ORIGINAL RESEARCH PAPER

Study on proximate analysis, phenolic content and antioxidant capacity of fish *singgang* extracts

ABSTRACT

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Aims: To evaluate the proximate analysis, phenolic content, flavonoid content and antioxidant capacity 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 until July 2019.
Methodology: Three type of *singgang* dishes were prepared, cooked and extracted with aqueous and ethanol (50%, 70% and 100%) each; namely chub mackerel *singgang* (ST), Indian mackerel *singgang* (SK) and control *singgang* (SC). Then, the extracts were analysed for their moisture and ash content, total phenolic content (TPC) using Folin-Ciocalteu reagent, total flavonoid content (TFC) using $AlCl_3$ colorimetric assay and antioxidant activity using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and total antioxidant capacity (TAC) assays.
Results: Results showed that 100% ethanol extract of SC gives the highest percentage yield (3.74%) while ST and SK gives lower moisture content (93.03%; 94.21%) and higher ash content (0.96%; 0.85%). The ST in 100% ethanol shows the highest TPC (315.04 mg GAE/100g) and TAC (8.87 T-AOC activity, U/mL) but the lowest in DPPH scavenging activity (12.18 %). While, 70% ethanol extract of SK shows the highest value of TFC with 6485.28 mg QE/100g compared to other. There is significant positive correlation of TFC and TPC with DPPH and TAC assays.
Conclusion: In conclusion, this study observed that ST extract provided the best antioxidant capacity as compared to other extracts.

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

1. INTRODUCTION

Singgang is one of the traditional dishes in Terengganu that was make by boiling the fish with a selected herbs and spices ingredient which is good for the health [1]. Commonly, chub mackerel and Indian mackerel fish are type of fish that used to cook *singgang* dish in Terengganu. In Asian, spices and herbs such as turmeric, galangal, garlic, *Garcinia atroviridis* and chillies are well known as flavour enhancer of the cooked food. Turmeric have antioxidant, antibacterial and anticancer activity [2]. Meanwhile, galangal have antioxidant, anti-cancer and anti-diabetic [3]. Garlic also has antioxidant, anti-carcinogenic, hypolipidemic effect and improves immune function [4].

In cooking preparation of fish *singgang,* it was believed that the mixture of fish and herbs or spices could become one of the nutritious meals that are high in antioxidants, high unsaturated fatty acids and highly susceptible to lipid oxidation [5]. Generally, fish quality depends on various aspects, such as season, weight, age, feeding patterns, maturity, environmental factors, topography and physiological composition [6 – 8]. On the other hand, manufacturing or cooking methods may also impact the nutritional value of fish [8].

Some authors have previously published on the proximity of raw fish, several cooked fish dishes, spices and herbs, towards phenolic content and antioxidant abilities [8 – 11]. Since the health benefits of each ingredient in *singgang* have been confirmed, analysis of whole *singgang* as traditional fish dishes in Malaysia could provide more insight. Therefore, this study aimed to investigate the proximate analysis, phenolic content and antioxidant capacity of several samples of fish *singgang* extracts.

2. MATERIAL AND METHODS

2.1 Sample preparation of *singgang* dishes

Dishes of *singgang* were prepared at Therapeutic Diet and Laboratory, UniSZA Gong Badak. Three types of *singgang* sample were prepared namely; chub mackerel *singgang* (ST), Indian mackerel *singgang* (SK) and control *singgang* (SC). The ingredients used for *singgang* dish were chub mackerel, Indian mackerel, 2 pieces of turmeric grounded roughly (15g), 2 inch size of galangal grinded roughly (15g), 5 pieces of fresh chillies (25g), 3 cloves of garlic (6g) and 3 pieces of *G. atroviridis* (10g) were prepared. The *singgang* dish were cooked by simmering all the ingredients in 600 mL of distilled water. Following that, fish (chub mackerel and Indian mackerel each), salt and sugar were added. After seasoning with salt and sugar, the *singgang* dish were further boiled for another 20 mins until the fish cooked properly. Next, the edible portion of the fish *singgang* dishes were weighed and blended to obtain the homogenous mixture using home blender (Phillips) for 2 mins and put aside until used.

2.2 Extraction procedure of *singgang* dishes

In this study, several adjustments was done with the extraction method represent by Mohd Adzim Khalili *et al.* [12]. A total of 30 g of each blended *singgang* dishes (ST, SK, SC) were soaked into four different solvents which were 100% ethanol, 70% ethanol, 50% ethanol and 100% aqueous with an extracting ratio of sample to solvent was 1:10 (w/v) for 24 h at room temperature (24-25^0 C). The supernatants for each solvent were filtered using nylon filter paper (pore size 0.45 µm) and evaporated using rotary evaporator under reduced pressure at 40^0 C using vacuum pump. Then, the crude extract were dried in the drying oven at 40^0 C. All the extracts were stored at -20^0 C prior analysis use.

2.3 Extraction yield procedure of *P. minus* extracts

The extraction yield was calculated according to the method of Jiang *et al*. [13] using the formula;

$$W2/W1 * 100\% \qquad [13]$$

W1= original weight of sample= 500 g,
W2= weight of dried extract.

2.4 Proximate analysis

2.4.1 Moisture content

The moisture content was measured using a moisture analyser machine (Sartorius). Following the instructions of the machine, five grams of sample was weighed in triplicate and placed in pans. The sample was dried out in the machine and the percentage moisture of the samples were automatically calculated.

2.4.2 Ash content

The AOAC 900.02 method was used to measure the ash content of the samples [14]. Five grams of the samples were weighed into crucible and the samples were dried at 550℃ for 12

hours. The crucible's weight was taken after the ash process completed and the control was carried out without samples. The ash was expressed as percentage of initial fresh sample weight. The percentage was calculated using the following method:

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

Where, W1 = Weight of sample (g)
W2 = Weight of ash (g)

2.5 Total phenolic content (TPC) assay

Total phenolic content was determined using Folin-Ciocalteu method by Alyaqoubi *et al*. [15] with slightly modification. Briefly, 1 mg sample of crude extract of each *singgang* dish was diluted into 1 mL of methanol to obtain 1 mg/mL of sample (stock solution). Then, a volume of 100 µL was obtained from the stock solution and mixed thoroughly with 0.4 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent. Then, the sample was left for 5 mins. After 5 mins, 1 mL of 7.5 % sodium carbonate was added and the samples were allowed to stand in a dark place for 2 hours. The absorbance was measured at 765 nm using a spectrophotometer (Genesys 20). This process was conducted in triplicate for each sample. To determine the activity potential of the samples, a calibration curve of Gallic acid was plotted. The findings were expressed as milligram of Gallic acid equivalents per 100 grams of sample (mg GAE/100 g of sample). Then, the following formulas were used to measure the TPC values:

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

Where, T = TFC content in mg GAE/100 g of extract
c = Concentration of the Gallic acid from calibration curve
V = Volume of solvent used to dissolve extract
M = Weight of extract used in gram (g)

2.6 Total flavonoid content (TFC) assay

The total flavonoid content of crude extract was determined by the aluminium chloride ($AlCl_3$) colorimetric method. Approximately, 1 mg of crude extract of each *singgang* dish was diluted with 1 mL of methanol to obtain 1mg/mL of sample (stock solution). Then, a volume of 100 µL was obtained from the stock solution and mixed thoroughly 500 µL of distilled water and 100 µL of 5% sodium nitrate. The solution was allowed to stand for 6 mins. Next, 150 µL of 10 % $AlCl_3$ solution and 200 µL of 1M sodium hydroxide were added. Again, it was left for another 5 mins. The absorbance of the spectrophotometer was measured and reported at 510 nm. For each sample, the same procedure was replicated in triplicate. The findings were expressed as quercetin equivalents (mg QE/100 g). Then, the following formulas were used to measure the TFC values:

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

Where, T = TFC content in mg GAE/100 g of extract
c = Concentration of the Gallic acid from calibration curve
V = Volume of solvent used to dissolve extract
M = Weight of extract used in gram (g)

2.7 Antioxidant capacity

2.7.1 DPPH activity

Using the method defined by Rohin *et al*. [16], the antioxidant activity was measured using DPPH radical scavenging activity assay. A total of 10 mg extract was dissolved in 1.0 mL methanol and the solution was applied at room temperature to a 1.0 mL DPPH solution. By using the UV-1601 Shimadzu spectrophotometer, the absorbance was measured at 517 nm. All measurements were done in triplicate and a standard curve was developed using Quercetin. The findings were represented by test samples as a percentage of the reduction in the initial DPPH absorption as follows:

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

Where, A0 = the absorbance of the control reaction
A1 = the absorbance of the sample

2.7.2 Total antioxidant capacity (TAC)

The total antioxidant activity was determined using antioxidant kit (E-BC-K136) from Elabscience, USA. This kit consisted of four reagents which were, reagent 1 (Buffer solution), reagent 2 (Chromogenic agent), reagent 3 (ferric salt stock solution) and reagent 5 (stop solution). Firstly, 1mL of reagent 1 were pipetted into sample tubes followed by 1mL of 10 mg/mL of samples, 2mL of reagent 2 and 0.5 mL of reagent 3. The solution was then fully mixed and incubated at 37℃ for 30 mins. Then, 0.2 mL of reagent 5 were pipetted into the solution. The solution was then fully mixed and left to stand for 10 mins. Lastly, the solution was measured using spectrophotometer at 520nm in 1cm cuvette. The procedure was repeated with control tube but the samples were put at the last step of the procedure after reagent 5. The result was expressed as T-AOC activity, *U*/mL, using the following formula:

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

Where:
Abs = absorbance
df = dilution factor of sample before tested

2.8 Statistical analysis

Data were analyzed using descriptive and inferential statistical analysis and the values were represented as means and standard deviation (SD). The statistical software, SPSS for Social Sciences version 20.0 (IBM, Armonk, NY, US) was employed for all the statistical analyses in this study. Two-tailed tests were performed in this study and significantly difference at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Extraction yield of *singgang* extracts

Phytochemicals from plant can be obtained in many steps such as milling, grinding, homogenization and extraction [17, 18]. Yet, extraction is the key stage between the above steps in the recovery and separation of phytochemicals from plants by contact with a solvent to form two immiscible phases [19]. The efficacy of the extraction is influenced by the whole extraction process which includes sample particle size, chemical composition of the phytochemicals, the solvent used and the presence of interfering substances as well [20]..

Nevertheless, the extraction yield then depends fairly on the polarity, pH, temperature, extraction time and sample composition of the solvent [18].

In this study, *singgang* extracts were attained by applying different polarity solvents of 100% ethanol, 70% ethanol, 50% ethanol and aqueous (Table 1). Based on the findings, the highest extraction yield for ST and SK samples were by 70% ethanol, followed by 50% ethanol, 100% ethanol and aqueous with 2.58%, 15.58%, 2.70% and 9.68% difference, respectively ($p<0.05$). Meanwhile, the highest extraction yield for SC was by 100% ethanol, followed by 70% ethanol, 50% ethanol and aqueous with 3.74%, 3.51%, 3.06%, and 2.10% respectively ($p<0.05$). Thoroughly, 100% ethanol extract of SC gives the highest extractive yield among other solvents and samples.

Table 1. Extraction yield of *singgang* extracts using 100% ethanol, 70% ethanol, 50% ethanol and aqueous.

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

From the findings, aqueous extract of each *singgang* sample showed significantly lower extraction yield than 100%, 70% and 50% ethanol extracts. While, 70% ethanol extract gives significantly higher extractive yield of ST and SK samples compared to 100% ethanol extract of SC sample ($p<0.05$). Do *et al.* [18] had detailed that the extraction yield of organic solvents such as acetone, methanol and ethanol will be improved with a higher water content, since the extracted compound could be soluble in both water and organic solvent. Consequently, aqueous and ethanol were used because it is considered to be a green solvent that is less harmful, flammable and did not affect the environment [21]. Solvent greenness assessments of instruments from Rowan University and ETH Zurich have shown that the ethanol score is similar in magnitude [22]. As both systems accept that ethanol is a green solvent and is not proposed as an alternative to any hydrocarbon solvent, ethanol is therefore included as a benchmark entry [21].

3.2 Proximate analysis – moisture and ash contents

As depicted in Table 2, the moisture and ash analysis in SC, ST and ST were assessed. Result showed SC has the highest amount of moisture which is 96.37% and ST has the lowest amount which is 93.03%. In contrast, ST has the highest amount of ash (0.96%) and SC has the lowest ash content (0.71%).

Table 2. Proximate analysis (moisture and ash content) of chub mackerel *singgang* (ST), Indian mackerel *singgang* (SK) and control *singgang* (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.

The analysed moisture content shows that SC has the higher content compared to ST and SK. The findings had been expected as SC was prepared and cooked without any fish ingredient while ST and SK samples were cooked with chub mackerel and Indian mackerel fish, respectively. In detail, Sankar [23] had observed that water portion of living fish varies between 65 – 90%, although it is typically in the range of 70-75%. Muscle water is also closely bound to the proteins in the structure of living fish in such a way that it cannot be easily expelled even under high pressure, thereby increasing the content of water [6]. However, the protein lost the ability to retain water due to denaturation effects and thermal destruction after a long freezing storage or cooking period, which allows water to be lost as a drip from the fish body [6, 24]. In this analysis, it was presumed that the thermal effects and destruction during cooking decreased the water content of the entire *singgang* dish compared to the SC sample without fish ingredient.

Nevertheless, fish is a strong source of iron, calcium, zinc, phosphorus, selenium, fluoride and others, including almost all minerals [25]. Then, it was also predicted to have higher ash content in the ST and SK samples compared to the SC sample. While other spice ingredients such as chillies and onion [26, 27] may provide *singgang* dishes with mineral content, even after the cooking process, the addition of fish ingredient would increase more due to its higher mineral content. Previously, Gokoglu *et al*. [8] had reported the range of ash values obtained for fresh rainbow trout is 1.35 – 1.66% and 0.95 – 2.50% for fresh silver catfish. Compared to the present study, due to their sensitivity to heat, lower result may cause many mineral distortions [7]. However, Nader *et al*. [7] had detailed that the best cooking methods were baking and boiling, taking into account overall indices of nutritional quality, vitamin and mineral content among other methods used.

3.3 Total phenolic and total flavonoid contents

Findings showed the higher TPC is observed in 70% ethanol (114.33 mg GAE/100g) of SC while of ST and SK is showed in 100% ethanol and aqueous which is 315.04 mg GAE/100g and 82.77 mg GAE/100g (Table 3). However, SC extracted by 100% ethanol shows the lower amount of TPC compare to all solvents in each sample, which is 20.00 mg GAE/100g. Besides that, SC and SK has the same amount of TPC in 50% ethanol which is 51.21 mg GAE/100g. By comparing all the samples extracted by each solvents, 100% ethanol extracted the highest amount of TPC for ST while aqueous extracted the highest amount of TPC for SK and 70% ethanol extracted highest TPC for SC. However, only SC and ST showed significance value of the TPC among type of the solvents used.

Table 3. Total phenolic (mg GAE/100g) and flavonoid content (mg QE/100g) of chub mackerel *singgang* (ST), Indian mackerel *singgang* (SK) and control *singgang* (SC).

Samples	Solvents	TPC (mg GAE/100g)	p-value	TFC (mg QE/100g)	p-value
SC	100% eth	20.00 ± 7.67 [a]	0.011*	5585.28 ± 1772.21 [a]	0.025*
	70% eth	114.33 ± 22.35 [b]		1757.50 ± 245.94 [b]	
	50% eth	51.21 ± 36.63 [a,c]		4429.72 ± 1216.45 [a,b,c]	

ST	aqueous	51.91 ± 9.03 ^{a,c,d}	< 0.001*	4321.39 ± 1027.89 ^{a,b,c,d}	0.847
	100% eth	315.04 ± 37.48 ^{a}		2935.28 ± 2076.76	
	70% eth	88.79 ± 50.18 ^{b}		3326.94 ± 958.53	
	50% eth	23.55 ± 5.35 ^{b,c}		2418.61 ± 719.87	
SK	aqueous	52.62 ± 17.33 ^{b,c,d}	0.608	2815.83 ± 731.20	0.608
	100% eth	74.61 ± 30.56		5374.17 ± 864.94	
	70% eth	27.09 ± 21.73		6485.28 ± 2051.36	
	50% eth	51.21 ± 46.68		2115.83 ± 639.23	
	aqueous	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.

Based on Table 3, the higher TFC value could be observed by 50% ethanolic extract of SC (4429.72 mg QE/100g) while in ST and SK by 70% ethanolic extract with 3326.94 mg QE/100g and 6485.28 mg QE/100g, respectively. Yet, 70% ethanol extract of SK gives the highest flavonoid content but 70% ethanol extract of SC gives the lower flavonoid content among all samples. By comparing types of solvent, 100% and 70% ethanol showed higher value of flavonoid content compared to 50% ethanol and aqueous. However, only SC had significance difference of TFC among the type of solvents used ($p<0.05$).

According to Dzoyem and Eloff [28], phenolics and flavonoids are still considered to be the main contributors to the antioxidant function of plant materials. Due to their redox properties, antioxidant activity occurs that may serve as reducing agents, singlet oxygen scavengers and others [29]. Therefore, the TPC and TFC in *singgang* dishes extracts were investigated in this study. TPC was quantified by the Folin-Ciocalteu method, which is an assay based on electron transfer, and gives reducing capacity expressed as phenolic content [30]. In the meantime, TFC was calculated by means of an $AlCl_3$ colorimetric assay; which capable of detecting flavonoids [31].

3.4 Antioxidant capacity (DPPH and TAC assay)

The mean DPPH scavenging activity of each samples and solvents were measured accordingly with values expressed in percentage (%) as illustrated in Table 4. Results showed that DPPH scavenging effect was higher in aqueous extract of SK sample (99.07 ± 24.48%), while lower DPPH scavenging effect showed by 100% ethanol extract of SK sample (12.18 ± 1.67%). There is significant difference between DPPH scavenging effect values between samples and solvents ($p<0.05$). Nevertheless, it can be seen that ST and SK samples gives increment pattern of DPPH scavenging values as aqueous > 50% ethanol > 70% ethanol > 100% ethanol, while SC sample showed contradict values.

Compared to the DPPH assay, 100% ethanol extract of SK gives the highest TAC which is 8.87 T-AOC activity, U/mL while 70% ethanol of SC gives the lowest TAC value with 0.44 T-AOC activity, U/mL (Table 4). The mean TAC value (U/mL) in each solvents of samples was significantly difference ($p<0.05$). Nevertheless, the result obtained shows there is increment pattern of TAC value with 50% ethanol < aqueous in each sample before results showed fluctuated pattern.

Table 4. DPPH scavenging (%) and TAC activity of chub mackerel *singgang* (ST), Indian mackerel *singgang* (SK) and control *singgang* (SC).

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

Following, antioxidants activity of *singgang* extracts is measured using DPPH and TAC assays. The DPPH is a free radical based on nitrogen and is commonly used to assess samples' of free radical scavenging ability [32]. On the other hand, TAC is known as an electron transfer method based on the 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonate (ABTS) principle, which is the most sensitive assay compared to the DPPH assay [33]. Based on Marecek *et al.* [34], DPPH radical only interacts with polyphenols, whereas phenolic acids and sugar are highly reactive to ABTS radical, which allows it the ability to react with a wider variety of antioxidant.

3.5 Correlation between TPC, TFC, DPPH and TAC assay

There are positive and significant correlation between TPC and TFC with antioxidant capacities (DPPH and TAC assays) of samples tested ($p<0.05$) (Table 5). Meanwhile, low to moderate correlation were found between antioxidant capacities (DPPH and TAC assays) and TPC, TFC in each samples tested.

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*

Based on the findings, it can be shown that the pattern of TPC and TFC values varies and fluctuated among different concentration solvents in each samples. However, result observed that 100% ethanol extract of ST gives the higher TPC and 70% ethanol extract of

SK gives the higher TFC values. Meanwhile, 100% and 70% ethanol extract each of SC shows the lower values for TPC and TFC compared to other samples. Nevertheless, the DPPH scavenging effect and TAC was higher in aqueous and 100% ethanol of SK, respectively. Yet, the lowest value of the DPPH scavenging effect and TAC was in 100% ethanol extract of ST and 70% ethanol extract of SC, respectively.

Previously, Tiwo *et al*. [9] had reported a phenolic content of 0.29 ± 0.02 mg GAE/100g in raw fish. While, Hanis Mastura *et al*. [10] had reported higher phenolic content of 352.58 mg GAE/100g in fish curry paste. In contrast, current studies have shown that *singgang* extracts containing chub mackerel and Indian mackerel fish ingredients provide a higher phenolic and flavonoid content than *singgang* extracts containing no fish ingredients. Therefore, previous studies have shown and endorsed that cooked fish dishes offer a higher phenolic and flavonoid content compared to raw fish and fish-free dishes.

Nevertheless, after heat therapy on fish curry paste and Thai red curry paste samples, Hanis Mastura *et al*. [10] and Inchuen *et al*. [11] reported substantial increases in TPC and antioxidant capacities (DPPH and FRAP). However, the rise in TPC after heating could be due to increased extractability of polyphenol compounds due to heat disruption of the cell wall, allowing some polyphenol compounds to be released from raw food samples [10]. A strong correlation between TPC and TFC with the DPPH and TAC assays was also found in the present analysis. Vice versa, there was a non-significant correlation between the DPPH, TAC assays and the TPC, TFC was observed.

Thoroughly, this can be explained in detail by animal metabolism, amino acid catabolism and microbiological activities during the cooking process [35]. In addition, the antioxidant ability of food samples is not only dependent on the level of antioxidant, but also on the synergies between antioxidant compounds and other component of plants [36]. Similarly, Tomaino *et al*. [37] proposed that other antioxidants containing other spices and herbal ingredients in the food sample could prevent thermal oxidative degradation of those antioxidants. These findings showed that the antioxidant activity of *singgang* extracts could be at least partially linked to the existence of high phenolic and flavonoid compound materials.

4. CONCLUSION

In conclusion, 100% ethanol extract of SC gives the highest percentage yield followed by 70% ethanol extract of SK and SC, respectively. Meanwhile, results showed fish *singgang* extracts of ST and SK had lower moisture and higher ash content due to protein thermal destruction during cooking process. Follows, it was observed that 100% ethanol extract of ST gives the higher TPC and 70% ethanol extract of SK gives the higher TFC values. Yet, DPPH scavenging effect and TAC was higher in aqueous and 100% ethanol of SK, respectively. The relationship then was confirmed with strong positive correlation between TPC and TFC with DPPH and TAC assays. Therefore, it was anticipated for further study on analysing the phytochemical screening in the *singgang* dish.

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