RECOVERY OF THE BIOLOGICAL ACTIVE COMPOUNDS OF *Musa* Sp. THROUGH MICROWAVE ASSISTED EXTRACTION

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ABSTRACT: This study set out to investigate the recovery of the biological active compounds of *Musa* sp. through microwave-assisted extraction (MAE) system. The aim of this paper is to critically analyze the effects of temperature, microwave power, irradiation time, and solid to liquid ratio on antioxidant activity and phenolic compounds. The extraction was conducted on the unripe peel, unripe pulp, ripe peel, and ripe pulp of *Musa* sp. The extraction process was carried out by utilizing distilled water as an extracting agent. The antioxidant activity and phenolic compounds for unripe and ripe *Musa* sp were observed to be extracted best at 70 ^{o}C, microwave power range of 500 W – 800 W, and 90 s irradiation time at 3:60 solid to liquid ratio. Overall, antioxidant and phenolic compounds were found to be significantly higher in the following order: unripe peel, ripe peel, unripe pulp, and ripe pulp.

ABSTRAK: Kajian ini adalah untuk mengkaji kedapatan sebatian aktif biologi dari *Musa* sp. mengguna sistem ekstrak mikrogelombang (MAE). Objektif kajian ini adalah untuk menganalisa dengan kritikal kesan suhu, kuasa gelombang mikro, tempoh sinaran gelombang dan nisbah pepejal kepada cecair terhadap aktiviti antioksida dan sebatian fenolik. Pengekstrakan dilakukan pada kulit yang belum matang, pulpa yang belum matang, kulit masak dan pulpa masak *Musa* sp. Proses pengekstrakan dilakukan dengan menggunakan air suling sebagai agen pengekstrak. Aktiviti antioksidan dan sebatian fenolik bagi *Musa* sp. yang masak dan tidak masak adalah paling baik diekstrak pada suhu 70 ^{o}C, kuasa gelombang mikro antara 500 W – 800 W, tempoh iradiasi selama 90 saat pada nisbah 3:60 pepejal kepada cecair. Keseluruhannya, sebatian antioksidan dan fenolik adalah lebih tinggi pada turutan berikut: kulit yang belum matang, kulit masak, pulpa yang belum matang dan pulpa masak.

KEYWORDS: *antioxidant; phenolic; microwave assisted extraction; Musa sp.; toxicity free solvent*

1. INTRODUCTION

Generally, *Musa* sp. is known as banana, and it is one of the most consumed worldwide fruits [1] and has also been classified as one of the antioxidative foods [2]. Results showed that *Musa* sp. pulp and peel contain various antioxidants such as vitamins, β-carotene as well as phenolic compounds such as catechin, epicatechin, lignin and tannins, including anthocyanins [3-5]. In addition, *Musa* sp. is also remarkably rich with minerals such as potassium and phosphorus [4, 6]. The peels possess higher phenolic compounds and antioxidant properties [3, 7] along with mineral contents compared to *Musa* sp. pulps. [8].

Antioxidants can be found either in plant materials or supplements (synthetic). There are zero side effects that have been reported on the use of natural antioxidants from plants, whereas synthetic antioxidants were found to have a genotoxic effect [9]. In addition, natural antioxidant agents have grabbed serious attention due to their ability to scavenge free radicals [10]. There is crucial interest on free radicals due to the development of a number of disorders, including cancer, neurodegeneration, and inflammation [11]. The presence of antioxidants such as phenolics and flavonoids in plants may provide protection against a number of diseases, in which the mortality from degenerative disorders has inversely led to the ingestion of natural antioxidants [12]. Hence, its application on foods allows rancidity to be minimized as well as retard the formation of toxic oxidation products, which helps maintain the nutritional quality and increase food shelf life [13].

Natural antioxidants are in high demand, especially to be applied in nutraceuticals, biopharmaceuticals, and food additive pertinent to consumer preferences. In recent years, apart from an antioxidant, phenolic compounds have also received much attention as a result of their potential ability and beneficial implications in human health [14].

Several studies have identified the extraction of antioxidants and phenolic compounds. Direct extraction using solvents is the most common technique employed to obtain extracts with high antioxidant activity. An extraction process is intended to achieve the maximum yield of substances and the highest quality of targeted compounds [15]. The solvent extraction has been widely used to extract bioactive components from the plants [16]. The standard solvents for extracting antioxidant include methanol, ethanol, and acetone, which are used either separately or in combination with an aqueous solution [5, 17-19]. For the past 10 to 15 years, the interest in MAE has significantly increased in regards of their advantages which include its ability to reduce the extraction time, solvent volume, and improve extraction yield [20-23] in comparison to the traditional extraction techniques (e.g. Soxhlet extraction and hot water extraction (HWE)). It is important to note that conventional extraction methods have been associated with high solvent requirements, longer extraction times and a higher risk of degradation of heat sensitive constituents. In addition, MAE is a green technology and considered as a potential alternative to the conventional solid-liquid extraction of bioactive compounds from plant matrices [24].

Furthermore, MAE is a process that uses microwave energy and solvents to extract the targeted compounds from the matrices. Microwave energy acts as a non-ionising radiation that causes rotation of the dipoles. The extremely high temperature at the restricted area of MAE was found to cause selective migration of target compounds from material to the surroundings at a higher rapid rate. MAE performs similar or better recoveries compared to the conventional extraction method. In MAE, the solvent and sample are contained in sealed extraction vessels under controlled temperature and pressure conditions. The closed vessels allow the temperature of the solvent to increase above its boiling point, which then shortens the extraction time and subsequently increases extraction efficiency. The efficiency of MAE depends on several variables which may not be suitable for all plant materials as a result of different nature of the existing bioactive phytochemicals [22, 25].

It has been observed that up to this date, there is no report on the use of a toxicity-free solvent in MAE on the maturity pulp and peel of *Musa* sp. Therefore, the specific objective of the study was to determine the effect of MAE method using distilled water as a toxicity free solvent in extracting antioxidant and phenolic compounds of ripe and unripe *Musa* sp. pulp and peel.

2. MATERIALS AND METHOD

2.1 Chemicals

Methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid (GA), Folin-Ciocalteu reagent, sodium bicarbonate was obtained from Sigma-Aldrich.

2.2 Sample Material and Preparation

The *Musa* sp. was obtained from a local supermarket at Kuantan, Pahang, Malaysia. An approximate of 2 kg unripe (green) and ripe *Musa* sp. (yellow) were washed and separated into pulps and peels, which was then sliced into a thickness of 2 mm. *Musa* sp. sliced sample was dried overnight at 60 ^{o}C using an oven prior to the extraction [26]. Meanwhile, fresh *Musa* sp. sample was prepared freshly.

2.3 Microwave-assisted Extraction (MAE)

The extractions of antioxidant activity and total phenolic content were performed using MAE (Brand Ethos E touch control, Milestone Corporation, Monroe, CT). A total of 30 g fresh and dried pulp *Musa* sp. was investigated at different extraction temperature (40 ^{o}C to 70 ^{o}C). Other parameters such as microwave power, distilled water, and irradiation time were fixed at 800 W, 600 ml, and 60 s, respectively.

A series of the experiment was conducted by manipulating other parameters which include microwave power (100 W to 800 W), irradiation time (30 s to 90 s), and solid to liquid ratio (1:60 to 3:60). The temperature was fixed at 70 ^{o}C. Fresh *Musa* sp. was examined during the experiment. On top of that, fresh *Musa* sp. was divided into 4 types throughout the process, namely unripe peel, unripe pulp, ripe peel, and ripe pulp. All samples were performed in triplicate.

2.4 Antioxidant Activity using Free Radical (DPPH)

This method requires 0.2 nM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol to be prepared. Next, 0.5 ml of the extract was added to 3 ml of methanol, followed by 0.3 ml of DPPH added in methanol. The mixture was known as the sample extract. The blank sample contains 3.3 ml of methanol and 0.5 ml of extract, while control sample contains 3.5 ml of methanol and 0.3 ml of DPPH in methanol. The mixture was shaken immediately and left standing at room temperature. This experiment was conducted in the dark. The mixture was then measured in a UV-VIS of absorbance at 517 nm after 30 min [27]. The antioxidant activity was measured by taking into account the inhibition of free radical (%) as shown in Eq. (1). Samples were analyzed in triplicate and average values were calculated.

$$\text{Inhibition of free radical (DPPH)}(\%) = \frac{A_o - A}{A_o} \times 100\% \quad (1)$$

A_o refers to the absorbance of DPPH solution without a sample, while A represents the absorbance of the test sample mixed with DPPH solution.

2.5 Total Phenolics Content Determination

The concentration of total phenolic compounds was determined using the Folin–Ciocalteu total phenol procedure with certain modifications as described by Spanos and Wrolstad [28]. Gallic acid (GA) standard solutions were prepared at 0.0 to 0.5 mg/ml. The extracts (0.1 ml) and the GA standards (0.1 ml) were transferred into 15 ml test tubes. 3.0 ml of 0.2 N Folin–Ciocalteu reagent were added to each test tube and mixed using a vortex

mixer. After 1 minute, 2.0 ml of 9.0% (w/v) sodium carbonate (Na_2CO_3) in water were added and mixed. The absorbance at 765 nm was determined using a UV-VIS spectrophotometer (Hitachi U-1800) after being left for 2 h at room temperature [29]. All samples were determined in triplicate. Total phenolic content (TPC) was expressed as mg GA per g fresh weight (FW) *Musa* sp.

3. RESULTS AND DISCUSSION

3.1 Effect of Fresh and Dried Pulp on the Temperature of % Scavenging Activity

The purpose of preliminary studies is to determine the antioxidant activity of fresh and dried pulp *Musa* sp. at different temperatures. Other parameters such as the ratio of solid to liquid, microwave power, and irradiation time were also fixed. The antioxidant activity was determined using methanol solution of DPPH reagent. The result showed that freshly prepared DPPH solution fades the deep purple color when antioxidant molecules extinguish DPPH free radicals and change them into a colorless or bleached product, thus resulting the absorbance to be reduced at 517 nm band [30]. As can be seen in Figure 1, the antioxidant activity is expressed in terms of percentage of inhibition of free radical (%). According to the same figure, both fresh and dried pulps show the highest antioxidant activity when the temperature is set at 70 ^{o}C. The antioxidant activity concentration shows major increment when the microwave temperature is increased from 40 ^{o}C to 70 ^{o}C. At 70 ^{o}C, the antioxidant activity of fresh pulp is 45% higher compared to the dried pulps represented by 58% and 40%, respectively. The dehydration of the samples caused a decrease of the antioxidant to about 50% on average. The difference of antioxidant activity might be caused by the process of drying performed on the dried pulp, which resulted in the breakdown of some of the antioxidant compound and denatured at high temperature (> 70 ^{o}C) or evaporated during the drying process. These results match those observed in earlier studies where antioxidants are heat sensitive, whereby prolong heat treatment may cause irreversible chemical changes to the antioxidant contents [31-33].

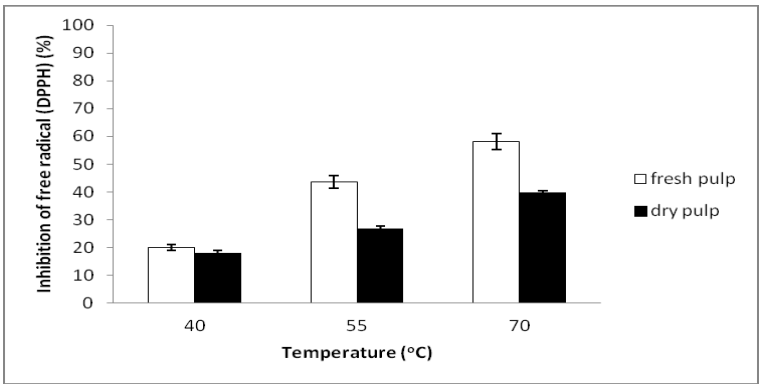

Fig. 1: Antioxidant activity of fresh and dried pulp *Musa* sp at different temperatures.

3.2 Effect of Microwave Power on Antioxidant Activity and Total Phenolic Content

The effect of microwave power was investigated on the antioxidant activity and total phenolic compounds of pulp and peel of ripe and unripe *Musa* sp. Results obtained for each element are illustrated in Fig. 2 and Fig. 3, respectively. Generally, the power applied in MAE was found to be significantly affecting the antioxidant activity of ripe and unripe *Musa* sp., pulp and peel (Fig. 2). As shown in Fig. 2, the trend of antioxidant activities of ripe and unripe pulp and peel *Musa* sp. is dissimilar. Meanwhile, the antioxidant activity

observed on ripe pulp is significantly higher at 500 W with 82% inhibition of free radical. The result is different for 800 W and 100 W, where the inhibition of free radicals is 52.1% and 52.4%, respectively (Fig. 2a). On the other hand, for the ripe peel, no significant difference of antioxidant activity is observed for both microwave power 500 W or 800 W (Fig. 2a).

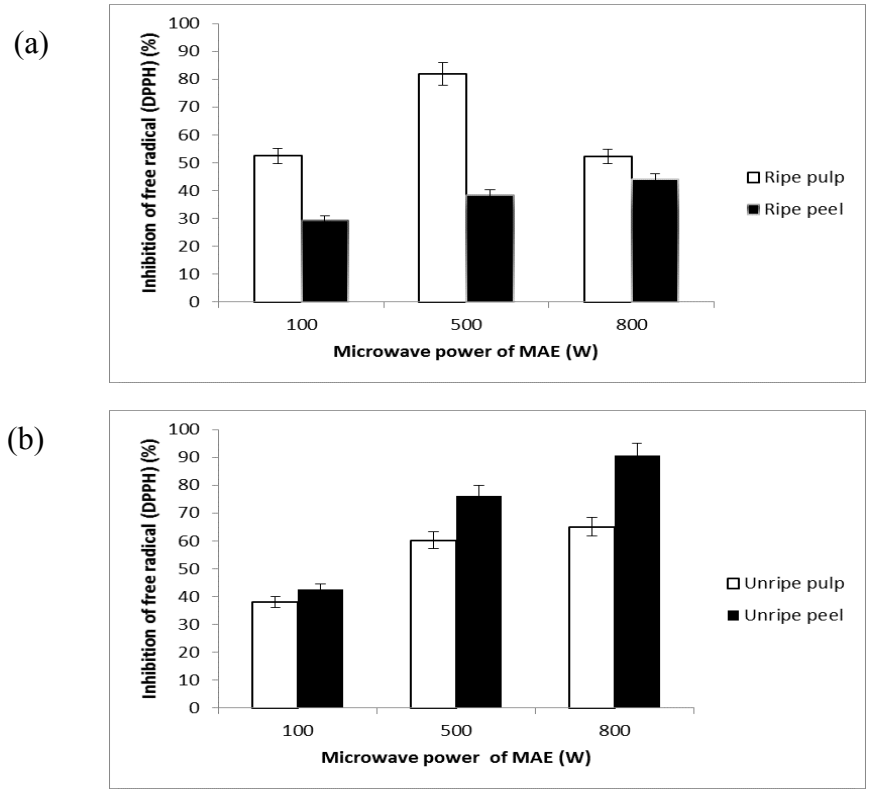

Fig. 2: Antioxidant activity of ripe pulp and peel (a) and unripe pulp and peel (b) at a different microwave power of MAE. Temperature, irradiation time and solid to liquid ratio were fixed at 70 ^{o}C, 60 s and 3:60, respectively.

On the other hand, the antioxidant activities were increased with the escalation of MAE power from 100 W to 800 W, for both the unripe pulp and peel. According to the results, the extract obtained from the peel contains higher antioxidant activity compared to the pulp, especially for the unripe *Musa* sp (Fig. 2b). This result is consistent with Fatemah et al. suggestion [34]. The increase of the microwave power of MAE resulted in higher antioxidant activity, whereby the inhibition of free radical is 65% and 91% on unripe pulp and peel at 800 W, respectively (Fig. 2b). The increased in the antioxidant activity was due to the increase of microwave power in MAE. This may be associated with the direct effects of microwave energy on biomolecules through ionic conduction and dipole rotation. The microwave energy tends to cause the power to be dissipated inside the solvent and plant material, which later generates molecular movement and heating. As a result, the cell walls may be ruptured, which enhances the extraction of antioxidant activity [35-37]. In addition, more electromagnetic energy was transferred to the extraction system, which also improved the extraction efficiency when the microwave power is increased from 100 W to 800 W. However, this phenomenon was not the case for ripe *Musa* sp. (Fig. 2a) that obtained higher antioxidant on the pulp. This might be caused by other compounds other than phenolics and flavonoids that were also involved in inhibiting the DPPH radicals. Compounds such as ascorbic acid, β-carotene, α-carotene, and

different xanthophylls [7, 38] were detected in *Musa* sp. and may influence the antioxidant activity of the extracts.

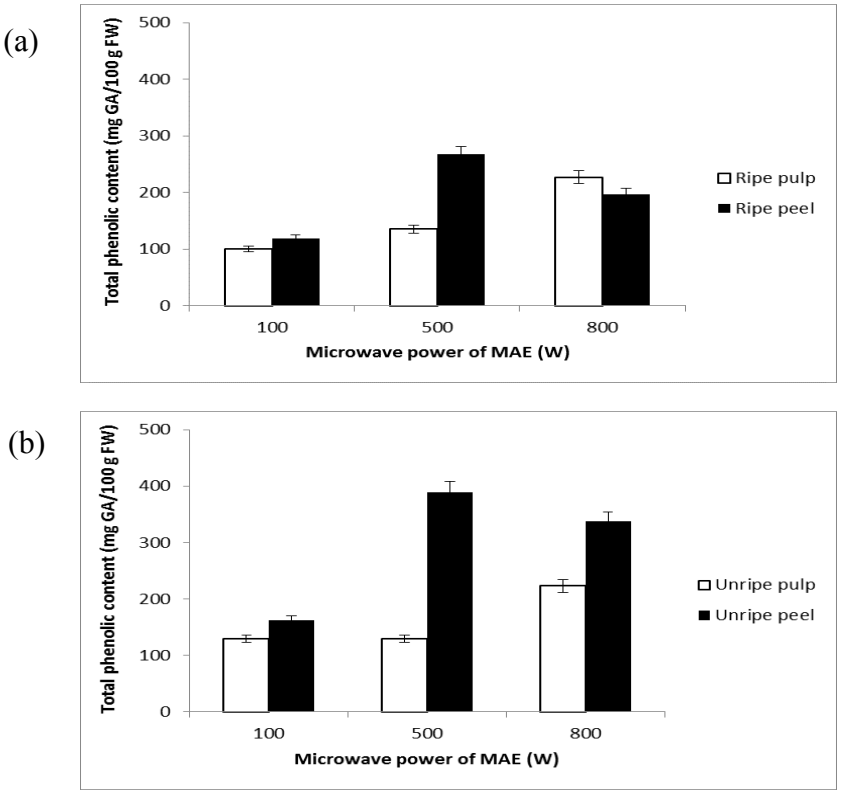

Fig. 3: Total phenolic content of ripe pulp and peel (a) and unripe pulp and peel (b) at a different microwave power of MAE. Temperature, irradiation time and solid to liquid ratio were fixed at 70 ^{o}C, 60 s and 3:60, respectively.

Figure 3 shows the total phenolic content of ripe and unripe *Musa* sp., pulp, and peel. Microwave power of MAE is observed to significantly affect the total phenolic content of ripe and unripe *Musa* sp. Moreover, a similar trend is observed for the ripe and unripe peel, whereby a higher total phenolic content is obtained at 500 W but no further increments are detected at 800 W, with 268 ± 13.4 mg GA/ 100 g FW and 389 ± 19.5 mg GA/ 100 g FW, respectively. By contrast, the total phenolic content of the pulp is found to be the highest at 800 W microwave power, for ripe and unripe (Figure 3). The dissimilarity of the trend might be the result of the complexity of the pulp matrix structure, where in MAE, the microwave power that contains irradiation energy enhances the penetration of the solvent into the pulp matrix. The electromagnetic field offers a rapid transfer of energy to the solvent and solid matrix through molecular interaction, thus allowing the components to be extracted [39]. Unlike the peel, the total phenolic content can be extracted at low power (500 W), which is believed to be the result of the less complex structure of the peel.

Phenolic compounds are essential to fruit constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals [40]. The findings show that the total phenolic content in the extracts was inversely correlated with the antioxidant activity, especially for the unripe *Musa* sp. (Fig. 3b and Fig. 2b), which further shows that the MAE conditions that obtain high antioxidant activity are not selective for phenolic content.

3.3 Effect of Irradiation Time on Antioxidant Activity and Total Phenolic Content

The yield of antioxidant activity and the total phenolic content were investigated on the pulp and peel of ripe and unripe *Musa* sp. Figures 4 and 5 demonstrate the antioxidant activity and the total phenolic content of pulp and peel of ripe and unripe *Musa* sp., respectively.

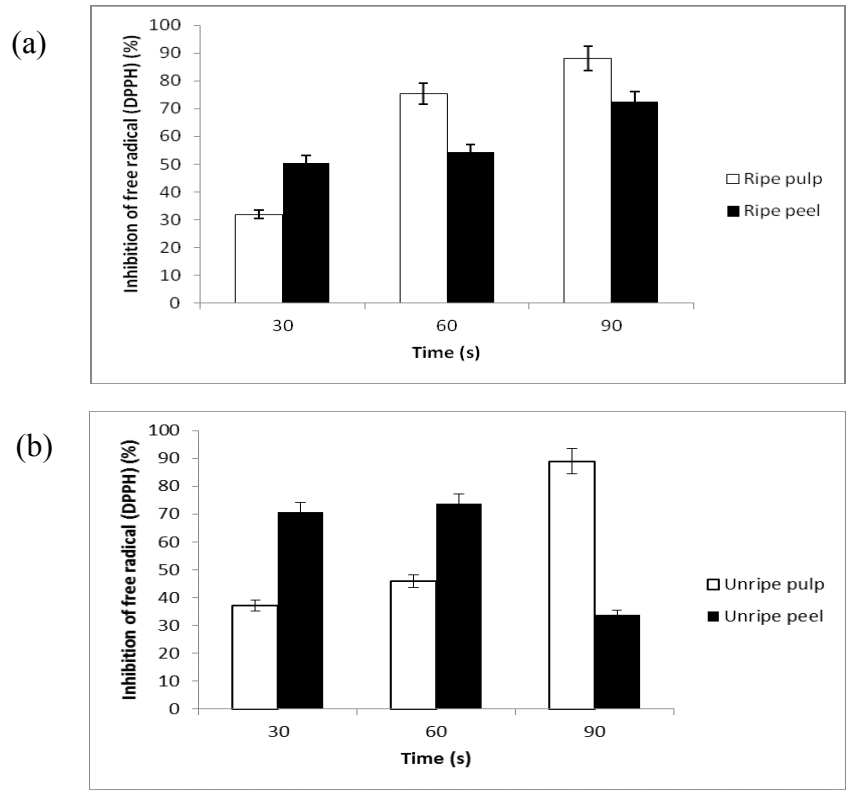

Fig. 4: Antioxidant activity of ripe pulp and peel (a) and unripe pulp and peel (b) at different extraction time of MAE. Temperature, microwave power and solid to liquid ratio were fixed at 70 ^{o}C, 800 W and 3:60, respectively.

In general, the longer the extraction time; hence, the greater the recovery of the antioxidant activity. The ripe pulp is represented by the trend shown in Fig. 4a. The antioxidant activity was high at a longer extraction time (90 s), where the inhibition of free radical was observed to increase from 32% to 88%, at 30 s to 90 s extraction time. However, prolonging the extraction time did not improve the antioxidant activity for an unripe peel (Fig. 4b). This phenomenon might be the result of the breakdown of antioxidant compounds in the less complex matrix structure of unripe peel. Longer extraction time coupled with higher microwave power might increase the temperature of the solvent, thus causing the cell wall of the peel to be ruptured [20]. In addition, longer exposure of the sample to the solvent and microwave irradiation may stimulate extraction of other components (such as polysaccharides and proteins) of the *Musa* sp., which then relatively reduced the antioxidant activity in extracts [41-42]. As a result, extending the irradiation time with a higher microwave power may lead to thermal degradation of the phenols [29].

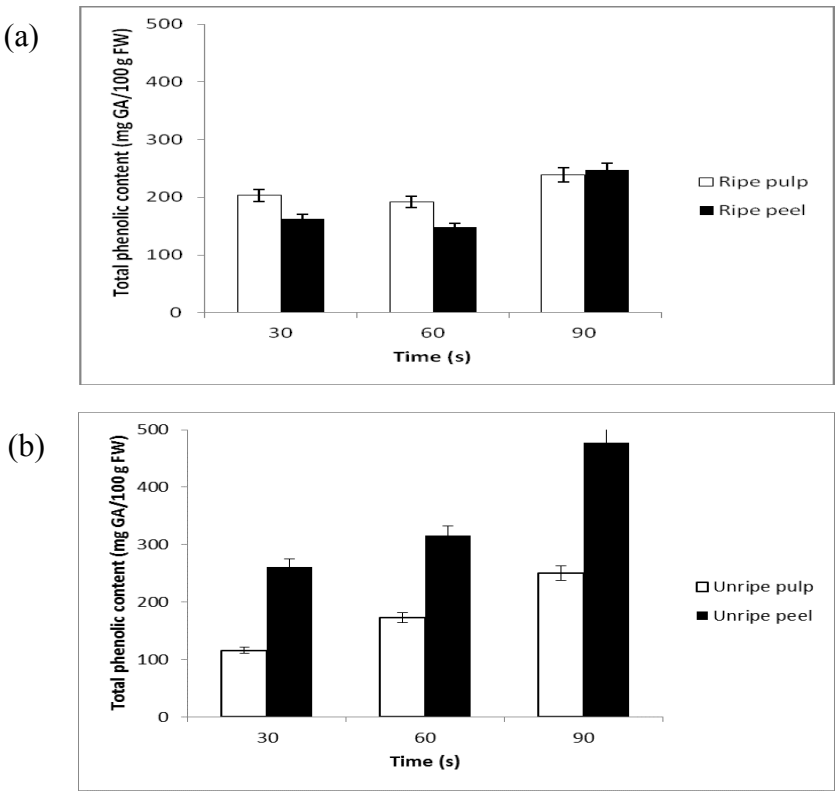

Fig. 5: Total phenolic content of ripe pulp and peel (a) and unripe pulp and peel (b) at different extraction time of MAE. Temperature, microwave power and solid to liquid ratio were fixed at 70 ^{o}C, 800 W and 3:60, respectively.

Figure 5 shows the total phenolic content of ripe and unripe *Musa* sp., pulp and peel at different extraction times. The figure reveals similar trend for ripe and unripe *Musa* sp., where the total phenolic content increased with the increase of the extraction time. From the figure, phenolic content was found to be significantly higher in unripe *Musa* sp. in contrast to ripe *Musa* sp., where the total phenolic contents were 477 ± 23.9 mg GA/ 100 g FW and 247 ± 12.4 mg GA/ 100 g FW, respectively. This finding is in agreement with Fatemah et al. (2012), where the total phenolic content was generally higher in the unripe than in ripe, whereas the peel contains higher total phenolic content than the pulp [34]. The variation in total phenolic content among different plant materials might be indicated by several factors such as natural chemical composition, maturity at harvest, soil state, and conditions of post-harvest storage [43].

3.4 Effect of Solid to Liquid Ratio on Antioxidant Activity and Total Phenolic Content

Another factor that significantly affected the amount of the antioxidant and total phenols is solid to liquid ratio. The effect of solid to liquid ratio in MAE was identified based on the yield of antioxidant activity as well as the total phenolic content of pulp and peel of ripe and unripe *Musa* sp. Figures 6 and 7 show the antioxidant activity and the total phenolic content of pulp and peel of ripe and unripe *Musa* sp., respectively.

The solid to liquid ratio of 2:60 of ripe *Musa* sp. resulted in higher antioxidant activity with the free radical inhibition of 82% when compared to the other ratios (ratio 1:60 and 3:60) (Fig. 6a). Hence, this further explains that the volume of solvent extraction was enough to swell the ripe *Musa* at the solid to liquid ratio of 2:60, which caused the cell of

ripe *Musa* to directly absorb the microwave energy. As a result, the cell was ruptured, and the antioxidant was consequently released to the surrounding [44]. In contrast, the antioxidant activity was increased with the increase of solid to liquid ratio in the unripe *Musa* sp. (Fig. 6b).

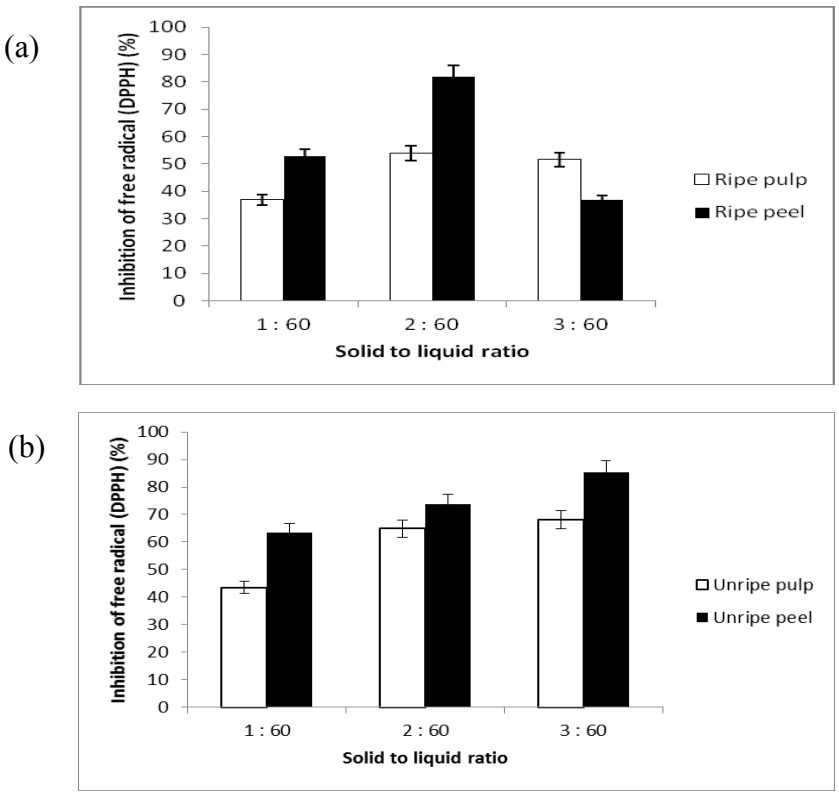

Fig. 6: Antioxidant activity of ripe pulp and peel (a) and unripe pulp and peel (b) at different solid to liquid ratio in MAE. Temperature, irradiation time and microwave power were fixed at 70 ^{o}C, 60 s and 800 W, respectively.

On the other hand, the total phenolic content of ripe and unripe *Musa* sp. was significantly increased with the increase of solid to liquid ratio (Fig. 7). The total phenolic content was increased from 200 ± 10 mg GA/ 100 g FW to 287 ± 14.4 mg GA/ 100 g FW, at solid to liquid ratio of 1:60 and 3:60 (unripe pulp), respectively. The experimental data suggested that the presence of an excessive amount of the solvent at the ratio of 1:60 (solid to liquid) may absorb more energy, and the material will reduce the microwave absorption. Therefore, the outcome leads to less efficient extraction and lower phenolic content in the *Musa* sp. extract [45].

Therefore, it is very crucial to find the best solvent mixture ratio in order to get higher extraction yield. An optimised sample to liquid ratio could pinpoint the importance of extracting the target compounds or extracts.

4. CONCLUSION

The results of this study indicate that *Musa sp*. has a great potential to be utilized as a natural antioxidant and phenolic compound sources. Higher antioxidant and total phenolic content were obtained from the peel in comparison to the pulp of *Musa* sp. The results also suggested that antioxidant and total phenolic content of *Musa* sp. extracted using MAE

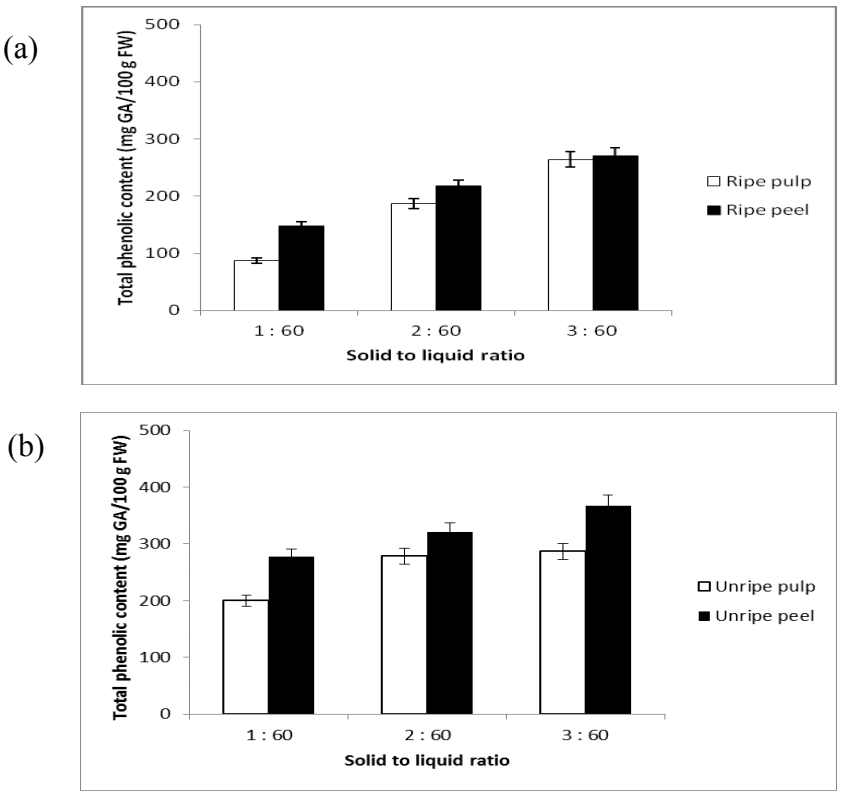

Fig. 7: Total phenolic content of ripe pulp and peel (a) and unripe pulp and peel (b) at different solid to liquid ratio in MAE. Temperature, irradiation time and microwave power were fixed at 70 ^{o}C, 60 s and 800 W, respectively.

with toxicity free solvent (distilled water) set in better extraction compared to the conventional extraction coupled with methanol as obtained by Shaida et al. [26]. The total phenolic content in this study (similar species to Shaida et al. [26]) was 87.3 mg GA/ 100 g FW, whereas the total phenolic content with methanol was found as 0.03 mg GA/ 100 g FW for ripe pulp. Hence, this experiment demonstrated that *Musa* sp. extracted using MAE with solvent-free toxicity is a better extraction method for natural antioxidant and phenolic content. Consequently, temperature, microwave power, irradiation time, and solid to liquid ratio play a huge role in affecting the efficiency of the MAE.

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