

# Antioxidant and $\alpha$ -Glucosidase Inhibitory Activities and Gas Chromatography–Mass Spectrometry Profile of Salak (*Salacca zalacca*) Fruit Peel Extracts

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## ABSTRACT

**Background:** *Salacca zalacca* or better known as salak fruit is widely distributed in tropical and subtropical countries, and it is traditionally used to treat diabetes. This study was aimed to investigate the salak peel extracts for their biological and chemical activities. Also, the chemical profile of the most promising extract was analysed on gas chromatography- mass spectrometry (GC-MS). **Materials and Methods:** The peel extracts were prepared by maceration process at room temperature with different ratio of ethanol/water. All the extracts were determined for their  $\alpha$ -glucosidase inhibitory activity using  $\alpha$ -glucosidase enzyme. The antioxidant activities of the extracts were determined through their Ferric reducing antioxidant power assay (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). The chemical constituents of salak peel extracts were analysed using gas chromatography–mass spectrometry (GC MS). **Results:** Phytochemical screening showed the presence of phenolic and flavonoid contents in all the extracts. About 100% ethanol extract shows the highest phenolic content ( $116.70 \pm 0.764 \mu\text{g/mL}$ ) while 60% ethanol extract has the lowest content  $18.65 \pm 1.155 \mu\text{g/ml}$  using gallic acid as a reference. 100% ethanol extract was observed to exhibit highest radical scavenging, ferric reducing antioxidant power (FRAP), and  $\alpha$ -glucosidase inhibitory activities ( $\text{IC}_{50}$ :  $49.45 \pm 3.87 \mu\text{g/mL}$ ,  $144.81 \pm 3.72 \mu\text{g AAE/g}$ ,  $\text{IC}_{50}$ :  $11.62 \pm 0.67^b \mu\text{g/mL}$ ), respectively. Water extracts had the lowest FRAP, radical scavenging activity as well as  $\alpha$ -glucosidase activity. The phytochemical investigation on GC-MS showed the presence of active compounds in salak fruit peel extracts. **Conclusion:** Salak fruit peels showed the highest antioxidant as well as  $\alpha$ -glucosidase inhibitory activities. Phytochemical analysis on GC-MS confirms the presence of gallic acid, linoelaidic acid, palmitic acid,  $\alpha$ -tocopherol, and steric acid which may contribute to  $\alpha$ -glucosidase inhibitory activity.

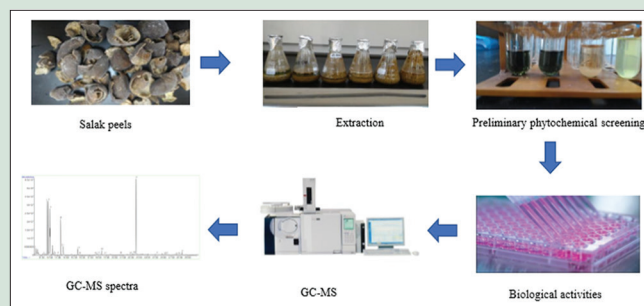
**Key words:** Antioxidant, phytochemical screening, Salak fruit, total phenolic,  $\alpha$ -glucosidase inhibitory activity

## SUMMARY

- Salak fruit peels were extracted with different concentration of ethanol/water.
- Phytochemical screening,  $\alpha$ -glucosidase inhibitory, and antioxidant activities

were evaluated.

- Results showed that ethanolic extract has the highest total phenolic contents,  $\alpha$ -glucosidase inhibitory, and antioxidant activities.
- GC-MS analysis of ethanolic extract has shown the presence of primary and secondary metabolites specifically carbohydrate, phenolic acid, fatty acid, sterol, and phytosterol.



**Abbreviations Used:** GC-MS: Gas Chromatography–Mass Spectrometry; FRAP: Ferric reducing antioxidant power assay; TPTZ: 2,4,6-tris (2-pyridyl)-s-triazine; DPPH: 2,2-diphenyl-1-picrylhydroxyl; MSTFA: N-methyl-N-(trimethylsilyl)-trifluoroacetamide; TPC: Total phenolic content; DMSO: Dimethyl sulfoxide; ANOVA: Analysis of variance.

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## INTRODUCTION

Plants are natural source of numerous phytochemicals which are the secondary metabolites produced to carry out various functions. Phytochemicals are useful in the treatment of many human ailments including diabetes, cancer, abnormalities in blood pressure, and others.

There are evidence of herbal medicine practice since ancient times, either at home or by the practitioners of traditional medicine. The trend continues until today as it grew as a cultural belief in different parts of the world. According to the report of the WHO, 80% of people in developing countries are still dependent on traditional herbal medicine derived from plants and animals to treat primary and secondary health issues. Kim *et al.* in 2015 report that the estimated market of herbal products by 2050 will rise to US\$ 5

trillion.<sup>[1]</sup> The scientific discoveries added scientific evidence of their pharmacological and biomedical potentials. Moreover, the high cost of medical treatments attracted the patients toward herbal

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medicine. Besides, the emergence of new diseases made researchers more willing to go for the herbal products in search of potential compounds and remedies.

*Salacca zalacca* Gaertn Voss., generally known as snake fruit and locally named as salak, is one of the species of palm tree which belongs to the Arecaceae family. Salak is distributed mainly in Southeast Asia regions such as Malaysia, Indonesia, Thailand, and Brunei.<sup>[2]</sup> The harvesting season in Malaysia and Thailand is about 4 months, from May to August. However, in Indonesia, salak is a year-round fruit. Salak fruit grows in such as palm, it is egg-like in shape, and the skin is brown with a diameter of 3–6 cm and weight ranging from 30 to 100 g.<sup>[3]</sup> The flesh is a pale yellow in color and pulp of ripe fruit used as edible having sweet and soft with a pleasant aroma.<sup>[4,5]</sup> Domestically people used immature salak fruit for making pickles.<sup>[4]</sup> The fruit has significant medicinal properties and it is already proven in a number of research studies, where its extracts exhibit anticancer, antioxidant, and antihyperuricemic properties. Compared to other fruits, such as apple, mango, kiwi, banana, watermelon, and papaya, salak has more antioxidant potential.<sup>[6]</sup> In one of the studies, Leontowicz *et al.* suggest that snake fruit and mangosteen apart of good taste have a high concentration of bioactive compounds and high antioxidant potential and positively affect plasma lipid profile and plasma antioxidant activity in rats fed with cholesterol-containing diets. Therefore, the degree of this positive influence is higher in rats fed diet supplemented with snake fruit. It is suggested that snake fruit-supplemented diet could be useful for a patient suffering from hypercholesterolemia.<sup>[7]</sup> According to Afrianti, isolated compounds from salak such as pyolite-2,4-dicarboxylic acid-methyl ester showed cytotoxicity activity when tested against T47D ( $IC_{50} = 1.1942 \mu\text{g/mL}$ ) and MCF-7 ( $IC_{50} = 45.414 \mu\text{g/mL}$ ) cell lines.<sup>[8]</sup> A study conducted by Kanlayavattanakul *et al.* on the peels of salak fruits suggests that the ethyl acetate fraction from 70% ethanol extract has the highest antioxidant activity; also reported noncytotoxic *in vitro* and normal human fibroblast cells. Peels of salak fruit are rich in secondary metabolite compounds such as a flavonoid, polyphenols, and phenolic.<sup>[9]</sup> These are rich sources of caffeic acid, chlorogenic, chlorogenic acid, quercetin, and gallic acids. Their antioxidant activity has been verified by two different *in vitro* studies.<sup>[9]</sup> However, the screening for antioxidant activity of peels of salak fruit is limited. Moreover, phytochemical screening of salak fruit peels and  $\alpha$ -glucosidase inhibitory activity remains unestablished.

Fruit, juices, and vegetable are significant sources of flavonoids and phenolic compounds which are important to human nutrition.<sup>[10]</sup> The excellent antioxidants such as phenolic, flavonoids, and polyphenols could have a significant role in the diseases induced by oxidative stress, such as cardiovascular and cerebrovascular diseases.<sup>[11]</sup> These are helpful in treating various forms of cancer,<sup>[12,13]</sup> especially those linked with the DNA damage due to oxidative stress.<sup>[14]</sup> In addition, the intrinsic  $\alpha$ -glucosidase inhibitory activity makes it a valuable aid for the metabolic disorders linked with carbohydrate metabolism (diabetes).<sup>[15]</sup>

Therefore, it is pertinent to investigate the antioxidant and  $\alpha$ -glucosidase inhibitory activities with proximate analysis and phytochemical profiling using gas chromatography–mass spectrometry (GC-MS) of salak fruit peel extracts.

## MATERIALS AND METHODS

### Chemicals and reagents

The chemicals and reagents used were methanol, glycine, and aluminum chloride ( $\text{AlCl}_3$ ) which were purchased by Merck Germany. Folin–Ciocalteu phenol reagent ( $\geq 95\%$  HPLC, solid), p-nitrophenyl- $\alpha$ -D-galactopyranoside (PNPG),  $\alpha$ -glucosidase, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), iron (III) chloride hexahydrate,

sodium acetate, quercetin, ascorbic acid, gallic acid, pyridine, N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), and methoxyamine hydrochloride (98%) were obtained from Sigma-Aldrich. Sodium carbonate, ethanol, and  $\alpha$ -glucosidase enzyme obtained from HmbG Chemicals Inc. (Hamburg, Germany) and Megazyme (Wicklow, Ireland), respectively. All chemicals and reagents were used of analytical grade.

### Sample preparation

Fresh fruit salak was harvested at a local farm in Terengganu, Malaysia. A sample was sent to herbarium for species identification at Kulliyyah of Pharmacy, International Islamic University Malaysia, and a documented voucher specimen (PIIUM 0269-2). The fruit was washed with tap water and dried with tissue paper. The peel of the fruit was lyophilized using a freeze dryer and pulverized using a grinder (KHIND BL1012) and stored at  $-80^\circ\text{C}$  before use.

### Extraction

Approximately 20 g of the pulverized sample was immersed in different concentrations of ethanol in water (1:3 w/v) (0%, 20%, 40%, 60%, 80%, and 100%, v/v).<sup>[16]</sup> The mixture was kept overnight at room temperature ( $20^\circ\text{C} \pm 2^\circ\text{C}$ ) and then filtered using filtration funnel. Each extraction was repeated until exhaustion. The filtrates from each solvent system were then combined and concentrated in vacuum using rotavapor (Buchi\*, Flawil, Switzerland) at  $40^\circ\text{C} \pm 1^\circ\text{C}$ . The extracts recovered were freeze-dried and then stored at  $-80^\circ\text{C}$  until further analysis.

### Preliminary phytochemical screenings

The tests to predict the bioactive constituents were carried out for the different extracts obtained from the peel of salak using standard procedures.<sup>[17]</sup>

### Test for phenol

About 2 mL of the test solutions was added with few drops of ferric chloride solution. Appearance of bluish green or red shows the presence of phenol.

### Test for tannin

About 2 mL of ferric chloride was added to 1 ml of various extracts. The appearance of green or blue indicates the presence of catechin tannins while blue-black shows gallic tannins.

### Test for alkaloids

About 2 mL of diluted hydrochloric acid was added to 2 mL of various extracts followed by 2–3 drops of Mayer's reagent consecutively. Formation of brown or white precipitate indicates the presence of alkaloids.

### Test for flavonoids

About 2 mL of the test solution was treated with few drops of sodium hydroxide solution formation yellow color which turns slowly colorless with the addition of sulfuric acid the presence of flavonoids.

### Determination of total phenolic contents

The total phenolic contents (TPCs) of the peel of salak fruit extracts were determined according to Lee *et al.*<sup>[18]</sup> Briefly, 50  $\mu\text{L}$  of the extract solution was pipetted in a 96-well micro plate, and then, it was mixed with 50  $\mu\text{L}$  of freshly prepared Folin–Ciocalteu reagent (10-fold

dilution). The mixture was left to react at room temperature for 5 min, and then, 50  $\mu$ L of 75% (w/v) sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) was added. After 45 min of incubation at room temperature, the absorbance was measured at 725 nm (Tecan, Männedorf, Switzerland). The standard curve was prepared using 50, 100, 150, 200, and 250  $\mu$ g/L solutions of gallic acid in methanol. The results were expressed as  $\mu$ g of gallic acid equivalents per milligram dry weight ( $\mu$ g gallic acid/mg dry weight).

### $\alpha$ -Glucosidase inhibitory activity

The *in vitro* assay used to evaluate  $\alpha$ -glucosidase inhibitory activity was developed by Collins *et al.* The determination of  $\alpha$ -glucosidase inhibitory activity was done by preparing alpha-glucosidase enzyme and substrate separately.<sup>[19]</sup> About 6 mg of the substrate PNPG was prepared in 20 ml of 50 Mm phosphate buffer pH 6.5. The  $\alpha$ -glucosidase enzyme was also dissolved in 50 Mm phosphate buffer pH 6.5. About 3 mg of hydroethanolic extracts were dissolved in 1 ml of dimethyl sulfoxide (DMSO). About 1 mg of quercetin also dissolved in 1 ml of DMSO was used as positive control. The inhibition potential of the extracts was measured by loading 100  $\mu$ L of 30 Mm phosphate buffer in each well, and then, 10  $\mu$ L of extract and 15  $\mu$ L of enzyme were added. The plate was incubated at RT for 5 min, and then, 75  $\mu$ L substrate was added to each well. After 15 min of incubation at room temperature, 50  $\mu$ L of (2M) glycine with pH 10 was added to stop the reaction. The final volume in each well was 250  $\mu$ L. Blank sample was prepared by adding 115  $\mu$ L of 30 Mm phosphate buffer, 10  $\mu$ L sample and 75  $\mu$ L substrate, while the control was added 10  $\mu$ L of DMSO. The absorbance of the sample was taken using spectrophotometer at 405 nm wavelength.

$$\% \text{ inhibition of sample} = \left\{ \frac{D_{\text{control}} - D_{\text{sample}}}{D_{\text{control}}} \right\} \times 100\%$$

Where  $D_{\text{control}}$  is the absorbance control and  $D_{\text{sample}}$  is the absorbance of sample.

### 2,2-Diphenyl-1-picrylhydrazyl free radical scavenging assay

The radical scavenging activity was determined as reported Maulidiani *et al.*<sup>[20]</sup> with slight modification. Briefly, 3.94 mg of DPPH was dissolved in 50 ml methanol. About 80  $\mu$ L of the methanolic solutions of DPPH (0.2 M) was added to 20  $\mu$ L extract in a microplate 96-well. An equal amount of alcohol was added to the control. Then, it was left at dark at room temperature, and the absorption was measured at 540 nm after 10 min. Ascorbic acid was used as a positive control. A control reaction was carried out without the test sample. Absorbance values were corrected for radical decay using blank solution. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula:

$$\% = \left\{ \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right\} \times 100\%$$

Where  $A_{\text{control}}$  is the absorbance control and  $A_{\text{sample}}$  is the absorbance of sample.

### Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was determined according to the reported method of Szydłowska-Czerniak *et al.*<sup>[21]</sup> A FRAP stock solution was prepared 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM  $\text{FeCl}_3$ , and 25 mL of 0.1 M acetate buffer pH 3.6. The mixture was incubated in the oven for 10 min at 37°C. About 20  $\mu$ L of each different extract of salak was added to 40  $\mu$ L of FRAP solution, and then, 140  $\mu$ L of distilled water in a 96-well plate produces a blue-colored solution. Then, it was left at room temperature for 20 min in the dark and then measured at 593 nm with a microplate reader and was used to plot the standard curve. The results were corrected for dilution and expressed as AA  $\mu$ g of ascorbic acid/mg.

### Derivatization for gas chromatography–mass spectrometry analysis

The samples were derivatized for GC-MS analysis according to the method Robinson *et al.*<sup>[22]</sup> About 25 mg ethanolic extract was dissolved in 50  $\mu$ L pyridine in a 2 ml centrifuge tube. Then it was vortexed for 5 min before sonicated for 10 min at 30°C. About 100  $\mu$ L of methoxyamine HCl (20 mg/mL in pyridine) was added and incubated for 2 h at 60°C. About 300  $\mu$ L of MSTFA was added and incubated again for 30 min at 60°C. Finally, the solution sample was filtered using a microsyringe filter and left overnight.

### Gas chromatography–mass spectrometry analysis

The GC-MS analysis was generated following the method described by Javadi *et al.* with slight modification.<sup>[16]</sup> The sample was analyzed on an Agilent 6890 gas chromatograph connected to an Agilent 5973 quadrupole and mass selective detector using an electron impact ionization. The analysis was performed using column DB-5MS, 5% phenyl methyl siloxane column with an inner diameter of 250  $\mu$ m, and a film thickness of 250  $\mu$ m attached with an autosampler (Agilent, Santa Clara, United States). About 1  $\mu$ L of the sample was injected in splitless mode into GC-MS. The initial oven temperature was set at 180°C and the hold time 10 min and then increased to 220°C at a rate of 20°C/min with the hold time of 5 min. The final oven temperature was 315°C at a rate of 30°C/min with the hold time of 10 min, and total run time was 30.16 min. Helium gas was used as carrier gas at a flow rate of 1.5 mL/min. The injector and detector were set to 330°C and 250°C, respectively.

Mass spectra were acquired using a full scan and a monitoring mode in a range of 50–550 m/z after a solvent delay of 6 min. Identification of compounds was identified by matching their peak spectra with the NIST14 library.

### Statistical analysis

The results were reported as mean  $\pm$  standard deviation experiments ( $n = 3$ ). Minitab 17 (Minitab Inc., State College, PA., USA) software was used one-way analysis of variance with a Turkey with a confidence interval of 95%. Correlation between  $\text{IC}_{50}$  value of extracts for DPPH, FRAP,

**Table 1:** 2,2-diphenyl-1-picrylhydrazyl, ferric reducing antioxidant power, and  $\alpha$ -glucosidase inhibitory

Extracts (ethanol-water) (%)	DPPH (IC50 $\mu$ g/mL)	FRAP ( $\mu$ g AAE/g)	$\alpha$ -glucosidase (IC50 $\mu$ g/mL)	Yield (%)
100	49.45 $\pm$ 3.87 <sup>b</sup>	144.81 $\pm$ 3.72 <sup>a</sup>	11.62 $\pm$ 0.67 <sup>b</sup>	3.68 $\pm$ 0.25 <sup>d</sup>
80	112.54 $\pm$ 5.09 <sup>c</sup>	132.44 $\pm$ 3.11 <sup>b</sup>	35.23 $\pm$ 3.15 <sup>c</sup>	6.09 $\pm$ 0.23 <sup>c</sup>
60	166.81 $\pm$ 8.94 <sup>d</sup>	120.66 $\pm$ 1.43 <sup>b,c</sup>	70.40 $\pm$ 5.13 <sup>d</sup>	7.75 $\pm$ 0.22 <sup>b,c</sup>
40	182.22 $\pm$ 7.54 <sup>e</sup>	126.63 $\pm$ 2.32 <sup>c,d</sup>	158.35 $\pm$ 10.11 <sup>e</sup>	8.78 $\pm$ 0.80 <sup>b</sup>
20	204.96 $\pm$ 11.43 <sup>f</sup>	112.70 $\pm$ 1.69 <sup>c,f</sup>	163.98 $\pm$ 10.96 <sup>f</sup>	9.37 $\pm$ 0.47 <sup>a,b</sup>
0	306.47 $\pm$ 13.18 <sup>g</sup>	109.53 $\pm$ 1.78 <sup>f</sup>	179.34 $\pm$ 12.45 <sup>g</sup>	10.697 $\pm$ 1.09 <sup>a</sup>
Quercetin	ND	ND	3.5 $\pm$ 0.43 <sup>a</sup>	ND
Ascorbic acid	19.4 $\pm$ 0.98 <sup>a</sup>	116.58 $\pm$ 1.73 <sup>d,c</sup>	ND	ND

Values represent the mean $\pm$ SD,  $n=3$ . Values in the same columns with different superscript letters are significantly different ( $P<0.05$ ), ND: Not determined; SD: standard deviation; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power

$\alpha$ -glucosidase inhibitory and TPC, and yield of ethanol-water. The differences were regarded statistically significantly ( $P < 0.05$ ).

## RESULTS

### Extraction yield

The average yield extracts from different solvent ratio of (ethanol-water) extraction by maceration technique are shown in Table 1. The yield of ethanol extract was found to be  $3.68 \pm 0.25$  (as % w/w of salak fruit peels on a dry weight basis). A sharp increase of yield was observed which almost double  $6.09 \pm 0.2$  is while using 20% ethanol-water. The yield gradually upsurges with the higher ratio of water in the ethanol-water.

### Phytochemical screening

The phytochemical screening of the salak fruit peels extracted with different solvent ratio of ethanol-water (0%, 20%, 40%, 60%, 80%, and 100%, v/v) are shown in Table 2, observing that 20%, 40%, 60%, 80%, and 100% extracts of salak fruit peels are rich in phenol and flavonoid contents. However, aqueous extract of salak fruit peels was showed the presence of flavonoid in abundance and least amount of phenols. However, phenol and flavonoid contents are less in a flesh compared to the peels,<sup>[23]</sup> and the presence of tannin and alkaloids was not observed in peels but was observed in flesh of salak.<sup>[24]</sup>

### Total phenolic contents of salak fruit peels

Phenolic compounds are secondary metabolites present in plants, vegetable, and fruits. They are known to possess high antioxidant and  $\alpha$ -glucosidase

activities. The TPC of the peels extracts is shown in Figure 1, expressed as gallic acid as equivalents per gram of dried samples. About 100% ethanol extract had the highest TPC,  $116.70 \mu\text{g GAE/mg}$ , whereas the 60% extract was observed  $18.65 \pm 1.15 \mu\text{g GAE/mg}$ . TPCs of salak fruit peels were significantly ( $P < 0.05$ ) different from other parts of salak, such as flash and seeds. TPCs were showed the trend of  $100\% > 80\% > 20\% > \text{water} > 40\% > 60\%$ .

### $\alpha$ -Glucosidase inhibitory activity

The  $\text{IC}_{50}$  value for antioxidant and  $\alpha$ -glucosidase inhibitory activities of salak fruit peel extracts obtained from the different ratio of ethanol-water is shown in Table 1. The extract has lower  $\text{IC}_{50}$  value which is desirable from higher antioxidant and  $\alpha$ -glucosidase activities.

All the extracted showed  $\text{IC}_{50}$  ranging from 11 to 179  $\mu\text{g/ml}$ . Ethanol extract has the highest  $\alpha$ -glucosidase inhibition with the values of  $\text{IC}_{50} 11.62 \pm 0.67 \mu\text{g/ml}$ . The water extract exhibited the lowest activity ( $\text{IC}_{50}$ :  $179.34 \pm 12.45 \mu\text{g/ml}$ ). Followed by 80%, 60%, 40%, and 20% extracts 35.23, 70.40, 158.35 and 163.98  $\mu\text{g/ml}$  respectively ( $P < 0.05$ ). As the water ratio decreased, the activity of salak peels increased significantly. The water extract displayed the lowest  $\text{IC}_{50}$ , 179.34  $\mu\text{g/ml}$ . The  $\alpha$ -glucosidase activity showed the trend of  $100\% > 80\% > 60\% > 40\% > 20\% > \text{water}$ .

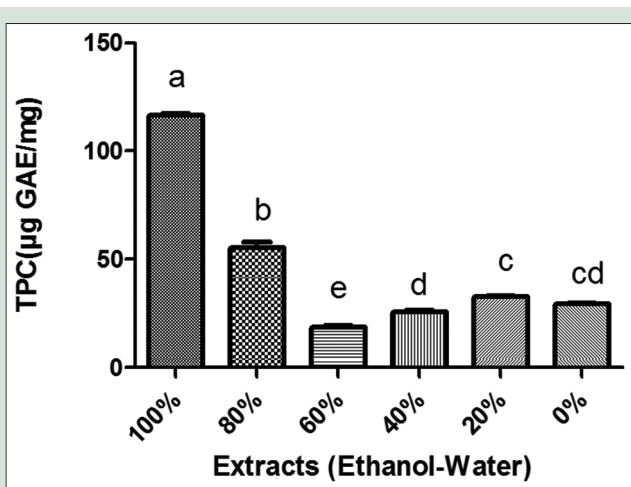
### Antioxidant activity

Decolorization of DPPH radical from purple to yellow is usually measured at 515 nm. Result obtained from DPPH radical scavenging activity of salak fruit peel extracts is shown in Table 1. The results were presented as the mean of three determinations. The  $\text{IC}_{50}$  value is defined as the concentration of peel extract required to scavenge 50% radical was tabulated as determined from the regression curve. Since it is a measure of inhibitory concentration, lower  $\text{IC}_{50}$  reflects higher antioxidant activity *vice versa*. As shown in Table 1, all the extracts except 100% ethanol extract displayed lower activity, with their  $\text{IC}_{50} > 100 \mu\text{g/ml}$  among all the extracts. Hence, 100% ethanol extract had the highest radical scavenging activity with  $\text{IC}_{50}$  (49.45  $\mu\text{g/ml}$ ) was significant ( $P < 0.05$ ) while the least activity observed to be water with  $\text{IC}_{50}$  306.47  $\mu\text{g/ml}$ . These results imply that polar extract of peels is not suitable for radical scavenging.

In FRAP assay, antioxidant compounds exert their effect by cleaving the free radical chain through hydrogen atom transfer. Result for FRAP assay is presented in Table 1, expressed in term of AA equivalent. Result obtained showed that ethanol 100% had highest ferric reducing power 144.81  $\mu\text{g AAE/g}$ , while water extract had the least. This also indicates that antioxidants from peels are relatively less polar compounds.

### Gas chromatography–mass spectrometry analysis

GC-MS analysis was considered to identify the compounds present in ethanol extract, based on the NIST14 database library. Figure 2 shows the spectra of GC-MS. This study was mainly focused on those compounds which a matching library scores 90% similarity index. Those compounds are primary and secondary metabolites such as phenolic, phytosterol, fatty acid, and organic acids. Table 3 shows that the ethanolic extract is abundant in the phenolic acid compound which had antioxidant and  $\alpha$ -glucosidase inhibitory activities. The GC-MS analysis was identified 27 compounds which belong to carbohydrate (i.e., sorbose, D-(-)-fructofuranose, d-mannose, D-fructose, D-(-)-tagatose, and L-sorbopyranose as carbohydrate and gallic acid as phenolic acid). In addition, fatty acid (i.e., linoleic acid, arachidic acid, 1-monopalmitin acid, palmitic acid, and heptadecanoic acid), sterol (i.e.,  $\beta$ -sitosterol), and phytosterol (i.e., campesterol) were also identified [Table 3].



**Figure 1:** Total phenolic content of extracts with different solvent ratio (ethanol-water). Values are mean  $\pm$  standard deviation of triplicate analyses. Values followed by different letter are significantly different ( $P < 0.05$ )

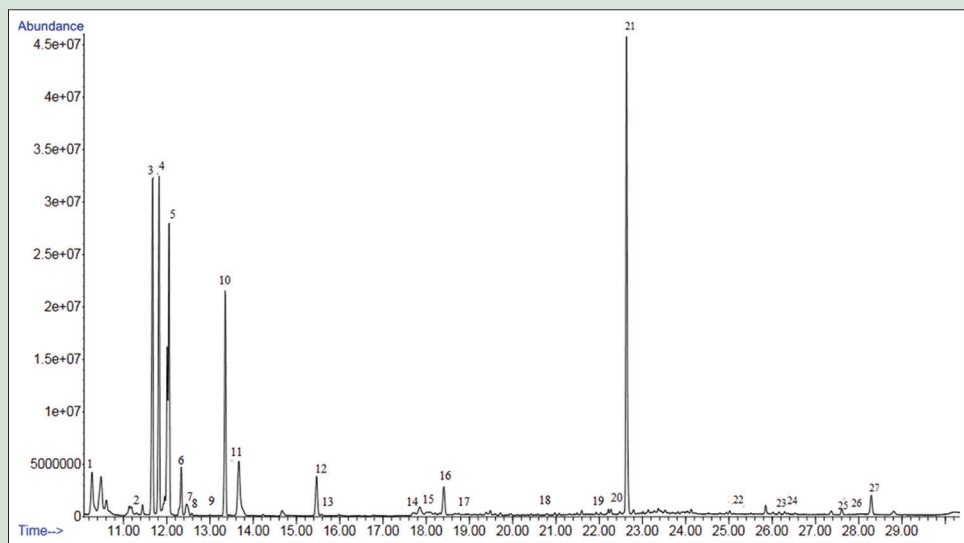
**Table 2:** Phytochemical screening of different ethanol-water extracts of peel of salak fruit

Phytochemical compounds	Extracts (ethanol-water)					
	100%	80%	60%	40%	20%	0%
Phenols	++	++	++	++	++	+
Tannin	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-
Flavonoids	+++	+++	+++	+++	+++	+++

(-) Not detected; (+) present in low amount; (++) present in moderate amount; (+++) present in high amount.

**Table 3:** Metabolites identified in ethanol extract of salak fruit peels

n	Retention time	Area (%)	Probability (%)	Tentative metabolites
1	10.276	2.67	91	D-(-)-Fructofuranose
2	11.305	0.17	90	L-Sorbopyranose
3	11.676	12.05	93	D-Fructose
4	11.825	11.05	95	D-(-)-Tagatose
5	12.013	5.25	95	$\alpha$ -D-Glucopyranose
6	12.339	2.01	91	d-Mannose
7	12.459	0.86	95	$\beta$ -D-Glucopyranose
8	12.579	0.11	93	D-Glucitol
9	13.002	0.04	98	Gallic acid
10	13.356	8.10	94	Galactopyranose
11	13.671	3.98	99	Palmitic acid
12	15.465	2.01	91	Myo-inositol
13	15.991	0.14	97	Heptadecanoic acid
14	17.712	0.31	96	Linoelaidic acid
15	17.854	0.81	95	13-Octadecenoic acid (E)
16	18.409	1.62	99	Stearic acid
17	19.592	0.15	92	Gluonic acid
18	20.975	0.13	97	Arachidic acid
19	21.929	0.17	90	Iron (0), (2-methylbutadiene)-1,2-bis (diisopropylphosphino) ethane
20	22.215	0.32	93	1-Monopalmitin
21	22.632	17.07	95	Sucrose
22	25.021	0.24	90	$\gamma$ -Tocopherol
23	26.022	0.20	95	Stigmastan-3,5-diene
24	26.159	0.22	95	$\alpha$ -Tocopherol
25	27.370	0.26	99	Campesterol
26	27.610	0.30	93	Stigmasterol
27	28.291	1.09	99	$\beta$ -Sitosterol



**Figure 2:** Gas chromatography–mass spectrometry chromatogram of metabolites in ethanol extract from salak fruit peels

## DISCUSSION

Tropical and subtropical fruits such as salak, papaya, star fruit mango, mangosteen, kiwi, and guava are found in many countries of Asia, Europe, and North and South America as they are rich in phenolic and flavonoid contents which belong to secondary metabolites and therefore responsible for good antioxidants.<sup>[9,24-26]</sup> In one of the studies on tropical fruits, Fitri *et al.* performed a study on tropical fruits. salak fruit peels were used for their antioxidant, TPC, and total flavonoid content (TFC) values. It was found that it contains highest radical TFC and TPC ( $6.4 \pm 1.8 \mu\text{g/ml}$ ,  $317.0 \pm 29.2 \text{ mg GAE}$ ,  $71.7 \pm 11.7 \text{ mg QE}$ ), respectively, followed by matoa peel, soursop

peel, papaya peel, and rambai peel.<sup>[27]</sup> There was a correlation between the phenolic contents and the  $\alpha$ -glucosidase and antioxidant. Furthermore, Kanlayavattanukul *et al.* reported that salak peel was an excellent source of antioxidants.<sup>[9]</sup> However,  $\alpha$ -glucosidase inhibition of salak peels has not been studied that as of our knowledge, the antioxidant and TPC of salak peels were significantly higher than flesh of salak fruit, their results were in accordance with antioxidant potential ( $46.7 \pm 4.7$  and  $72.9 \pm 7.4 \mu\text{mol TE/g}$ ) and content of polyphenols ( $14.9 \pm 1.5$  and  $9.2 \pm 0.8 \text{ mg GAE/g}$ ).<sup>[6]</sup> GC-MS analysis was carried out to identify chlorogenic acid, myo-inositol, gallic acid, linoelaidic acid, palmitic acid, shikimic acid, and xylitol. Based on

literature, searches documented that the identified compounds have reported for various biological activity, considered as an antioxidant, anti-inflammatory, antibacterial, and antidiabetic.<sup>[27-30]</sup> The higher phenolic contents in ethanol extracts may be attributed to the high phenolic compounds present in the extracts. The high antioxidant and  $\alpha$ -glucosidase inhibitory properties of *S. zalacca* indicated that the fruit possessed potential health benefits. Phenolic compounds are used for the reducing of digestive enzymes and also help to oxidize fats of the body owing to their thermogenic properties. On the other hand, both terpenes and terpenoid are known for their antihyperglycemic properties.<sup>[31,32]</sup>

## CONCLUSION

The study reveals that the ethanolic extract of salak fruit peels showed the highest antioxidant as well as  $\alpha$ -glucosidase inhibitory activities compared to other extracts and rich in phenolic and flavonoid contents. Phytochemical analysis on GC-MS confirms the presence of gallic acid, linoelaidic acid, palmitic acid,  $\alpha$ -tocopherol, and stearic acid which may contribute to  $\alpha$ -glucosidase inhibitory activity. Promising results of this experiment will grant a new hope in exploring the peel biochemical activities through *in vitro* and *in vivo* studies.

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## Conflicts of interest

There are no conflicts of interest.

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