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Effect of Operating Conditions of Supercritical Carbon Dioxide on *Piper Betle* Leave Oil Yield and Antioxidant Activity

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Abstract

Background: Piper betle leaves, a member of family Piperaceae is an edible plant. The leaves of Piper betle have been traditionally used in India for prevention of oral diseases. Scientific research shows that the leaves possess many biological activities with a good medicinal and commercial value. The studies on natural products are one of the most sought after research in this world. Nowadays, advance technologies have been used to improve high quality products. This study focuses on supercritical fluid extraction technology which carbon dioxide, CO_2 act as a solvent. In addition, Soxhlet extraction was used as a comparison method. Objective: The aim of this study was to investigate the effects of supercritical carbon dioxide (SC-CO_2) extraction process variables, namely pressure (10–30 MPa) and temperature (40–80 °C) on oil yields and antioxidant activities and to compare the different between SC-CO_2 extraction process with soxhlet extraction. Results: The result shows that the oil yield increases as the pressure decrease while the temperature increase. Furthermore, it was found that the antioxidant activity gradually decreased with the increase of the extraction temperature at the constant pressure, while the antioxidant activity increased significantly with the pressure enhancement at the constant temperature. In addition, the comparison between SC-CO_2 extractions with soxhlet extraction shows that SC-CO_2 extraction process gave the best process compared to soxhlet extraction. Conclusion: Despite the highest oil yield of SC-CO_2 extraction (6.456%) was lower than Soxhlet extraction (10.419%), the SC-CO_2 extraction method was more selective compared to the Soxhlet extraction as the percentage of antioxidant activity by SC-CO_2 were higher than that obtained by the Soxhlet extraction method. Therefore, SC-CO_2 extraction was the best method than Soxhlet extraction method.

Keywords— Piper Betle Leaves, Supercritical Carbon Dioxide, Antioxidant

I. INTRODUCTION

Piper betel leaves are a dioecious, evergreen creeper that grows in moist, tropical and subtropical regions where it is used widely as a traditional medicine in different countries such as Malaysia, Indonesia, Philippines, Thailand, China and many other western countries [1]. This plant originated from the central and eastern part of peninsular Malaysia [2]. From ancient time betel are chewed along with Areca nut, slaked lime, cardamom and clove in many Asian countries [3]. In Malaysia, *Piper Betle* leaves have been used for chewing, wound healing and marriage tradition. Due to limited storage time before quality is affected and the use of a poor transportation system, many betel leaves are disposed of as waste every year, while during the rainy season, leaf production is so high that the leaves remain unsold or sold at a throwaway price [4].

Therefore, manufacturing of essential oil, talc, medicinal compounds, perfumes, beverages and food additives may be practical in stabilizing the market price of the crop year round. This requires the attention of researchers for the development of value-added products [5]. To date, the numerous studies carried out on the essential oil composition of *Piper betle* Leaves have identified five chemical groups depending on the predominance of particular compounds: (a) alkaloid/amide group [6]; (b) propenylphenol group [7]; (c) terpene/sesquiterpene group [8]; (d) steroid group [8]; (e) prenylated hydroxybenzoic acid group [9]. Hydroxychavicol (HC) and eugenol (EU) have been regarded as the major compounds belonging to the propenylphenol group.

Extraction and product recovery were considered as the most essential steps in the evaluation of target molecules from various plant parts [10]. Most of the extraction processes are time consuming, laborious and involve lengthy operational techniques, bulk amounts of solvents and ultimately result in the thermal decomposition of the target molecules at continuous high temperature. The high temperatures used in the extraction method often lead to the degradation of heat-sensitive compounds. Moreover, traces of toxic solvents are rarely removed from the extracts, which directly influence the quality of the products. Therefore, alternative extraction techniques with better selectivity and efficiency are highly desirable. Products obtained by supercritical fluid extraction (SFE) are free from toxic residues and generally possess a higher quality than products obtained by conventional techniques [11]. Hence, to fulfill the current pursuit of clean technology, it is necessary to demonstrate to investors that in addition to being technically viable, SFE is indeed an attractive choice for an extraction process.

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Hamid [12] stated that oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent that produces free radicals. Previous study shows that the presence of phenolic compounds can act as antioxidants. In addition, *Piper Betle* leaves have traditionally known with its biological properties that contain a lot of antioxidant activity. Thus, the leaves can be used to react with the free radical chemicals and also can be used as a future antioxidant for some disease especially cancer. Recently, many research works show the *Piper Betle* leaves contained rich carotenes (80 IU/g fresh wt.), ascorbic acid (1.94 mg/g fresh wt.) and phenolics (Pin *et al.*, 2010). Data on the phenolic compounds of this plant is related to chavicol chavibetol, chavibetol acetate and eugenol.

2.0 MATERIALS AND METHOD

2.1 Sample Preparation

The fresh leaves of *Piper Betle* were collected from Banting, Selangor, Malaysia in bulk. The leaves were separated from the stalks and washed thoroughly with clean water to remove any dirt and dust present. The clean leaves were cut into small pieces and were dried at 40 °C for 12 hours. The dried leaves were ground just before extraction in a grinder (mill machine, Pulverisett 14, Ider- Oberstein, Germany) to produce a powder with an approximate particle size of355µm and stored at 0−5 °C prior to use.

2.2 Supercritical Carbon Dioxide System

Supercritical fluid extraction was performed using SFX TM 220 extraction system (ISCO, Lincoln, NE, US) consisting of high pressure syringe pump (Model: OM51K40GN-CW2), extractor chamber, 10 mL stainless steel extraction cell and extractor controller (Model: TESCOM 10000) was used in this study. Others parts involved were a chiller (Model: Wisezcircu). CO_2 with 99.99% purity (MOX Gases Sdn. Bhd. Selangor) was used as a solvent throughout the process. The equipment set up of the extraction process SFX TM 220 is shown in Figure 1.

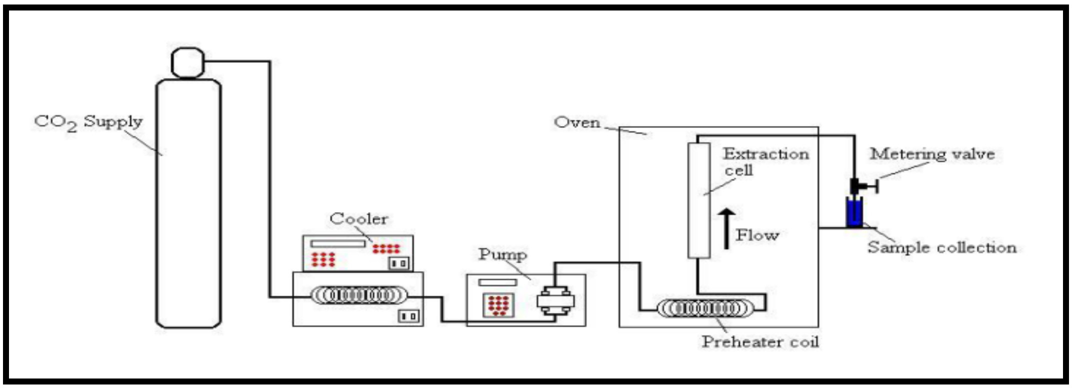

Figure 1 The schematic diagram for Supercritical Fluid Extraction

5.0 g of ground dried *Piper betle* leaves that have reached room temperature were loaded into the extraction cell and tightly sealed. Next, the extraction cell was placed in the extraction chamber and let the system to reach the desired condition. The extraction started after reaching the desired condition. Volume of CO_2 used and extracted oil was recorded every 30 minutes of the extraction process. The extraction duration was 3 hours and 30 minutes with a flow rate of CO_2 at 4mL/min. Extracts are finally separated from the CO_2 phase and collected in collector at ambient temperature and atmospheric pressure. The CO_2 gas was depressurized in order to remove from the separator.

2.3 Soxhlet Extraction

Soxhlet extractions were performed using 10 g weighed of powdered *Piper betle* leaves and were placed in a thimble holder. Soxhlet extraction was carried out using 150 mL of solvents (Hexane). The heating power was set slightly above the boiling point of the solvent used. The arrangement is such that the vapors of the solvent were generated from the round-bottomed flask, pass through the thimble and get condensed in the condenser. The condensed solvent comes in contact with the *Piper betle* leaves in the thimble, where extraction occurs. When the liquid reaches the overflow level in the thimble, the liquid moves through the siphon back into the round-bottomed flask, carrying extracted solutes into the bulk liquid. The solvent was removed by the rotary evaporator at a temperature slightly above the boiling point of the solvent.

2.4 Analysis of Antioxidant Activity using Ultraviolet-Visible Spectrometry (UV-VIS)

The antioxidant activities of the supercritical fluid extracts were determined using the DPPH free radical scavenging activity assay. Activity was measured according to the method of Maisuthisakul [14] with slight modifications. 5ml of 0.135mM of DPPH solution in methanol was mixed with 5ml of distilled water as a reference standard. Next, the sample was diluted with 5ml of methanol and vortex thoroughly for 30s. After that, place the mixtures in the dark for 30 minutes at room temperature. The absorbance of the solution is obtained by using UV-Vis spectrophotometer with wavelength of 517 nm against the reference standard. The result obtained in percentage of scavenging activity.

Scavenging Activity, % = 1 - X 100

Where:

AA = absorbance of the DPPH solution mix with sample

AC = absorbance of solution without nothing added

3.0 RESULTS AND DISCUSSION

3.1 Effect of Pressure and Temperature on the Percentage Oil Yield

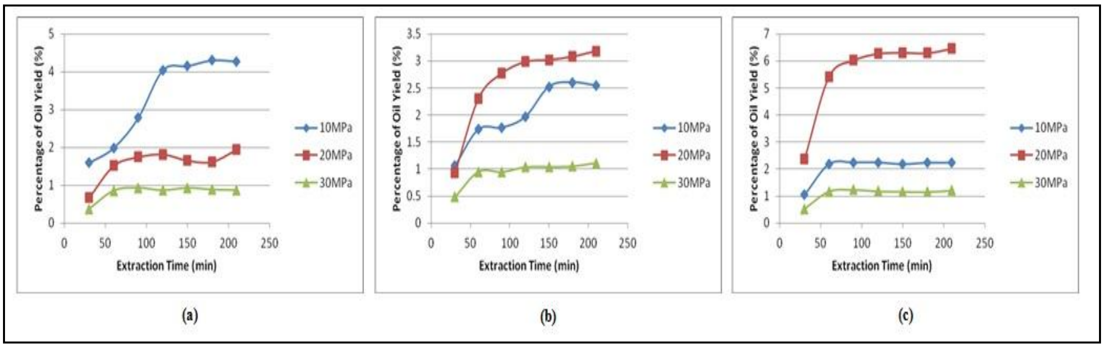

Figure 2 Effects of Pressure and Temperature on Percentage Oil Yield. (a) at 40°C, (b) at 60°C and (c) at 80°C

Figure 2 presents the effect of pressure and temperature on extraction oil yield of *Piper betle* leaves in SC-CO_2 at different pressure for the ranges from 10MPa to 30MPa and different temperature at the ranges from 40°C to 80°C. It can be seen that the percentage of oil yield decreases when the pressure is increased. These phenomena can be explained in terms of competition of solvent density and solute pressure. According to Siti Machmudah [15], the density of the solvent increases with an increase in pressure at constant temperature, however the vapor pressure of the solute decreases with an increase in pressure.

In addition, at elevated pressure, the change in solvent density becomes smaller and the solute vapor pressure change becomes more effective and can easily overcome the effect of solvent density change on the extraction rate [15]. On the other hand, these results happen also due to the diffusion rates of the extracted compounds from the plant matrix to the supercritical fluid medium [16]. The fluid density of CO_2 ascends as the pressure increase, which will decrease in the diffusion coefficient and then reduce the interaction between CO_2 and the sample [17]. Therefore, it can be concluded from this effect of pressure is higher oil yield of 6.456% obtained at pressure of 20MPa.

From Figure 2, it shows that higher temperature resulted in higher oil yield. These phenomena occurred due to the increase of the oil volatility in this condition was more dominant compared to the drop in the density of CO_2. Besides, the solvent approached the gas-like as the temperature increased. This facilate in the increase of extraction rate that produced a faster extraction to reach asymptotic yield [18]. However, from the result obtained at Figure 2, it can be observed that higher temperature resulted in a lower percentage of oil yield. This phenomenon was due to the increase of temperature can decrease fluid density ensuing in the reducing of extraction efficiency [19].

According to the Span and Wagner [20], the extraction efficiency increases with an increase in temperature can be dependent on the molecular mass that related to the binary diffusion coefficient of solute molecules in SC-CO_2. Thus, it can be concluded from this figure is higher oil yield (6.456%) obtained at higher temperatures (80^{o}C). The extraction rate increases with increasing temperature because of increasing vapor pressure of the components.

3.2 Effect of Pressure and Temperature on the Antioxidant Activity

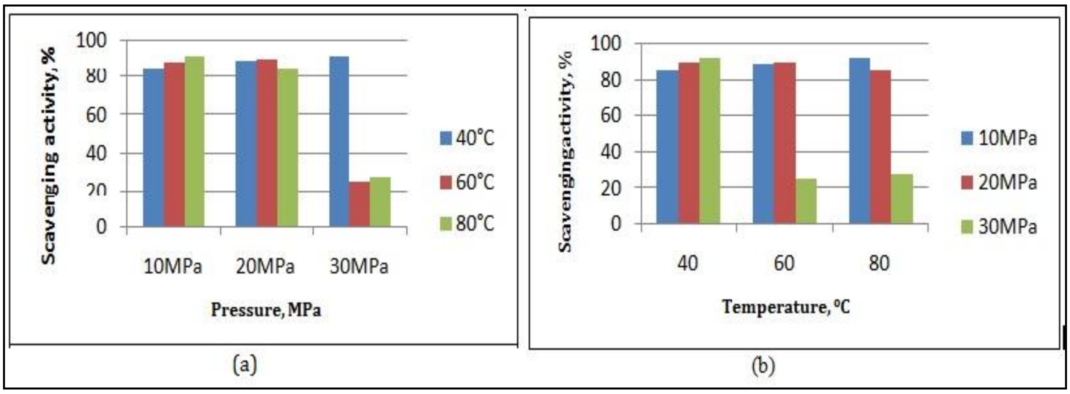

Figure 3 Effects of Pressure and Temperature on Antioxidant Activity. (a) at constant temperature and (b) at constant pressure

Figure 3 presents the effect of pressure and temperature on antioxidant activity of *Piper betle* leaves oil in SC-CO_2 at different pressure for the ranges from 10MPa to 30MPa and different temperature at the ranges from 40^{o}C to 80^{o}C. It can be seen that the highest antioxidant activity is 92.241% with the extraction pressure of 10MPa and temperature of 80°C and followed by 92.081% at the extraction pressure of 30MPa and temperature of 40°C. The figure also shows that the lowest antioxidant activity is 27.797% of oil yield with the extraction pressure at 30MPa and temperature 80°C.

The graphs that have been plotted in Figure 3 were about the effect of pressure and temperature on antioxidant activity. The smallest value of observance shows the high antioxidant activity and strong antioxidant compound. From the observation during the experiments, the color will be changed from purple to yellow. However, some operating extraction which operated at pressure of 30MPa and temperature of 60^{o}C and 80^{o}C does not change the color to the yellow color. This observation resulted in the higher antioxidant activity percentage.

Based on the Figure 3, it was found that the antioxidant activity gradually decreased with the increase of the extraction temperature at the constant pressure. Meanwhile from the Figure, it shows that the antioxidant activity increased significantly with the pressure enhancement at the constant temperature. Temperature is one of the most significant factors affecting antioxidant activity. As the temperature increase, the density of CO_2 is reduced and leading to reduce the solvent power of SC-CO_2 [21]. Besides, Reblova [22], on the research of the effect of temperature on the antioxidant activity of phenolic acids also stated that the lowest antioxidant activity was obtained in the increasing extraction temperature. This is because the easily oxidisable antioxidants show an antioxidant activity decreases with increasing temperature at a slower rate than the less oxidisable ones.

3.3 Comparison of SC-CO_2 extraction and Soxhlet extraction on Oil Yield

The extraction of *Piper Betle* oil in this research has been done by using two extraction methods which are SC-CO_2 extraction and Soxhlet extraction. The results obtained for the oil yield percentage and antioxidant activity percentage are compared to both methods.

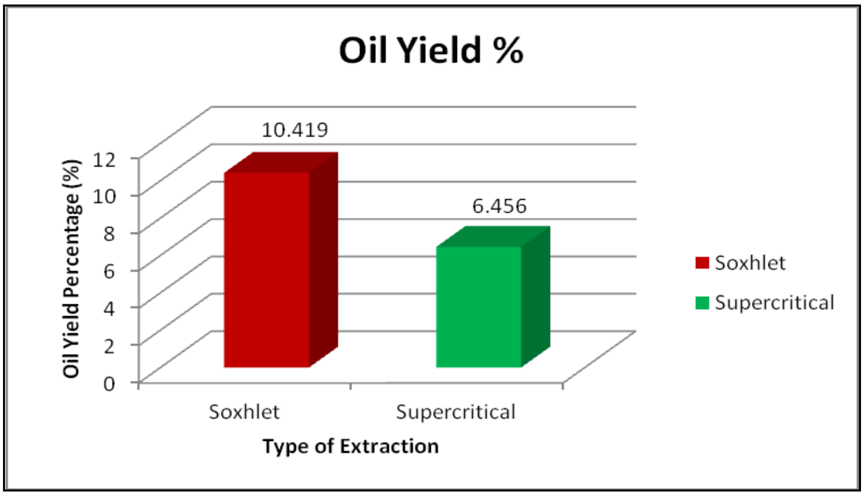

Figure 4 Comparison between SC-CO_2 and soxhlet extraction on oil yield

Figure 4 shows the comparison of oil yield obtained from Soxhlet extraction and SC-CO_2 extraction. From the figure above, 6.456% oil yield extracted by SC-CO_2 extraction is lower than extracted by Soxhlet extraction of 10.419%. In general, for comparison of oil yield percentage that obtained from both methods, the Soxhlet extraction method will give more oil yield compare to the SC-CO_2 extraction. The same result was observed in the previous researcher that the extracted oil yield obtained by SC-CO_2 extraction was lower than Soxhlet extraction [17].

This is because the high oil solubilization capability than hexane soxhlet as well as capable to extract the groups of oleo resin and waxes (typically waxy materials in the outer part of the seed). According to Taylor [23] in the Stahl's extraction rules with pure CO_2, the more strongly polar (e.g. amino acid) only could be extracted in the range above 40.00 MPa. Therefore, at the higher SC-CO_2 extraction conditions, CO_2 solvent was able to extract both, the polar and non-polar compounds.

3.4 Comparison of SC-CO_2 extraction and Soxhlet extraction on antioxidant activity.

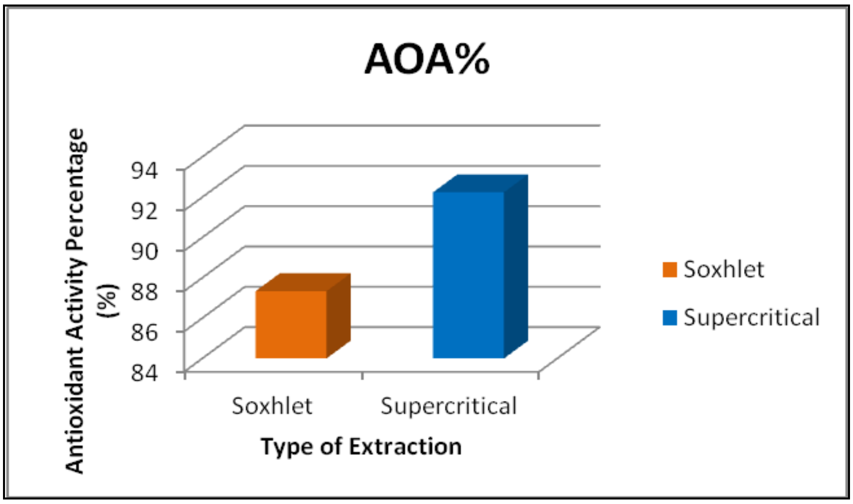

Figure 5 Comparison between SC-CO_2 and soxhlet extraction on oil yield

Figure 5 shows the comparison of antioxidant activity in *piper betle* oil from Soxhlet extraction and SC-CO_2 extraction, where it was clearly shown that the different value of antioxidant activity percentage for both extraction methods. From the result obtained, it obviously shows that the percentage of antioxidant activity that obtained from SC-CO_2 are higher compared to the Soxhlet extraction.

Based on this comparison, in order to obtain the higher value of antioxidant activity in oil yield, it shows that the SC-CO_2 extraction is a better method than Soxhlet Extraction. This is because CO_2 is non-polar solvent and it conforms the fundamental principle of ‘like dissolve like’, SC-CO_2 extraction can extract more non-polar compound of Piper Betle oil ensuing in the larger amount of antioxidant activity in oil yield. Furthermore, according to the research of [24], the SC-CO_2 are given more advantage in respect to antioxidant activity in specific or the selected plant compare to the Soxlet Extraction.

Overall, although the result shows the percentage of oil yield that obtain from Soxhlet extraction is remarkable higher than extracted by SC-CO_2 extraction, it still does not demonstrate high antioxidant activity in oil yield [25] and also cannot illustrate that Soxhlet extraction better than SC-CO_2 extraction [26]. This is because SC-CO_2 extraction has different advantages over Soxhlet extraction methods. Qingyong [27] stated that SC-CO_2 extraction is a less expensive process at laboratory scale compared to the Soxhlet extraction. Besides, SC-CO_2 has shorter time taken during the extraction process and high selectivity in the extraction of oil yield [21]. Moreover, the use of CO_2 as a solvent is safer comparing the use of n-hexane as a solvent.

4.0 CONCLUSIONS

Generally, there have many methods to extract the *Piper Betle* oil. In this research, SC-CO_2 extraction and Soxhlet extraction have been applied to extract oil yield from *Piper Betle* leaves. The effects of extraction conditions such as pressure and temperature on extraction yield and also antioxidant activity were investigated. The highest yield (6.456%) is obtained by using SC-CO_2 at the extraction condition of 80^oC and 20MPa. Meanwhile the low yield (0.874%) was obtained at the operating condition of 40^oC and 30MPa. Furthermore, the highest antioxidant activity of 92.241% is obtained at the extraction pressure of 10MPa and temperature of 80°C. Based on the Soxhlet extraction results, non-polar n-hexane solvent shows a higher percentage of oil yield (10.419%) and obtain antioxidant activity of 87.336%.

The oil yield and antioxidant activity obtained by SC-CO_2 and Soxhlet extraction were also compared. Despite the highest oil yield of SC-CO_2 extraction (6.456%) was lower than Soxhlet extraction (10.419%), the SC-CO_2 extraction method was more selective compare to the Soxhlet extraction as the percentage of antioxidant activity by SC-CO_2 were higher than that obtained by the Soxhlet extraction method. Therefore, SC-CO_2 extraction was the best method than Soxhlet extraction method.

5.0 ACKNOWLEDGEMENT

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REFERENCES:

[1] Ramji, N., R, Lyer and S, Chandrasekaran., 2002. Phenolic antibacterials from *Piper betle* in the prevention of halitosis. Journal Ethnopharmacology, 83: 149 - 152.

[2] Anon. 2002. Compendium of Medicinal Plants used in Malaysia 2002, Herbal Medicine Research Centre, Institute for Medical Research, Kuala Lumpur. 225 - 226.

[3] Walter, T.M., and Sofia, H.N. (2007). Effect of consumption of thamboolam (conventional betel chewing) in traditional siddha medicine. http://openmed.nic.in/2223/01/Thamboolam.pdf.

[4] Pin, K.Y., T, G, Chuah., A, Rashih., C,L, Law., M,A, Rasadah and T,S,Y, Choong., 2009. Drying of Betel Leaves (*Piper betle* L.): Quality and Drying Kinetics. Drying Technology, 27: 149–155.

[5] Rayagura, K., M.K. Khan, G.R. Sahoo and U.S. Pal., 2007. Post harvest practices of betel leaves in Orissa. Agricultural Mechanization in Asia, Africa, and Latin America, 38(3): 33–37.

[6] Stöhr, J.R., P.-G. Xiao and R. Bauer. 2001. Constituents of Chinese *Piper* species and their inhibitory activity on prostaglandin and leukotriene biosynthesis in vitro. J. of Ethnopharmacol. 75(2–3): 133-139.

[7] Sarkar, D., P. Saha, S. Gamre, S. Bhattacharjee, C. Hariharan and S. Ganguly. 2008. Antiinflammatory effect of allylpyrocatechol in LPS-induced macrophages is mediated by suppression of iNOS and COX-2 via the NF-*k*B pathway. International Immunopharmacology 8(9): 1264–1271.

[8] Parmar, V.S., S.C. Jain, K.S. Bisht, R. Jain, P. Taneja, A. Jha, O.D. Tyagi, A.K. Prasad, J. Wengel, C.E. Olsen and P.M. Boll. 1997. Phytochemistry of the genus *Piper*. Phytochemistry 46: 597−673.

[9] Flores, N., I.A. Jimannez, A. Gimralnez, G. Ruiz, D. Gutianrrez, G. Bourdy, I.L. Bazzocchi. 2009. Antiparasitic activity of prenylated benzoic acid derivatives from Piper species. Phytochemistry 70(5): 621 627.

[10] Rayaguru, K., W. Routray and S.N. Mohanty. 2011. Mathemathical modeling and quality parameters of air dried Betal leaf (*Piper betle* L.). J. Food Process. Pres. 35: 394–401.

[11] Handley, A.J. 1999. Extraction Methods in Organic Analysis. Sheffield Academic Press. Sheffield, UK.

[12] Hamid, A. A., Aiyelaagbe, O. O., Usman, L. A., Ameen, O. M. and Lawal, A. (2010). Antioxidants: Its medicinal and pharmacological applications. African Journal of Pure and Applied Chemistry. 4(8): 142-151.

[13] Pin, K. Y., Luqman Chuah, A., Abdull Rashih, A., Mazura M. P., Fadzureena, J., Vimala S and Rasadah, M. A. (2010). Antioxidant and Anti-inflammatory Activities of Extracts of Betel Leaves (Piper betle). Journal of Tropical Forest Science 22(4): 448-455.

[14] Maisuthisakul, P. (2011). Phenolic Antioxidants from Betel Leaf (Piper betel Linn.) Extract Obtained with Different Solvents and Extraction Time, 56.

[15] Siti Machmudah, Amalia Peterson, Bhupesh, C. Roy, Motonobu Goto, Mitsuru Sasaki and Tsutomu Hirose (2006). Extraction of essential oil from geranium (Pelargonium graveolens) with supercritical carbon dioxide. Journal of Chemical Technology and Biotechnology. 81: 167-172.

[16] Rezaei, K. and Temelli, F. (2000). Using supercritical fluid chromatoghraphy to determine the binary diffusion coeffiecient of lipids in supercritical CO_2. J Supercritical Fluids. 17: 35-44.

[17] Mohd Azizi Che Yunus (2007). Extraction, identification and separation of vitamin E and djenkolic acid from Pithecellobium jiringan (jack) Prain seeds using supercritical carbon dioxide, PhD Thesis. Universiti Sains Malaysia.

[18] Mohd Azizi, C. Y., Nur Husnina A., Salman Z., Zuhaili I., Siti Hamidah, S. and Ana Najwa, M. (2013). Effect of Supercritical Carbon Dioxide Condition on Oil Yield and Solubility of Pithecellobium Jiringan (Jack) Prain Seeds. Jurnal Teknologi (Science & Engineering). 60: 45-50

[19] Xiao, J.B., Chen J. W. and Xu, M. (2007). Supercritical fluid CO_2 extraction of essential oil from Marchantia convoluta: global yields and extract chemical composition. Journal of Biotechnology. ISSN: 0717-3458. 10(1).

[20] Span, R. and Wagner, W. (1996) A new equation of state carbon dioxide covering the region from triple-point temperature to 1100 K at pressure up to 800 MPa. Journal of Physical and Chemical, 25, 1509- 1596.

[21] Mandana, B., Russly, A. R., Farah Saleena T. Ali G., Liza M. S., Jinap S., Azizah, H. and I.S.M. Zidul. (2011). Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (Mentha spicata L.) leaves. Food and Bioproducts Processing. 89:67-72.

[22] Reblova, Z. (2012). Effect of temperature on the antioxidant activity of phenolic acids. Czech journal of food science. 30(2): 171-177.

[23] Taylor, T. L. 1996. *Supercritical Fluid Extraction: Technique in Analytical Chemistry*. New York: Wiley-Interscience.

[24] Beatriz Diäaz-Reinoso, Andreä S Moure, Herminia Domiänguez, Juan Carlos Parajo. (2006). Supercritical CO_2 Extraction and Purification of Compounds with Antioxidant Activity. J. Agric. Food Chem. 54, 2441−2469

[25] Liza, M. S., Hasmida, M. N., Nur Syukriah, A. R., and Mohd Azizi, C. Y. (2014). Effect of different extraction techniques on total phenolic content and antioxidant activity of *Quercus infectoria* galls. International Food Research Journal. 21(3): 1075-1079.

[26] Mohd Azizi, C. Y., Zuhaili I. and Noor Azian M. (2015). Optimisation of squalene from palm oil mesocarp using supercritical carbon dioxide

[27] Qingyong Lang, C. M. W. (2001). Supercritical fluid extraction in herbal and natural products studies-a practical review. Talanta, 38: 771-782