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MICROWAVE-ASSISTED EXTRACTION OF PHENOLIC COMPOUND FROM PINEAPPLE SKINS: THE OPTIMUM OPERATING CONDITION AND COMPARISON WITH SOXHLET EXTRACTION

(Pengekstrakan Sebatian Fenolik daripada Kulit Nenas dengan Bantuan Gelombang Mikro: Pengoptimuman Keadaan Pengendalian dan Perbandingan dengan Pengekstrakan Soxhlet)

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Abstract

A new method of extraction by using a microwave has been widely used in the extracting of bioactive compound from plants. In this research**,** the pineapple (*Ananas comosus L., Merr*) wastes (namely skin) has been chosen as it contains a very high phenolic compound and provide a good source of antioxidant to human's health. The three parameters varied were the microwave power, the types of solvent extraction and the extraction temperature. Each of the samples was evaluated for the Total Phenolic Compound (TPC) and Antioxidant Activity (AA). The aims of this study are to obtain the optimum operating condition of Microwave-Assisted Extraction (MAE) in the extraction of phenolic compound from pineapple skins and to compare the extraction yield between MAE and Soxhlet Extraction (SE) method. From the results, it was found that the optimum condition was at 750 W microwave power, 60 °C operating temperature and with the solvent ratio of ethanol: water (50-50) by volume**.** At this optimum condition, the TPC observed was 207.72 mg GAE/g dw, whereas for the EC_{50}, DPPH value obtained was the lowest, 13.2 mg/mL. MAE has proven that this method is comparable to SE, with the TPC obtained was 28.78 mg GAE/g dw and EC_{50} of 2.78 mg/L, respectively.

Keywords: pineapple skin, microwave-assisted extraction, microwave power, total phenolic compound, antioxidant activity

Abstrak

Satu kaedah baru pengekstrakan dengan menggunakan gelombang mikro telah digunakan secara meluas di dalam pengekstrakan sebatian bioaktif daripada tumbuhan. Di dalam kajian ini, sisa (kulit) nenas (*Ananas comosus L., Merr*) telah dipilih kerana ia mengandungi sebatian fenolik yang tinggi dan menyediakan sumber antioksidan yang baik kepada kesihatan manusia. Tiga parameter boleh ubah iaitu kuasa gelombang mikro, jenis pelarut pengekstrakan dan suhu pengekstrakan. Setiap sampel telah dinilai untuk jumlah sebatian fenolik (TPC) dan aktiviti antioksidan (AA). Tujuan kajian ini adalah untuk mendapatkan keadaan pengendalian optimum oleh bantuan gelombang mikro (MAE) di dalam pengekstrakan sebatian fenolik daripada sisa nenas dan membandingkan hasil pengekstrakan di antara MAE dan pengekstrakan Soxhlet (SE). Daripada keputusan, telah didapati bahawa keadaan optimum adalah pada kuasa gelombang mikro 750 W, pengendalian suhu 60 °C dan nisbah pelarut etanol: air (50-50) mengikut isipadu. Pada keadaan optimum ini, TPC yang dicerap adalah 207.72 mg GAE/g dw, manakala untuk EC_{50}, DPPH telah didapati pada nilai yang paling rendah, iaitu 13.2 mg/mL. MAE telah membuktikan bahawa kaedah ini setanding dengan SE, dengan TPC diperoleh adalah masing – masing 28.78 mg GAE/g dw dengan EC_{50} 2.78 mg/L.

Kata kunci: kulit nenas, gelombang mikro terbantu, kuasa gelombang mikro, jumlah sebatian berfenol, aktiviti antioksidan

Introduction

Phytochemicals are the major bioactive compounds which usually available in fruits and vegetables. Usually, the plants phenolics are found in both edible and non-edible parts of the plants and was reported to have multiple biological effects, including antioxidant activity [1]. In the recent years, the phenolics compound, or known as polyphenolics have become one of the interest due to their ability to act as a powerful antioxidant [2]. An antioxidant is a group of compounds which are able to delay or inhibit the oxidation lipids or other biomolecules and hence could prevent or repair the damaged body cells that is oxidized by the oxygen [3,4] and it has beneficial effects on metabolism.

The source of antioxidants can be synthetic or natural based. Due to the adverse effect of the synthetic antioxidant, there is an increasing attention to develop a new approach by using natural antioxidant, which is mostly, comes from fruits and vegetables [5,6]. Studies have revealed that daily intake of natural antioxidant will help to reduce the risk of those common diseases such as cancer, cardio and cerebro-vascular diseases [7].

In industry, normally the processing fruits will leave behind a large amount of fruits residue, such as peels, seeds, pulp, bean, rind and skin. The disposal of these fruit wastes can create the pollution problem to the environment [8]. In order to overcome this problem, the recovery of the fruit residues is one of the best alternatives. This is because the output from this recovery could be used in pharmaceutical industry, food and cosmetic [9]. In the past few years, the research on the phenolic compounds and antioxidant activity in fruits residues has been conducted on guava leaves [4], mango seed kernel [10], cantaloupe residue (leaf, stem, skin, seed and flesh) [11], bean [12], peanut skins [13], peels of mangosteen, rambutan and pomegranate [14] and citrus mandarin peels [15] and the residue of Kinnow (seed and peel), Litchi (seed and pericarp), grape seed and banana peel [8].

The pineapple, also known as *Ananas comosus L., Merr*, by-products is not exempted because it consists of peels, stem, pulp and leaves as it's residual. As one of the most important fruits in the world, the pineapple is the leading edible member of the *Bromeliacea*'s family [16]. It contains a good nutrient which gives benefit to human's health. This is because the pineapple contains a very high amount of phenolics compound [6, 16 – 20]. Several studies have been conducted in the extracting of phenolic compound from pineapple with different methods such as by using Soxhlet extraction unit [8, 21], magnetic stirrer [6, 19, 22] and orbital shaker (incubator shaker) [20].

In the recent technology of extraction, the microwave-assisted extraction (MAE) has been introduced. As compared to the conventional method, namely Soxhlet extraction unit, the MAE has a lot of advantages in the extraction process such as higher extraction rate, less extraction time, less solvent needed and can produce better product with lower cost [23]. This extraction method has been done on *Berberis asiatica* leaves [24], chokeberries (*Aronia melanocarpa*) [25], bark extracts [26], asparagus, cauliflower, celery, and chicory wastes [27], longan peel [28], bean [29], citrus mandarin peel [30], peanut skin [31] and many more.

This research is the extension research which has been done before where the pineapple wastes (namely skin) has been extracted for the analysis of phenolic compounds and antioxidant activity [17]. In this preliminary research, the parameters varied were only the temperature of extraction and the type of solvent used in the extraction, whereas the microwave power has been fixed at 250 W. From this research, it was found that the optimum condition at 250 W of microwave power was at 30 °C by using the deionized water as the extraction solvent, with the optimum value of phenolic compound and antioxidant activity (EC_{50}) were 206.46 mg GAE/g dw and 13.65 mg/mL, respectively. It indicated that the higher the phenolic value, the lower the EC_{50}, the better the antioxidant property. Thus, it was proven that the pineapple could serve as the potential candidate in becoming one of the best natural antioxidant [17]. However, based on the previous study the microwave power was just fixed at one value only, which was at 250 W. Thus, the effect of the variation in the usage microwave power has not been studied. Therefore, in this research, the microwave power will be varied, as well as the extraction temperature and the types of solvent used. The outcome of this result should reveal the optimum condition of MAE in the extracting of pineapple wastes and the results will be compared with the Soxhlet Extraction Method.

Materials and Methods

Material

The skin of the pineapple (waste) was peeled for the sample preparation [17].

Sample preparation

The samples (skin) obtained were washed, frozen, dried and grounded. Each sample was weight at 5 g each before the extraction process [17].

Microwave-assisted extraction

The extraction process was conducted at three different parameters, namely, the microwave power (500 W, 750 W and 1000 W), temperature (30 °C, 60 °C and 90 °C) and the type of solvent used (100% ethanol, 50% ethanol in deionized water and 100% deionized water). About 1 mL of sample was mixed with 50 mL of solvent. The extraction time was 20 minutes. The extract obtained was then analyzed for total phenolic compound and antioxidant activity [17].

Total phenolic compound

The total phenolic compound was measured using the Folin-Ciocalteu method [32]. The results were expressed in milligram of Gallic Acid Equivalent (GAE)/gram of dry weight sample of pineapple (mg GAE/g dw). All samples were analyzed in duplicates [17].

Scavenging activity on DPPH radical

The scavenging activity or antioxidant activity of the extract was analyzed by 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) [33,34,35]. All tests were performed in duplicates [17].

Sample statistical analysis

All the experiments were conducted in duplicates and the mean was calculated using MS Excel [17].

Soxhlet extraction

A total volume of solvent 250 mL (50 mL of ethanol in 200 mL of deionized water) was used in the Soxhlet experiment. The solvent was mixed with 5 g of sample and refluxed for 5 hours at 78 °C. After the extraction process, the supernatant was separated from the solvent by using the rotary evaporator for 15 minutes. The supernatant was then tested for total phenolic content and scavenging activity on DPPH radical analysis.

Results and Discussion

Effect of microwave power

The MAE is the process whereby the polar solvent which in contact with the solid samples is heated by using microwave energy [36], thus reducing the extraction time and amount of solvent consumed. The mechanism of MAE in the extracting of phenolic compound can be explained by a phenomenon. In the extraction process, the dried plant used normally will contain small traces of moisture. When the microwave energy is absorbed, this energy will be change to heat and thus the moisture starting to evaporate. This evaporated water will cause the pressure to increase inside the cell wall and lead to the rupture of the cell in the sample. Eventually, the active compound will be released from the rupture cell into the surrounding solvent and increasing the extraction yield [37].

In this research, there were three effects that have been studied. The first effect or parameter was the microwave power used in the extraction. The values were ranged from 250 W to 1000 W. For each of the microwave power value, the total phenolic compound and antioxidant activity of the pineapple waste were evaluated. Based on the previous research, a standard calibration curve was prepared to get the value of phenolic compound. The graph showed a linear relationship, whereby the gallic acid were ranged from 50 – 500 mg/L, with a correlation coefficient (R^2) of 0.97 [17].

From the preliminary study, the optimum condition at 250 W microwave power had been obtained at 30 °C operating temperature by using deionized water as solvent. The study has proven that at 120 °C operating

temperature, both phenolic compound and EC_{50} (defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals, is a parameter widely used to measure antioxidant activity value from antioxidant activity) marked a deteriorated value [17]. Therefore, in this study the temperatures were varied from 30 °C to 90 °C only.

Table 1 showed the extraction condition at 500 W microwave power. At 30 °C operating temperature by using ethanol as the solvent, the phenolic compound was 165 mg GAE/g dw, whereas the antioxidant value, which reported as EC_{50}, the value was 21.68 mg/mL. Meanwhile, by using the deionized water at 30 °C operating temperature, the phenolic compound increased to 177.24 mg GAE/g dw. The EC_{50} value at this condition was 15.07 mg/mL. As the temperature increased to 60 °C, there was a remarkable increase for the phenolic compound, 200.63 mg GAE/g dw and a sharply decrease for EC_{50} value, 14.59 mg/mL. The solvent used was ethanol in deionized water (50-50). At the highest operating temperature, which was at 90 °C the phenolic compound and EC_{50} recorded were 184.56 mg GAE/g dw and 14.84 mg/mL, respectively. The trends showed that as the temperature increase, the phenolic compound increased until 60 °C but then decrease at 90 °C. This was contradicting with the trends in EC_{50}, whereby as the temperature increase, the EC_{50} dropped at 60 °C but then inclined to 90 °C. From 500 W microwave power, the optimum value was obtained at 60 °C by using ethanol in deionized water (50-50). The phenolic compound and EC_{50} value recorded at this condition were 200.63 mg GAE/g dw and 14.59 mg/mL, respectively. EC_{50} value is a parameter to measure the antioxidant activity. It is also known as the concentration of antioxidant required for 50% scavenging of DPPH radicals. The smaller value of EC_{50}, the higher antioxidant activity of the plant extracts [38]. Therefore, the higher the phenolic compound, the lower the EC_{50} value, the better the antioxidant property of the pineapple skins.

Table 1. Total phenolic compound and DPPH radical scavenging of the extract at the microwave power of 500 W with different temperatures and solvents

Temperature (°C)	Types of Solvent	Phenolic Compound (mg GAE/g dw)	EC_{50} (mg/mL)
30	Ethanol	165.23	21.68
30	Water	177.24	15.07
60	Ethanol-Water (50-50)	200.63	14.59
90	Ethanol-Water (50-50)	184.56	14.84

The extraction at 750 W microwave power was shown in Table 2. As compared to the operating microwave power at 500 W, generally the readings of phenolic compound at 750 W were much higher. The phenolic compound recorded at 30 °C using ethanol as solvent was 186.25 mg GAE/g dw and the EC_{50} value obtained was 16.51 mg/mL. Similar trends go to the operating temperature of 30 °C using deionized water, the phenolic compound was higher compared to the solvent with pure ethanol. In general, the phenolic compound at 30 °C operating temperature using deionized water was higher both in 250 W and 500 W microwave power. Meanwhile, by using the ethanol in deionized water as a solvent, the phenolic compound at 60 °C and 90 °C were 207.72 mg GAE/g dw and 182.34 mg GAE/g dw, respectively. The value of EC_{50} was recorded at 13.2 mg/mL for 60 °C operating temperature. At 90 °C, the EC_{50} was a slightly higher at 14.38 mg/mL. Thus, the optimum condition at 750 W was at 60 °C by using ethanol in deionized water.

On the other hand, Table 3 showed the total phenolic compound and EC_{50} value at 1000 W microwave power of extraction. Overall, the readings of phenolic compound at 1000 W microwave power were slightly decreased as compared to 750 W. At 30 °C operating temperature, the phenolic compound and EC_{50} value were recorded at 165.54 mg GAE/g dw and 84.03 mg/mL, respectively. Those values were analysed by using ethanol as the solvent. Meanwhile, at 60 °C operating temperature by using deionized water as the solvent, there was a small inclined in the phenolic compound reading, 187.74 mg GAE/g dw. The EC_{50} value for the antioxidant activity was recorded at 16.41 mg/mL. There were a tremendous decreased in the phenolic compound value for both at 60 °C and 90 °C

operating temperature, which recorded at 163.32 mg GAE/g dw and 79.8 mg GAE/g dw, respectively. The same pattern of depreciation could be observed in the EC_{50} value which depicted at 22.15 mg/mL and 65.96 mg/mL for 60 °C and 90 °C operating temperature, respectively. The optimum value at 1000 W microwave power was obtained at 30 °C operating temperature by using deionized water as solvent.

Table 2. Total phenolic compound and DPPH radical scavenging of the extract at the microwave power of 750 W with different temperatures and solvents

Temperature (°C)	Types of Solvent	Phenolic Compound (mg GAE/g dw)	EC_{50} (mg/mL)
30	Ethanol	186.25	16.51
30	Water	195.68	15.02
60	Ethanol-Water (50-50)	207.72	13.20
90	Ethanol-Water (50-50)	182.34	14.38

Table 3. Total phenolic compound and DPPH radical scavenging of the extract at the microwave power of 1000 W with different temperatures and solvents

Temperature (°C)	Types of Solvent	Phenolic Compound (mg GAE/g dw)	EC_{50} (mg/mL)
30	Ethanol	165.54	84.03
30	Water	187.74	16.41
60	Ethanol-Water (50-50)	163.32	22.15
90	Ethanol-Water (50-50)	79.80	65.96

In any plant extraction, the extraction yield might be reduced if the high microwave power is applied. This is due to the degradation or decomposes of thermal sensible compound. Generally, as the microwave power increase, the extraction yield will be increase linearly, up to a certain limit, before it starts to decrease or become insignificant [39 – 42]. Microwave power supplies the heating directly to the sample and will assist the MAE to break down the plant cell so that the active compound (analyte) could diffuse from the plant and dissolve in the solvent [43].

From the four-optimized microwave power that have been discussed above based on the reading of phenolic compound and EC_{50} value, the most optimum microwave power was determined at 750 W. The operating temperature was at 60 °C and ethanol in deionized water was used as the solvent. At this optimum condition, the highest value of phenolic compound was recorded at 207.72 mg GAE/g dw, whereas the antioxidant activity, EC_{50} value was obtained at the lowest value of 13.20 mg/mL.

Effect of operating temperature

The second effect observed in this study was the effect of the extraction temperature. The value of temperature was ranged from 30 °C up to 90 °C for all the samples. The temperature and microwave power are interconnected. This is because as the temperature increases, the solvent power will be increases as well due to the decreasing in viscosity and surface tension [37]. However, there will be always a limit of the operating temperature for all the plants and fruits tested before the phenolic compound starts to degrade. A report has claimed that at a very high microwave power such as 1000 W, the extraction yield of the flavonoids from Radix astragali roots has been decreased if the extraction temperature was higher than 110 °C [40]. Another investigation found that the phenolic compound extracted from Oolong tea has been increased with the increasing extraction temperature and the optimum temperature was obtained at 170 °C [44].

According to our preliminary research, the reading of the phenolic compound was increased with the increasing temperature, but slightly decreased at 90 °C and declined sharply at 120 °C due to the thermal degradation [17, 43]. Since the extraction at 120 °C has proven to be the worst among all the temperatures tested, therefore in this research the focus of temperatures was between 30 °C to 90 °C. In general, the trends in operating temperature were the same for all the microwave power tested. This is proved by the result from Table 1, 2 and 3. Nevertheless, out of all the operating temperature, the value of the antioxidant activity, EC_{50} showed the highest value at 30 °C by using ethanol as the extraction solvent for all microwave power. This showed that the condition was not suitable to extract the phenolic compound from the pineapple skins. Therefore, the most suitable temperature to extract the phenolic compounds from the pineapple wastes was at 60 °C at 750 W microwave power.

Effect of solvent extraction

Among the most commonly used solvent for the extraction of phenolics compound from fresh fruits or plants at different concentration in water are such as ethanol, methanol, ethyl acetate, propanol, acetone and dimethylformamide [45,46]. The percentage of polyphenolic yield produced from plant materials is depending on the solubility of the phenolic compounds in the solvent used for the extraction. In other words, the solvent types used in the extraction process is very important as well as its polarity [22]. For example, in the extraction of phenolic compounds from grape skin and seed, methanol has been used as the solvent and it has been proven that the yield of polyphenolic was higher as compared to ethanol, but the latter extract had stronger antioxidant properties [47]. Generally, ethanol is the most common solvent used and acts as a good microwave absorber to extract the bioactive compounds from plants [48]. Based on the result, by referring to the all powers and temperatures shown in Table 1, 2 and 3, the highest yield of phenolic compounds was obtained by using ethanol in deionized water as the solvent, at 750W microwave power and 60 °C of operating temperature. The EC_{50} value recorded was also the lowest at this condition. A study has been reported that by addition of water to the solvent has increased the yield [49]. Several organic solvents should be prepared in the aqueous solution form before it is used as a solvent extraction. This is because with the presence of water in the organic solvent, it can improve the penetration of the solvent into the plant cell and hence, could increase the heating efficiency [50].

Comparison between microwave-assisted extraction method and the soxhlet extraction method

From the study of the three effects that have been discussed, the optimum condition for microwave power, operating temperature and solvent extraction were 750 W, 60 °C and ethanol in deionized water, respectively. This condition was then compared with the conventional method, namely Soxhlet extraction, by using the ethanol in deionized water as a solvent. The time taken for the Soxhlet extraction was 2 hours [51], whereas for the MAE needed only 20 minutes of extraction time.

Based on Figure 1, there was a significant different in phenolic compound value by using Soxhlet extraction, which was recorded at 28.78 mg GAE/g dw as compared to the MAE [51]. The results of phenolic compound are comparable since the value obtained by using the MAE was much higher at 207.72 mg GAE/g dw. On the other hand, Figure 2 shows that the value for EC_{50} for Soxhlet extraction was lower, 2.78 mg/mL compared to 13.20 mg/mL by using MAE. As discussed in the previous section, the lower the EC_{50}, the greater the antioxidant activity. Even though the EC_{50} value of Soxhlet extraction is lower compared to MAE, but based on the value of phenolic compound obtained, it has proven that MAE is an effective method compared to Soxhlet extraction. This is in line with the advantages of MAE which only consumed less solvent and has shorter extraction time [52]. Higher extraction rate in fruits, vegetables, foods and beans have been reported in several studies by using MAE compared to the Soxhlet extraction [53,54,55]. Lower yield, high amount of solvent used and continuous operation time of 6 or 12 hours make the Soxhlet is not the best extraction method. The long operating hours will lead to the increasing of the operational cost [56].

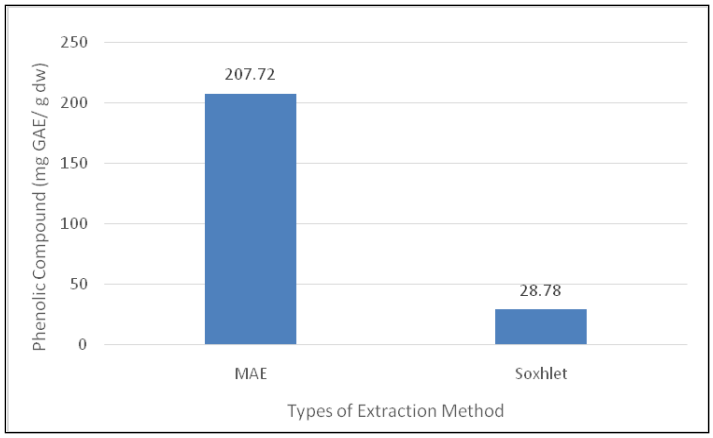

Figure 1. Comparison of total phenolic compound extracted using microwave-assisted extraction and soxhlet method

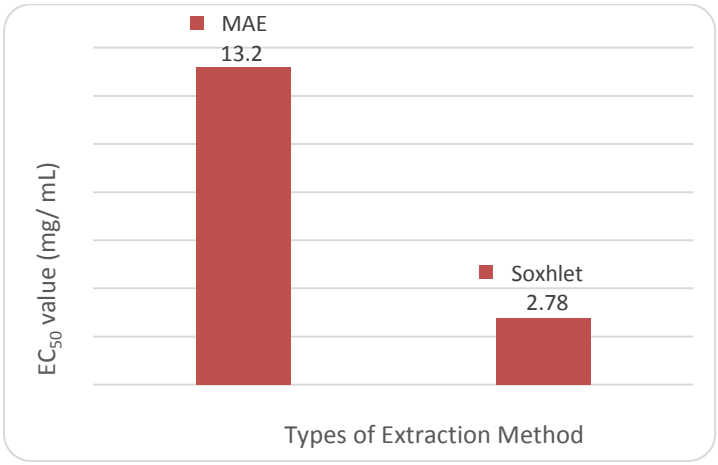

Figure 2. Comparison of DPPH radical scavenging value using microwave-assisted extraction and soxhlet method

Conclusion

Microwave-assisted extraction (MAE) is an alternative technique of extraction which provides higher yield, shorter time and less solvent used. In this research, three parameters were varied, namely, the microwave power, extraction temperature and solvent type. Based on the results, the optimum condition has been achieved at 750 W of microwave power, 60 °C of operating temperature and ethanol in deionized water was used as the solvent. At this optimum condition, the phenolic compound was obtained at the highest yield, which was 207.72 mg GAE/g dw, whereas the antioxidant activity or EC_{50} value was at the lowest, 13.2 mg/mL. The higher the phenolic compound, the lower the EC_{50} value, the better the antioxidant properties of the fruits or plants. Thus, the pineapple skins could become one of the good sources of antioxidant, which are renewable and environmental friendly compared to the commercial antioxidant. As compared to the Soxhlet extraction method, MAE exhibits more efficient method with a higher extraction of phenolic compound value.

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