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Investigation of Antimicrobial Activity and Phytochemical Analysis of Thorns of *Ceiba petandra* Plant

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Thorns of *Ceiba petandra* or "kekabu" plant are widely used in complementary medicine and have been recommended for the treatment of many diseases such as bronchitis, diarrhoea and skin disease. The extraction process of this plant (thorn part) was investigated at various conditions such as different types of solvents and solid to solvent ratio during the extraction process. The research attempts to get higher yield of the extracted oil and at the same time to ensure that all important phytochemicals such as flavanoids, alkaloid and tannin are presence in the extracted oil sample. In addition, the antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* were also observed. It is found that the optimum condition is obtained from the sample which extracted at 1:15 using methanol as solvent. Based on the Gas Chromatography Mass Spectrometry (GC-MS) analysis, it was proved that the extracted oil by using methanol contain high amount of active compound compare to sample extract using ethanol due to the polarity of the solvents. Results showed that the methanol extract gave higher degree of inhibition zone than ethanol extract against *Escherichia coli* and *Staphylococcus aureus*.

Key words: *Ceiba petandra*, phytochemical screening, optimum condition, antimicrobial.

Ceiba petandra, in English also called as called kapok tree or silk cotton. The plant has a spreading crown, huge trunk and large palm shaped leaves (Rameshwar *et al*., 2014) .In traditional system of medicine, the whole plant (leaves, stems, roots, seeds and thorns) of *Ceiba petandra* have been recommended for the treatment of bronchitis, diabetes, diarrhoea, skin disease,painful eye diseases, chronic fever and insect bite (Elumalai, 2012). The world Health Organization has estimated about 80% of the populations in the developing countries still relies on traditional medicines. Plants extracts can help in reducing drug resistance by providing an alternative mechanism which previously the pathogens were not adapted to resist (Clarence Rubaka, 2014).

In Malaysia, the application of the *Ceiba petandra* as a medicine is not commercially used. Anosike *et al*. (2012) reported that the extraction of *Ceiba petandra* can be used as antimicrobial while Odinma (2013) presented the work on extraction of stem of *Ceiba petandra* consists of major phytochemical which are flavanoids, tannins and alkaloids. The tannins or phenolic compounds possess biological properties such as anti-inflammation, ant-aging as well as anti-carcinogen (Prashant, 2011). Besides, these phytochemical were also effective as antioxidant and shows strong anticancer activities (Okwu, 2004).

There have been investigations on the presence of phytochemicals (carbohydtaes, protein, alkaloids, tannins) in seed of *Ceiba*

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petandra which potentially used as anti-inflammatory (Alagawadi, 2011). Most of the researchers used stem, seeds and leaves compared to the thorns. It is found that thorn of *Ceiba petandra* has potential for the extraction of usefull phytochemical compound (Dangi *et al*., 2014). Alkaloids have been associated with medicinal uses for certain country and have antibacterial properties (Yadav, 2011). The presence of flavanoids in the thorns of plant indicates their medicinal value. Hence, the flavanoids are antioxidants and free radical which prevent oxidation. They have strong anticancer activity and also protect the cell against all the stage of carcinogenesis. Meanwhile, tannins possess astringent properties which help the process of healing for wounds or cuts.

Antimicrobial is an agent that kills or inhibits the growth of the microorganism. Microbial medicines can be grouped according to the microorganism they are act primarily against. For example, antibacterial is used against bacteria and antifungal is used against fungi. They are many types of microorganism can be used in order to check for the antimicrobial activity (Doughari, 2009). For the *Ceiba petandra* plant, most of the present researchers used *Staphylococcus aureus* for the antimicrobial activity analysis (Cushnie *et al*., 2005). Many researchers have reported that the oil extracted from stem bark of *Ceiba petandra* plant are potentially used as antimicrobial due to the presence of of flavanoids, saponins and alkaloids (Anosike *et al*,. 2012, Doughari, 2009 ; Odinma 2013). However, most of the researchers only studied on the presence of this bio active compound without presenting the quantitative analysis of the compound exist in *Ceiba petandra* plant.

Therefore, the aim of this research was to explore the potential of utilizing indigenously available thorns of *Ceiba petandra* oil for the phytochemical study by using Soxhlet extraction apparatus. The work involved in investigating the optimum operating condition for extraction process by varying the types of the solvent and solid to solvent ratio. The is conducted by determining the condition which can give the highest yield of extraction of benificial phytochemical component and ensure all the main phytochemical presence at this condition. For this reasons, phytochemical constituents contents in the extracted oil from the thorns of *Ceiba petandra* was analysed in this study by using Gas Chromatography Mass Spectrometry (GC-MS) analysis.

MATERIALS AND METHODS

Materials

Material used in this study was thorns of *Ceiba petandra* plant obtained from the rural area at Muadzamshah, Pahang. All chemicals and reagents used were methanol, ethanol, hydrochloric acid, iodine, potassium iodide, sodium hydroxide and ferric chloride.

Methods

Collection and preparation of plant

The thorns of *Ceiba petandra* plant were dried in the oven at 60 °C within 20 minutes to reduce the moisture content and grinded into a fine powder form by using grinder. Then, the powder was stored in an air tight container and was kept in the chillers at temperature of 4 °C. The method for sample preparation for extraction was done by modifying the method used by Ezigbo *et al*., (2013).

Extraction of plant

The powder of thorns of *Ceiba petandra* was prepared via drying and grinding process. Then, the sample was extracted with methanol as a solvent, using a Soxhlet apparatus for 6 hours at 65 °C. The percentage of yield of the extraction process was calculated by measuring the weight of the thimble before and after the extraction process. The extracted oil was filtered and evaporated under decrement pressure by using rotary evaporator at 70 °C. Then, the extracted oil was stored in the sample bottle and kept in the chillers for further used. The process was repeated with different type of solvent (ethanol) and the temperature of 80°C is chosen as it very closed to the the boiling point of the ethanol.

Phytochemical Screening

Three phytochemical analyse were carried out on the extracted oil from sample by using two different type of solvent (methanol and ethanol). This process was conducted to detect the presence of major bioactive compounds in thorns of *Ceiba petandra* plant (flavanoids, tannins and alkaloids). The procedures outlined were carried out based on the work of Prashant Tiwari (2011).

Gas Chromatography-Mass Chromatography Analysis

Gas Chromatography-Mass Chromatography analysis was carried out on the extracted oil sample which obtained at optimum operating condition for both type of solvents during the extraction process. Extracted oil were diluted in solvent (GC-MS grade solvent) where the volume ratio of extraction product and solvent is 1:10 (v/v). Solution mixture was then filtered by using syringe filter, before it can undergo the analysis process using the GC-MS equipment. The sample was run under GC-MS based on standard procedure (Devendran and Balasubramaniam, 2011).

Preservation of Culture

Two types of bacteria were used which are *Escherichia coli* and *Staphylococcus aureus*. *E. coli* was obtained from Centre Laboratory of UMP while *S. aureus* was purchased from Chromadex Company. The cultures were stored in the chiller at 4-6 °C, transferred to an agar plate and incubated for 24 hours at 30 °C.

Culture Preparation

The 30 g of Muller Hinton agar was mixed with 1 litre of distilled water and the stabilized in autoclave at 121 °C for 20 minutes. The sterilized media was poured into petri dish and allowed to solidify at room temperature in sterile environment.

Evaluation of Antimicrobial Activity

The antimicrobial test was carried out on the extracted oil by using paper disc diffusion test. In this test, 5 mm of the sterile paper disk were soaked in the extracted oil obtained from extraction process by using different type of solvents (methanol and ethanol) for 20 minutes. The bacteria was spread onto the surface of agar plate and incubated for 35 °C and observed for the growth of inhibition zone after 24 hours.

RESULTS AND DISCUSSIONS

Percentage of extractions of thorns of *Ceiba petandra*

The effect of the different type of solvent (methanol and ethanol) in the extraction process are presented in Figure 1.

In overall, the result shows that the methanol is the best solvent choosed compare to the ethanol as its gave higher yield of extracted oil at all the ratio. This observation is due to the high polarity of methanol compare to ethanol which accelerate the extraction process (Farooq Anwar, 2012).The highest yield was obtained at 1:20 ratio, using methanol as a solvent while the lowest yield is obtained at ratio 1:10 by using ethanol as solvent. Andri Cahyo Kumuro (2009) reported that, higher yield of extracted oil was obtained from the extraction process which employed the polar organic solvents containing hydroxyl group that have similar solubility properties (Andri Cahyo Kumuro, 2009).

Table 1. Phytochemical screening of extracted oil from the thorn of *Ceiba petandra*

Solvent	Ratio (w:v)	Phytochemical		
		Alkaloids	Flavanoids	Tannins
Methanol	1:20	+	-	-
	1:15	+	+	+
	1:10	+	+	+
Ethanol	1:20	+	-	-
	1:15	+	+	+
	1:10	+	+	+

Notes: +: Presence, -: Absence

Table 2. Antimicrobial activity of extracted oil from thorns part of *Ceiba petandra* plant

Microorganism	Zone of inhibition (mm)	
	Methanol	Ethanol
Escherichia coli	2	1
Staphylococcus aureus	4	2

Both methanol and ethanol show that the percentage of yield was increased as the ratio of solid to solvent increase.The increase in the yield with the increased of solvent to solid ratio occurred due to the increased in concentration gradient in the sample, which acted as a driving force for the diffusion process (Ibrahim Buiduk, 2015).

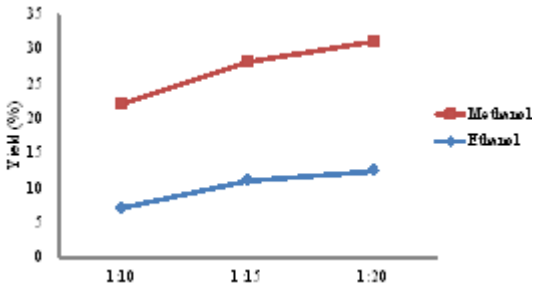

Fig. 1. Percentage of yield of extraction for thorns of *Ceiba petandra* plant

Phytochemical screening of thorns of *Ceiba petandra* plant

Three major phytochemical constituents presence in thorns of *Ceiba petandra* are summarized in Table 1.

Based on Table 1, alkaloids was presence in all samples for both type of solvents (methanol and ethanol). According to the results reported by Iroka *et al*., it was found that alkaloids is the highest phytochemical constituents on the stem of *Ceiba petandra* followed by flavanoids and tannins (Iroka *et al*., 2014). However, in this recent study, flavanoids and tannins only presence in the sample with the solid to solvent ratio at 1:10 (w: v) and 1:15 (w: v). It is no detected at all at ratio 1:20 (w:v). This result is might due to the increased in volume of the solvent cause the component (flavanoids

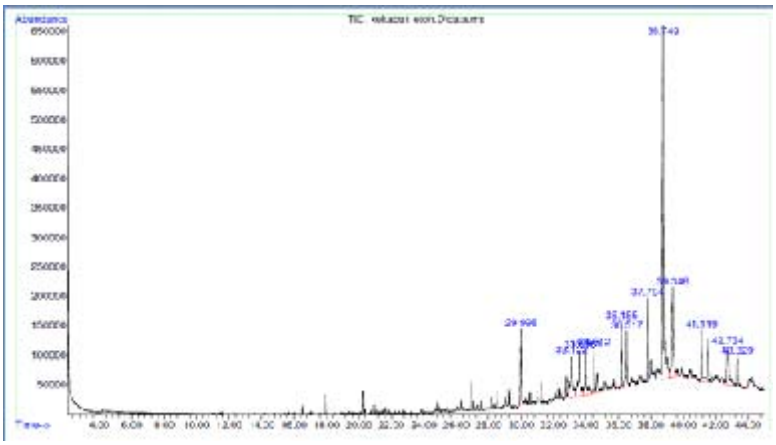

Fig. 2. GC-MS analysis of phytochemical for optimum condition 1:15 in methanol extract

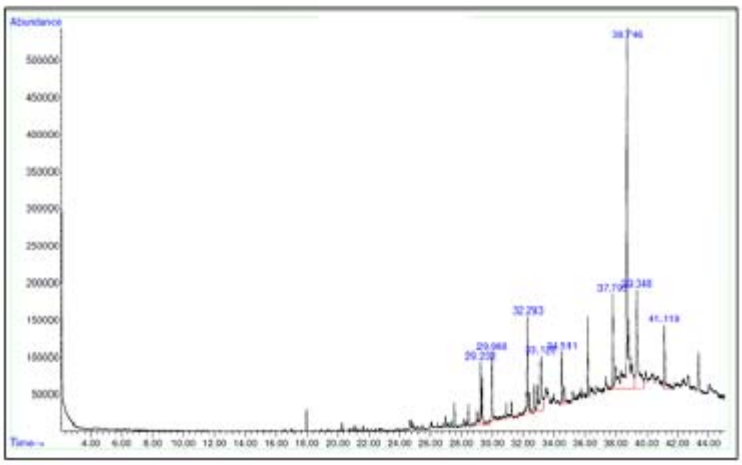

Fig. 3. GC-MS analysis of phytochemical for optimum condition in ethanol extract

and tannins) evaporated easily because the components (flavanoids and tannins) have low molecular weight. Therefore, the optimum solid to solvent ratio for both type of solvent is obtained at 1:15 solid to solvent ratio which presence all the three phytochemicals.

GCMS Analysis of Phytochemical Constituents in *Ceiba petandra*

Fig. 2 and 3 show the GC-MS analysis of *Ceiba petandra* thorns. It is revealed that there are 23 active constituent's compounds exist in the sample extracted in methanol and only 18 active compounds presence in sample extracted in ethanol. The identification of the phytochemical compound was confirmed based on the peak area, retention time and molecular weight based on National Institute of Standards and Technology (NIST).

Antimicrobial activity

The results of antimicrobial activity for *Ceiba petandra* extract oil in two different type of solvent (methanol and ethanol) are shown in Table 2. Result has revealed that both types of solvents have potential used as an antimicrobial agent against both type of bacteria (*Escherichia coli* and *Staphylococcus aureus*). *Staphylococcus aureus* showed higher degree of inhibitions zone for both solvent compared to *Escherichia coli*. In the traditional medicine practices, *Ceiba petandra* plant is used to cure skin infection (Wadood *et al*, 2013). Therefore the results from this study proved that extracted oil from the thorn of *Ceiba petandra* plant can be acted as antimicrobial agent against *Staphylococcus aureus* bacteria, which responsible for skin disease to human or animal.

CONCLUSION

From the result, it can be concluded that methanol was appeared as the best solvent in the extraction of *C. petandra* plant because the higher yield and bigger inhibition zone was obtained against the bacteria. The optimum ratio of solid to solvent was obtained at 1:15 as revealed the presence of the important phytochemical at this condition.

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