Malaysian Journal of Microbiology, Vol 18(5) 2022, pp. 505-513
DOI: http://dx.doi.org/10.21161/mjm.221548

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Toxicity evaluation of the ethanolic *Jambu bol* [*Syzygium malaccense* (L.) Merr. and Perry] leaves extract and mechanisms underlying its antibacterial action

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Received 13 June 2022; Received in revised form 19 August 2022; Accepted 1 September 2022

ABSTRACT

Aims: Many plants and their derivatives are widely used in food manufacturing because of their biological activities. They play a significant role as food additives to control microbial growth and the occurrence of oxidation reactions. *Syzygium malaccense* L. is a well-known plant with biological activities such as antimicrobial and antioxidant activities. Thus, the aims of this study were to evaluate the toxicity of the ethanolic leaves extract of *S. malaccense* and to study its antibacterial mode of action.
Methodology and results: The toxicity assessment of *S. malaccense* leaves extract was determined using the brine-shrimp larvae model. The action mechanisms against bacterial membrane were determined by studying the intracellular material leakage by means of nucleic acid (DNA and RNA) release, crystal violet dye uptake and cellular protein leakage. The present findings proved the extract's safety as indicated by a high dose of 7.402 mg/mL for lethal concentration (LC_{50}) against brine-shrimp larvae. On the other hand, the ethanolic extract caused a severe membrane permeability towards all the tested bacteria as indicated by the increased intracellular material leakage in a concentration-dependent manner.
Conclusion, significance and impact of study: The current study provides valuable information regarding the safety and antibacterial action mechanism of *S. malaccense* ethanolic leaves extract, thus paving the way for its utilization as a natural preservative in a wide range of food products.

Keywords: Brine-shrimp, Bradford, cell's constituent release, crystal violet, *S. malaccense*

INTRODUCTION

Natural goods, such as those derived from plants, animals or marine sources, are increasingly used as health supplements, disease prevention agents and flavoring agents in food. Synthetic food preservatives and sanitizers are widely used to prevent microbial growth in food, but the reported side effects of using those preservatives and sanitizers are cause for concern. Because of their long history of human use, herbal products are frequently thought to be harmless or low in toxicity (Pariyani *et al.*, 2015). Even though safety is still a major concern with the use of plant extracts in food, toxicity tests must be performed to determine their safety profile. As a result, assessing the toxicological effects of any plant extract intended for use as a food preservative or food sanitizer is essential in its risk evaluation. Toxicity testing is required because some herbs consumed can be harmful and numerous cases of toxicity caused by long-term herb consumption have been reported (Ugwah-Oguejiofor *et al.*, 2019). Currently, the lethality assay with brine-shrimp, also known as *Artemia salina*, is broadly applied to determine the cytotoxicity of bioactive compounds. It involves testing the cytotoxicity of dental materials and nanostructures in addition to the toxicity of plant extracts, fungal toxins, heavy metals, cyanobacteria toxins and pesticides. As a result, it is suggested that it can be established using an animal model. Some of the other benchtop experiments include the inhibition of crown gall tumours on potato tuber discs, the control of frond proliferation in duckweed and yellow fever mosquito larvae fatality tests. The brine-shrimp lethality test is the simplest, inexpensive and effective among them (Sarah *et al.*, 2017). The brine-shrimp lethality bioassay is faster (less than 24 h), simple to execute (no aseptic procedures necessary) and inexpensive, requiring only a small amount of test material (2-20 mg or less). The bioassay is strongly associated with cytotoxic and pesticide activity in

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ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(5) 2022, pp. 505-513
DOI: http://dx.doi.org/10.21161/mjm.221548

various solid human tumours. This test was established by Michael *et al.* (1956). Since its inception, this *in vivo* lethality test has been used as a primary screening tool that can be followed up with more specific and complex bioassays once active compounds have been identified (Apu *et al.*, 2010).

Antibacterial properties of certain plant species have been extensively studied. Cinnamon, garlic, basil, curry, ginger, sage and mustard, among other herbs, have antibacterial effects in crude extracts against a broad spectrum of Gram-positive and Gram-negative bacteria. Additionally, it has been proven that extracts of Chinese chives and cassia successfully inhibit the growth of *Escherichia coli* and other germs while preserving meat, juices and milk. Many medicinal plant extracts are well known to have antimicrobial activity and understanding the mechanism of antimicrobial activity is essential in optimizing their use as natural food preservatives to increase food quality and extend the service life (Gonelimali *et al.*, 2018). *Syzygium malaccense* leaves extract was reported to be toxic to brine-shrimp when methanol, hexane and ethyl acetate were used as extraction medium (Itam and Anna, 2020). The antibacterial activity of *S. malaccense* leaves extract was proven by previous research (Savi *et al.*, 2020). However, the mechanism of action is not yet identified. In this study, the toxicity of ethanolic leaves extract of *S. malaccense* was analyzed using the brine-shrimp assay and the mechanism of action of the antibacterial activity of the extract was determined based on the bacterial cell membrane integrity.

MATERIALS AND METHODS

Plant sampling and extraction

Syzygium malaccense leaves were collected in February 2020 from Taman Pertanian, Universiti Putra Malaysia (UPM). The chosen leaves were uniform in shape, fresh, healthy and mature. Samples were cleaned under running tap water to clean them up from dust and dirt and then washed again with distilled water. The extraction method was performed using the maceration technique as described by Rukayadi *et al.* (2013), with slight modifications. Cleaned leaves were dried in the shade for 2 days, then complete drying was done in a hot-air oven (Euroasaia, Penang, Malaysia) at 43 ± 2 °C temperature for 3 h. Dried leaves were crushed to a fine powder using stainless steel grinder. A hundred grams of powdered leaves were soaked in 400 mL of absolute ethanol (RandM Chemicals, Essex, UK) in a universal bottle and incubated for 24 h in a water bath incubator shaker (Heidolph, Schwabach, Germany) at 40 ± 3 °C with 110 revolutions per minute (rpm). The ethanolic solution was filtered using Whatman filter paper size no. 2 (Whatman International Ltd., Middlesex, England) by using a continuous vacuum filtration machine and then it was concentrated using a rotary evaporator (Heidolph, Schwabach, Germany) at 40 ± 3 °C and a speed of 110 rpm. The crude extract was diluted in dimethyl sulfoxide (DMSO) to obtain 100 mg/mL and then further diluted in 1:10 (v/v) distilled water to get 10 mg/mL stock solutions.

Toxicity analysis of *S. malaccense* leaves extract

Brine-shrimp eggs of species *A. salina* sp. (JBL Artemio Mix, Germany) were commercially provided and were purchased from a marine specialist shop in Seri Kembangan, Selangor, Malaysia. The following hatching procedures were followed based on the product description. The hatching process starts with preparing artificial seawater by dissolving 35 g of table salt in 1 liter (L) of tap water, making the salt percentage 3.5, which is similar to the salt percentage of natural seawater. The pH of the artificial seawater was adjusted to pH 8.1, the natural pH of seawater. Then, 15-20 g of brine-shrimp eggs were added to the artificial seawater and then incubated for 24-48 h, where a source of light was directed to the flask that contained brine-shrimp eggs. After hatching brine-shrimp eggs, the larvae are harvested by attracting the larvae to the light, which separates them from their eggs. The larvae were pipetted into universal bottles and then counted for toxicity analysis using the light Petri plate reader (Syahmi *et al.*, 2010). The brine-shrimp lethality assay method described by Apu *et al*. (2010) was used to determine the toxicity of *S. malaccense* leaves extract. Initially, 10 mL of different concentrations of the extract and positive control potassium dichromate (R and M Marketing, Essex, UK) ranged from 10-0.001 mg/mL and 0.625-0.005 mg/mL, respectively and 10% of DMSO as negative control were prepared using the artificial. Then, ten alive larvae were added to the different prepared solutions of extract, positive and negative controls, and the results were recorded based on observing the survived larvae hourly. The graph of mean percentage mortality (%) was plotted against the logarithm of concentrations and the value of LC_{50} of the extract and potassium dichromate were calculated based on the following formulas: $y = 4.379x + 17.586$ (R^2=0.9237) and $y = 145.6x + 9.2768$ (R^2=0.9821), respectively.

Antibacterial mode of action of *S. malaccense* leaves extract

Cell's constituent release

Changes in the bacterial cell membrane result in the release of constituents such as DNA and RNA into suspension, which can be quantified using an optical density spectrometer with a wavelength of 260 nm (OD_{260}). Toa *et al*. (2014) described the method used to determine the constituents of bacterial cells released into suspension with slight modifications. Briefly, Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and Gram-negative bacteria *E. coli* and *Salmonella* Typhimurium were transferred to 40 mL of freshly prepared MHB and incubated for 24 h. Using the 0.5 MacFarland standard, the log of bacteria obtained was adjusted to 10^6-10^8 CFU/mL. Using a centrifuge

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(5) 2022, pp. 505-513
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machine (Kubota, Osaka, Japan), the inoculums were centrifuged at 3000× *g* for 20 min, resulting in the bacteria adhering to the bottom of the centrifuge tubes. The bacteria were washed 3 times and suspended in 0.1% phosphate buffer saline (PBS pH 7.0). The suspensions were then incubated at 37 °C in the presence of the extract at various concentrations 0.00, 2.50, 5.00, 10.00 and 20.00 mg/mL (0× MIC, 0.5× MIC, 1× MIC, 2× MIC and 4× MIC) for 0.5, 1, 2 and 4 h in an incubator shaker (200 rpm). The samples were added to centrifuging machine (Beckman counter, California, USA) and centrifuged for 15 min at 13400× *g*. The supernatant was used to measure the absorbance of released constituents at 260 nm with a UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan).

Crystal violet assay

Crystal violet (CV) assay is the method that was used to determine the alteration in bacterial cell's membrane permeability as described by Halder *et al*. (2015) but with some modifications. Briefly, the suspensions of the following bacteria *B. subtilis*, *E. coli*, *S. aureus* and *S.* Typhimurium, as previously prepared in the cell constituents' release step, were incubated at 37 ºC in an incubator shaker (200 rpm) for 0.5, 1, 2 and 4 h in the presence of the extract at different concentrations (0× MIC, 0.5× MIC, 1× MIC, 2× MIC and 4× MIC). Then, the samples were centrifuged at 9300× *g* for 5 min and then the supernatants were disposed of. After, 1 mL of prepared crystal violet dye (Sigma-Aldrich, Louis, USA) (10 µg/mL) was added to each sample and then incubated in an incubator shaker (200 rpm) for 10 min. Next, the samples were centrifuged at 13400× *g* for 15 min. Then the OD of the supernatants was measured at a wavelength of 590 nm. The OD value of the crystal violet solution was considered 100%. The analysis of crystal violet uptake was done by using the following formula:

Percentage uptake of crystal violet = 100 - (OD value of sample/OD value of CV solution) × 100%

Bradford protein assay

The integrity of the cell membrane was examined using the Bradford assay method (Bradford, 1976). The principle of this method is that the protein that leaked from the cell membrane into the supernatant produces a dye-binding reaction with Bradford reagent. In order to prepare its reagent, the Bradford assay needed to dissolve 100 mg of Coomassie Brilliant Blue G-250 (Sigma-Aldrich, Louis, USA) in 50 mL of 95% ethanol and then 100 mL of 85% (w/v) phosphoric acid was added to the solution. After the dye was dissolved completely, the solution was diluted in 1 L of deionized water and then filtered through Whatman filter paper no. 1. Next, the prepared suspensions of bacteria, as mentioned in the preparation for cell constituents' release test, were incubated at 37 ºC in an incubator shaker (200 rpm) for 0.5, 1, 2 and 4 h in the presence of the extract at different concentrations (0× MIC, 0.5× MIC, 1× MIC, 2× MIC and 4× MIC). Then, the samples were centrifuged at 13400× *g* for 15 min. To examine the protein leakage of bacterial cells, 25 µL of supernatants were removed to a 96-well microplate and then 200 µL of Bradford reagent were mixed and left in the dark for 10 min. Protein dye binding was measured at 595 nm using a BIO-RAD 170-6930 Benchmark plus Microplate Spectrophotometer (Bio Rad, California and United States). In a standard protein (known) test tube, 0.1-1 mL of bovine serum albumin (BSA) solution (10%) was taken. The concentration of protein in the supernatant of bacteria before and after treatment with the extract was calculated using the standard curve of optical density versus concentrations (mg/mL).

Statistical analysis

The results of cell constituents' releases, crystal violet assay and Bradford assay that presented the antibacterial mode of action of *S. malaccense* leaves extract was presented as mean ± standard deviation of triplicate experiments (n = 3 × 1) and analyzed by using Microsoft Excel version 16.0.

RESULTS

Toxicity of *S. malaccense* leaves extract on brine-shrimp

The toxicity of *S. malaccense* leaves extract was determined by measuring the lethal concentration (LC_{50}) that was responsible for killing 50% of the living larvae during the experimental time. The LC_{50} value was calculated using the best-fit-line that was plotted to present killed larvae in percentage versus concentrations of *S. malaccense* leaves extract. The determined LC_{50} values of the extract and potassium dichromate (Table 1) were 7.402 mg/mL within 5 h and 0.28 mg/mL within 4 h, respectively.

Table 1: Toxicity of *S. malaccense* leaves extract using brine-shrimp lethality assay.

Sample	LC_{50} (mg/mL)
S. malaccense leaves extract	7.402
Potassium dichromate	0.28

Cell constituents' releases

Cell constituents' release is one of the most common methods used to determine the mechanism of action of antimicrobial substances. By the method, the leaked constituents of bacterial cells into the supernatant were determined by the measurement of the absorbance at 260 nm. The results of cell constituents released when the pathogens *B. subtilis*, *E. coli*, *S.* Typhimurium and *S. aureus* were treated for 4 h with *S. malaccense* leaves extract are shown in Figure 1 (a), (b), (c) and (d). The pathogens *B. subtilis* and *S. aureus* were recorded as

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(5) 2022, pp. 505-513
DOI: http://dx.doi.org/10.21161/mjm.221548

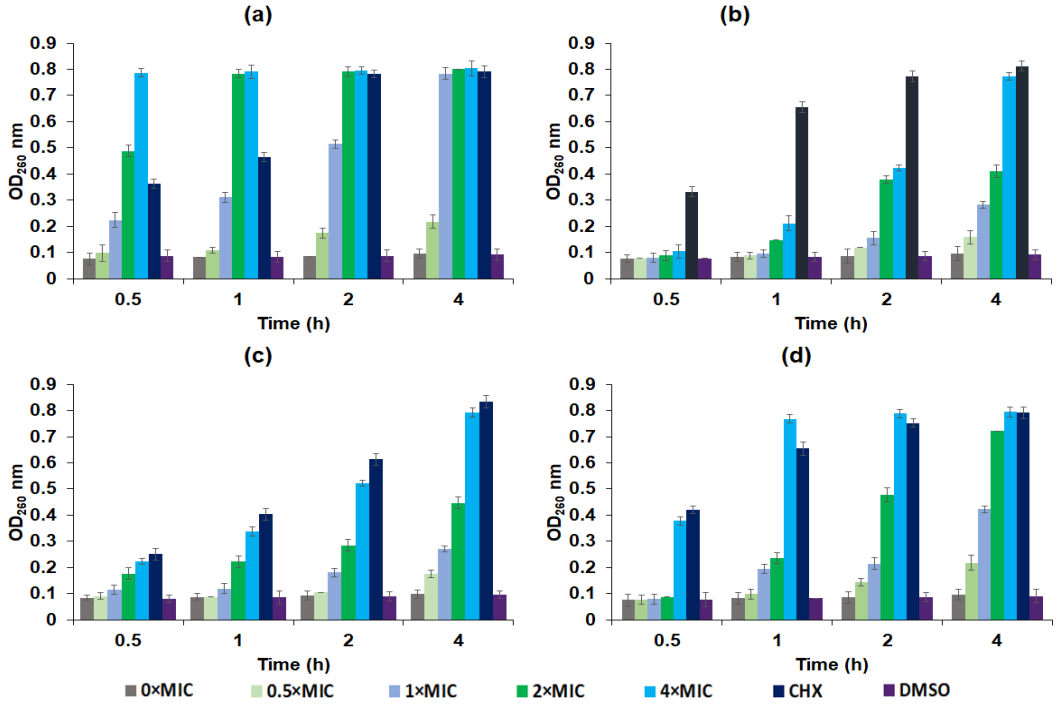

Figure 1: Cell's constituent release analysis of (a) *B. subtilis*, (b) *E. coli*, (c) *S.* Typhimurium and (d) *S. aureus* after treated with *S. malaccense* leaves extract at the concentrations of 0× MIC, 0.5× MIC, 1× MIC, 2× MIC and 4× MIC, respectively.

faster constituents released into the supernatant at 4× MIC within 0.5 h and 1 h with an absorbance value of 0.787 and 0.768, respectively. The highest released cell constituents were recorded for S. Typhimurium and *E. coil* at 4× MIC with an absorbance of 0.792 and 0.774, respectively, within 4 h. *B. subtilis* had recorded high constituents' release at 2× MIC and 1× MIC where the absorbance records were 0.784 and 0.780 within one h and four h, respectively. The positive control had recorded higher constituents' release than the extract in both *E. coli* and S. Typhimurium, where the absorbance values were 0.812 and 0.833 within 4 h, respectively.

Crystal violet assay

The Crystal violet assay is widely used for cytotoxicity and cell viability studies with adherent cell cultures, in addition to studies on the mechanisms of action of antimicrobial agents (Sasirekha *et al.*, 2015). In the present study, the supernatant of *B. subtilis*, *E. coli*, *S.* Typhimurium and *S. aureus* treated with the extract exhibited different absorbance values for crystal violet dye uptake, which were highly influenced by the concentration of the extract and the duration of treatment (Figure 2 a, b, c and d). In the untreated group (absence of the extract), the crystal violet uptake of *B. subtilis*, *E. coli*, *S.* Typhimurium and *S. aureus* were 24.61%, 22.53%, 24.17% and 20.42%, respectively. A significant increment in the uptake of crystal violet was observed after the treatment with the ethanolic extract and reached the maximum of 93.47%, 88.95%, 91.81% and 90.77% for *B. subtilis*, *E. coli*, *S.* Typhimurium and *S. aureus*, respectively.

Bradford assay

The leakage of protein from bacterial cells into the supernatant was measured using the Bradford method. The results of treated bacterial cells, including *B. subtilis*, *E. coli*, *S.* Typhimurium and *S. aureus* at different concentrations of *S. malaccense* leaves extract indicated a significant protein leakage compared to the non-treated bacteria (control group). The untreated bacterial cells exhibited a negligible protein leakage into the supernatants, which recorded leakage values in the range of 0.010 to 0.012 mg/mL for all the tested bacteria. The protein leakage of treated bacterial cells displayed a remarkable increase, in which the highest leakage into the supernatant was recorded at 0.072 mg/mL for *B. Subtilis*, followed by 0.088 mg/mL, 0.095 mg/mL and 0.092 mg/mL for *E. coli*, *S.* Typhimurium and *S. aureus*, respectively, as shown in Figure 3 (a), (b), (c) and (d).

DISCUSSION

The extraction process employed in the current investigation showed that *S. malaccense* leaves extract is considered safe for human consumption, as shown by the very high lethal concentration against larvae. The result obtained in this study is superior to several previously published research that worked on the same plant as this

ISSN (print): 1823-8262, ISSN (online): 2231-7538

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DOI: http://dx.doi.org/10.21161/mjm.221548

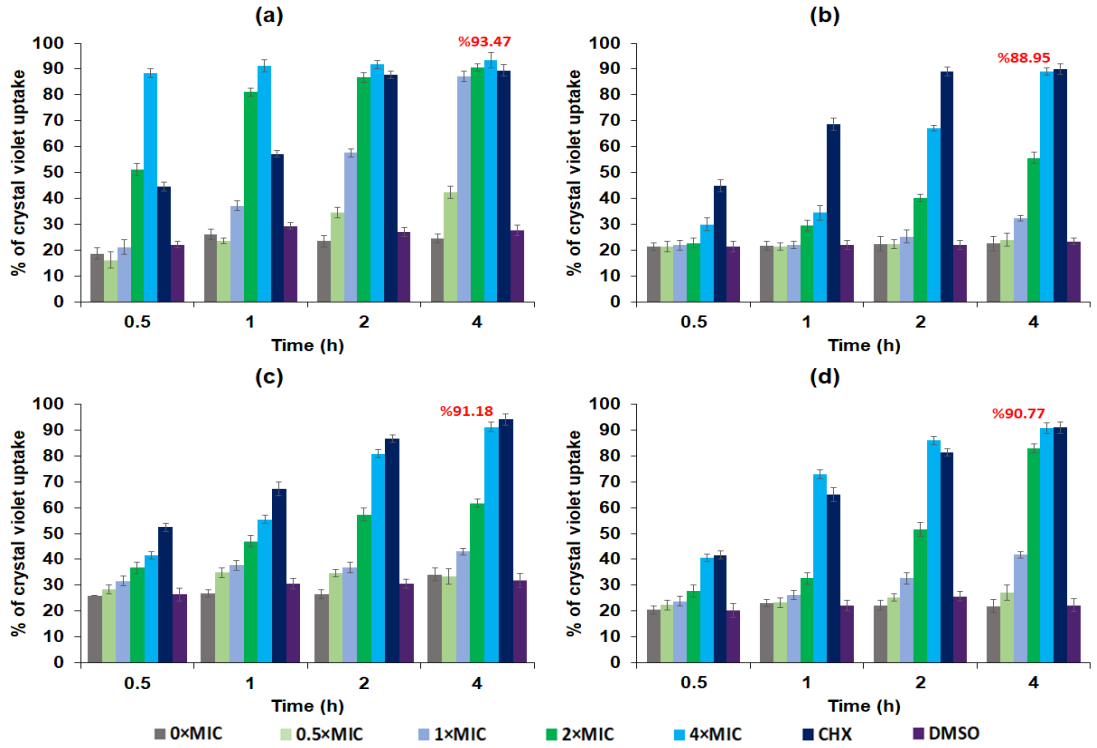

Figure 2: Crystal violet assay of (a) *B. subtilis*, (b) *E. coli*, (c) *S.* Typhimurium and (d) *S. aureus* after treated with *S. malaccense* leaves extract at the concentrations of 0× MIC, 0.5× MIC, 1× MIC, 2× MIC and 4× MIC, respectively.

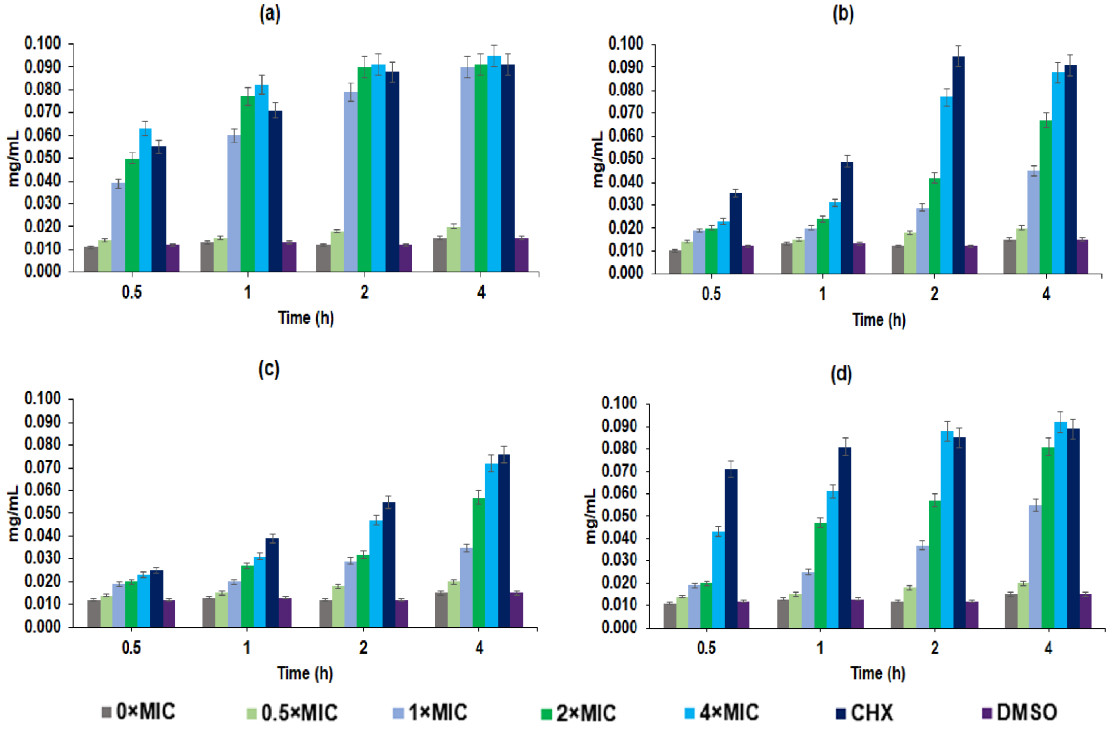

Figure 3: Bradford assay of (a) *B. subtilis*, (b) *E. coli*, (c) *S.* Typhimurium and (d) *S. aureus* after treated with *S. malaccense* leaves extract at the concentrations of 0× MIC, 0.5× MIC, 1× MIC, 2× MIC and 4× MIC, respectively.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

study. Itam and Anna (2020) reported that *S. malaccense* leaves extract had different toxicity values, which were highly dependent on the solvents used for the extraction process. For example, ethyl acetate had the highest toxicity effect with an LC_{50} value of 70 µg/mL, while other extracts using hexane and methanol showed a toxic effect at only 204 µg/mL and 329 µg/mL, respectively. Meyer *et al*. (1982) had set the toxicity consideration of plant extracts based and their LC_{50} and he reported that plant extract with an LC_{50} value greater than 1 mg/mL is considered safe or non-toxic, while LC_{50} value of less than 1 mg/mL is considered significantly toxic. It is noteworthy that our findings obtained in the current study signify that the ethanolic extract of *S. malaccense* leaves could be considered safe for its development as natural preservatives as it recorded an LC_{50} value of more than 1 mg/mL compared to other extracts produced by different solvents and potassium dichromate which were significantly toxic as they achieved LC_{50} values at a concentration of less than 1 mg/mL. Potassium dichromate is commonly used in this assay due to its well-known toxicity. The loss of cell constituents could be interpreted as irreversible damage to the bacterial cytoplasmic membranes. Accordingly, these results suggested that pathogens exposed to the leaves extract released their cell constituents more readily with the increased concentration of the extract and longer incubation time compared to the control group (untreated strains). According to Ramli *et al*. (2017), the highest cell release of *S. aureus* was indicated by the highest absorbance, which was at 0.551 after being exposed to *Syzygium polyanthum* leaves extract for 4 h. Karsha and Lakhsmi (2010) reported the leakage of *S. aureus* contents when treated with *Piper nigrum* (Black Piper) extract for 1.5 h, where the absorbance was 0.340. The results discussed the possibility of the black piper extract altering the membrane permeability of bacterial cells, resulting in the leakage of the cells' constituents of those absorbed at an OD_{260} nm wavelength, such as DNA, RNA and protein. By comparison, in this study, *S. malaccense* leaves extract exhibited a better antibacterial effect against *S. aureus* with an absorbance of 0.768 nm at the same concentration and incubation time.

According to Papuc *et al.* (2017) and Górniak *et al.* (2019), the antibacterial activity of polyphenols and flavonoids compounds was contributed to some important factors, including salt concentration, availability of nutrients for pathogen growth and multiplication, bacterial cell surface properties and the structure of the specific phytochemical compounds, such as the number and positions of OH groups bonded to both the aromatic rings and the oxygen substituted ring. In general, interactions between pathogenic microbes and polyphenolic compounds are non-specific. They are influenced by hydrogen groups and hydrophobic effects, which can significantly affect the formation of covalent bonds and lipophilic forces (Taguri *et al.*, 2006; Górniak *et al.*, 2019). As a result, several extracts' antibacterial properties were attributed to a variety of mechanisms of action. Cell membrane integrity is crucial for osmotic protection, transport activities and cell biosynthesis, and its disruption can result in bacterial death (Taguri *et al.*, 2006). According to Yi *et al*. (2014), tea extract has a diversity of polyphenolic compounds, which can increase the permeability of bacterial cell membranes and cause some damage, allowing biological molecules to seep out. Ren *et al.* (2019) have reported that the pterostilbene produced from Xinjiang wine grape could affect the cell membranes of *S. aureus* and *E. coli*, inducing cell membrane depolarization.

According to several studies, the unsaturated fatty acids located in the cell membrane are targeted by ROS, which causes lipid peroxidation and subsequently, cell membrane disintegration, which can lead to the apoptosis of cells (Hwang and Lim, 2015; Li *et al.*, 2016; Qian *et al.*, 2016). Apoptosis is a sort of programmed cell death involving the biological regulation of critical cellular activities and its activation is regarded as one of the antibacterial mechanisms. (Sun *et al.*, 2013; Li *et al.*, 2016). As an apoptosis factor, an increase in ROS levels in treated cells with *S. malaccense* leaves extract would most likely result in *S. aureus* cell apoptosis via a membrane-mediated apoptosis pathway combined with cell membrane permeability and integrity test results (Zhang *et al.*, 2020).

According to Guo *et al*. (2016), the crystal violet uptake of *E. coil* and *S. aureus* supernatants was 21.1% and 30.2%, respectively, in the absence of *Fructus forsythia* essential oil (FEO). However, they increased their signal to 56.4% and 91.5% after being treated with FEO. In comparison, *S. malaccense* leaves extract had a significantly stronger effect on *E. coli* cell membrane permeability that led to higher crystal violet uptake than FEO, while the dye uptake in *S. aureus* in the extract in FEO was convergent. A study by Ramli *et al*. (2017) mentioned that the exposed *S. aureus* to *S. polyanthum* leaves extract had recorded crystal violet uptake of 91.5%, which is close to the obtained results in this study. According to Halder *et al*. (2015), the uptake of crystal violet by *S. aureus* was 76% after being exposed to cetyl trimethyl ammonium bromide (CTAB) within 1 h of incubation. This result showed that *S. malaccense* leaves extract was more effective against *S. aureus* than CTAB.

Bacteria's membranes expand, their fluidity and permeability rise, their membrane-embedded proteins are disturbed, their respiration is inhibited, and their ion transport pathways are altered as a result of the extraction of lipophilic and hydrophobic properties from an aqueous phase (Trombetta *et al.*, 2005). Generally, crystal violet has been known to exhibit weak penetration of the outer cell membrane, while it easily enters into cells with weakened or damaged membranes. Thus, the crystal violet assay could be considered an indicator for recognizing damaged cell membranes and provides valuable information regarding altered membrane permeability (Halder *et al.*, 2015). In this study, *S. malaccense* leaves extract was found to exhibit a concentration-dependent and time-dependent augmentation in the uptake of crystal violet, where the higher concentration and longer incubation will lead to a

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

higher uptake of crystal violet. Batista *et al*. (2017) reported that flavonoids are found in this leaves extract, which mainly comprises flavanols. Flavanols contain hydroxyl groups and phenolic rings, which make them hydrophobic and accumulate in the cell membrane, thus increasing cell interactions with the proteins and bacterial cell wall to form complexes, penetrate the cellular system, and cause membrane disruption and death (Zongo *et al.*, 2011).

The protein leakage from treated bacteria was highly influenced by the extract concentration and exposure time, where a higher concentration led to more protein leakage into the supernatant, and no further leakage was observed during a prolonged treatment period. According to Moyo and Mukanganyama (2015), *Triumfetta welwitschii* extract at 1 mg/mL concentration had an antibacterial effect against *E. coli* where the protein leakage was 0.048 mg/mL. Although *S. malaccense* was slightly less active than *T. welwitschii*, in this study, *S. malaccense* extract showed concentration and time-dependent increases in the leakage of protein, where 1.25 mg/mL of the extract leaked 0.045 mg/mL of protein that was found in *E. coli* cytoplasm within 4 h and when the concentration was doubled, the leaked protein from bacterial cells was measured to be 0.042 mg/mL and 0.067 mg/mL within 2 h and 4 h, respectively. According to Senthil *et al.* (2017), the leaked protein from *S. aureus* and *E. coli* cells was significantly higher when the bacteria were treated with green synthesized silver nano-particles of the ethanolic leaves extract of fenugreek (*Trigonella foenum-graecum*) and the cause of protein leakage was believed to be related to an alteration of the cell membrane of bacteria that happened when treated by an extract or antibacterial agent.

Generally, the exact antibacterial action mechanisms of plant-based extracts are not fully understood and still need more investigations for better understanding. In spite of this, distinct antimicrobial groups were tested for different mechanisms of action. Since it is not easy to recognize the same action site where many interactions occur simultaneously, different thoughts on proposed mechanisms of action have been reported (Holley and Patel, 2005; Gyawali and Ibrahim, 2012). One of the mechanisms of action of plant extracts as antimicrobial agents is membrane-disrupting, causing leakage of cellular content, interference with active transport or metabolic enzymes, or dissipation of cellular energy in ATP form (Davidson, 1997). Various phytochemical compounds in plants have antibacterial properties; these compounds can interrupt the cell membrane integrity, disrupt cell enzyme systems, impair a bacterial cell's genetic material or create hydroperoxidase from unsaturated fatty acids through oxygenation (Burt *et al*., 2007). The antimicrobial activity of phytochemicals such as phenols and flavonoids found naturally in plants is significant due to the ability of these compounds to penetrate the cell membrane of bacteria (Davidson and Naidu, 2000). A variety of cellular activities, including metabolism, membrane potential, cell growth, substance movement across the surface membrane, cytoskeleton polymerization state and the capacity of muscle cells to contract, can be affected by membrane damage and variations in intercellular pH (Valtierra-Rodriguez *et al*., 2010).

CONCLUSION

In contrast to previous studies, the ethanolic leaves extract of *S. malaccense* was found to be safe, as shown by the high LC_{50} towards brine-shrimp. The extract caused a significant alteration to the membrane of *B. subtilis*, *E. coli*, *S.* Typhimurium and *S. aureus*, and this could be a possible mode of action, which led to severe damage to their cell membrane and subsequently caused leakage of the intracellular material including protein and nucleic acids into the supernatant.

ACKNOWLEDGEMENTS

The authors acknowledge the Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, for the technical support.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538