore higher IC_{50} values were expected when compared to the standard drugs. To the best of our knowledge glutathione S-

transferase and β-glucuronidase enzymes inhibitory activities of finger millet extracts have not been reported previously and this is the first report revealing glutathione S-transferase and β-glucuronidase enzymes inhibitory activities of any extract of any finger millet variety worldwide. Besides, cytotoxic effects of finger millet against human lung and breast cancer cells have not been reported previously and to the best of our knowledge this is the first report of cytotoxic effects of any extract of any Sri Lankan finger millet variety against any cancer cell line. The specific compounds which are responsible for each activity will be studied in future studies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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