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Solvent Selection in Extraction of Essential Oil and Bioactive Compounds from *Polygonum minus*

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Abstract: This preliminary study evaluated the biological activity of *Polygonum minus* (kesum) using two bioassays: antioxidant and antibacterial assay. *P. minus* was extracted using maceration method (solvent extraction) to study the effect of solvent type and concentration in biological activity of *P. minus*. Seven solvents (water, 50% (v/v) methanol, 50% (v/v) ethanol, 70% (v/v) methanol, 70% (v/v) ethanol, methanol and ethanol) were used in order to select the best solvent to be used in supercritical fluid extraction (SFE). The antioxidant capacity was evaluated using four different assays: Total Phenolic content assay (TP), Total Flavonoid content assay (TF), Ferric Reducing/antioxidant Power (FRAP) assay and free radical-scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Seventy percent methanol extract showed the highest polyphenol and flavonoid content. The highest TP and TF were from 70% methanol extract (11.3±0.06 mg gallic acid equivalents/g extract (mg GAE g^{-1}) and 22.5±0.07 mg catechin equivalents/g extract (mg CAE g^{-1} extract, respectively). Seventy percent methanol extract also showed the highest FRAP value and percentage of DPPH radical inhibition which were 377.6±5.40 μMol Fe (II)/g sample and 80.3±0.10%. The high antioxidant capacity was significantly correlated with high TP/TF content. Agar-well diffusion method was used to investigate the antibacterial activity where result showed that 70% methanol extract exhibited the largest inhibition zone against *Staphylococus aureus* (Gram+), 16.2±0.30 mm in diameter.

Key words: *Polygonum minus*, supercritical fluid extraction, solvent effect, essential oil, antioxidant, antibacterial

INTRODUCTION

Essential oils have a complex composition, containing especially hydrocarbons such as terpenes and sesquiterpenes and also oxygenated compounds such as alcohols, aldehydes, phenols, ketones, acids, oxides, lactones, ethers and esters (Anitescu *et al*., 1997). The bioactive components in the complex composition made the essential oil from herbal plant are in a high demand. There are several methods to extract essential oil containing bioactive components from herb and spices like steam distillation, hydrodistillation and solvent extraction. Solvent extraction has been widely used to extract bioactive components from plants (Musa *et al*., 2011). Besides, the study of the solvent effects on the extraction of active components from herbs is very important for the screening and selection of the solvent for the extraction, fractionation and purification steps in the herbal processing. Among all the variables investigated (pre-treatment of the sample, solvent/sample ration, type of solvent, time and temperature of extraction) to ensure the efficiency of extraction, type of solvent has been the most studied factor (Spigno *et al*., 2007).

Herb plants are well known to be associated with many medicinal properties. That is why in this study we chose a commonly grown herb plant in Malaysia, namely *Polygonum minus* (known as kesum). A study by Huda-Faujan *et al*. (2009) indicated that *P. minus* showed the highest phenolic content and the greatest ability in reducing ferric ion compared to several herbs such as *Murraya koeniigi* (curry leaves), *Cosmos caudatus* (ulam raja) and *Centella asiatica* (pegaga). Therefore, the main objectives of this study were to determine the antioxidant capacity and antibacterial activity of *P. minus* and to select the best solvent to be used in SFE process by examining the efficiency of different solvent systems for the extraction of antioxidant and antibacterial compounds using solvent extraction.

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MATERIALS AND METHODS

Sample preparation and extraction: Fresh *Polygonum minus* samples was obtained from Ulu Yam, Selangor. The fresh samples were cleaned and washed using running tap water and then the leaf part was separated. The leaf samples were dried using an oven (Sheldon Manufacturing, Inc., FX2-2, USA) at 40°C and then ground for approximately 2-3 minutes using a grinder (*Multifunction disintegrator* SY-04, Golden Bull). Dried and ground samples (10 g) were then immersed in 200 mL solvents (namely water, 50% (v/v) methanol, 50% (v/v) ethanol, 70% (v/v) methanol, 70% (v/v) ethanol, methanol and ethanol) at room temperature. After three days, the solutions were filtered using filter paper. The collected extracts were then evaporated using a vacuum rotary evaporator (Yamato Scientific Co., Ltd, RE 600, Japan) to yield a viscous mass. The crude extracts were weighed and diluted before being stored at 0-4°C for further analysis.

Antioxidant assay

Determination of total phenolic content (TP): The total phenolic content (TP) of the *P. minus* extracts was determined using the Folin-Ciocalteu reagent (FC) as described by Singleton and Rossi (1965). A calibration curve was prepared using a standard solution of gallic acid (20, 40, 60, 80 and 100 mg L^{-1}, $r^2 = 0.960$). Properly diluted *P. minus* extract solution (20 μL) was mixed with 100 μL of FC reagent in the dark. After the reagent stood for 3-8 minutes at room temperature, 80 μL of sodium carbonate solution (7.5% w/v) was added. The solutions were mixed and allowed to stand in the dark for 2 hours at room temperature for the reaction to occur. The absorbance at 765 nm was measured. The results were expressed on a fresh weight basis as mg gallic acid equivalents (GAE) g^{-1} sample.

Determination of total flavonoid content (TF): Total flavonoid contents (TF) of the extracts were determined according to the calorimetric assay developed by Zhishen *et al.* (1999) with a slight modification in term of solution volume. A calibration curve was prepared using a standard solution of catechin (20, 40, 60, 80 and 100 mg L^{-1}, $r^2 = 0.972$). Twenty microliter of properly diluted extract was mixed with 80 μL of distilled water. At zero time, 6 μL of (5% w/v) $NaNO_2$ was added. After 5 min, 6 μL of (10% w/v) $AlCl_3$ was added. At 6 min, 40 μL of 1 M solution of NaOH were added. After that, the volume was made up to 200 μL, immediately by the addition of 48 μL of distilled water. The absorbance of the mixture was read at 510 nm. The results were expressed on a fresh weight basis as mg catechin equivalents (CEQ) g^{-1} sample.

Ferric reducing/antioxidant power (FRAP) assay: The FRAP assay was performed according to a modified method described by Benzie and Strain (1999). FRAP reagent was freshly prepared by mixing 5 mL 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) solution (10 mM) in 40 mM hydrochloric acid solution with 5 mL $FeCl_3.6H_2O$ solution (20 mM) and 50 mL acetate buffer solution (0.3 M, pH 3.6) and incubated at 37°C after the mixing. A calibration curve was prepared using an aqueous solution of ferrous sulphate ($FeSO_4.7H_2O$ at 200, 400, 600, 800 and 1000 μM, $r^2 = 0.948$). Properly diluted *P. minus* extract (50 μL) was mixed with 1.5 mL of FRAP reagent under dark conditions. The absorbance at 593 nm of 200 μL of the mixture was determined against a blank. FRAP values were expressed on a fresh weight basis as micromoles of ferrous equivalent Fe (II) per gram of sample.

DPPH free radical-scavenging assay: The antioxidant capacity was studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method proposed by De Ancos *et al.* (2002). Diluted extract (20 μL) was mixed with 80 μL of methanol and 200 μL of 0.1 mM DPPH. The mixture was kept in the dark for 30 min before the absorbance at 515 nm was measured against a control solution of methanol and DPPH without extracts. The results were expressed as percentage of the DPPH radical. The percentage of the DPPH radical was calculated according to following equation:

$$\% \text{ inhibition of DPPH} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \quad (1)$$

where, $A_{control}$ is the absorbance of DPPH without extract, while A_{sample} is the absorbance of the extracts.

Antibacterial assay using agar-well diffusion method: The antibacterial assay was conducted using the agar-well diffusion method described by Lorian (2005) and Hernnadez *et al.* (2005) with a slight modification. Fourty microliter from each bacterial strain (*Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* with $OD_{600} = 0.1$) were mixed into 20 mL Mueller-Hinton agar (MHA) before being solidified. The mixture was then transferred into a petri dish and circular wells with 6 mm diameter were cut from the agar. Eighty microliter of the extracts were filled in the well. The plates were inverted and incubated for 24 h at 37°C. For each bacterial strain, controls were maintained where solvents were used instead of the extract. Clear inhibition zones around the discs indicated the presence of antibacterial activity.

RESULTS AND DISCUSSION

Antioxidant assay: Four antioxidant assays were conducted on maceration extracts. The antioxidant and extraction yield results were shown in Table 1. Methanol produced the highest yield of extract followed by ethanol, 70% methanol, 70% ethanol, 50% methanol, 50% ethanol and the lowest was distilled water extracts. The results showed that the extraction yield have positive correlation with all antioxidant assays except for pure methanol and ethanol. Total phenolic content (TP) of the extracts measured using Folin-Ciocalteu's colorimetric method. The highest TP obtained from 70% methanol extracts (11.3±0.06 mg GAE g^{-1}). The Total Flavonoid content (TF) of this plant was determined using aluminium chloride colorimetric assay. It showed the same trend with TP result where the highest TF obtained from 70% methanol extracts. From the result, TP and TF values showed the same trend and from it, it can be said that the correlation between TP and TF existed. This correlation indicates that flavonoids are the important phenolic group in representing the antioxidant capacity of *P. minus*.

The antioxidant capacity of the different *P. minus* extracts was studied using the ferric reducing/antioxidant power (FRAP) test and the DPPH free radical inhibition test. The FRAP value and percentage of DPPH inhibition show the same trend where a positive correlation exists between the antioxidant activity and the reducing capability of the extracts. This similar trend has also been reported in banana, pineapple and guava plants (Alothman *et al*., 2009). The relationship between extract reducing power and antioxidant activity may be due to the same reaction mechanism for both the FRAP and DPPH assays. These two methods are concerned with the capability of the antioxidant to reduce the involved radicals (ferric ion and DPPH free radical). On the other hand, the antioxidant power is related to the phenolic antioxidant activity (Yildirim *et al*., 2000). That is why FRAP and DPPH also showed the same trend with TP value.

The solvent type and concentration are key factors in this extraction process where as solvent polarity will play a key role in increasing phenolic solubility (Naczk and Shahidi, 2006). Besides, solvent polarity affects both the kinetics of phenolic release from the solid matrix and the antioxidant activity of the extract (Mussatto *et al*., 2011). The best results of TP and TF as well as antioxidant power (FRAP and DPPH) were achieved on methanol extracts. Previous study showed that alcohols like methanol and ethanol were more efficient than water in extracting phenolics from citrus peel and also medicinal plants (Zia, 2006; Jamal *et al*., 2010). As can be observed for both methanol and ethanol, concentration of 70% (v/v) extracted more phenolics and flavonoids and also exhibited the highest antioxidant power compared with other concentrations. Previous studies (Lapornik *et al*., 2005; Musa *et al*., 2011; Mussatto *et al*., 2011) reported that, the use of water in combination with an organic solvent contributing to the creation of a moderately polar medium that insures the extraction of phenolics, giving better results than when using a pure organic solvent.

Antibacterial assay: The antibacterial activity of both maceration and SFE extracts was tested on three bacteria (*Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli*); the results were shown in Fig. 1. All the extracts tested showed antibacterial activity against all tested strains except for distilled water extracts against *B. subtilis and S. aureus*. 70% methanol extract displayed the great antibacterial activity againsts all strains with the highest inhibition zone was on *S. aureus* (16.2 mm in

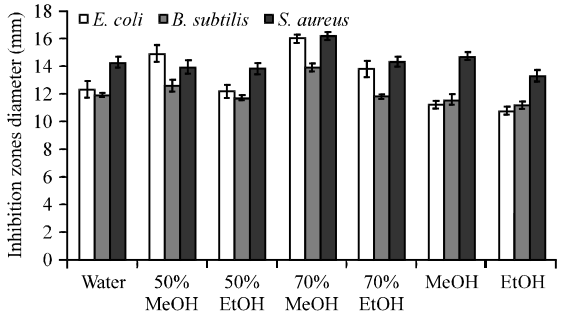

Fig. 1: Inhibition zone diameter of *E. coli*, *B. subtilis* and *S. aureus*

Table 1: Antioxidant capability of maceration extracts using four antioxidant assay

Solvent	Yield of extract (%)	Total phenolic (mg GAE g^{-1} extract)	Total flavonoid (mg CAE g^{-1} extract)	FRAP value (µMol Fe (II) g^{-1})	DPPH inhibition (%)
Water	21.6±0.4	6.3±0.05	14.9±0.18	217.7±1.41	45.5±1.00
50% methanol	26.7±0.2	10.0±0.06	12.1±0.12	323.1±2.30	66.9±0.18
50% ethanol	23.4 ±0.5	7.6±0.08	10.4±0.08	304.0±0.12	53.0±0.03
70% methanol	28.2±0.3	11.3±0.06	22.5±0.07	377.6±5.42	80.3±0.12
70% ethanol	26.1±0.2	8.2±0.07	14.6±0.28	255.5±1.77	73.8±0.06
Methanol	31.2±0.3	6.5±0.07	7.0±0.08	224.4±0.39	60.6±0.22
Ethanol	28.7±0.4	5.1±0.04	6.0±0.04	189.7±0.89	54.7±0.71

*Standard deviation for 3 replicates

diameter). These differences in bacterial inhibition may be attributed, possibly due to the effect of various phytochemicals such as polyphenols which are known to cause damage to cell membranes, causing leakage of cellular materials and ultimately the microorganism death (Bernheim, 1972; Mshvildadze *et al*., 2000; Abdel Ghani *et al*., 2008). Previous study by Polydoro *et al*. (2004) also reported that flavonoids act as an inhibitor toward the superoxide anion and the hydroxy and peroxy radicals to inhibit the key enzyme in mitochondrion respiration that possess antimicrobial properties (Fogliani *et al*., 2005). Therefore, the high TP and TF content for all extracts as discussed before could be the best explanation of antibacterial activity for *P. minus*.

CONCLUSION

The result of this study showed that the type of solvents and their concentration affecting the extraction yield and antioxidant activity as well as antibacterial activity. There was a positive correlation between total phenol and antioxidant capacity of the *P. minus* extracts. The higher the total phenolic content, the higher were the FRAP and DPPH values. This study also indicated that the existence of antibacterial compounds in *P. minus* extracts towards all tested strains especially *S. aureus*. 70% methanol was observed to exhibit the properties of good extraction ability as compared to other solvents in the both assays. Methanol is the most commonly used extraction solvent due to its high polarity which could produce high extraction yields. However, its toxic characteristic could be judgmental when it wants to be used in the food and pharmaceutical applications. Regardless of that, the use of methanol in the present study was very useful in obtaining and verifying the antioxidant and antibacterial compounds from *P. minus*.

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