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RESEARCH ARTICLE

Antimicrobial Activity of *Syzygium polyanthum* Wight (Walp.) Extract against Foodborne Pathogens in Food: A Potential Antimicrobial Agent for Natural Food Washing Solution

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Abstract Developments of natural preservatives derived from plant sources instead of chemical preservatives are gaining more attention nowadays. In this study, the effect of *Syzygium polyanthum* (Wight) Walp.] leaves extract against foodborne pathogens on raw chicken meat and cherry tomato was evaluated using the method of washing treatment by extract solution. Results found that *Syzygium polyanthum* (Wight) Walp. leaves extract have significant effect ($P < 0.05$) in reduction of foodborne pathogens in all tested food samples at 5 mg/mL of extract for 5 min soaking period. Moreover, *S. polyanthum* extract (10 mg/ml and 50 mg/ml) able to reduce yeast, mould and *E. coli* in raw meat chicken after storing at 4 ± 2°C for 5 days and reduce *Pseudomonas* species in cherry tomato after storing for 15 days at 4 ± 2°C. Antimicrobial property of *S. polyanthum* might be due to the presence of pyrogallol, β-Sitosterol, phytol, hexadecanoic acid, gallic acid, bergenin, quercetin 3-(6''-galloylgalactoside), madecassic acid, quillaic acid and asiatic acid that were detected by GC-MS and LC-MS. In conclusion, *S. polyanthum* extract exhibited antimicrobial activity, thus it has potential to be developed as antimicrobial agent for natural food washing solution.

Keywords: Natural antimicrobial activity, *S. polyanthum* leaves, foodborne pathogens, raw food, food washing solution.

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Introduction

Foodborne pathogens are the major causes of foodborne diseases. Foodborne diseases occur cause of consumption of foods that contaminated by foodborne pathogens. The spoiled foods include colour changes, off-flavours and off-odours are mainly caused by the growth of microbes and excess of their metabolisms in the food products [1]. An illness occurred due to the ingestion of food contaminated with pathogens has a wide economic and public health impact worldwide. The symptoms of foodborne diseases were included nausea, vomit, diarrhea and fever. Meanwhile, severe foodborne diseases may lead to death [2,3]. Generally, foodborne illnesses occur are due to improper food handling and poor hygienic practices, especially during food preparation and storage periods [4]. Many food decontamination treatments have been applied to eliminate or substantially decrease bacterial populations both in fresh produce and raw poultries. A number of antimicrobial treatments that had been studied including chlorine, organic acids, bacteriocins, and hydrogen peroxide. Although chemical food sanitizers and provide a good effect on the reduction of microbial growth in food materials, however, some of these applications sanitizers may lead to food quality deterioration, a decrease of product stability, potential health risk and high processing cost [5, 6].

The application of hurdle technologies in food preservation also initiates food safety issues even though the technique is sometimes successful in controlling the growth of foodborne pathogens. For instance, the long-term use of syntetic sanitizer such as chlorine sanitizer may cause several carcinogenic effects [7]. Thus, studies on natural antimicrobial agents from plants are gaining attention nowadays. Natural antimicrobials are gaining wide interest because most of them are classified as Generally Recognized as Safe (GRAS) status which has been discovered to have higher levels of food safety [3,8]. Many studies reported that most of the medicinal plants, including spices and herbs, have strong antimicrobial compounds such as phenolic compounds, essentials oils and saponins were found in the roots or leaves of plant [9-11]. Flavonoid, alkaloid, triterpenes, and phenolic acid) fergenin and quercetin.

S. polyanthum grows wildly on lowlands and is widely distributed in the temperate, subtropical and tropical regions in the world [12]. This plant can be found in the western part of peninsular Malaysia and Western Indonesia [13]. The height of *S. polyanthum* trees may reach about 25 m and they have large straight root, round trunk with a smooth surface as well as small, white, and fragrant flowers. *S. polyanthum* leaf is about 2.5 to 8 cm long, with flat margins and blunt tip [14]. Indeed, the leaf of *S. polyanthum* has been used traditionally as medicine or therapeutic agents. It is being utilized as ingredients in the Indonesian traditional medicine called *Jamu*. This plant is effective against ulcers, hypertension, diabetes, hyperuricemia, diarrheal, gastritis, skin diseases and inflammation. This plant also has a diuretic and analgesic effect, besides having the ability to neutralize hang over caused by over-consumption of alcohol [15]. Furthermore, *S. polyanthum* are effective in fighting the symptoms of cold, flu and infections. The dried mature leaves of *S. polyanthum* are used in culinary as a spice due to their aromatic smell and sour taste. The leaves are used as ingredients in meat dishes and commonly used in the preparation of rice (*nasi liwet*). The young shoots of *S. polyanthum* are consumed as a fresh salad (*ulam*) [16]. In fact, the presence of caryophyllene oxide, D-nerolidol, n-decanal, patchoulin, octanal, cyclohexane, 3, 7-dimethyl-1-octene and 3, 7-dimethyl-1-octene in *S. polyanthum* which lead to its antimicrobial property.

However, no study has been reported on the effect of *S. polyanthum* extract to inhibit the microorganism growth in raw chicken meat and cherry tomato. Hence, this study was done to identify the bioactive compounds in *S. polyanthum* extract and the effect of *S. polyanthum* extract on the microorganisms in several raw food samples at different concentrations of extract and exposure time. Therefore, this study is important to identify the potential of this plant extract in inhibiting microbial activity in raw food.

Materials and Methods

Plant sample collection

S. polyanthum leaves were purchased from Herbal Market, Pasar Baru, Bandung, Indonesia. The sample was processed in the Laboratory of Natural Products, Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), Serdang, Malaysia. Plant taxonomic identification was done by Dr. Shamsul Khamis, a Herbarium Officer in Institute of Bioscience (IBS), Universiti Putra Malaysia, Serdang, Malaysia under the voucher specimen number of SK 3047/16.

Raw food samples

The raw food samples which were chicken meat and cherry tomato were bought from Giant Hypermarket, Kuala Lumpur. All raw samples were kept in a cooler box (2°C - 5°C) between the time of purchase and initiation of experiments. Chicken meats were cut into a cube size of approximately 10 g each before used for treatment. Since cherry tomato were in various shapes in nature and impossible to cut into a cube shape, there were only weighed with approximately 10 g each for treatment [18].

Preparation of crude plant extract

Plant extraction was done according to Rukayadi *et al*. [19], with some modifications. One hundred grams of dried *S. polyanthum* was ground and extracted with 400 mL of absolute ethanol (99.8%) (R & M Marketing, Essex, UK), sonicated for 30 min [20]. Then, Whatman No. 2 filter paper (Whatman International Ltd., Middlesex, England) was used to filter the plant materials. The filtrate was concentrated using a rotary vacuum evaporator (Heidolph VV2011, Schwabach, Germany) at 50°C and a speed of 150 rpm for about 2 to 3 h. The crude extract was diluted in distilled water to 10 mg/mL (10 000 µg/mL) stock solutions. Finally, *S. polyanthum* crude extract was collected and stored in the fridge (- 20°C) for use in further studies.

Identification of active compound by using Gas chromatography-mass spectrometry (GC-MS)

S. polyanthum extract was dissolved in methanol HPLC grade to yield 5 mg/mL. An aliquot of the extract was injected into QP2010 Ultra gas chromatograph-mass spectrometer equipped with BP5MS column (30.0 m long, 0.25 mm i.d, film thickness 0.25 µm) for compound separation. The carrier gas used was Helium with the flow rate of 0.8 mL/min. The operating conditions of the oven were: initial temperature at 50°C, increase rate at 3°C/min to 300°C and hold for 10 min. The injection temperature and ion-source temperature was 200°C respectively. The peaks were analysed by comparing their retention times and mass fragments patterns with standard spectra available in Shimadzu GC-MS NIST/ Wiley library.

Identification of active compound by using Liquid chromatography-mass spectrometry (LC-MS)

In LC-MS analysis, 2 mg/mL of *S. polyanthum* extracts was prepared and the analysis was done using Agilent 1290 Infinity LC system coupled to Agilent 6520 Accurate-Mass Q-TOF with dual, positive and negative ionization mode (ESI). The analyte (injection volume, 1.0 µL) was separated using Agilent Zorbax Eclipse XDB-C18 column (internal diameter of 2.1 with mm, length of 150 mm) with linear gradient of mobile phase that consist of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) at 0.5 mL/ min flow rate. The mass ranges for both positive and negative ion polarity were 100-1000 *m/z* and 115-1000 *m/z* respectively. The compounds were analysed using Agilent MassHunter Qualitative Analysis software version B.05.00.

Preparation of *S. polyanthum* extract for treatment solutions

In the preparation of *S. polyanthum* treatment solution, four types of concentration were prepared by diluting with deionized water (DIW). Firstly, 10 g of crude extract was dissolved in distilled water to make the concentration of 100 mg/mL. Then, it was further diluted to 1% concentration by taking out 10 mL of 100 mg/mL and diluted in 90 mL of DIW. Besides that, from the concentration of 100 mg/mL of extract, 4 mL was taken out and dissolved in the separate universal bottles containing 36 mL of deionized water (DIW) which then make the concentration to 50 mg/mL. The next concentrations were 5 mg/mL and 0.5 mg/mL were prepared by taking out 4 mL (0.5 mg/mL concentration) and 4 mL (5 mg/mL concentration) into 36 mL of DIW in two separated universal bottles, as respectively [18]. From these dilution processes, there were three types of solutions with different degrees of concentrations have been prepared (0.5 mg/mL, 5 mg/mL, 10 mg/mL and 50 mg/mL) for the treatment analysis.

Preparation of selective media

There were six types of agars were used including the plate count agar (PCA), potato dextrose agar (PDA), *Pseudomonas* agar, eosin methylene blue agar (EMB) and xylose lysine deoxycholate agar (XLD). The preparations for each of them were according to the formulation as attached to the media bottle. Selective agars were chosen based on their ability to grow the microorganisms and make them distinct with other for easier and faster detection [21]. According to Leininger *et al*. [22], EMB is a reliable, simple, rapid, and inexpensive medium for the differentiation of *E. coli* from other Gram-negative pathogens.

Washing treatment of raw food materials with *S. polyanthum* extract solutions

Washing treatment of raw food materials with *S. polyanthum* extract solution was done according to Yusoff *et al*. [18] with slight modification. A 10 g from each of the raw food samples (chicken meat and cherry tomato) were weighed separately to prevent any cross-contamination of microflora that occurred between the food samples. Then, each of the 10 g food samples were immersed in 50 mL of filtered tap water and *S. polyanthum* treatment solutions (0.5 mg/mL, 5 mg/mL, 10 mg/mL and 50 mg/ml) at different time exposure (5 min, 10 min and 15 min).

Microbiology analysis

The treated samples were collected and dried on the sterile filter paper to remove the excess of treatment solutions before diluted into the stomacher bag which contained 90 mL of phosphate saline buffer solution. The mixture was homogenized using the stomacher machine (BagMixer 400-P Interscience, France) at 250 rpm for 2 min. After the homogenization process, serial dilutions were performed by taken out 1 mL of the mixture into the 9 mL of phosphate saline buffer solution to made up for dilutions; 10^{-2}, 10^{-3} and 10^{-4}. All universal bottles containing different dilutions were vortex first before were spread onto the selective agar separately. The agar plates were then incubated overnight at 37°C for bacteria growth and 35°C for fungi growth and results were analysed by identifying and counting the presence of colonies

from each plate. Untreated samples were used as control. All experiments were conducted at room temperature (23 ± 2°C).

Effect of *S. polyanthum* extract washing treatment during storage

The storage study was done on chicken meat and cherry tomato samples. The samples were soaking with the extract and store at refrigerator temperature (4ºC) for 5 days and 15 days, respectively. Samples were randomly withdrawn at everyday interval for analysis. Microbiological analysis was conducted. Results were compared with the set of untreated samples [18].

Statistical analysis

All experiments were performed three times with three replications each. The application of MINITAB software was used to analyse the data for the analysis of variance (ANOVA). The significance of difference ($p<0.05$) between the treatments was analyse using Turkey's test. Results were interpreted as means ± standard deviation (SD) of replicate analysis.

Results and Discussion

GCMS and LCMS based profile of bioactive compounds in *S. polyanthum* extract

A total of 25 peaks were identified in GC-MS chromatogram of *S. polyanthum* extract (Figure 1). Upon comparing of their mass-spectral databases with Wiley and NIST libraries, these phytochemical compounds were identified and characterized as listed in Table 1.

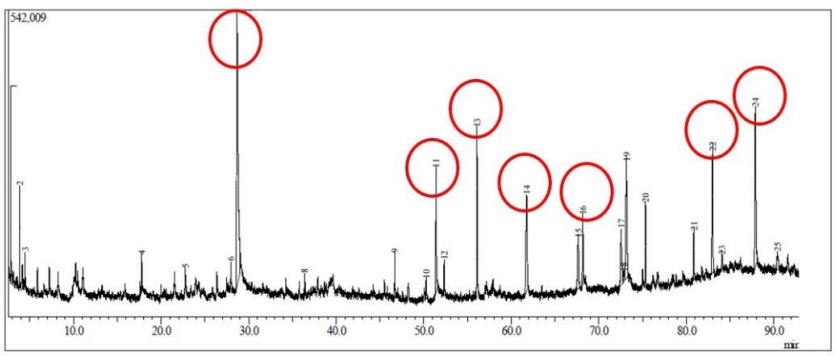

Figure 1. Chromatogram of phytochemical compounds of *S. polyanthum* extract

Table 1. Identification of phytochemical compounds in *S. polyanthum* leaves extract using GC-MS

Peak no.	Compounds name	Peak (%)	RT(Min)
1	Hydroxyacetone	2.20	2.84
2	2-Propynol	1.64	3.84
3	Methylglyoxylic acid methyl ester	0.90	4.40
4	3,5-dihydroxy-2-methyl-5,6-dihydropyran	1.60	17.78
5	1,2,3-Propanetriol, 1-acetate	1.37	22.77
6	Decanoic acid	1.04	27.97
7	Pyrogallol	23.90	28.67
8	Nerolidol	0.83	36.38
9	Neophytadiene	1.51	46.70
10	Unknown compound	1.13	50.27

Peak no.	Compounds name	Peak (%)	RT(Min)
11	Hexadecanoic acid	5.79	51.41
12	Palmitic acid ethyl ester	1.06	52.34
13	Phytol	6.64	56.09
14	2-Pentanone, 1-(2,4,6-trihydroxyphenyl)	8.23	61.78
15	Unknown compound	4.12	67.66
16	2-hydroxy-1-(hydroxymethyl)ethyl ester	3.50	68.17
17	Unknown compound	3.58	72.57
18	Unknown compound	0.20	72.86
19	Unknown compound	8.26	73.16
20	Squalene	2.96	75.35
21	β-Tocopherol	1.70	80.86
22	α-Tocopherol	5.35	82.97
23	Unknown compound	0.94	84.06
24	β-Sitosterol	10.31	87.88
25	Unknown compound	1.235	90.42

RT: retention time

As shown in Figure 1, there were six major compounds (Table 2) identified from the *S. polyanthum* extract chromatogram. The most abundant compounds were pyrogallol (23.90%) (Peak no. 7), followed by β- Sitosterol (10.31%) (Peak no. 24), unknown compound with retention time of 73.16 (8.26%) (Peak no. 19), 2-Pentanone, 1-(2, 4, 6-trihydroxyphenyl) (8.23%) (Peak no. 14), phytol (6.64%) (Peak no. 13), hexadecanoic acid (5.79%) (Peak no. 11) and α-Tocopherol (5.35%) (Peak no. 22). The other compounds were present by the amount of less than 4%. The unknown compound at retention time of 73.16 was needed further analysis for characterization in the future study. Interestingly, all the major compounds in *S. polyanthum* extract were believed to exhibit antimicrobial activity based on previous study [25, 27]. These phytochemical compounds were characterized as listed in Table 2.

Table 2. Identification of antimicrobial active compounds in *S. polyanthum* leaves extract by using GC-MS

Peak number	Peak concentration (%)	Compound name	Similarity index	Molecular weight (g/mol)	Molecular formula	Kovalt Retention Index (KI)
7	23.903	Pyrogallol	94	126	$C_6 H_6 O_3$	1389
11	5.789	Hexadecanoic acid	90	330	$C_{16} H_{32} O_2$	1971
13	6.644	Phytol	92	296	$C_{20} H_{40} O$	2114
22	5.352	α-Tocopherol	92	430	$C_{29} H_{50} O_2$	3148
24	10.311	β-Sitosterol	82	414	$C_{29} H_{50} O$	3357

Liquid chromatography- mass spectrometry (LC-MS) has been widely used to analyse complex mixtures like biological samples. This method is effective for identifying various bioactive compounds [23]. Figures 2 and 3 show the compounds chromatogram for both positive and negative ion mode detection, respectively. Positive ion mode was used generally for detection of saponins and aldehyde groups and

negative ion mode was used for estimation of organic acids and OH containing organic bioactive components [24].

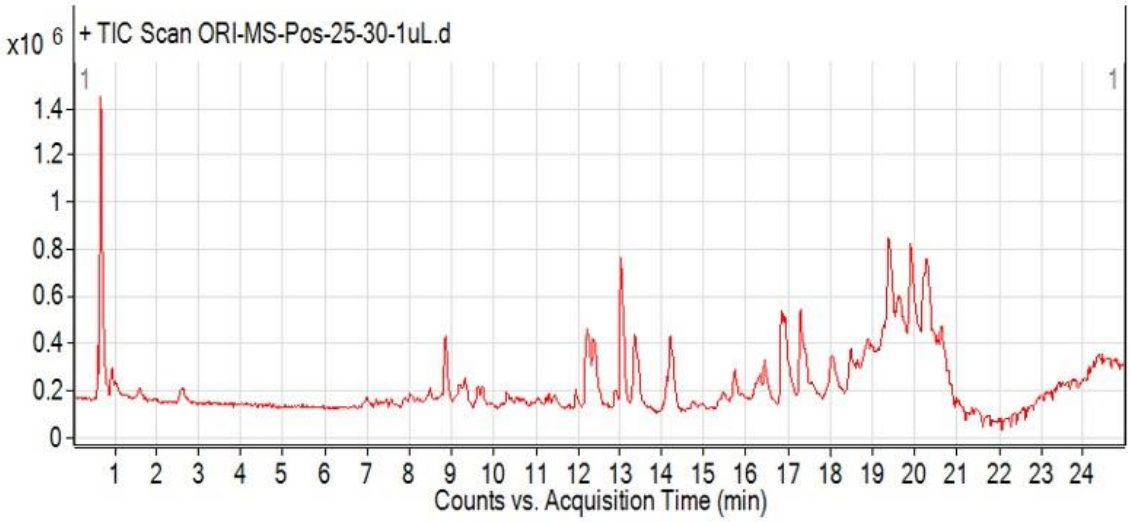

Figure 2. Chromatogram of compounds in *S. polyanthum* extract in positive ion mode

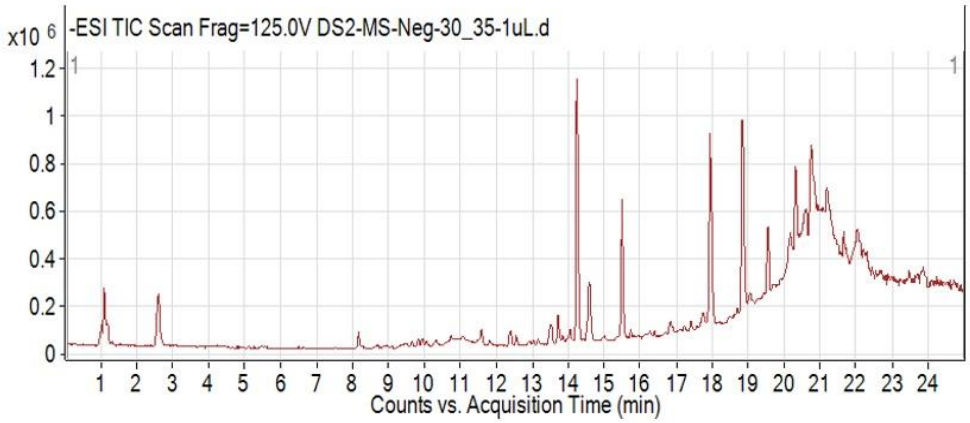

Figure 3. Chromatogram of compounds in *S. polyanthum* extract in negative ion mode

The data was analysed by Agilent MassHunter Qualitative Analysis B.05.00. Compound lists were then screened against mass databases; the Metlin metabolomics database. LC-MS characterization and chemoprofile of extract revealed the presence of nearly 32 compounds based on their molecular mass as shown in Table 3.

Table 3. Identification of phytochemical compounds in *S. polyanthum* leaves extract by using LC-MS

No.	Compound name	Molecular mass	*m/z* value	Ion (+/-)
1	2-Amino-3-methyl-1-butanol	103.09	104.09	[M+H]+
2	3-Ethylmalate	162.05	163.05	[M+H]+
3	Gallic acid	170.02	171.02	[M+H]+
4	Levulinic Acid	204.07	205.07	[M+H]+
5	Dodecanedioic acid	230.15	229.15	[M-H]-
6	(R)-(Homo)3-citrate	234.07	235.07	[M+H]+
7	(1R,6R)-6-Hydroxy-2-succinylcyclohexa-2,4-diene-1-carboxylate	240.06	241.06	[M+H]+

No.	Compound name	Molecular mass	*m/z* value	Ion (+/-)
8	Palmitic amide	255.25	256.25	[M+H]+
9	N6-Methyl-2'-deoxyadenosine	265.12	266.12	[M+H]+
10	Zidovudine	267.10	268.10	[M+H]+
11	dihydrophaseic acid	282.15	281.15	[M-H]-
12	4,2',4'-Trihydroxy-3-methoxydihydrochalcone	288.10	289.10	[M+H]+
13	Trichodermin	292.17	293.17	[M+H]+
14	methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate	310.18	309.18	[M-H]-
15	Phytosphingosine	317.29	318.29	[M+H]+
16	Idebenone Metabolite (QS-8)	324.16	324.17	[M+H]+
17	5'-O-beta-D-Glucosylpyridoxine	331.13	332.13	[M+H]+
18	13,14-dihydroxy-11-mulinen-20-oic acid	350.25	349.25	[M-H]-
19	Bergenin	328.08	327.08	[M-H]-
20	trans-BTP Dioxolane	406.16	405.16	[M-H]-
21	Vernoflexuoside flavo	408.18	407.19	[M-H]-
22	Demethylphylloquinone	436.34	437.34	[M+H]+
23	Laserpitin	450.26	449.26	[M-H]-
24	Gallocatechin 3-O-gallate – flavonoid	458.09	459.09	[M+H]+
25	Prednisolone tebutate	458.27	457.27	[M-H]-
26	Propapyriogenin A2	484.32	485.32	[M+H]+
27	Quillaic acid	486.33	487.33	[M+H]+
28	Asiatic acid	488.35	487.35	[M-H]-
29	Madecassic acid	504.35	503.35	[M-H]-
30	Pheophorbide a	592.27	593.27	[M+H]+
31	Amaroswerin	602.16	603.16	[M+H]+
32	Quercetin 3-(6'' galloylgalactoside)	616.11	617.11	[M+H]+

Out of 32 compounds, six compounds were predicted to possess antimicrobial activity based on previous study [25-34] . There are gallic acid, bergenin, quillaic acid, asiatic acid, madecassic acid and querce-tin 3-(6''-galloylgalactoside).

The phytochemical analysis for these six compounds are shown in Table 4.

Table 4. Identification of antimicrobial active compounds in *S. polyanthum* leaves extract using LC-MS.

Compound	Retention Time (RT)	Molecular mass	*m/z* [M+H]+/[M-H]-	Score (DB)	Molecular formula
Gallic acid	2.559	170.02	171.02	98.29	$C_7 H_7 O_5$
Bergenin	8.148	328.07	327.07	97.38	C14 H15 O9
Quillaic acid	12.88	486.33	487.33	99.44	$C_{31} H_{43} N_4 O$
Asiatic acid	14.57	488.35	487.35	99.53	$C_{30} H_{49} O_5$
Madecassic acid	12.40	504.34	503.34	97.83	$C_{31} H_{45} N_4 O_2$
Quercetin 3-(6''-galloylgalactoside)	9.73	616.11	617.11	99.58	$C_{28} H_{25} O_{16}$

Based on GCMS result analysis, this extract had several compounds that contribute to its antimicrobial activity including pyrogallol, β- Sitosterol, phytol, hexadecanoic acid and α-Tocopherol whereas from LCMS result, we found some compounds such as gallic acid, bergenin, quillaic acid, asiatic acid, madecassic acid and quercetin 3-(6''-galloylgalactoside). According to Kocacaliskan *et al*. [25], pyrogallol was effective as antimicrobial agents. It able to inhibit the growth of *B. cereus*, *E. coli*, *K. pneumoniae*,

P. aeruginosa and *S. aureus* [26]. Antimicrobial activity of *S. polyanthum* also due to the present of β-Sitosterol. β-Sitosterol able to prevent the growth of foodborne pathogens including *S. aureus, B. subtilis, S. pyrogene, K. pneumoniae, S. dysentariae* and *C. krusei* [27]. Besides that, the present of phytol, hexadecanoic acid [28], and α-Tocopherol [29] also contribute to antimicrobial properties of *S. polyanthum*.

In fact, gallic acid was also able to inhibit the growth of *S. aureus*, *E. coli*, *P. aeruginosa* and *S.* Typhimurium [30], whereas bergenin was proved had antifungal activity [31]. On the other hand, quillaic acid, asiatic acid [32], madecassic acid [33] and quercetin 3-(6''-galloylgalactoside) [34] had antimicrobial activity and had been confirmed in both mentioned studies. Therefore, all the compounds that had been identified from *S. polyanthum* extract by using GCMS and LCMS in this study were responsible to antimicrobial property of *S. polyanthum* extract.

Microbiology analysis

A preliminary study was conducted to determine the presence and concentration level of each bacterial in raw food samples as shown in Table 5. Results showed that in chicken meat, TPC, yeast and mould and *E. coli* were detected at 6.17 ± 0.12, 4.25 ± 0.34 and 5.90 ± 0.15 Log_{10} CFU/mL, respectively. Meanwhile *Salmonella*, *S. aureus, Pseudomonas* spp. and *Vibrio* spp. were not identified. In cherry tomato TPC, yeast and mould, *E. coli* and *Pseudomonas* spp. were present with concentration 5.90 ± 0.09, 3.11 ± 0.04, 5.59 ± 0.23, 3.26 ± 0.21 Log_{10} CFU/mL, respectively.

Table 5. Total plate count and number of natural microbial detected in raw food samples using different selective media agar (Log_{10} CFU/mL)

Natural microflora / Food sample	TPC	Yeast and mould	*E. coli*	*Pseudomonas* spp.	*Salmonella* spp.	*S. aureus*	*Vibrio* spp.
Chicken meat	6.17 ± 0.12	4.25 ± 0.34	5.90 ± 0.15	n.d	n.d	n.d	n.d
Cherry tomato	5.90 ± 0.09	3.11 ± 0.04	5.59 ± 0.23	3.26 ± 0.21	n.d	n.d	n.d

n.d: Not detected

Both *Salmonella* and *S. aureus* was not found in all food samples. *E. coli* populations were detected in all tested raw foods might be due to their ability to survive in various environmental conditions and survive with extended time. As reported by Lynch *et al*. [35], *E. coli* is the common microorganism populations found especially on fresh produce foods which then caused the foodborne outbreak. The result was supported by Chang and Fang [36] who had found *E. coli* and *Salmonella* spp. in lettuce while Doyle and Erickson [37] isolated this *E. coli* from poultry. Besides that, *E. coli* had also been found in fruits and vegetables. According to Razzaq *et al*. [38], *E. coli* was detected in 48% of street vendor of vegetables in Pakistan. Meanwhile, in Korea, 2.8% of vegetables and fruits was contaminated with this pathogen [39]. *Pseudomonas* spp. was detected in cherry tomato. The concentration of *Pseudomonas* spp. in cherry tomato was 3.26 ± 0.21 Log_{10} CFU/mL. *S. aureus* was not identified in chicken meat and cherry tomato. Finding was a contradiction with Akbar and Anal [40] where they investigated that most of *S. aureus* species easily be found in poultry meat. On the other hand, *Salmonella* spp. was not detected in all tested food samples.

In chicken meat sample (Table 6) the presence of total plate count, yeast and mould and *E. coli* were detected.

Table 6. Effects of different concentration and exposure time of *S. polyanthum* extract on natural microorganisms in chicken meat (Log_{10} CFU/mL)

Sample	Chicken meat											
Bacterial species	TPC			Yeast and mould			*E. coli*			*Pseudomonas* spp.		
ET / Treatment	5 min	10 min	15 min	5 min	10 min	15 min	5 min	10 min	15 min	5 min	10 min	15 min
Control	6.17 ± 0.12^{aA}	6.18 ± 0.12 ^{aA}	6.17 ± 0.12^{aA}	4.25 ± 0.34^{aA}	4.25 ± 0.34^{aA}	4.25 ± 0.34^{aA}	5.90 ± 0.15^{aA}	5.90 ± 0.15^{aA}	5.90 ± 0.15^{aA}	5.90 ± 0.15^{aA}	5.90 ± 0.15^{aA}	5.90 ± 0.15^{aA}
Tap water	6.15 ± 0.32^{aA}	6.17 ± 0.01^{aA}	6.12 ± 0.02^{aA}	4.23 ± 0.02^{aA}	4.22 ± 0.71^{aA}	4.23 ± 0.32^{aA}	5.10 ± 0.01^{aA}	5.02 ± 0.01^{aA}	5.03 ± 0.01^{aA}	5.10 ± 0.01^{aA}	5.02 ± 0.01^{aA}	5.03 ± 0.01^{aA}
0.5 mg/ml	6.11 ± 0.72^{aA}	6.13 ± 0.01^{aA}	6.08 ± 0.02^{aA}	4.23 ± 0.01^{aA}	4.21 ± 0.01^{aA}	4.19 ± 0.21^{aA}	5.03 ± 0.16^{aA}	5.03 ± 0.03^{aA}	5.06 ± 0.12^{aA}	5.03 ± 0.16^{aA}	5.03 ± 0.03^{aA}	5.06 ± 0.12^{aA}
5 mg/ml	5.77 ± 0.33^{bA}	5.71 ± 0.43^{bA}	5.68 ± 0.01^{bA}	3.87 ± 0.13^{bA}	3.74 ± 0.03^{bA}	3.52 ± 0.12^{bA}	5.15 ± 0.33^{aA}	4.74 ± 0.16^{bA}	4.69 ± 0.02^{bA}	5.15 ± 0.33^{aA}	4.74 ± 0.16^{bA}	4.69 ± 0.02^{bA}
10 mg/ml	5.37 ± 0.36^{bA}	5.73 ± 0.01^{bA}	5.32 ± 0.61^{bA}	3.78 ± 0.51^{bA}	3.61 ± 0.31^{bA}	3.12 ± 0.21^{bB}	3.98 ± 0.02^{bA}	3.54 ± 0.22^{cA}	3.22 ± 0.72^{cB}	3.98 ± 0.02^{bA}	3.54 ± 0.22^{cA}	3.22 ± 0.72^{cB}
50 mg/ml	4.95 ± 0.04^{cA}	4.13 ± 0.51^{cB}	3.42 ± 0.01^{cC}	3.33 ± 0.02^{bA}	3.15 ± 0.01^{bA}	0.00 ± 0.00^{cB}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{dA}	0.00 ± 0.00^{dA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{dA}	0.00 ± 0.00^{dA}

TPC: Total plate count. Values with different small letters within the same columns are significantly different ($p < 0.05$). Values with different capital letters within the same rows are significantly different ($p < 0.05$). ET: Exposure Time.

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Potential contamination in chicken meat by the foodborne pathogens can be caused through the unhygienic practices in handling, cooking or post-cooking and storage of products. Moreover, cross contamination also might be from environmental factors such as water, litter, air and also originate from the animals itself [41]. Furthermore, the storage temperature to which the meat is exposed may favour the proliferation of pathogenic bacteria for humans [42]. Before chickens are shipped to the market or the further processing plants for cooking, they may have already passed through various stages of processing. Some stages are very critical to the microbial quality of chicken, such as immersion scalding and irradiation [43]. Thus, controlling microbial contamination in poultry meat during slaughtering, processing, storage, handling, and preparation becomes a great challenge. In this study, the number of TPC was reduced significantly ($p<0.05$) starting at 0.50% treatment during 5 min of soaking time, which was from 6.17± 0.12 Log_{10} CFU/mL to 5.77 ± 0.33 Log_{10} CFU/mL. The same pattern was shown for yeast and mould where the reduction was significantly started at treatment 0.50% of extract for 5 min exposure. However, the population of *E. coli* was started to decrease significantly ($p<0.05$) after exposure at 0.50% for 10 min with from 5.90 ± 0.15 to 4.74 ± 0.16 Log_{10} CFU/mL. At the maximum concentration and exposure time (5% for 15 min), TPC reduced from 6.17 to 3.42 Log_{10} CFU/mL while yeast and mould and *E. coli* was not detected at this condition (Log_{10} 0.00 ± 0.00 CFU/mL). This means *S. polyanthum* extract significantly ($p<0.05$) affect TPC, yeast and mould and *E. coli* population started at 0.50% for 5 min exposure time, while the highest reduction for the foodborne pathogens population was after exposure at 5% of extract for 15 min treatment. Besides that, different time of exposure has influenced the reduction of microbial count which was shown by the count of TPC after exposing to 5.00% of extract where the plate count was reduced to 4.95 ± 0.04, 4.13 ± 0.51 and 3.42 ± 0.01 Log_{10} CFU/mL after washing with extract for 5, 10 and 15 min, respectively. Yeast and mould as well as *E. coli* also showed similar observation, where the reduction of the microbial count was significantly ($p<0.05$) different at a different time of exposure.

For cherry tomato sample, the total plate count, yeast and mould, *E. coli* and *Pseudomonas* spp. started to reduce significantly ($p<0.05$) after treatment at 0.50% extract for 5 min treatment time (Table 7).Yeast and mould and *Pseudomona*s spp. were not identified started at 0.50% at 5 min exposure. However, the population of *E. coli* was not identified in the cherry tomato samples after exposed at 1% extract for 5 min. Meanwhile, the highest reduction for total plate count was observed at 5.00% concentration of *S. polyanthum* for 15 min exposure time with from 5.90 ± 0.09 to 3.05 ± 0.31 Log_{10} CFU/mL. In this study, treatment with tap water is referring to the common washing method applied by the household. Capability of tap water to reduce the total bacterial count around 2 to 3 Log_{10} CFU/mL [44]. However, in this study, the treatment with tap water only showed a slight reduction compared to previous study. Brackett [45] had reported that the use of tap water for washing cannot completely remove the bacterial populations on food materials. Besides, there are limitations of using tap water in washing food materials which is due to the presence of chlorine residues in treated tap water. Chlorine residues have become a concern in food safety due to their potentiality to produce carcinogenic compounds such as trihalomethanes, haloacetic acids, haloketones and chloropicrin when reacting with organic matter.

Furthermore, washing with tap water alone will sometimes increase the growth of foodborne pathogens [46]. Re-using of processing water as sanitizer will make the tap water as another source of cross-contamination [47]. Besides, the microflora in food samples which was immersed in DIW (0.00% extract) found no significant difference even though at different exposure times [18]. The number of bacteria survivals was decreased with the increase of *S. polyanthum* extract concentrations. Finding was similar to Abadias *et al*. [48], who also reported that the reduction of microbial populations were increased as the concentration of sanitizer and washing time increased. However, some of them reported differently which stated that the soaking time of sanitizer was not influenced the reduction number of bacteria populations in raw food materials. A study conducted by Tornuk *et al*. [49] proved this situation where thyme sanitizer which analysed at the same concentration but with different exposure time did not gave a significant reduction ($p>0.05$) on the bacterial populations in apple fruits. Based on the finding of the bacterial reduction in treated food samples, it was noticed that the reduction of microbial populations was proportional to the increasing of *S. polyanthum* extract concentration and soaking time. The relative influence in terms of microbial inactivation was: Tap water < 0.05% < 0.50% < 1.00 < 5.00%. Generally, *S. polyanthum* had significantly ($p<0.05$) antimicrobial activity against natural microflora in tested food samples started at 0.50% for 5 min exposure. Higher concentrations of *S. polyanthum* with longer soaking time found more powerful to inhibit the growth of microorganisms. However, the effect of higher concentration of extract against colour and texture needs to investigate because consumer preferences on types of food are categorized in various aspects including food nutrition value, food safety, food appearance and physical characteristics [50].

Table 7. Effects of different concentration and exposure time of *S. polyanthum* extract on natural microorganisms in cherry tomato (Log_{10} CFU/mL)

Sample	Cherry tomato											
Bacterial species	TPC			Yeast and mould			*E. coli*			*Pseudomonas* spp.		
ET / Treatment	5 min	10 min	15 min	5 min	10 min	15 min	5 min	10 min	15 min	5 min	10 min	15 min
Control	5.90 ± 0.09 ^{aA}	5.90 ± 0.09 ^{aA}	5.90 ± 0.09 ^{aA}	3.11 ± 0.04 ^{aA}	3.11 ± 0.04 ^{aA}	3.11 ± 0.04 ^{aA}	5.59 ± 0.23 ^{aA}	5.59 ± 0.23 ^{aA}	5.59 ± 0.23 ^{aA}	3.26 ± 0.21 ^{aA}	3.26 ± 0.21 ^{aA}	3.26 ± 0.21 ^{aA}
Tap water	5.76± 0.10^{aA}	5.82± 0.02^{aA}	5.73± 0.12^{aA}	3.11± 0.21^{aA}	3.05± 0.06^{aA}	3.04± 0.06^{aA}	5.44± 0.20^{aA}	5.44± 0.11^{aA}	5.36± 0.11^{aA}	3.20± 0.07^{aA}	3.23± 0.12^{aA}	3.13± 0.10^{aA}
0.5 mg/ml	5.69± 0.11^{aA}	5.67± 0.03^{aA}	5.63± 0.18^{aA}	3.03± 0.06^{aA}	3.07± 0.05^{aA}	3.10± 0.40^{aA}	5.34± 0.10^{aA}	5.40± 0.17^{aA}	5.31± 0.10^{aA}	3.23± 0.03^{aA}	3.10± 0.07^{aA}	3.06± 0.05^{aA}
5 mg/ml	5.02± 0.11^{bA}	5.17± 0.04^{bA}	5.07± 0.14^{bA}	0.00± 0.00^{bA}	0.00± 0.00^{bA}	0.00± 0.00^{bA}	3.85± 0.05^{bA}	3.27± 0.12^{bA}	3.33± 0.06^{bA}	0.00± 0.05^{bA}	0.00± 0.06^{bA}	0.00± 0.00^{bA}
10 mg/ml	4.96± 0.07^{bA}	4.70± 0.18^{bA}	3.43± 0.17^{cB}	0.00± 0.00^{bA}	0.00± 0.00^{bA}	0.00± 0.00^{bA}	0.00± 0.08^{cA}	0.00± 0.20^{cA}	0.00± 0.10^{cA}	0.00± 0.00^{bA}	0.00± 0.00^{bA}	0.00± 0.00^{bA}
50 mg/ml	4.08± 0.04^{cA}	3.09± 0.05^{cB}	3.05± 0.31^{cB}	0.00± 0.00^{bA}	0.00± 0.00^{bA}	0.00± 0.00^{bA}	0.00± 0.00^{cA}	0.00± 0.00^{cA}	0.00± 0.00^{cA}	0.00± 0.00^{bA}	0.00± 0.00^{bA}	0.00± 0.00^{bA}

TPC: Total plate count. Values with different small letters within the same columns are significantly different ($p < 0.05$). Values with different capital letters within the same rows are significantly different ($p < 0.05$). ET: Exposure Time.

Effect of *S. polyanthum* extract washing treatment during storage

Figure 4 (a), (b) and (c) show the total plate count, yeast and mould count and *E. coli* population in untreated (control) and treated chicken meat during storage at -4℃ for 5 days, respectively. The initial count of total plate count was 6.17 ± 0.12 Log_{10} CFU/mL. After the end of storage, the population increased to 8.26 ± 0.60 Log_{10} CFU/mL in untreated samples. After treatment with 1% of the extract, the total plate count also increased slightly with the end reading was 6.25 ± 0.04 Log_{10} CFU/mL.

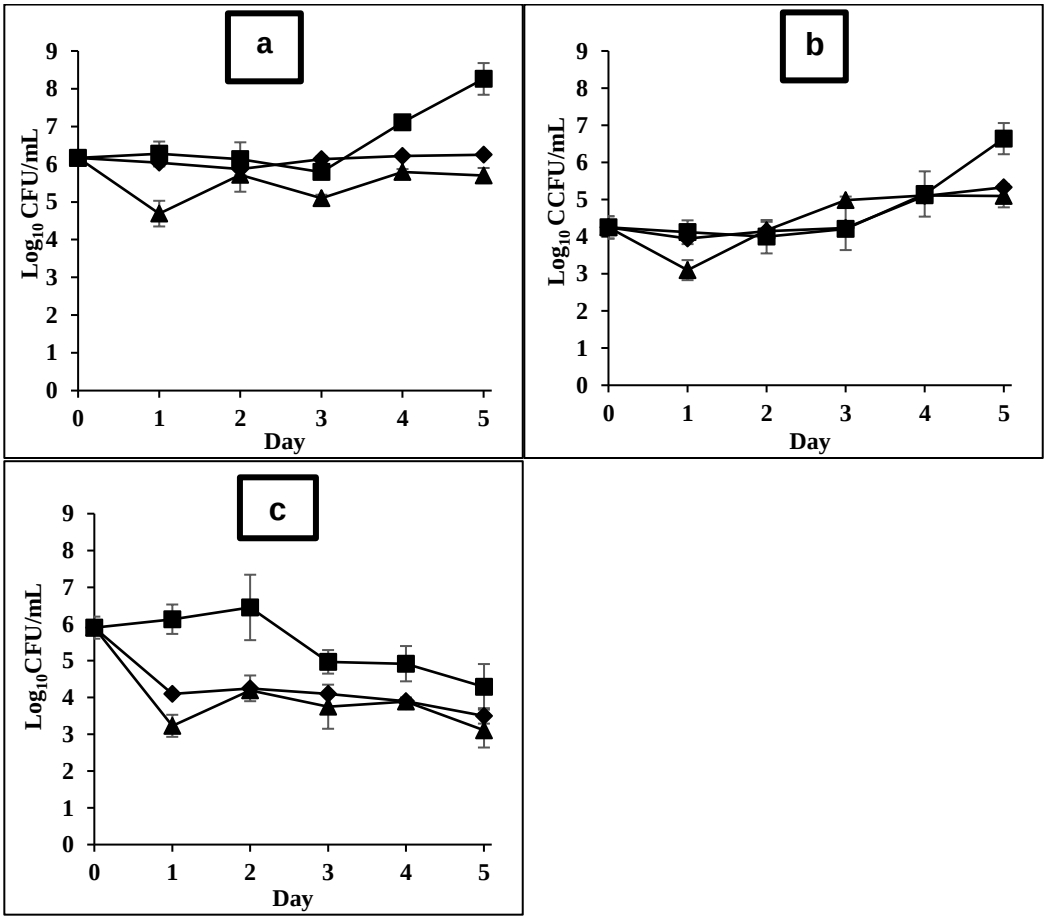

Figure 4. Representative the (a) total bacterial, (b) yeast and mould and (c) *E. coli* counts on the chicken meat exposed to different concentration of *S. polyanthum* extract during storage at temperature 4 ± 2°C for 5 days. (■) Control, (♦) 10mg/ml extract and (▲) 50mg/ml extract.

However, this increment was lower than the control. Chicken meat treated with 5% of *S. polyanthum* extract showed the reduction of total plate count with 5.70 ± 0.02 Log_{10} CFU/mL on the 5th day of storage. Therefore, the increasing of bacterial count be due to the storage temperature (4^{o}C) where microorganisms still able to grow at this temperature [51].

Yeast and mould count increased from 4.25 ± 0.34 to 5.33 ± 0.34 and 4.75 ± 0.31 Log_{10} CFU/mL for treated samples with 1 and 5% of extract, respectively. However, the untreated samples showed the highest increment for yeast and mould's population with 6.64 ± 0.40 Log_{10} CFU/mL. Besides, the increment of this population was started on 3rd day of storage. On the other hand, *E. coli*'s population displayed a reduction on the last day of storage. Five percent of the extract showed the best concentration of the treated chicken meat during the storage due to the lowest reduction with 3.11 ± 0.02 Log_{10} CFU/mL. For the control samples, the population shows the increment on the 1st day of storage from 5.90 ± 0.15 to 6.45 ± 0.89 Log_{10} CFU/mL, whereas the treated samples showed the reduction to 4.10 ± 0.01 (1%) and 3.23 ± 0.3 Log_{10} CFU/mL (5%) on the same day, respectively. However, throughout the storage, 5% of extract gave the lowest reduction of *E. coli*. (The 5% extract is the highest concentration in this analysis and have strong inhibition property against *E. Coli*. Therefore, during the storage time, the population of *E. coli* is not able to survive due to the present of extract.)

Figure 5 (a), (b), (c) and (d) show the growth of total plate, yeast and mould, *E. coli* and *Pseudomonas* spp. on cherry tomato during stored at 4 ± 2°C for 15 days. Total plate count in cherry tomato after stored at chiller temperature (°C) increased to 7.46 ± 0.39 Log_{10} CFU/mL.

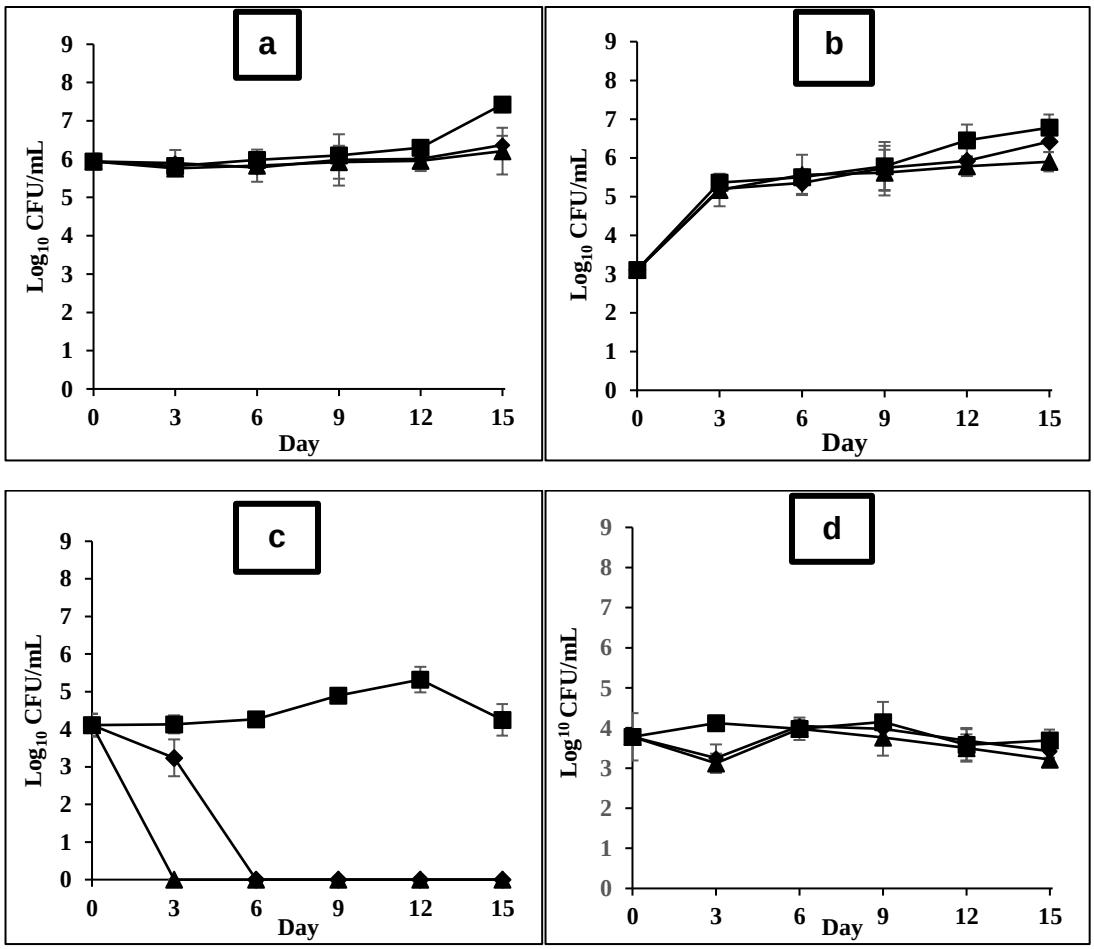

Figure 5. Representative the (a) total bacterial, (b) yeast and molud, (c) *E. coli* and (d) *Pseudomonas* spp. counts on cherry tomato exposed to different concentration of *S. polyanthum* extract during storage at 4 ± 2°C for 15 days (■) Control, (♦) 10 mg/ml extract and (▲) 50/ml extract

Meanwhile, the treated samples also showed the increment in total plate count. However, the increment was lower compared to control samples. On the other hand, from the observation, even though the population of yeast and mould had been increased, the treated samples showed lower increment and this proved that *S. polyanthum* extract could inhibit the yeast and mould growth. The growth of *E. coli* increased until day 12, and then the population dropped to 4.25 ± 0.25 Log_{10} CFU/mL in control samples. Meanwhile, the treated samples demonstrated that, *E. coli*'s population was not detected on the 6th day of storage for treated samples at 1% extract, while 5% extract showed the most effective effect on the reduction of *E. coli* where the population was not found since day 3. *Pseudomonas* spp. in control samples observed slightly reduced until the last day of storage. Nevertheless, there was *Pseudomonas* spp. had been still detected on the end day of storage for both treated samples with 1% and 5% of the extract. However, the population reduction was higher than in untreated samples. This finding showed that treatment with extract help to extend the shelf life of cherry tomato. Figure 6 and Figure 7 show physical appearances treated chicken meat and cherry tomato after storage, respectively.

Morshedy & Sallam [52] reported that chicken carcasses in zero-day storage, average for mesophilic microorganisms score of 4.62 Log_{10} CFU/mL but obtained higher score in the six and eight days of storage at 2°C (8.63 Log_{10} CFU/mL). Fallah *et al*. [53] studied chicken breast meat stored at 4°C and found 5.59 Log_{10} CFU/g in the samples of aerobic mesophilic bacteria. After 15 days of storage, it was 7.88 Log_{10} CFU/g for the same microorganisms. The mesophilic bacteria are considered the best indicators of microbiological quality of foods and it can provide indications of the hygienic conditions for

their preparation and storage as well as the potential health risks to consumers [54]. According to Pizato *et al*. [54], the population of *Staphylococcus* in cooked chicken meat was reduced after stored at 4ºC for about 10 days. Meanwhile, the species was increased until to 5 Log after chicken meat was store at 20ºC for 24 h. Microbial contamination of tomatoes can be a serious cause of their deterioration and represents a health hazard to the consumer. The most common microorganisms found in post harvested tomatoes are mesophyll, coliforms and moulds and yeast. At 25ºC the, coliform was increased from 2.2×10^5 to exponentially to 1.8×10^8 CFU/g and mould from 4.6×10^2 to 3.6×10^5 CFU/g [55]. On the other hand, *Pseudomonas* is known to be resistant at cold storage [56]. This present study also showed that the population of *Pseudomonas* spp. in cherry tomato still had been detected until the end of storage time at 4ºC. In addition, the population was increased in room temperature due to the favorable condition for its survival.

A strawberry jam which was treated by pomegranate extract achieved a mean reduction of 0.71, 0.52, and 0.58 Log units for aerobic mesophylls, lactose acid bacteria, yeasts, and moulds, respectively while after treated by a combination of pomegranate and lemon extract, the reductions were 1.52, 1.34, and 1.38 Log units during storage at room temperature [57]. This showed that, the extract had antimicrobial properties and help the extend food's shelf life during storage. Refrigeration is employed to control the rate of certain chemical and enzymatic reactions as well as the rate of growth of food microorganism [58]. The food spoilage process was reduced since the mobile ability of food spoilage bacteria within the food matrices was limited, hence it reduce the food deterioration [59]. Besides that, freezing causes mechanical damage in the cell walls and membranes due to the formation of intracellular crystals [60], which leads microorganisms to death or leave them injured. However, perishable food will deteriorate, even at refrigerator temperature, due to microorganisms, enzymes and oxidation [61].

Conclusions

Findings indicated that the significant reduction of natural microbial populations in treated food samples was dependent on the concentrations and time of exposure. The reduced number of natural microbial populations in tested food materials was significant starting at concentration of 5 mg/ml of *S. polyanthum* extract for 5 min of exposure. Moreover, the treated samples found lower microbial count throughout the storage time compared to untreated samples. Therefore, *S. polyanthum* extract had potential to be developed as antimicrobial agent for natural food washing solution.

Conflicts of Interest

The authors have declared there are no competing interests.

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References

[1] Lulietto, M. F., Sechi, P., Borgogni, E., & Cenci-Goga, B. T. (2015). Meat spoilage: A critical review of a neglected alteration due to ropy slime producing bacteria. *Ital. J. Anim. Sci.*, *14*, 316-326.
[2] Lorenzo, J. M., Munekata, P. E., Dominguez, R., Pateiro, M., Saraiva, J. A., & Franco, D. (2018). *Main groups of microorganisms of relevance for food safety and stability. Innovative Technologies for Food Preservation*. Massachusetts, United States: Academic Press.
[3] Wong, J. X., Ramli, S., & Chen, S. N. (2021). Assessment of toxic effect of *Centella asiatica* extract and its application as natural preservative in fresh cut mango, pear and cabbage. *J. Food Process Pres.*, *45(10)*, e15824.
[4] Wong, J. X., & Ramli, S. (2021). *Standard Food Safety Practices from Receiving to Cleaning in the Restaurant*. Pennsylvania, United States: IGI Global Publishing Company.
[5] Ding, T., Rahman, S. M. E., & Oh, D. (2011). Inhibitory effects of low concentration electrolyzed water and other sanitizers against foodborne pathogens on oyster mushroom. *Food Control*, *22*(2), 318-322.
[6] Putnik, P., Pavlić, B., Šojić, B., Zavadlav, S., Žuntar, I., Kao, L., Kitonić, D., & Kovačević, D. B. (2020). Innovative hurdle technologies for the preservation of functional fruit juices. *Foods*, *9*, 699.

[7] Harjan, I., Harjan, A., Haider, M., & Zaidan, K. (2019) health effects of chlorinated water: A review article. *Pakistan J. Biotech.*, *16(3)*, 163-167.
[8] Sharif, Z., Mustapha, F., Jai, J., Mohd Yusof, N., & Zaki, N. (2017). Review on methods for preservation and natural preservatives for extending the food longevity. *Chem. Eng. Res. Bull.*, *19*, 145-143.
[9] Ndjateu, F. S. T., Tsafack, R. B. N., Nganou, B. K., Awouafack, M. D., Wabo, H. K., Tene, M., Tane, P., & Eloff, J. N. (2014). Antimicrobial and antioxidant activities of extractsand ten compounds from three Cameroonian medicinal plants: *Dissotis perkinsiae* (Melastomaceae), *Adenocarpus mannii* (Fabaceae) and *Barteria fistulosa* (Passifloraceae). *S. Afr J. BOT.*, *91*, 37-42.
[10] Limsuwan, S., Subhadhirasakul, S., & Voravuthikunchai, S. P. (2009). Medicinal plants with significant activity against important pathogenic bacteria. *Pharm. Biol.*, *47*, 683-689.
[11] Wong, J. X., Ramli, S., Rukayadi, Y., Juhari, N. K. K., Radu, S., & Wahid, N. B. A. (2022). Antifungal activity of ethanolic extract of *Syzygium polyanthum* (Wight) Walp. leaves extract against several types of filamentous fungi and *Candida* species. *Malays. J. Microsc.*, *18(1)*, 136-146.
[12] Perumal, S., Mahmud, R., Piaru, S. P., Cai, L. W., & Ramanathan, S. (2012). Potential antiradical activity and cytotoxicity assessment of *Ziziphus mauritiana* and *Syzygium polyanthum*. *Int. J. Pharmacol.*, *8*, 535-541.
[13] Othman, A., Mukhtar, N. J., Ismail, N. S., & Sui, K. C. (2014). Phenolics, flavonoids content and antioxidant activities of 4 Malaysian herbal plants. *Int. Food Res. J.*, *21*, 759-766.
[14] Lau, K. Y., Zainin, N. S., Abas, F., & Rukayadi, Y. 2014. Antibacterial and sporicidal activity of *Eugenia polyantha* Wight against *Bacillus cereus* and *Bacillus subtilis. Int. J. Curr. Microbiol. Appl. Sci*., *3(12)*, 499-510.
[15] Ismail, A., Mohamed, M., Sulaiman, S. A., & Wan Ahmad, W. A. N. (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. Complement. Altern. Med*., *201*, 1-17.
[16] Sumono, A., & Wulan, A. S. (2008). The use of bay leaf (*Eugenia polyantha* Wight) in dentistry. Dentistry J., *41(3)*, 147-150.
[17] Abd Rahim, E. N. A., Ismail, A., Omar, M. N., Rahmat, U. N., & Wan Ahmad, W. A. N. (2018). GC-MS analysis of phytochemical compounds in *Syzygium polyanthum* leaves extracted using ultrasound-assisted method. *Pharmacol. J*., *10(1)*, 110-119.
[18] Yusoff, N. A. H., Noor, N. F., & Rukayadi, Y. (2015). Effects of *Cosmos caudatus* Kunth. (*Ulam raja*) extract on microflora in raw chicken meat. *Int. J. Curr. Microbiol. App. Sci*., *4(2)*, 426-435.
[19] Rukayadi, Y., Shim, J. S., & Hwang, J. K. (2008). Screening of Thai medicinal plants for anticandidal activity. *Mycoses*, *51(4)*, 308-312.
[20] Annegowda, H. V., Bhat, R., Min-Tze, L., Karim, A. A., & Mansor, S. M. (2012). Influence of sonication treatments and extraction solvents on the phenolics and antioxidants in star fruits. *J. Food Sci. Technol*., *49(4)*, 510-514.
[21] Lee, M. (2009). *Basic skills in interpreting laboratory data* (4th ed). Maryland, United States: American Society of Health-System Pharmacist, Inc.
[22] Leininger, D. J., Roberson, J. R., & Elvinger, F. (2001). Use of eosin methylene blue agar to differentiate *Escherichia coli* from other gram-negative mastitis pathogens. *Vet. Diagn. Invest*., *13(3)*, 273-275.
[23] Spacil, Z., Novakova, L., & Solich, P. (2010). Comparison of positive and negative ion detection of tea catechins using tandem mass spectrometry and ultrahigh performance liquid chromatography. *Food Chem*., *123(2)*, 535-541.
[24] Biswas, D., & Roymon, M. (2013). G. LC/ TOF/ ESI/ MS based detection of bioactive compounds present in leaf and bark extract of *Acacia arabica*. *Recent Res. Sci. Technol*. *5(4)*, 37-40.
[25] Kocacaliskan, I., Talan, I., & Terzi, I. (2006). Antimicrobial activity of catechol and pyrogallol as allelochemicals. *J. Biosci*., *61*, 639-642.
[26] Namuli, A., Abdullah, N., Sieo, C. C., Zuhainis, S. W., & Oskoueian, E. (2011). Phytochemical compounds and antibacterial activity of *Jatropha curcas* Linn. extracts. *J. Med. Plant Res*., *5*, 3982-3990.
[27] Odiba, J. O., Aliyu, M. M., Halimatu, S. H., Sani, M. Y., & Ephraim, I. O. (2014). Antimicrobial activity of isolated Stigmast- 5-en-3β-ol (β-Sitosterol) from honeybee propolis from North-Western, Nigeria. *Int. J. Pharm. Sci.*, *5(12)*, 345-350.
[28] Krishnan, K. R., James, F., & Mohan, A. (2016). Isolation and characterization of n-hexadecanoic acid from *Canthium parviflorum* leaves. *J. Chem. Pharm*., *8*, 614-617.
[29] Ventaka-raman, B., Samuel, L. A., Pardha-Saradhi, M., Narashimha-Rao, B., Naga, V. K. A., Sudhakar, M., & Radhakrishnan, T. M. (2012). Antibacterial, antioxidant activity and GCMS analysis *Euphatorium odoratum*. *Asian J. Pharm. Clin. Res*., *5(2)*, 99-106.
[30] Fu, L., Lu, W. Q., & Zhou, X. M. (2016). Phenolic compounds and in vitro antibacterial and antioxidant activities of three tropic fruits: persimmon, guava, and sweetsop. *BioMed. Res. Int*., *2016*, 1-9.

[31] Karunai Raj, M. K., Duraipandiyan, V., Agustin, P., & Ignacimuthu, S. (2012). Antimicrobial activity of bergenin isolated from *Peltophorum pterocarpum* DC. flowers. *Asian Pac. J. Trop. Biomed*., *2(2)*, 901-904.
[32] Liu, W. H., Liua, T. C., and Mong, M. C. (2015). Antibacterial effects and action modes of asiatic acid. *Biomedicine*, *5(3)*, 22-29.
[33] James, T. J., & Dubery, I. A. (2009). Pentacyclic triterpenoids from the medicinal herb, *Centella asiatica* (L.). *Molecules*, *14(10)*, 3922-3941.
[34] Nitiema, L.W., Savadogo, A., Simpore, J., Dianou. D., & Traore, A. S. (2012). *In vitro* antimicrobial activity of some phenolic compounds (coumarin and quercetin) against gastroenteritis bacterial strains*. Int. J. Microbiol. Res.*, *3(3)*, 183-187.
[35] Lynch, M. F., Tauxe, R. V., & Hedberg, C. W. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiol. Infect*., *137(3)*, 307-315.
[36] Chang, J. M., & Fang, T.J. (2007) Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovars Typhimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against *E. coli* O157:H7. *Food Microbiol*., *24*, 745-751.
[37] Doyle, M. P., & Erickson, M.C. (2006). Reducing the carriage of foodborne pathogens in livestock and poultry. *Poult. Sci*., *85(6)*, 960-973.
[38] Razzaq, R., Farzana, K., Mahmood, S., & Murtaza, G. (2014). Microbiological analysis of street vended vegetables in Multan city, Pakistan: A public health concern. *Pak. J. Zool.*, *46(4)*, 1133-1138.
[39] Hong, C. K., Seo, Y. H., Choi, C. M., Hwang, I. S., & Kim, M. S. (2012). Microbial quality of fresh vegetables and fruits in Seoul, Korea*. J. Food Hyg. Saf*., *27(1)*, 24-29.
[40] Akbar, A., & Anal, A. K. (2013). Prevalence and antibiogram study of *Salmonella* and *Staphylococcus aureus* in poultry meat. *Asian Pac. J. Trop. Med*., *3(2)*, 163-168.
[41] Cohen, N., Ennaji, H., Bouchrif, B., Hassar, B., & Karib, H. (2007). Comparative study of microbiological quality of raw poultry meat at various seasons and for different slaughtering processes in Casablanca (Morocco). *J. Appl. Poult. Res*., *16*, 502-508.
[42] Gill, C. O., and Badoni. M. (2005). Recovery of bacteria from poultry carcasses by rinsing, swabbing or excision of skin. *Food Microbiol*., *22(1)*, 101-107.
[43] Al-Dughaym, A. M., & Altabari. A. F. (2010). Safety and quality of some chicken meat products in Al-Ahsa markets-Saudi Arabia. *Saudi J. Biol. Sci*., *17(1)*, 37-42.
[44] Ukuku, D. O., & Fett, W. E. (2004). Method of applying sanitizers and sample preparation affects recovery of native microflora and *Salmonella* on whole cantaloupe surfaces. *J. Food Protect*., *67***,** 999-1004.
[45] Brackett, R. E. 1992. Shelf stability and safety of fresh produce as influenced by sanitation and disinfection. J. Food Protect. *55*(10): 808-814. Doi: 10.4315/0362-028X-55.10.808
[46] Klaiber, R. G., Baur, S., Wolf, G., Hammes, W. P., & Carle, R. (2005). Quality of minimally processed carrots as affected by warm water washing and chlorination. *Innov. Food Sci. Emerg. Technol*., *6(3)*, 351-362.
[47] Gil, M. I., Selma, M. V., Lopez-Galvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash disinfection: problems and solutions*. Int. J. Food Microbiol*., *134*(1-2), 37-45.
[48] Abadias, M., Canamas, T. P., Asensio, A., Anguera, M., & Vinas, I. (2006). Microbial quality of commercial '*Golden Delicious'* apples throughout production and shelf-life of Lleda (Catalonia, Spain). *Int. J. Food Microbiol*., *108*(3), 404-409.
[49] Tornuk, F., Cankurt, H., Ozturk, I., Sagdic, O., Bayram, O., & Yetim, H. (2011). Efficacy of various plant hydrosols as natural food sanitizers in reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on fresh cut carrots and apples. *Int. J. Food Microbiol*., *148*(1), 30-35.
[50] Lazarova, R., & Krystallis, A. (2010). Consumer's perception of food quality and its relation to the choice of food. Master Thesis. University Aarhus, Vennelyst Blvd, Aarhus Demark.
[51] Hwang, C. A. (2016). Effect of mayonnaise pH and storage temperature on the behavior of *Listeria monocytogenes* in ham salad and potato salad. *J. Food Prot*., *68*(8), 1628-1634.
[52] Morshedy, A. E. M. A., & Sallam, K. I. (2009). Improving the microbial quality and shelf life of chicken carcasses by trisodium phosphate and lactic acid dipping. *Int. J. Poult. Sci*., *8*(7), 645-650.
[53] Fallah, A. A., Saei-Dehkordi, S., & Rahnama, M. (2010). Enhancement of microbial quality and inactivation of pathogenic bacteria by gamma irradiation of ready-to-cook Iranian barbecued chicken. *Radiat. Phys. Chem*., *79*(10), 1073-1078.
[54] Pizato, S., Cortez-Vega, W. R., & Prentice, C. (2015). Quality assessment of cooked chicken breast meat at different storage temperatures. *Int. Food Res. J*., *22*(1), 143-154.
[55] Vinha, A. F., Barreira, S. V. P., Castro, A., Costa, C., & Oliveira. M. B. P. P. (2013). Influence of the storage conditions on the physicochemical properties, antioxidant activity and microbial flora of different tomato (*Lycopersicon esculentum* L.) cultivars. *J. Agric. Sci*., *5*(2), 119-128.
[56] Beal, S. L. (2001). Ways to fit a PK model with some data below the quantification limit. *J. Pharmacokinet. Pharmacodyn*., *28*, 481-504.
[57]G omez, F., Igual, M., Camacho, M. M., & Pagan. M. J. (2013). Effect of the addition of plant

extracts on the microbiota of minimally processed strawberry jam and its physicochemical and sensorial properties. *CyTA-J. Food*, *11*(2), 171-178.
[58] Srivastava, R. P., & Kumar, S. (2002). *Fruit and Vegetable Preservation: Principles and Practices*. Lucknow, India: International Book Distributing Company.
[59] Whitman, W. C., Johnson, W. M., & Tomczyk, J. A. (2005). *Refrigeration and Air Conditioning Technology* (5th ed). New Delhi, India: Thomson Publications.
[60] Geiges, O. (1996). Microbial processes in frozen food. *Adv. Space Res*., *18*(12), 109-118.
[61] Tanoj, K.S and Keith,R. C. (2003). The shelf life of foods. An overview: Freshness and Shelf Life of Foods ACS Symposium Series. Washington, United States: American Chemical Society, p. 10-14.