Emirates Journal of Food and Agriculture. 2020. 32(6): 461-468
doi: 10.9755/ejfa.2020.v32.i6.2116
http://www.ejfa.me/

RESEARCH ARTICLE

Toxicity analysis of *Syzygium polyanthum* (Wight) Walp. leaves extract and its stability against different pH and temperature

Suzita Ramli[a,b], Son Radu[b], Khozirah Shaari[c,d], Yaya Rukayadi[b,d*]

[a]Department of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak Darul Ridzuan, Malaysia, [b]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia, [c]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia, [d]Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

ABSTRACT

Food sanitizer is important to reduce or eliminate microbial population on the food surface and also to removes dirt. Nowadays, food sanitizer is basically from chemical base. Therefore, this study was to evaluate the toxicity level of *S. polyanthum* and its stability against different pHs and different temperatures in determining the suitability of leaves extract as natural food sanitizer. Leaves extract was adjusted to pH 3, pH 7, pH 11 conditions and exposed to temperatures at 30°C, 50°C and 80°C for 15 min each. Treated extracts were tested for their antimicrobial properties in term of MICs and MBCs. Toxicity level of *S. polyanthum* was determined by brine shrimp lethality assay. As a result, antimicrobial activities of *S. polyanthum* extract was not affected by different pHs and temperatures while toxicity study demonstrated that *S. polyanthum*, extract was not toxic to *Artemia salina* with LC_{50} was 75.85 mg/mL. Therefore, *S. polyanthum* had the potential to be developed as antimicrobial agent in food sanitizer.

Keywords: *Syzygium polyanthum*; pH; Temperatures; Stability; Toxicity; *Artemia salina*

INTRODUCTION

Food products can be subjected to contaminate by bacteria and fungi. The growth of these microorganisms in food products can cause foodborne illness. To overcome this problem, prevention should be done at the early stage of food processing such as sanitizing. Commonly, chemical sanitizers had been applied in food industry. However, the application of these chemicals for long term will affected human health. Sanitizer is used to reduce the number of harmful microorganisms to the safe level for human health. It does not eliminate the entire population of microorganisms, but helps minimize it to a number considered as safety standard without altering the product's quality or its safety (Maria et al., 2009).

S. polyanthum grows wildly on lowlands and is widely distributed in the temperate, subtropical and tropical regions in the world (Perumal et al., 2012). This plant can be found in the western part of peninsular Malaysia and Western Indonesia (Othman et al., 2014). *S. polyanthum* has large straight root, round trunk with white and fragrant flowers (Lau et al., 2014) and its leaves have a fragrant smell and astringent taste (Sumono and Wulan, 2008). *S. polyanthum* has been used traditionally as medicine or therapeutic agents. This plant is effective against ulcer, hypertension, diabetes, hyperuricemia, diarrheal, gastritis, skin diseases and inflammation (Ismail et al., 2013; Sumono and Wulan, 2008). Antimicrobial property of *S. polyanthum* against pathogenic bacteria such as *Salmonella* spp., *B. cereus*, *B. subtilis, E. coli*, *P. fluorescens* and *S. aureus* is due to the presence of active compounds including triterpenoids, saponins, citral, eugenol and lectins (Setiawan, 2002).

There were studies on the toxicity level of other Syzygium species. According to Selma et al. (2011), hydroalcoholic extract of *S. cumini* does not show acute toxic or chronic effect against rats after treated orally. On the other hand,

***Corresponding author:**
Yaya Rukayadi (Assoc. Prof. Dr.), Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia. **Phone:** +60-3-8946-8519. **Fax:** +60-3-8942-3552. **E-mail:** yaya_rukayadi@upm.edu.my

Received: 27 March 2020; **Accepted:** 30 May 2020

a study by Manaharan et al. (2014), *S. aqueum* did not show any visible signs of toxicity to Sprague-Dawley (SD) rats by given a single dose of 2000 mg/kg of the *Syzygium aqueum* was given by oral-gavage. However, based on the study by Lekhnath and Cheng (2013), *Syzygium aromaticum* had a toxicity effect. Besides, finding by Sautron and Cock (2014), indicate that *Syzygium australe* and *Syzygium leuhmannii* fruits displayed significant toxicity (<1000 μg/mL) in the *Artemia salina* analysis while *Syzygium jambos* leaves extract also showed toxicity effect in the *Artemia franciscana* bioassay with LC_{50} = 387.9 ± 38.8 μg/mL.

According to Hada and Sharma (2017), garlic extract that had been stored at room temperature showed a low antimicrobial activity towards the growth of *B. cereus*. On the other hand, the garlic extract retained its antimicrobial property after store at 8 °C. Besides that, Tyneca et al. (1993), reported that storage of *Allium ursinum* juice at 4° C had reduced its antimicrobial property. Lee et al. (2004) mentioned that antimicrobial activity of Chinese leaf extract had been reduced after exposed to heat treatment at above 75 °C. However, this extract showed its stability at pH between 2.0 to 8.0. Study by Doughari (2006) showed that bioactivity of *Carica papaya* root extract increase with the increasing of temperature while its antimicrobial property was reducing with increasing of pH. *Allium sativum* extract showed good antimicrobial activity after keep at room temperature up to 7 days but it showed moderate inhibitory activity after store at 4° C for 60 days. Moreover, *Allium sativum* demonstrated the decreasing of its bioactivity at alkaline condition (Srinivasan et al, 2009). Ghogare et al. (2015), stated that high pH condition effect the inhibitory activity of *Zingiber officinale* and *A. sativum* extract by reduced its ability to inhibit microorganisms. Therefore, it is very important to determine the effect of physical factors on extract to maintain its efficacy at different conditions.

Hence, in the present study, the effect of different pH and temperature on the minimum inhibition concentration and minimum bactericidal inhibition had been determined. In addition, this study also to evaluate the toxicity level of *S. polyanthum* leaves extract in order to determine its suitability as natural sanitizer and also can be applied as antimicrobial agent in food industry.

METHOD AND MATERIALS

Plant sample collection

S. polyanthum leaves had been collected from Herbal Market, Pasar Baru, Bandung, Indonesia. The sample was dried and processed in the Laboratory of Natural Products, Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM). Plant taxonomic identification was done and the voucher specimen number is SK 3047/16.

Preparation of crude leaves extract

Leaves extraction was done by referring to Rukayadi et al. (2008). One hundred gram of dried *S. polyanthum* was weighed and ground by using dry blender. Then, the leaves was soaked with 400 mL of absolute ethanol for 7 days (Suzita et al., 2012). Then, Whatman No. 2 filter paper was used to filter the leaves powder. The filtrate was concentrated by using rotary vacuum evaporator at 50°C and speed of 150 rpm for about 2 to 3 h. The crude extract was diluted in DMSO to obtain 100 mg/mL and then further diluted in 1:10 (v/v) distilled water to get 10 mg/mL (10 000 μg/mL) stock solutions.

Microbial strains and maintenance

There were 8 types of bacteria strains and 8 types of fungi strains that had been tested in this study. The bacteria strains were included *Escherichia coli* O157:H7 (ATCC 43895), *Klebsiella pneumoniae* (ATCC 13773), *Listeria monocytogenes* (ATCC 19112), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus mirabilis* (ATCC 21100), *Salmonella Typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 29737) *and Vibrio parahaemolyticus* (ATCC 17802) while the fungi strains were *Aspergillus flavus* (ATCC 22546), *Aspergillus niger* (ATCC9029), *Rhizopus oligosporus* (ATCC 22959), *Rhizopus oryzae* (ATCC 22580), *Candida albicans* (ATCC 10231), *Candida glabrata* (ATCC 2001) and *Candida krusei* (ATCC 32196). Bacteria strains were maintained by subculturing them on the fresh Muller Hinton agar (MHA), filamentous fungi on the fresh Potato dextrose agar (PDA) and *Candida* spp., strains were cultured and maintained on Sabouraud dextrose agar (SDA). (Addgene, 2014).

Stability of *S. polyanthum* extract at different pH and temperature

The stability of *S. polyanthum* extract at different pHs and temperatures were evaluated according to the method described by Durairaj et al. (2009), with slight modifications. Briefly, the stability of *S. polyanthum* extract was determined by adjusting the pH of extract in the range of pH 3, pH 7 and pH 11, using 0.1 M of hydrochloric acid or 0.1 M of sodium hydroxide. While for the stability of temperature, *S. polyanthum* extract was exposed to various temperatures starting from 30°C, 50°C and 80°C for 15 min each. After treatment, each of the treated extracts was tested for their antimicrobial property by using MICs and MBCs/MFCs analysis. Untreated extract with pH 6 at room temperature (25 ± 2°C) was used as control.

Minimum inhibition concentration (MIC)

Minimum inhibition concentration (MIC) was conducted in 96-well U-shaped microtiter plate using two fold standard

broth microdilution methods. The inoculum concentration was approximately 10^6 - 10^8 CFU/mL (bacteria and *Candida* spp.) and 10^4 (filamentous fungi). *S. polyanthum* leaves extract with a concentration of 10 mg/mL was mixed and two-folds diluted was done in the medium containing inoculum. The highest concentration of extract was 5 mg/mL while the lowest concentration of extract is 0.0097 mg/mL (Zainin et al., 2013).

Minimum bactericidal concentration/ minimum fungicidal concentration (MBC/MFC)

The minimum bactericidal/fungicidal concentration (MBC/MFC) was determined by sub-culturing 10 µL of each of the suspension from the microtiter plates (MIC analysis) onto the MHA/SDA/PDA and incubated at 37°C for 12 - 24 h (bacteria), 24 - 48 h (yeast) and 35°C, 3-7 days for filamentous fungi. The least concentration which showed no visible growth was considered as the MBC value (Andrews, 2001).

Toxicity analysis of *S. polyanthum* extract by using brine shrimp lethality assay

The toxicity of *S. polyanthum* extract was determined using brine shrimp lethality assay. Two-fold dilution method of *S. polyanthum* extract was performed to get the concentration ranged between 0.01 mg/mL - 10 mg/mL. Potassium dichromate was used as the positive control. Twenty larvae added into each of the bottles and incubated for 24 h. Results were recorded by observing the survival of the larvae in each bottle. The graph of mean percentage mortality (%) was plotted against the logarithm of concentrations and the value of LC_{50} was calculated based on the plotted graph (Syahmi et al., 2010).

RESULTS AND DISCUSSION

The stability of extract was tested on different pHs and temperatures. Both parameters were the most influencing factors on the efficacy of extract as antimicrobial agent in food sanitizer (Rivera, 2002). The pH of chlorine based wash water systems is an important factor in the reduction and inactivation of bacteria. The lethal effects of chlorine were observed at pH range of 6.0-7.5 (Sapers, 2003). In addition, by referring to Schuler (1999), the factors to be considered in the application of sanitizer are the length of time the sanitizer will be in contact with the surface to be sanitized, the temperature at which the sanitizer will be used and also the sanitizer's pH. It is important to know the pH of the solution in which the sanitizer will be expected to act. Moreover, according to Haute et al. (2015), the efficacy of sanitizer depends on several parameters such as dosage, concentration, contact time, temperature and pH. Therefore, the stability of extract at different pHs and temperatures had been studied.

The original pH of *S. polyanthum* extract is at pH 6. Generally, all the tested foodborne pathogens showed similar MIC and MBC values after exposed with the treated extract (pH 3, pH 7 and pH 11) as shown in Table 1. *S. Typhimurium* and *V. parahaemolyticus* showed the most similar MIC and MBC values with 1.25 mg/mL, respectively after exposed with treated extracts. *L. monocytogenes* and *S. aureus* showed that a small difference on antimicrobial activity of *S. polyanthum* extract after treated with different pH where it is a decrease in increasing the pH value. Food spoilage microorganisms also showed the same pattern where all the MIC and MBC are quite similar after exposed with treated extract (Table 2). The range of MIC was between 0.63-1.25 mg/mL and

Table 1: Stability of *S. polyanthum* extract at different pH on the MIC and MBC of pathogenic bacteria

Strain	mg/mL	pH 3	pH 6[a]	pH 7	pH 11
E. coli	MIC	2.50	1.25	5.00	2.50
	MBC	2.50	2.50	5.00	2.50
K. pneumoniae	MIC	0.63	1.25	1.25	1.25
	MBC	1.25	2.50	1.25	1.25
L. monocytogenes	MIC	0.63	0.63	0.63	1.25
	MBC	1.25	0.63	1.25	1.25
P. aeruginosa	MIC	1.25	1.25	1.25	1.25
	MBC	1.25	2.50	1.25	1.25
P. mirabilis	MIC	2.50	1.25	2.50	2.50
	MBC	2.50	2.50	2.50	2.50
S. aureus	MIC	0.63	0.63	0.63	1.25
	MBC	1.25	1.25	1.25	1.25
S. Typhimurium	MIC	1.25	1.25	1.25	1.25
	MBC	1.25	1.25	1.25	1.25
V. cholera	MIC	1.25	1.25	1.25	1.25
	MBC	1.25	1.25	1.25	1.25
V. parahaemolyticus	MIC	1.25	1.25	1.25	1.25
	MBC	1.25	1.25	1.25	1.25

[a]: Control

Table 2: Stability of *S. polyanthum* extract at different pH on the MIC and MFC of food spoilage fungi

Strain	mg/mL	pH 3	pH 6[a]	pH 7	pH 11
A. flavus	MIC	1.25	1.25	1.25	1.25
	MBC	2.50	5.00	5.00	5.00
A. niger	MIC	1.25	1.25	1.25	1.25
	MBC	2.50	5.00	2.50	2.50
R. oligosporus	MIC	1.25	1.25	1.25	1.25
	MBC	2.50	5.00	5.00	5.00
R. oryzae	MIC	1.25	1.25	1.25	1.25
	MBC	5.00	5.00	5.00	2.50
C. albicans	MIC	1.25	1.25	0.63	1.25
	MBC	1.25	1.25	1.25	1.25
C. glabrata	MIC	0.63	0.63	1.25	1.25
	MBC	1.25	1.25	1.25	1.25
C. krusei	MIC	0.63	0.63	0.63	0.63
	MBC	1.25	1.25	0.63	1.25
C. parapsilosis	MIC	0.63	0.63	1.25	1.25
	MBC	1.25	0.63	1.25	1.25

[a]: Control

MFC within ranged 1.25 - 5.00 mg/mL. However, a slight difference of MIC was observed in *C. glabrata* and *C. parapsilosis* where the value was increase from 0.63 to 1.25 mg/mL in alkaline condition. This showed that antimicrobial activity of *S. polyanthum* was slightly decreased in alkaline condition. This finding was similar to Anees et al. (2015), showed that treating the garlic extract at various pH decrease in the antibacterial activity with an increase in pH. In addition, Doughari and Manzara (2008), supported this finding where, they also reported antimicrobial activity of *Mangifera indica* leaves extract decreased in alkaline condition. However, Lin et al. (2004), reported that no changes in phytochemical properties of oregano and cranberry extract at neutral condition (pH 7). On the other hand, this observation was contradiction with Molan (1992) who stated that the activity of phytochemical compounds in plant extract can increase in acidic condition. Generally, although the antibacterial effect of *S. polyanthum* decreases with increasing of pH, however, the antibacterial activity was still sustained. Therefore, it can be concluded that generally, *S. polyanthum* extract was stable after exposed to different pH.

The influence of temperature on *S. polyanthum* extract also was investigated in this study. Results show that the antimicrobial activity of *S. polyanthum* extract also was not affected at temperature 25 ± 2˚C, 30˚C, 50˚C, and 80˚C against all the tested pathogenic bacteria (Table 3) where the MIC and MBC were in ranged 0.63 - 1.25 mg/mL and 0.63 - 2.50 mg/mL, respectively. Table 4 also shows that antimicrobial activity of *S. polyanthum* against food spoilage microorganisms almost similar at the different temperature where the MIC was between 0.63 - 2.50 mg/mL and while MBC was in the ranged of 1.25 - 5.00 mg/mL. This result was supported by Mahfuzul et al. (2008) who reported the stability of clove and cinnamon extracts when treated with higher temperature where it shows similar antimicrobial activity against *L. monocytogenes*, *E. coli* and *S. Enteritidis*. Moreover, according to Ahmed et al. (2015), garlic retains its antibacterial effect even at a higher temperature. Meanwhile, this finding was a contradiction with Arabshahi et al. (2007) who found a significant decrease in biological activity when drumstick leaves extract was treated under heat processing.

Generally, the result showed that exposure of extract to different temperatures (24 ± 2°C, 30°C, 50°C and 80°C), did not effect on the antimicrobial activity to *S. polyanthum* extract, while the activity slightly decreases when the pH increased. However, *S. polyanthum* extract still showed antimicrobial activity at alkaline condition. This observation was similar to Salama and Marraiki (2010), where they found that *Polygonum aviculare* extract was stable and not affected after exposure to different temperature,

Table 3: Stability of *S. polyanthum* extract at different temperatures on the MIC and MBC of pathogenic bacteria.

Strains	mg/mL	25±2°C [a]	30°C	50°C	80°C
E.coli	MIC	1.25	1.25	1.25	1.25
	MBC	2.50	2.50	1.25	1.25
K. pneumonia	MIC	0.63	0.63	0.63	1.25
	MBC	1.25	0.63	0.63	2.50
L. monocytogenes	MIC	0.63	0.63	0.63	1.25
	MBC	0.63	0.63	0.63	1.25
P. aeruginosa	MIC	1.25	1.25	1.25	1.25
	MBC	2.50	1.25	1.25	1.25
P. mirabilis	MIC	1.25	2.50	1.25	1.25
	MBC	2.50	2.50	2.50	2.50
S. aureus	MIC	0.63	1.25	1.25	1.25
	MBC	1.25	1.25	1.25	1.25
S. Typhimurium	MIC	1.25	1.25	1.25	1.25
	MBC	1.25	1.25	1.25	1.25
V. cholerae	MIC	1.25	1.25	1.25	1.25
	MBC	1.25	1.25	1.25	1.25
V. parahaemolyticus	MIC	1.25	2.50	1.25	1.25
	MBC	1.25	2.50	1.25	2.50

[a]: Control

Table 4: Stability of *S. polyanthum* extract at different temperatures on the MIC and MFC of food spoilage fungi

Strains	mg/mL	24±2°C[a]	30°C	50°C	80°C
A. flavus	MIC	1.25	1.25	1.25	1.25
	MBC	5.00	5.00	5.00	5.00
A. niger	MIC	1.25	1.25	1.25	2.50
	MBC	5.00	2.50	5.00	5.00
R. oligosporus	MIC	1.25	1.25	1.25	1.25
	MBC	5.00	5.00	5.00	5.00
R. oryzae	MIC	1.25	1.25	2.50	2.50
	MBC	5.00	5.00	5.00	5.00
C. albicans	MIC	1.25	1.25	1.25	1.25
	MBC	1.25	1.25	1.25	2.50
C. glabrata	MIC	0.63	1.25	1.25	1.25
	MBC	1.25	1.25	1.25	2.50
C. krusei	MIC	0.63	1.25	1.25	1.25
	MBC	1.25	1.25	2.50	1.25
C. parapsilosis	MIC	0.63	0.63	1.25	0.63
	MBC	0.63	1.25	1.25	1.25

[a]: Control

meanwhile, the antibacterial activity was slightly decreased at alkaline condition. From the results, *S. polyanthum* extract was generally stable when exposed to different ranged of pH and different degrees of temperature. However, the effects only tested solely on *S. polyanthum* crude extract. Results might be different when this extract is tested after added to any foodstuffs. According to Talcott et al. (2003), the presence of other polyphenolic compounds in food matrix may help to stabilize the polyphenolic compounds. Therefore, it is important to choose the optimum technological condition and processing factors in order to keep the biological activity of plant extract when applied

in food and in biological systems (Arabshahi et al., 2007).

Toxicity analysis of *S. polyanthum* using brine shrimp lethality assay

Brine shrimp lethality assay is one of the toxicity assays. The brine shrimp lethality assay is crucial to determine the safety and effectiveness of medicinal plant used by providing an early hint on the level of toxicity of the targeted plant. The toxicity of the plants may originate from different contaminants or from plant chemical compounds that are part of the plant (Hamidi et al., 2014). As this *S. polyanthum* extract was intended to be applied as food decontamination, its toxicity level is important to be determined to confirm the safety of this plant extract on human health. Many other toxicity tests can be applied to determine the toxicity of plants including zebra fish test, cell lines or white mouse but this brine shrimp test is more rapid and economic practice (Rajabi et al., 2015). As stated by Ramachandaran et al. (2011), brine shrimp assay is preferable compared to others due to its fastness, inexpensive and simple biassay in testing toxicity of plant extracts. This method was firstly introduced by Michael et al. (1956) and has been widely used particularly in toxicity study of plant extracts (Syahmi et al., 2010; Ramachandran et al., 2011). In addition, this technique is economic and utilizes a small amount of test material (Pisutthanan et al., 2004). Since its introduction, this *in vivo* test has been successively employed for bioassay-guide fractionation of active cytotoxic and antitumor agents (Ramachandran et al., 2011). Additionally, several studies demonstrated that there is a good correlation between the results for the lethal concentration that kills 50% of the exposed population (LC_{50}) obtained with the brine shrimp lethality assay and the results of the acute oral toxicity assay in mice (Arlsanyolu and Erdemgil, 2006).

According to Meyer et al. (1982), plant crude extracts resulting in LC_{50} value less than 1 mg/mL was considered as significantly toxic, while the LC_{50} more than 1 mg/mL is considered safe for human use. Potassium dichromate was used as a positive control in this bioassay due to its well-known toxicity and several studies had been reported that LC_{50} of this chemical is around 0.28 to 0.30 mg/mL (Sahgal et al., 2010; Syahmi et al., 2010). In the present study, the value of LC_{50} was obtained from the best-fit line plotted of the percentage of nauplii killed against the concentration of *S. polyanthum* extract (Figs. 1 and 2). The result was presented in Table 5. The mortality rate of the brine shrimp nauplii was founded to increase with the increasing concentration of the *S. polyanthum* extract. The result of brine shrimp lethality assay showed that *S. polyanthum* extract display no toxic effects to brine shrimp after 24 h with LC_{50} was 75.85 mg/mL and thus, concluded that *S. polyanthum* extract is also biologically safe and not toxic to humans. On contrary, potassium dichromate exhibit toxic effects to brine shrimp after 24 h with LC_{50} was 0.060 mg/mL. Potassium dichromate is commonly used in this assay due to its well-known toxicity. For the negative control, there was no death in the vial containing the highest concentration of DMSO (10%) tested in this assay. DMSO is widely used solvent for the reconstitution of evaporated plant extracts, because brine shrimp nauplii show no significant sensitivity to this solvent up to 11% concentration (Musa, 2012; Kamba and Hassan, 2010). The cytotoxic result of *S. polyanthum* extract obtained is in accordance to Perumal et al. (2012) who reported that *S. polyanthum* leaves extract is non-cytotoxic to normal mammalian cell line. Moreover, Kusuma et al. (2011) also reported that the extract of *S. polyanthum* leaves had LC_{50} of more than 1 mg/mL and thus, non-cytotoxic.

Table 5: Toxicity of *S. polyanthum* extract using brine shrimp lethality assay

Sample	LC_{50} (mg/mL)
S. polyanthum extract (24 h)	75.85
Potassium dichromate (24 h)	0.06

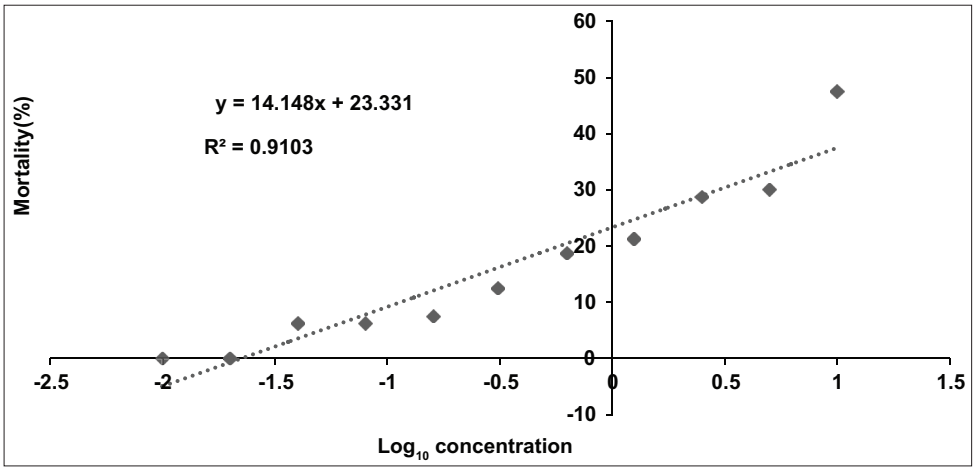

Fig 1. Standard curve of brine shrimp lethality assay after treated with *S. polyanthum* extract for 24 h.

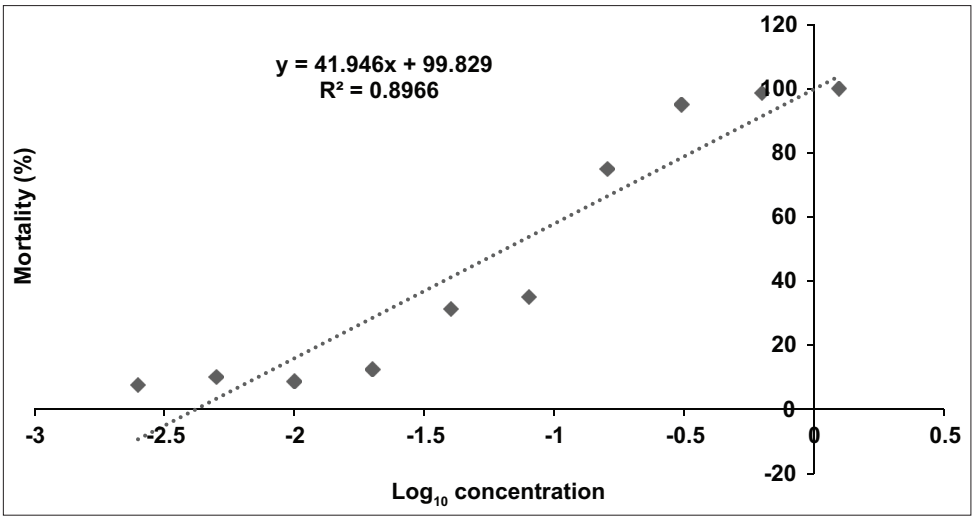

Fig 2. Standard curve of brine shrimp lethality assay after treated with potassium dichromate for 24 h.

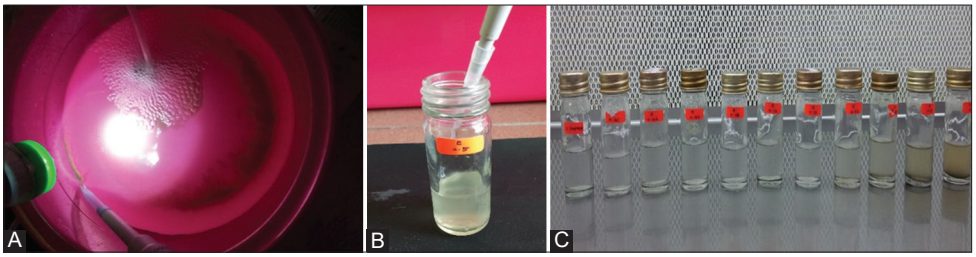

Plate 1. A: Brine shrimp hatching process; B: 20 brine shrimp in each bottle; C: Series of dilution for toxicity analysis.

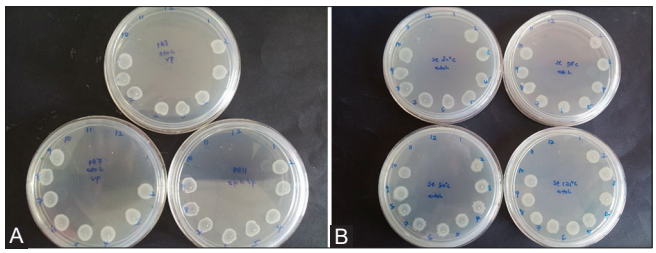

Plate 2. A: MBC analysis of extract at different pH; B: MBC analysis of extract at different temperature.

CONCLUSION

S. polyanthum extract was generally stable after been exposed to different ranges of pHs (pH 3, pH 7 and pH 11) and degrees of temperatures (30°C, 50°C and 80°C). The extract maintained its antimicrobial property by showing similar MICs and MBCs values after the treatments. In addition, *S. polyanthum* extract also exhibited no toxic effect (LC_{50} = 75.85 mg/mL) on *A. salina* spp. while potassium dichromate showed high toxic effect with LC_{50} = 0.060 mg/mL. Therefore, it can be concluded that *S. polyanthum* extract was safe for human consumption. Thus, due to its stability and did not have a toxic effect, this extract had the potential to be used in industry especially in food industry.

Authors' contributions

Suzita Ramli was conducted the whole experiments and wrote the first draft of the manuscript.

Yaya Rukayadi idealized the work, guided the first author and contributed greatly for the preparation of the manuscript. Son Radu and Khozirah Shaari contributed in helping during manuscript revision process.

REFERENCES

Ahmed, A., Z. K. Labu, S. K. Dey, A. Hira, M. S. I. Howlader M. H. Hossain and J. Roy. 2015. Phytochemical screening, antibacterial and cytotoxic activity of different fractions of *Xylocarpus mekongensis* Bark. Ibnosina J. Med. Biomed. Sci. 5: 206-213.

Andrews, J. M. 2001. Determination of minimum inhibitory concentration. J. Antimicrob. Chemoth. 1: 5-16.

Anees, A. M., S. Ravi and P. Ghogare. 2015. Studies on antimicrobial activity of spices and effect of temperature and pH on its antimicrobial properties. IOSR J. Pharm. 10: 99-102.

Arabshahi, D. S., D. D. Vishalakshi and A. Urooj. 2007. Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. Food Chem. 100: 1100-1105.

Arlsanyolu, M. and F. Z. Erdemgil. 2006. Evaluation of the antibacterial activity and toxicity of isolated arctiin from the seeds of *Centaurea sclerolepis*. J. Pharm. Pharmacol. 35: 103-109.

Doughari, J. H. and S. Manzara. 2008. *In vitro* antibacterial activity of crude leaf extracts of *Mangifera indica* Linn. Afr. J. Microbiol. Res. 2: 67-72.

Durairaj, S., S. Srinivasan and P. Lakshmanaperumalsamy. 2009. *In vitro* antibacterial activity and stability of garlic extract at different pH and temperature. Electron. J. Biol. 5: 5-10.

Doughari, J. H. 2006. Antimicrobial activity of *Tamarindus indica* Linn. Trop. J. Pharm. Res. 5: 597-603.

Doughari, J. H. and S. Manzara. 2008. *In vitro* antibacterial activity of crude leaf extracts of *Mangifera indica* Linn. Afr. J. Microbiol. Res. 2: 67-72.

Ghogare, P., A. M. Anees and R. Srivinas. 2015. Studies on antimicrobial activity of spices and effect of temperature and pH on its antimicrobial properties. IOSR J. Pharm. Biol. Sci. 10: 99-102.

Hada, D. and S. Kanika. 2017. Effect of different physical factors on *Cassia fistula* fruit pulp extract and their herbal formulation efficacy. J. Pharm. Pharm. Sci. 4: 1-7.

Hamidi, M. R., B. Jovanova and T. K. Panovska. 2014. Toxicological evaluation of the plant products using brine shrimp (*Artemia salina* L.) model. Macedonian Pharm. Bull. 60: 9-18.

Haute, V., J. Lopez-Galvez, V. M. Gomez-Lopez, M. Eriksson, F. Devlieghere, A. Allende and I. Sampers. 2015. Methodology for modeling the disinfection efficiency of fresh-cut leafy vegetables wash water applied on peracetic acid combined with lactic acid. Int. J. Food Microbiol. 208: 102-113.

Ismail, A., M. Mohamed, S. A. Sulaiman and W. A. N. Wan Ahmad. 2013. Autonomic nervous system mediates the hypotensive effects of aqueous and residual methanolic extracts of *Syzygium polyanthum* (Wight) Walp. var. *polyanthum* leaves in anaesthetized rats. Evid. Based Complement. Altern. Med. 2013: 716532.

Kamba, A. S. and L. G. Hassan. 2010. Antibacterial screening and brine shrimp (*Artemia salina*) toxicity over *Securidaca longepedunculata* (*Polygalaceae*) root bark. Afr. J. Pharm. Sci. Pharm. 1: 85-95.

Kusuma, I. W., K. Harlinda, T. A. Enos, A. Farida, Y. Min, J. Kim and Y. Kim. 2011. Biological activity and phytochemical analysis of three Indonesian medicinal plants, *Murraya koenigii, Syzygium polyanthum* and *Zingiber Purpurea.* J. Acupunct. Meridian Stud. 4(1): 75-79.

Lau, K. Y., N. S. Zainin, F. Abas and Y. Rukayadi. 2014. Antibacterial and sporicidal activity of *Eugenia polyantha* Wight against *Bacillus cereus* and *Bacillus subtilis.* Int. J. Curr. Microbiol. Appl. Sci. 3: 499-510.

Lee, C. F., C. K. Han and J. L. Tsau. 2004. *In vitro* activity of Chinese leek extract against *Campylobacter* species. Int. J. Food Microbiol. 94: 169-174.

Lekhnath, K. and J. L. Cheng. 2013. Toxicity and repellency of compounds from Clove (*Syzygium aromaticum*) to red imported fire ants *Solenopsis invicta* (*Hymenoptera*: *Formicidae*). *J. Ecol. Entomol.* 106: 131-135.

Lin, Y. T., R. G. Labbe and K. Shetty. 2004. Inhibition of *Listeria monocytogenes* in fish and meat systems by use of oregano and cranberry phytochemical synergies. Appl. Environ. Microbiol. 70: 5672-5678.

Mahfuzul, H. M., M. L. Barib, K. Vijay and S. Kawamoto. 2008. Antimicrobial activity of cloves and cinnamon extracts against food borne pathogens and spoilage bacteria, and inactivation of *Listeria monocytogenes* in ground chicken meat with their essential oils. J. Pure Appl. Chem. 72: 9-21.

Manaharan, T., S. Chakravarthi, A. K. Radhakrishnan and U. D. Palanisamy. 2014. *In vivo* toxicity evaluation of a standardized extract of *Syzygium aqueum* leaf. Toxicol. Rep. 1: 718-725.

Maria, I. G., V. S. Maria, F. Lopez-Galvez and A. Allende. 2009. Fresh-cut product sanitation and wash water disinfection: Problems and solutions. Int. J. Food Microbiol. 134: 37-45.

Meyer, B. N., N. R. Ferrigni, J. E. Putnam, L. B. Jascobsen, D. E. Nichols and J. L. McLaughlin. 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Plant Med. 45: 31-34.

Michael, A. S., C. G. Thompson and M. Abramovitz. 1956. *Artemia salina* as a test organism for a bioassay. Science. 123: 464.

Molan, P. C. 1992. The antibacterial activity of honey-the nature of antibacterial activity. Bee World. 73: 5-28.

Musa, A. A. 2012. Cytotoxicity activity and phytochemical screening of *Cochlospermum tinctorium* Perr Ex A. rich rhizome. J. Appl. Pharm. Sci. 2: 155-159.

Othman, A., N. J. Mukhtar, N. S. Ismail and K. C. Sui. 2014. Phenolics, flavonoids content and antioxidant activities of 4 Malaysian herbal plants. Int. Food Res. J. 21: 759-766.

Perumal, S., R. Mahmud, S. P. Piaru, L. W. Cai and S. Ramanathan. 2012. Potential antiradical activity and cytotoxicity assessment of *Ziziphus mauritiana* and *Syzygium polyanthum*. Int. J. Pharm. 8: 535-541.

Pisutthanan, S., P. Plianbangchang, N. Pisutthanan, S. Ruanruay and O. Muanrit. 2004. Brine shrimp lethality activity of Thai medicinal plants in the family *Meliaceae*. *Naresuan Uni. J*. 12: 13-18.

Rajabi, S., A. Ramazani, M. Hamidi and T. Naji. 2015. *Artemia salina* as a model organism in toxicity assessment of nanoparticles. DARU J. Pharm. Sci. 23: 20-21.

Ramachandran, S., M. Vamsikrishna, K. V. Gowthami, B. Heera and M. D. Dhanaraju. 2011. Assessment of cytotoxic activity of *Agave cantula* using brine shrimp (*Artemia salina*) lethality bioassay. Asian J. Sci. Res. 4: 90-94.

Rivera, E. V. 2005. A Review of Chemical Disinfection Methods for Minimally Processed Leafy Vegetables, Master Thesis. Kansas State University. Manhattan, Kansas, United State.

Rukayadi, Y., J. S. Shim and J. K. Hwang. 2008. Screening of Thai medicinal plants for anticandidal activity. Mycoses. 51: 308-312.

Salama, H. M. H. and N. Marraiki. 2010. Antimicrobial activity and phytochemical analyses of *Polygonum aviculare* L. (*Polygonaceace*), naturally growing in Egypt. Saudi J. Biol. Sci. 7: 185-189.

Sapers, G. M. 2003. Washing and sanitizing raw materials for minimally processed fruits and vegetables. In: J. S. Novak, G. M. Sapers and V. K. Juneja. (Eds.), Microbial Safety of Minimally Processed Foods. CRC Press, Boca Raton, FL, pp. 221-253.

Sahgal, G., S. Ramanathan, S. Sasidharan, M. N. Mordi, S. Ismail and S. M. Mansor. 2010. Brine shrimp lethality assay and acute oral toxicity studies on *Swietenia mahagoni* (Linn.) Jacq. seed methanolic extract. Pharmacogn. Res. 2: 215-220.

Sautron, C. and I. Cock. 2014. Antimicrobial activity and toxicity of *Syzygium australe* and *Syzygium leuhmannii* fruit extracts.

Pharmacogn. Comm. 4: 53-60.

Schuler, G. A., M. P. Nolan, A. E. Reynolds and W. C. Hurst. 1999. Cleaning, Sanitization and Pest Control in Food Processing, Storage and Service Area. University of Georgia College of Agricultural and Environmental Sciences and the U.S. Department of Agriculture cooperating, pp. 11-1.

Selma, N., G. Silva, C. Iracelle and H. Borges. 2012. The toxicity evaluation of *Syzygium cumini* leaves in rodents. Rev. Bras. Farmacog. 22: 121-125.

Setiawan, C. P. 2002. Effect of chemical and physical treatment of the antimicrobial activity of leaves (*Syzygium polyanthum* (Wight) Walp). Thesis. Faculty of Agricultural Technology, Bogor Agricultural University, Bogor.

Srinivasan, D., S. Srinivasan and P. L. Perumalsamy. 2009. *In vitro* antibacterial activity and stability of garlic extract at different pH and temperature. Electron. J. Biol. 100: 5-10.

Sumono, A. and A. S. Wulan. 2008. The use of bay leaf (*Eugenia polyantha* Wight) in dentistry. Dental J. 41: 147-150.

Suzita, R., R. Son, S. Khozirah and R. Yaya. 2017. Antibacterial activity of ethanolic extract of *Syzygium polyanthum* L. (*Salam*) leaves against foodborne pathogens and application as food sanitizer. BioMed Intr. Res. 2017: 1-13.

Syahmi, A. R. M., S. Vijayarathna, S. Sasidharan, L. Y. Latha, Y. P. Kwan, Y. L. Lau, L. N. Shin and Y. Chen. 2010. Acute oral toxicity and brine shrimp lethality of *Elaeis guineensis* Jacq. (oil palm leaf) methanol extract. Molecules. 15: 8111-8121.

Talcott, S. T., S. S. Percival, J. Pittet-Moore and C. Celoria. 2003. Phytochemical composition and antioxidant stability of fortified yellow passion fruit (*Passiflora edulis*). J. Agri. Food Chem. 51: 935-941.

Tyneca, Z., Z. Syczesniak and L. Gelowniak. 1993. The effect of various environment conditions on the antimicrobial activity of *Allium ursinum*. Planta. Med. 59: 701-706.

Zainin, N. S., K. Y. Lau., M. Zakaria, R. Son, A. F. Abdull Razis and Y. Rukayadi. 2013. Antibacterial activity of *Boesenbergia rotunda* (L.) Mansf. A. extract against *Escherichia coli.* Int. Food Res. J. 20: 3319-3323.