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The Effects of Varying Solvent Polarity on Extraction Yield of *Orthosiphon stamineus* Leaves

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Abstract: *Orthosiphon stamineus*, also known as Misai Kucing, is one of the invaluable medicinal plants originated from Southeast Asia. *O. stamineus* leaves were used in plenty of applications related to the medicinal purposes and are believed to cure certain disease such as hypertension, gout and fever. However, very little research done with regards to ascertain the extraction yield based on solvent polarity. Thus, the influence of solvents with different polarities on extraction yield was investigated. Water (H_2O), ethyl acetate (EA), Hexane (Hex) and ethanol (EtOH) were selected as the extraction solvents with 40°C temperature and four hours extraction time using Solid Liquid Extraction (SLE). The highest extraction yield was obtained from H_2O, gave 33.69 wt.% of extraction yield compared to EA (6.05 wt.%), EtOH (4.42 wt.%). and Hex (3.08 wt.%). Currently, the information on the extraction of *O. stamineus* based on solvent polarities was rather scarce. Thus, this study will provide a good source of information for particular extraction processes in different polarities of solvent.

Key words: Medicinal plants, extraction yield, extraction process, extraction solvent, *O. stamineus,* anti-inflammatory

INTRODUCTION

O. stamineus, locally known as Misai Kucing, is belongs to Lamiaceae family and originates from Southeast Asia (Olah *et al*., 2003). It is also known as Java Tea, Kumis Kucing, Remujung or Cat's whiskers due to characteristic of its flower. *O. stamineus* traditionally used to treat hypertension, diabetes, urinary disorders, rheumatism, tonsillitis and menstrual disorders (Sriplang *et al*., 2007).

O. stamineus contains several active constituents such as terpenoids (diterpenes and triterpenes), polyphenols and sterols (Tezuka *et al*., 2000). The medicinal properties of *O. stamineus* leaves were attributed to its polyphenol, the most important compound in the leaf which were reported to be effective on reducing oxidative stress by inhibiting the formation of lipid peroxidation products in biological systems (Akowuah *et al*., 2005). The polyphenols in *O. stamineus* is dominant by caffeic acid derivatives such as rosmarinic acid (RA) and polymethoxylated flavones namely sinensetin (SEN) (Olah *et al*., 2003).

A study by Huang and Zheng (2005) reported that RA shows several bioactivities including anti-bacterial, anti-inflammatory and anti-carcinogenic activities. SEN present in *O. stamineus* leaves had a rare structural formula, with a methoxy group at C-5 and classified as a unique flavonoids (Akowuah *et al*., 2005). It is also reported that SEN have high chemo synthesizing effect which is used for the synthesis of the multi- drug resistance (MDR) cell for anti-cancer drugs (Ahmad *et al*., 2008). This study was aimed to investigate the yield and quality extract of *O. stamineus* leaves from solvent with different polarity. The results provided information of selection solvents for Solid-liquid Extraction (SLE) of *O. stamineus* leaves in order to obtain highest yield extract as well as bioactive compounds.

MATERIALS AND METHODS

Raw material: The dried leaves of *O. stamineus* were provided by Natural Product Division, Forest Research Institute Malaysia (FRIM). The dried leaves were stored in Raw Material Storage Room of Herbal Technology Centre, FRIM.

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Chemicals: Ethanol was purchased from HmBg Chemicals Inc. (Germany), Ethyl acetate from RM Chemicals (UK) and High Performance Liquid Chromatography (HPLC) grade methanol from Merck Co. (Germany). Orthophosphoric acid was bought from Fisher Chemicals. Standard of compounds namely rosmarinic acid (RA) and sinensetin (SEN) were purchased from HWI ANALYTIK GmbH (Germany) and Indofine Chemicals Company Inc. (New Jersey), respectively.

Extraction process: Four different types of solvents were used in the experiment including water (H_2O), ethanol (EtOH), ethyl acetate (EA) and hexane (Hex). Ten grams of *O. stamineus* dried leaves were extracted with 100 mL of solvent at 40°C for 4 h in water bath (Memmert WNB 45, Germany) (Akowuah *et al*., 2005). The extract was filtered through filter paper (Whatman No. 1) with Buchner filter under vacuum. H_2O extract were kept in freezer at -20°C prior to freeze dry process and organic solvent extract stored at room temperature before solvent recovery process. The extractions were done in triplicate.

H_2O extract product then freeze-dried for a total of 72 h in order to remove the solvent. The extract from EtOH, EA and Hex were recovered using rotary evaporator (Model RE 300, Yamato, Japan) under vacuum. The evaporation process was conducted at 40°C to minimize any possible degradation of the phytochemicals in the samples. Extraction yield for both water and organic solvent were calculated using following equation (Pin *et al*., 2010):

$$Y = \frac{W_d}{V_e} \times R_{ss} \times 100 \quad (1)$$

where, W_d is the weight of dried extract (g), V_e is the volume of aqueous filtered (mL) and R_{ss} is the ratio of solvent to solid (mL g^{-1}). All experiments were conducted in triplicates.

HPLC analysis: The dried extracts from H_2O (1.0 mg) were dissolved in 1.0 mL of water. The dried extracts (1.0 mg) from EtOH, EA and Hex were dissolved in 1.0 mL of methanol (MeOH). The solutions were then filtered using syringe filter (diameter: 17 mm, porosity: 0.45 μm, polyvinylidene fluoride membrane) before HPLC analysis. The filtrate was injected into HPLC analysis to determine its chemical profiles and concentrations of RA and SEN. The HPLC analysis was carried out using Waters 600 System Controller coupled with Waters 2996 Photodiode Array detector and Waters 717+Autosampler. A Phenomenex Luna C18 100A column (250×4.6 mm, 5 μm particle size) was used as stationary phase. The mobile phase was in gradient mode and consisted of 0.1% orthophosphoric acid (H_3PO_4) and 100% MeOH.

Statistical analysis: Statistical comparisons were made using one way analysis of variance (ANOVA) with SPSS statistical program (version 17.0). Only variables with a confidence level ranking to 95% ($p<0.05$) were considered as significant.

RESULTS

Extraction yield on different extraction solvents: Figure 1 shows the comparison of extraction yield for four types of solvents. The order of increasing yield in different solvent extraction system was Hex<EtOH<EA<H_2O. From the ANOVA analysis, the extraction yield of H_2O was significant ($p<0.05$) compared to the others.

Phytochemicals content on different extraction solvents: Both targeted compounds, namely RA and SEN, were detected in all the extracts but with different amounts (Fig. 2a-d). The order of increasing amount of RA was Hex<EA<EtOH<H_2O. The order of increasing concentration of SEN in different solvent extracts in increasing order was H_2O<Hex<EtOH<EA.

DISCUSSION

Extraction yield on different extraction solvents: The result suggests that the major phytochemicals in *O. stamineus* leaves are mostly high in polarity and soluble in water. The basis of selection of solvent is based on their polarity. The polarity index of the selected solvent were shown in Table 1. An extraction of *Phyllanthus niruri* resulted that highest extract yield obtained from water compared to ethanol, n-hexane and petroleum ether (Markom *et al*., 2007). This observation

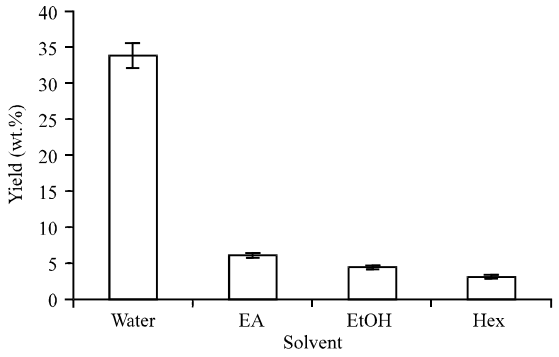

Fig. 1: Yield of extraction from different solvents

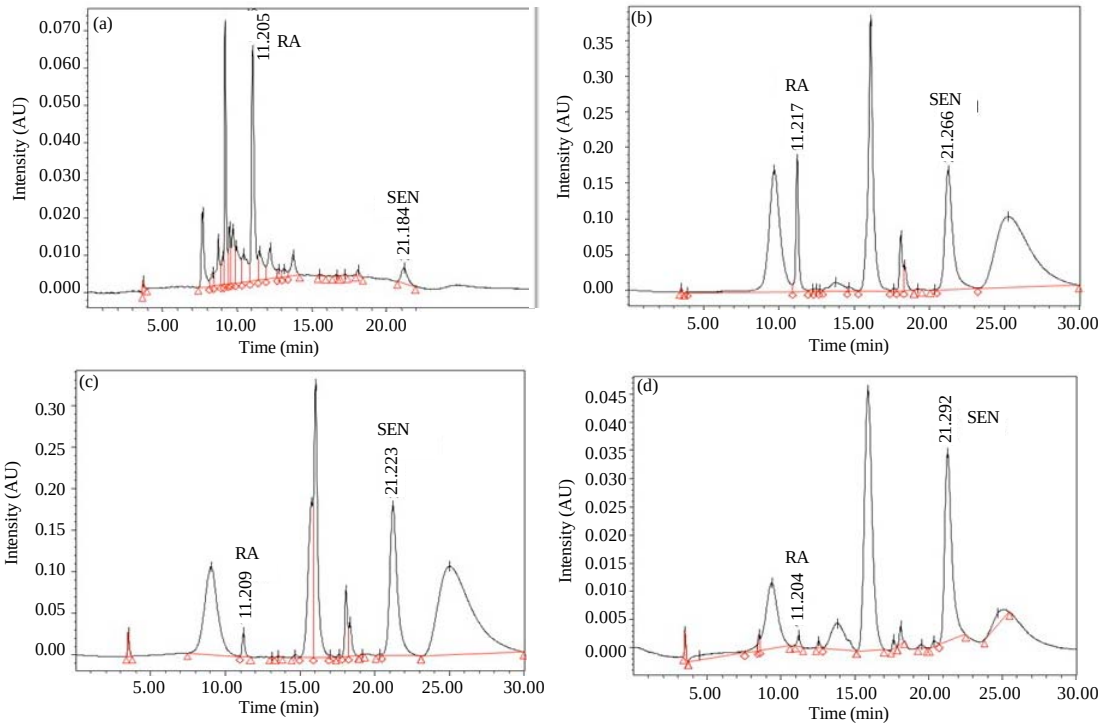

Fig. 2(a-d): HPLC chromatogram of (a) H_2O, (b) EtOH, (c) EA and (d) Hex extracts

Table 1: Polarity index for selected solvents

Extraction solvent	Snyder polarity index[a] (P')
Water	9.0
Ethanol	5.2
Ethyl acetate	4.3
Hexane	0.0

[a]Snyder (1974)

Table 2: Number of functional group in RA and SEN

	No. of functional group[a]	
Functional group	Rosmarinic acid (RA)	Sinensetin (SEN)
Carboxylic acid	1	0
Hydroxyl	4	0
Carbonyl	0	1
Ester	1	0
Ether	0	1
Methoxy	0	5

[a]Akowuah *et al.* (2005)

means that polar compounds are easier to be extracted compared to non-polar compounds. Although both water and ethanol contain hydroxyl group that can form hydrogen bonding with the solute, water is more effective in extracting the solute because it has higher polarity and shorter chain (Pin *et al.*, 2010). These characteristics of water improved its capability to extract the polar compounds. This thus explains the significant observed between H_2O and EtOH. The difference in yields for other solvents may be due to other factors including phytochemicals in plants, extraction temperature, extraction time and solvent to solid ratio.

Phytochemicals content on different extraction solvents: RA contains four hydroxyl and a carboxylic groups and is more soluble in the high polar solvents such as water. It can be deduced that the hydroxyl and carboxylic groups in RA are preferably extracted by H_2O through hydrogen bonding. The number of functional groups in RA and SEN were tabulated in Table 2 based on studied of skeletal structure of RA and SEN.

This was also found in the extraction of *P. niruri* where gallic acid contains carboxylic acid group and it was most soluble in water (Markom *et al.*, 2007). The carboxylic acid and ester group in RA have the nucleophilic ability in the carbonyl group and can react with both H_2O and EtOH (Pin *et al.*, 2010). Higher polarity in EtOH also gave advantages to it to extract more RA than EA. Concentration of RA was lowest in Hex extract because Hex is non-polar solvent and non- soluble for RA.

The presence of four methoxy group in SEN renders them to be lipophilic. It means that SEN is more soluble in lipophilic or less polar solvents. However, using Hex (non- polar solvent), the concentration of SEN is lower compared to less polar solvents.

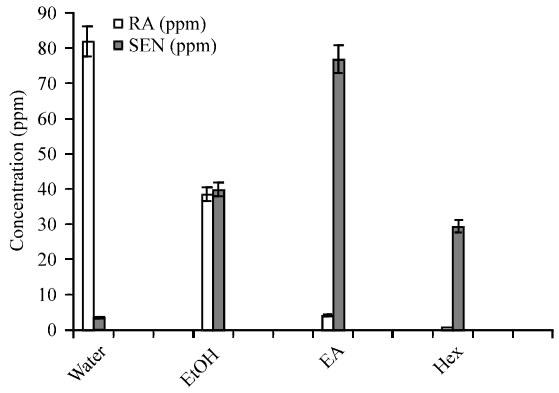

Fig. 3: Concentrations of RA and SEN in the extracts from different solvents

EA has the lowest polarity and more effective in extracting SEN in this study (Fig. 3). Previous research done were reported that EA extract gave the highest SEN yield for *Orthosiphon aristatus* among H_2O, MeOH, EtOH and n- Hex (Nawawi *et al*., 2006).

Hex gave lowest concentration of SEN among other solvents. This can be deduced that SEN not soluble in non- polar solvents. However, there are others factor that contributed to this including column used, flow rate of HPLC mobile phase and column temperature. The low concentration in H_2O extract is due to the high polarity of H_2O which did not allow it to be able to extract the SEN well.

CONCLUSION

Water gave the highest extraction yield and concentration of rosmarinic acid among the selected solvents. Rosmarinic acid is the compound that is believed to cause beneficial activity including anti-oxidant and anti-inflammatory. Hence, in this study, water is more suitable and effective solvent to obtain *O. stamineus* leaves extract.

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