ORIGINAL RESEARCH PAPER

Study on total phenolic, flavonoid and antioxidant capacity of fish *singgang* extracts

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ABSTRACT

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Aim: To evaluate the ash and moisture contents, total phenolic content, total flavonoid content, and antioxidant potential of Terengganu singgang extracts.
Study design: Experimental study.
Place and Duration of Study: Central Laboratory, Tissue Culture Laboratory, Universiti Sultan Zainal Abidin, Terengganu between April 2019 and July 2019.
Methodology: Samples comprised three types of singgang dishes, which were prepared, cooked, and then extracted with distilled water and ethanol (EtOH) in different strengths, 50%, 70%, and 100%.These singgang samples were chub mackerel (ST), Indian mackerel (SK), and a control sample with no fish(SC). Extracts were analyzed for their moisture and ash content. Also, the total phenolic content (TPC) was assayed using Folin-Ciocalteu reagent, while total flavonoid content (TFC) using $AlCl_3$ colorimetric assay, and antioxidant activity using 1,1-Diphenyl-2-picrylhydrazyl (DPPH). The total antioxidant capacity (T-AOC) was also evaluated.
Results: Experimental assays showed that the SC sample extracted in 100% EtOH produced the highest yield (3.7%). SK samples were lower than SC and ST in moisture content and ash content with 94.21%, 96.37% and 93.03% moisture content and 0.85%, 0.71%, and 0.96% ash content. Meanwhile, the extract of ST in 100% EtOH yielded the highest TPC (315.0 mg GAE/100g) and T-AC (8.8 U/mL) but the lowest in DPPH scavenging activity (12.2%). On the other hand, the extract of SK in 70% EtOH gave the highest TFC with 6485.3 mg QE/100g. The correlation of TFC and TPC with DPPH and T-AOC assays was positively significant.
Conclusion: In conclusion, the ST extract yielded the best antioxidant capacity.

Keywords: Antioxidant, DPPH, flavonoid, phenolic, singgang dish

1. INTRODUCTION

In Terengganu, singgang is a traditional fish dish, which is considered good for health [1]. It is commonly cooked by boiling the chub mackerel or Indian mackerel with selected herbs and spices, such as turmeric, galangal, garlic, sour plum, and chilies. These spices are well-known flavor enhancers for food. In general, turmeric is known to have antioxidant, antibacterial, and anticancer activity [2], galangal has the antioxidant, anticancer, and antidiabetic potential [3]. Also, garlic has antioxidant, anticarcinogenic, hypolipidemic effects while improving our body's immune function [4].

It is believed that the mixture of fish and herbs or spices would make the singgang dish one of the most nutritious meals with high antioxidants and high unsaturated fatty acids that are highly susceptible to lipid oxidation [5]. Generally, fish quality depends on various aspects, such as climatic season, fish weight, age, feeding patterns, maturity, environmental factors, topography, and physiological composition [6 – 8]. On the other hand, the cooking methods used may also impact the nutritional value of fish [8].

The phenolic content and antioxidant potential of several raw fish, cooked fish dishes, spices, and herbs had been well quantified [8 – 11]. Thus, the health benefits of each ingredient used in the preparation of singgang are considered well established.This study aimed to investigate the total phenolic content, total flavonoid content, and antioxidant potential fish singgang dishes. Analysis of the whole singgang might help promote the health of Terengganu people while preserving a traditional cuisine.

2. MATERIAL AND METHODS

2.1 Sample preparation of *singgang* dishes

In this study, dishes of singgang were prepared at the Therapeutic Diet and Laboratory of Universiti Sultan Zainal Abidin (UniSZA), Gong Badak (Terengganu), representing three types of samples namely, the chub mackerel singgang (ST), Indian mackerel singgang (SK), and the control singgang (SC). The SC sample was prepared without fish, i.e., comprising herbs and spices only. The herb and spices of each sample comprised 15 g grounded turmeric, 15 g grounded galangal, 25 g fresh chilies, 6 g garlic, and 10 g sour plum, and simmered in 600 mL distilled water for 5 min. Then, 5 g salt and 3 g sugar were added and followed by 500 g chub mackerel and 500 g Indian mackerel to ST and SK, respectively. Upon seasoning for 2 min, each singgang dish was boiled for 20 min, after which 400 g of the edible portion (fish and gravy) of singgang dishes was blended using a kitchen blender (HR2027/75, Koninklijke Phillips, N. V.) for 2 min to generate a homogenous mixture for each sample (ST, SK, and SC). The homogenous mixture was stored at -20^0 C until the nutrient extraction.

The extraction was largely based on the method of Mohd Adzim Khalili *et al.* [12], in which 30 g of each blended singgang dish sample (ST, SK, SC) was soaked in four different solvents, i.e., 100% ethanol (EtOH), 70% EtOH, 50% EtOH, and distilled water at room temperature for 24 h at an extracting ratio of sample to solvent 1: 10 (w/v). Altogether, 12 samples (three types of singgang x four solvents) were prepared. The supernatants of each sample were then filtered with nylon filter papers (pore size 0.45 µm) and evaporated using a rotary evaporator (BUCHI, R-215, Labortechnik AG) connected to a vacuum pump for 60 min at a reduced pressure (2300 - 5830 Pa) at 40 ^{0} C to yield the crude extract. The crude extract of each sample was dried in a drying oven at 40 ^{0} C for 60 min and then frozen at -20 ^{0} C before chemical analysis.

The extraction yield (Y) was calculated using the formula below [13].

$$Y\ (\%) = W2/W1 * 100\%$$

Where W1 = the original weight of the sample (after the blending), and W2 = weight of the dried extract.

2.2 Physicochemical analyses (Moisture and ash content)

Each crude extract's moisture content was measured using a moisture analyzer (MA 35, Sartorius, UK), in which 5 g of each sample was dried in the analyzer at 125 ℃ for 10 min to automatically generate the moisture content. The sample was analyzed in triplicate. Meanwhile, the ash content was analyzed using the method of Association of Official Analytical Chemists (AOAC) 900.02 [14], in which 5 g crude extract of each sample was dried at 550 ℃ for 12 h. The ash content was expressed as a percentage of the fresh sample weight (after blending) and calculated using the following formula [14]:

$$\text{Percentage of ash } (\%) = \frac{W2}{W1}\ x\ 100$$

Where, W1 = sample weight (g), and W2 = weight of ash (g)

2.3 Total phenolic content (TPC) assay

On the other hand, the gallic acid equivalence method was used to assay the total phenolic content (TPC) [15] of each sample. Briefly, 1 mg crude extract of each sample was diluted into 1 mL methanol (MeOH) to produce a stock solution of 1 mg/mL, and 100 µL of this stock solution was mixed thoroughly with 400 µL distilled water and 500 µL Folin-Ciocalteu indicator, and allowed to react for 5 min. Then, 1 mL of 7.5% sodium carbonate was added to the reacting mixture and allowed to settle in the dark for 2 h. The absorbance was recorded in triplicate for each sample at 765 nm via a UV-visible spectrophotometer (Genesys 20, Thermo Fisher Scientific, country). A calibration curve of gallic acid was plotted to determine the activity potential of samples, which was expressed as mg of gallic acid equivalence (GAE) per 100 g sample (mg GAE/100 g sample). The TPC value of the sample was calculated using the following formula [15]:

$$\mathrm{TPC} = \frac{\mathrm{cV}}{\mathrm{M}} \times 100$$

Where, c = concentration of the gallic acid from the calibration curve (mg/mL), V = volume of solvent used to dissolve the extract (mL), and M = weight of the extract used (g).

2.4 Total flavonoid content (TFC) assay

Also, the total flavonoid content (TFC) of crude extract was determined for each sample using the aluminum chloride ($AlCl_3$) colorimetric method. Briefly, 1 mg crude extract was diluted with 1 mL MeOH to produce a stock solution of 1 mg/mL, and 100 µL of this stock solution mixed thoroughly with 500 µL distilled water and 100 µL of 5% sodium nitrate and allowed to stand for 6 min. Then, 150 µL of 10% $AlCl_3$ solution and 200 µL of 1M sodium hydroxide were added to the reacting mixture and reacted for 5 min. The absorbance was measured in triplicate at 510 nm using the same spectrophotometer. The activity potential of samples was expressed as the quercetin equivalence (mg QE/100 g), and the TFC of each sample was calculated using the following formula [15]:

$$\mathrm{TFC} = \frac{\mathrm{cV}}{\mathrm{M}} \times 100$$

Where, c = concentration of the quercetin from the calibration curve (mg/mL), V = volume of solvent used to dissolve the extract (mL), and M = weight of the extract used (g).

2.5 Antioxidant capacity (DPPH and T-AOC assay)

The antioxidant activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [16], in which 10 mg crude extract of each sample was dissolved in 1.0 mL MeOH and reacted with 1.0 mL x 1 M DPPH. The absorbance was measured in triplicate at 517 nm using a UV-visible spectrophotometer (UV-16-1, Shimadzu, Japan). A standard curve was developed using the quercetin, which served as a positive control and prepared in the same concentration as the crude extract. The DPPH scavenging effect was calculated using the formula below.

DPPH scavenging effect (%) = 100 - [(A0-A1/A0) x100]

Where, A0 = the absorbance of the positive control, and A1 = the absorbance of the sample.

Meanwhile, the total antioxidant activity (T-AOC) was evaluated with an antioxidant kit (E-BC-K136, Elabscience, USA), in which, the reacting mixture, consisting of 1 mL buffer

(reagent 1), 2 mL chromogenic agent (reagent 2), and 0.5 mL ferric salt solution (reagent 3), was incubated at 37 ℃ for 30 min. Then, 0.2 mL stop solution (reagent 5) was added into the reacting mixture, thoroughly mixed, and left to stand for 10 min. The absorbance of samples and the negative control (without the samples) were then measured at 520 nm. The T-AOC activity, U/mL, using the following formula:

$$\text{T-AOC activity} = \frac{\text{Abs sample} - \text{Abs control}}{0.01} \div 30 \text{ (min)} \times \frac{\text{Total volume of reaction system (mL)}}{\text{The volume of sample (mL)}} \times \text{df}$$

Where, Abs = absorbance, and df = the dilution factor of the sample.

2.6 Statistical analysis

Both descriptive and inferential statistical analyses were used to analyze the data. The software Statistical Package for Social Sciences (SPSS, version 20.0, IBM, Armonk, USA) was used to perform two-tailed tests at the significance level of 0.05.

3. RESULTS AND DISCUSSION

3.1 Extraction yield, moisture and ash content

Table 1 shows the yield of SK, ST, and SC samples extracted in various solvents. For SK, 70% EtOH gave the highest extraction yield, i.e., 3.54%, and followed by 50% EtOH, 100% EtOH, and distilled water with 2.84%, 2.56%, and 2.26% yield, respectively. For ST, 70% EtOH gave the highest extraction yield, i.e., 3.45%, and followed by 50% EtOH, distilled water, and 100% EtOH with 3.32%, 2.63%, and 2.49% yield, respectively. Meanwhile, the highest extraction yield for SC was shown by 100% EtOH and followed by 70% EtOH, 50% EtOH, and distilled water with 3.74%, 3.51%, 3.06%, and 2.10% yield, respectively, and they significantly differed from each other ($p < 0.05$). Overall, the SC sample extracted in 100% EtOH gave the highest yield among all samples (3.74%).

Table 1. Extraction yield of *singgang* extracts using 100% EtOH, 70% EtOH, 50% EtOH and distilled water.

Samples	Solvents	Extraction yield (%)	F statistics (df)	p value
ST	100% EtOH	2.63 ± 0.01	11.81 (3,8)	<0.001*
	70% EtOH	3.45 ± 0.01		
	50% EtOH	3.32 ± 0.01		
	distilled water	2.49 ± 0.01		
SK	100% EtOH	2.56 ± 0.01	99.59 (3,8)	<0.001*
	70% EtOH	3.54 ± 0.01		
	50% EtOH	2.84 ± 0.01		
	distilled water	2.26 ± 0.01		
SC	100% EtOH	3.74 ± 0.01	46.97 (3,8)	<0.001*
	70% EtOH	3.51 ± 0.01		
	50% EtOH	3.06 ± 0.01		
	distilled water	2.10 ± 0.01		

Data are expressed as mean ± standard deviation.
Values shown are means of 3 independent experiments.
**Post hoc analysis: the extraction yield is statistically different from each other*

Also, extractions in distilled water gave a significantly lower yield than that of 100%, 70%, and 50% EtOH for each sample. In contrast, extraction in 70% EtOH gave a significantly

higher ($p < 0.05$) yield for ST and SK samples than 100% EtOH. While, 50% EtOH similarly gives higher yield in SK, ST and SC samples compared to distilled water. In general, the yield of extractions in organic solvents such as acetone, methanol, and ethanol would be improved at higher water content because the extracted compound might be soluble in both water and organic solvent [17]. However, contradicted results of the study indicated several parameters such as sample particle size, chemical composition of the phytochemicals and others might affected overall result [17, 18].

Table 2. Proximate analysis (moisture and ash content) of ST, SK, and SC.

Analysis	Samples		
	ST	SK	SC
Moisture Content (%)	93.03 ± 0.05	94.21 ± 0.05	96.37 ± 0.09
Ash Content (%)	0.96 ± 0.11	0.85 ± 0.46	0.71 ± 0.23

Data are expressed as mean ± standard deviation.

Table 2 shows the moisture and ash contents for SC, SK, and ST, in which SC had the highest moisture content, i.e., 96.4%, and ST the lowest, i.e., 93.0%. The high moisture content in SC (no fish) was expected because in SK and ST (with fish), the thermal effect (cooking) would decrease the water content of the entire *singgang* dish. The loss of water content happened because the fish protein lost the ability to retain water due to denaturation and thermal destruction after long freezing storage and cooking period, allowing the water to be lost as a drip from the fish meat [6, 19]. In contrast, ST had the highest amount of ash (0.96%) and SC has the lowest ash content (0.71%).

3.3 Total phenolic and total flavonoid contents

Table 3 shows the TPC for the extraction of SK, ST, and SC samples in various solvents. For SK, distilled water gave the highest TPC, i.e., 82.77 mg GAE/100 g, and followed by 100% EtOH, 50% EtOH, and 70% EtOH with 74.61, 51.21, and 27.09 mg GAE/100 g, respectively. For ST, 100% EtOH gave the highest TPC, i.e., 315.04 mg GAE/100 g, and followed by 70% EtOH, distilled water, and 50% EtOH with 88.79, 52.62, and 23.55 mg GAE/100 g, respectively. Meanwhile, the highest TPC for SC was shown by 70% EtOH and followed by distilled water, 50% EtOH, and 100% EtOH with 114.33, 51.91, 51.21, and 20.00 mg GAE/100 g, respectively. Strangely, SC and SK extracted in 50% EtOH had the same TPC, i.e., 51.21 mg GAE/100 g. In general, extraction in 100% EtOH gave the highest TPC for ST, while extraction in distilled water gave the highest TPC for SK, and extraction in 70% EtOH yielded the highest TPC for SC. Overall, the SC sample extracted in 100% EtOH had the lowest TPC (20.0 mg GAE/100 g) among all extractions. However, only SC and ST showed a significant difference in TPC for the same type of extraction.

Also, Table 3 shows the TFC for the extraction of SK, ST, and SC samples in various solvents. For SK, 70% EtOH gave the highest TFC, i.e., 6485.28 mg QE/100 g, and followed by 100% EtOH, distilled water, and 50% EtOH with 5374.17, 3515.83, and 2115.83 mg QE/100 g, respectively. For ST, 70% EtOH gave the highest TFC, i.e., 3326.94 mg QE/100 g, followed by 100% EtOH, distilled water, and 50% EtOH with 2935.28, 2815.83, and 2418.61 mg QE/100 g, respectively. On the other hand, the highest TFC for SC was shown by 100% EtOH and followed by 50% EtOH, distilled water, and 70% EtOH with 5585.28, 4429.72, 4321.39, and 1757.50 mg QE/100 g, respectively. Overall, extractions in 100% and 70% EtOH yielded higher TFC than that of 50% EtOH and distilled water. However, only SC samples (in all solvents) gives significantly different ($p < 0.05$) from other samples in TFC contents.

Table 3. Total phenolic (mg GAE/100g) and flavonoid content (mg QE/100g) of ST, SK, and SC.

Sample	Solvents	TPC (mg GAE/100g)	p-value	TFC (mg QE/100g)	p-value
SC	100% EtOH	20.00 ± 7.67 [a]	0.011*	5585.28 ± 1772.21 [a]	0.025*
	70% EtOH	114.33 ± 22.35 [b]		1757.50 ± 245.94 [b]	
	50% EtOH	51.21 ± 36.63 [a,c]		4429.72 ± 1216.45 [a,b,c]	
	distilled water	51.91 ± 9.03 [a,c,d]		4321.39 ± 1027.89 [a,b,c,d]	
ST	100% EtOH	315.04 ± 37.48 [a]	< 0.001*	2935.28 ± 2076.76	0.847
	70% EtOH	88.79 ± 50.18 [b]		3326.94 ± 958.53	
	50% EtOH	23.55 ± 5.35 [b,c]		2418.61 ± 719.87	
	distilled water	52.62 ± 17.33 [b,c,d]		2815.83 ± 731.20	
SK	100% EtOH	74.61 ± 30.56	0.608	5374.17 ± 864.94	0.608
	70% EtOH	27.09 ± 21.73		6485.28 ± 2051.36	
	50% EtOH	51.21 ± 46.68		2115.83 ± 639.23	
	distilled water	82.77 ± 49.65		3515.83 ± 1736.88	

Data are expressed as mean (standard deviation).
**p < 0.05 (one-way ANOVA test)*
[a,b,c,d] p<0.05 using Turkey or Dunnet T3 post hoc test.

The TPC (315.04 mg GAE/ 100 mg) value in this study was moderately high when compared to other food items, such as cooked asam pedas paste, tom yam paste and masak merah paste with 314.70, 257.38, and 302.23 mg GAE/ 100 mg, respectively [10]. Similarly, the TFC (6485.28 mg QE/ 100 g) in this study was high when compared to other food items, such as Kua-khling paste, turmeric powder, and shallot with 81.62, 2.17, and 11.18 mg QE/ 100 mg, respectively [20]. The moderately high TPC and TFC values in this study were apparently due to the use of herbs and spices in cooking the singgang dishes. The phenolic compounds and flavonoids in the herbs and spices might have contributed to the antioxidant function [21], probably due to their redox properties as reducing agents and singlet oxygen scavengers, thereby enabling antioxidant reactions [22].

3.4 Antioxidant capacity (DPPH and TAC assay)

Results observed in Table 4 shows the DPPH scavenging effect of SK, ST and SC samples in various solvents. For SK, distilled water gave the highest DPPH value of 99.07%, and followed by 50% EtOH, 100% EtOH, and 70% EtOH with 37.54%, 24.86% and 22.29%. For ST, distilled water gave the highest DPPH value with 62.04%, followed by 50% EtOH, 70% EtOH, and 100% EtOH with 26.50%, 18.09%, and 12.18%. On the other hand, the highest DPPH value for SC was shown by 70% EtOH and followed by 50% EtOH, 100% EtOH, and distilled water with 60.54%, 49.07%, 40.17%, and 36.18%. Overall, the DPPH scavenging effect differed significantly ($p < 0.05$) between samples and solvents.

Table 4. DPPH scavenging (%) and T-AOC activity of ST, SK and SC.

Samples	Solvents	DPPH (%)	p-value	T-AOC activity (U/mL)	p-value
SC	100% EtOH	40.17 ± 2.80 [a]	0.008*	1.39 ± 0.27 [a]	0.002*
	70% EtOH	60.54 ± 7.54 [b]		0.44 ± 0.09 [b]	
	50% EtOH	49.07 ± 8.21 [a,b,c]		4.24 ± 0.27 [c]	
	distilled water	36.18 ± 5.91 [a,c,d]		3.93 ± 0.18 [c,d]	
ST	100% EtOH	12.18 ± 1.67 [a,b]	< 0.001*	3.23 ± 0.27 [a]	0.022*
	70% EtOH	18.09 ± 2.78 [b]		5.07 ± 0.90 [b]	
	50% EtOH	26.50 ± 4.06 [c]		4.88 ± 0.63 [b,c]	
	distilled water	62.04 ± 0.86 [d]		2.09 ± 0.45 [d]	
SK	100% EtOH	24.86 ± 0.12 [a]	< 0.001*	8.87 ± 1.07 [a]	0.009*
	70% EtOH	22.29 ± 0.33 [a,b]		4.75 ± 0.99 [b]	
	50% EtOH	37.54 ± 3.55 [a,b,c]		4.37 ± 0.99 [b,c]	
	distilled water	99.07 ± 24.48 [d]		2.60 ± 0.27 [d]	

Data are expressed as mean ± standard deviation.
**p < 0.05 (one-way ANOVA test)*
[a,b,c,d] p<0.05 using Turkey or Dunnet T3 post hoc test.

Meanwhile, the highest T-AOC activity, (8.87 U/mL) was shown in the SK sample extracted in 100% EtOH, while the lowest in the SC sample extracted in 70% EtOH, i.e., 0.44 U/mL (Table 4). The mean T-AOC value differed significantly ($p < 0.05$) among various solvents within each sample. In general, T-OAC values increased when the concentration of solvent decreased from 50 to 0% EtOH (distilled water), after which the T-AOC values fluctuated among samples and solvents.

3.5 Correlation between TPC, TFC, DPPH and TAC assay

Table 5 showed that both TPC and TFC provides positive and negative correlation with R values ranging from -1.00 to +1.00 with antioxidant capacities, DPPH and T-AOC assays for each sample tested. Upon heating (cooking), both fish curry paste and Thai red curry paste samples showed substantial increases in TPC and antioxidant capacities (DPPH and Ferric Reducing Antioxidant Power) [10, 11]. The same scenario was likely to be true also for the singgang dish. However, the rise in TPC after heating might due to the increased extractability of polyphenol compounds as the heat would disrupt the cell wall of the herbs and spices, releasing some polyphenol compounds [10]. The antioxidant potential of food samples also depends on the synergies between antioxidant compounds and other plant components [23]. Spices and herbal ingredients used in the cooking, such as turmeric, garlic, chilies, sour plum, and galangal could prevent thermal oxidative degradation of antioxidants [24]. Thus, the antioxidant activity of singgang extracts could at least be partially linked to the high phenolic and flavonoid compounds.

Table 5. The correlation between TPC, TFC, DPPH and TAC assays.

	Samples	TPC	TFC	DPPH	TAC
TPC	SC	*	*	*	*
	ST	*	*	*	*
	SK	*	*	*	*
TC	SC	-0.50	*	*	*
	ST	0.16	*	*	*
	SK	-0.30	*	*	*
DPPH	SC	0.75*	-0.52	*	*
	ST	-0.53	-0.07	*	*
	SK	0.19	-0.44	*	*
TAC	SC	-0.40	-0.29	-0.42	*
	ST	-0.21	0.29	-0.61	*
	SK	-0.10	0.49	-0.64	*

Pearson's correlation test
**high positive correlation (p<0.05*

While, Table 6 showed there are no significant main effect of samples (F=0.73, p > 0.001) and type of extracts (F= 1.81, p > 0.001) on the DPPH value. Post-hoc multiple comparisons using Bonferroni test indicated that there is no significant difference of DPPH value between each samples and type of extracts. There is no significant interaction between factors. The model fits well and only 53.5% of DPPH value variation is explained by samples and type of extracts.

Table 6. Adjusted mean and 95% CI of the main effects of factors on DPPH value.

Factors	Adjusted mean (95% CI)	F-stat (df)	p-value
Sample			
SC	46.49 (19.08, 73.90)		
SK	29.70 (2.29, 57.12)	0.73 (2)	0.523
ST	45.94 (18.53, 73.35)		
Type of extracts			
100%EtOH	25.74 (-5.92, 57.39)		
70%EtOH	33.64 (1.98, 66.29)	1.81 (3)	0.245
50%EtOH	37.70 (6.05, 69.36)		
distilled water	12.94 (34.10, 97.42)		

Multifactorial ANOVA test was applied
Post-hoc test using Bonferroni's test was applied

The antioxidant potential of turmeric could be mediated through direct scavenging of oxygen radicals and stimulating antioxidant responses by nuclear factor erythroid 2-related factor 2 (Nrf2) activation [25]. Meanwhile, garlic could protect cells against oxidative stress by inducing the expression of several antioxidant enzymes, such as HO-1 and GCLM subunit through Nrf2- antioxidant response element (ARE) pathway [26]. The metalloenzyme and superoxide dismutase enzyme in chilies could also impart defense against oxidative stress by converting superoxide radical anion into hydrogen peroxide [27]. Besides, the antioxidant ability of sour plum could help against oxidative damage through lipid peroxidation, a chain reaction that caused multiple breakdowns of molecules, such as malondialdehyde [28]. Additionally, the alcoholic extract of galangal improved the antioxidant status [29]. Therefore,

it was strongly believed that spices and herbs such as turmeric, galangal, garlic, sour plum, and chilies also had contributed to the antioxidant potential of each singgang dish.

4. CONCLUSION

Overall, the sample SC extracted in 100% EtOH gave the highest yield (3.74%) and followed by samples SK (3.51%) extracted in 70% EtOH and SC (3.51%) extracted in 70% EtOH. Meanwhile, the ST sample had the lowest moisture content (93.03%), probably due to thermal destruction of proteins during the cooking process while the ST sample had the highest ash content (0.96%). On the other hand, the ST sample extracted in 100% EtOH yielded the highest TPC (315.04 mg GAE/100 g), while extraction of SK in 70% EtOH generated the highest TFC (6485.28 mg QE/100 g). However, the DPPH scavenging effect was the highest for all samples extracted in distilled water, while the T-AOC activity was the highest in the SK sample extracted in 100% EtOH (8.87 U/mL).The correlations between TPC and TFC and DPPH and TAC assays were ranging from negative to positive correlation. Further study on analyzing the phytochemicals contributing to the antioxidant activities in the singgang dish would be essential.

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