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Effects of Solvents and Extraction Methods on Herbal Plants *Phyllanthus niruri, Orthosiphon stamineus* and *Labisia pumila*

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Abstract

Background/Objectives: *Phyllanthus niruri, Orthosiphon stamineus* and *Labisia pumila* are the three herbs listed in Malaysian National Key Economic Areas (NKEA). This study was conducted to determine the herbs extract yields and activities on antioxidant and cytotoxicity properties. **Methods/Statistical Analysis:** Cold maceration and soxhlet were the extraction methods employed and water, ethanol and 50% ethanol (v/v) were chosen as solvents. **Findings:** The results showed 50% ethanol (v/v) was the best solvent for all three herbs in terms of extraction yield. For cold maceration, the extract yields were 14.3%, 17.4% and 7.6% for *P. niruri, O. stamineus* and *L. pumila*, respectively. Whilst for soxhlet method, the same trend was observed where 50% ethanol (v/v) gave the highest extract yield of 21.2%, 14.3% and 6.8% for *P. niruri, O. stamineus* and *L. pumila*, respectively. Total phenolic content and antioxidant capacity were also highest using 50% ethanol and soxhlet method for all herbs. **Application/Improvements:** This shows that the food-grade solvents at a certain concentration are suitable for the best extraction of selected herbs.

Keywords: *Labisia pumila,* Maceration*, Orthosiphon stamineus, Phyllanthus niruri,* Soxhlet

1. Introduction

Malaysia is one of the well-known South East Asia (SEA) country for it richness in medicine herbs along with Thailand and Indonesia. The uses of herbs for medicinal purposes and daily consumptions are getting more attention by consumers and researchers due to their great benefits. Therefore, Malaysia government listed six types of herbs under EPP1 (Entry Point Projects: High Value Product) NKEA (National Key Economic Area) development program. *Phyllanthus niruri, Orthosiphon stamineus* and *Labisia pumila* are recognized and listed as NKEA herbs.

Phyllanthus niruri or commonly called as Dukung Anak is extensively used to cure constipation, gonorea, bronchitis, diabetes and jaundice. Scientific research upon this herb began in 1960's on determining its phytochemicals composition. Lignan, glycoside, flavanoid, alkaloid, ellagitannins, terpene and flavanol are contained in leaf, stem and root of the herb[1]. Elalgitannin is one of the hydrolysable tannin of which its constituents are corilagin, ellagic acid and gallic acid[2]. This research was conducted to determine the suitable solvent to extract hydrolysable tannins with may yield antioxidant anti-cancer and hepatitis B virus scavenging properties.

Orthosiphon stamineus Benth or locally called MisaiKucing can be found in other Southeast Asia countries such as Thailand and Indonesia. Since ancient times, leaf of *O. stamineus* has been taken as beverage use or called as Java Tea[3]. The tea prepared from the leaves is claimed to improve health, to treat diabetes, edema and gout[4-6]. Benefits provided by this herbal plant indeed attract

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much attention of researchers worldwide. Researchers revealed *O. stamineus* exhibits antioxidant, antibacterial as well as anti-inflammatory properties[7-10]. It is found to be rich of more than 20 phenolic compounds namely rosmarinic acid, 3'-hydroxy-5, 6, 7, 4'-tetramethoxyflavone, sinensetin and eupatorin.

Labisiapumila (Myrsinaceae) is also known as Kacip Fatimah is an herb has been widely applied by Malaysia communities as well as in other Southeast Asia regions to induce and facilitate childbirth. From medical aspect, it is used to treat rheumatism, gonorea and intestine infection[11]. Nevertheless, *L. pumila* var. alata species is widely used in traditional medicine[12]. Previous researches indicate that *L. pumila* constitutes of highly amount of phenolic acid and flavanoid[13-15]. It was found to contain gallic acid, caffeic acid, methyl gallate, chlorogenic acid and catechin. Hence, antioxidant assay screening was performed and leaves of *L. pumila* exhibit highest antioxidant properties than other parts of the plant[15].

Previous research conducted was mostly upon identification and biological screening of *P. niruri, O. stamineus*and *L. pumila*. However, not much study performed to compare the extraction techniques and solvent effects on extracting phenolic components from these herbs are reported. Different solvents will affect the biological activity of plant extracts[16]. In addition, ratio of sample to solvent also plays big role on obtaining extract yield[17]. Therefore, the study of the solvent effects and extraction techniques on extract yield are conducted prior to phytochemical identification and bioassays.

2. Materials and Methods

2.1 Cold Maceration

10 grams of each plant sample was immersed in a 200 ml of different type of solvents comprises of water, ethanol and 50% (v/v) ethanol. All samples were left at room temperature for three days. Then, samples were filtered using filter paper and concentrated using vacuum rotary evaporator (Yamato Scientific Co., Ltd RE 600, Japan) at 80°C prior to drying process in freeze dryer (Martin Christ Alpha). Crude extracts were collected until thick and viscous paste or powder of extract is visible. The experiment was conducted in three replicates. Extraction yield of all extracts were calculated using the following equation below:

$$\text{Total extract yield, } Y_T\ (\%) = \frac{\text{Total mass of extract}}{\text{Total mass of sample}} \times 100 \quad (1)$$

2.2 Soxhlet Extraction

10 grams of each plant sample was loaded in a 30 x 80 mm cellulose extraction thimble (AquaLab CT3080) and 200 ml volume of solvent was used. Time span of extraction was set to six (6) hours and the temperature of the heating mantle was adjusted so that four (4) cycles per hour was achieved. Then, solvents obtained were collected and concentrated using vacuum rotary evaporator (Yamato Scientific Co., Ltd RE 600, Japan) at 80°C prior to drying process in freeze dryer (Martin Christ Alpha). Crude extracts were collected until thick and viscous paste or powder of extract is visible. Extraction yield of all extracts were calculated using equation (1).

2.3 Total Phenolic Content

Folin-Ciocalteu reagent method was used to determine the total phenolic content (TPC). Gallic acid was used as reference standard. Equipment used was a spectrophotometer (HACH DR 2800) and the absorbance wavelength was set at 765 nm and measured in replicates. The TPC was calculated as gallic acid equivalent (GAE) using method[18].

2.4 Antioxidant Assay

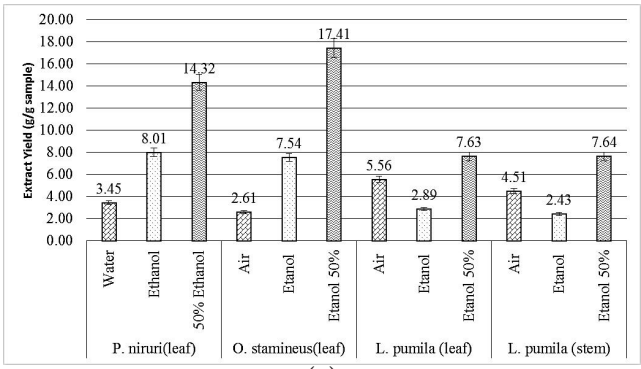
The antioxidant capacity for each extract was determined using the free radical inhibition test or DPPH, which was developed[19]. The absorbance wavelength was at 515 nm and measured by a spectrophotometer. The readings were recorded as percent of free radical inhibition and measured in replicates.

3. Results and Discussion

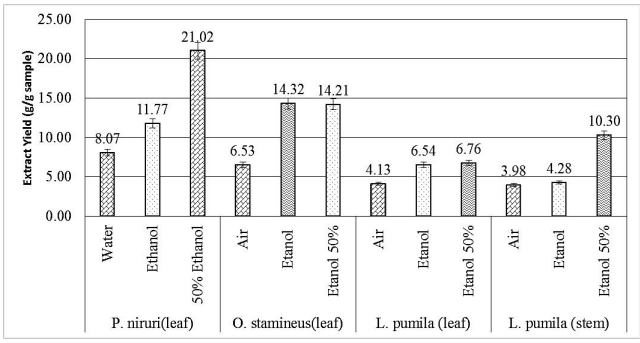
3.1 Effects of Solvent and Extraction Method on Extraction Yield

Extraction step is the initial step prior to analysing phytochemical component of the herbs. Two methods of extraction are performed; cold maceration and soxhlet extraction. Hence, effects of solvents and extraction methods are studied in terms of extraction yield as shown in Figure 1.

The results indicated a wide range of extraction yield for different solvents (2.43 - 21.02%). Among all of the solvents selected, all herbs showed a tendency to dissolve in 50% ethanol followed by water and ethanol. In cold maceration, *O. stamineus* yields highest percentage



(**a**)



(**b**)

Figure 1. Effects of solvents on extraction yield using (**a**) cold maceration (**b**) soxhlet.

of phenolic content at 17.41%, followed by *P. niruri* at 14.32% and *L. pumila* at 7.63 -7.64 %. Aside from cold maceration, soxhlet extraction is another option on extracting herbs at shorter time. The results indicate soxhlet extraction is capable to yield higher percentage of phenolic content from all three herbs.

In contrast, *P. niruri* exhibits the highest percentage yield at 21.02%, followed by *O. stamineus* at 14.21 % and *L. pumila* at 6.76-10.30 %. However, soxhlet only works at the boiling point of solvent which will then be recycled upon its completion. In addition, six (6) hours with four (4) cycles per hour was selected to obtain maximum yield. Colour reduction of the herb is expected at the end of extraction. However, it must be noted that degradation of herbs might occur if plant material is exposed at high temperature at such long time. Hence, cold maceration is still a better option because some of herbal components are heat sensitive.

The component separation by a solvent is dependent upon the polarity and molecule structure of the solvent. An addition of water in ethanol will increase extract yield since polar and non-polar components are extracted together.

However, according to a study conducted[20], *P. niruri* extraction exhibits higher extract yield by using water (26.2%) than 50% ethanol (22.5%).[21] reported extraction of *O. stamineus* also gave the highest yield using water (34%) followed by ethanol (5%). In their study, it is stated rosmarinic acid is present in *O. stamineus* abundantly of which it extraction is favoured by very polar solvents.[22] also reported water as the best solvent for *L. pumila* (leaf) extraction with the highest yield at 13.42% compared to ethanol at 5.96%. Therefore, the selection of best solvent is very much dependent on the component profile and distribution in the herbal plant.

3.2 Effects of Solvent and Extraction Method on Phenolic Content

Total phenolic contents for different solvents using maceration and soxhlet methods are shown in Figure 2. Phenolic compounds contain hydroxyl groups which may have ties to the aromatic compounds and are capable in destroying free radicals. For both *P. niruri* dan *O. stamineus*, the 50% ethanol extracts by soxhlet showed the highest phenolic contents, which were 5.54 ± 0.00 mg GAE/ g for *P. niruri* extract and 5.45 ± 0.001 mg GAE/ g for *O. stamineus* extract, respectively. For *L. pumila* extracts, the soxhlet method using 50% ethanol produced the highest phenolic contents for both leaf and stem parts, whereby the leaf extract gave the higher phenolic content of 6.10 ± 0.001 mg GAE/ g extract compared to the stem extract (3.43 ± 0.001 mg GAE/ g extract).

In the study conducted[23], the water extract of *P. niruri* showed the highest phenolic content (3.76 mg GAE/ g extract) in contrast to this study. Whereas for *L. pumila*, different results were obtained[24] where the methanol extract gave the highest phenolic content (0.468 mg

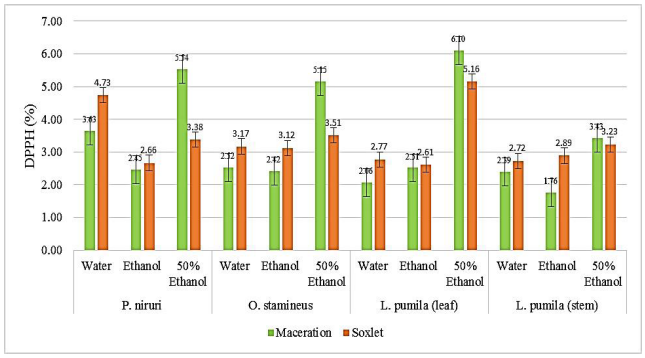


Figure 2. Effects of solvents and extraction methods on phenolic contents.

GAE/g extract compared to water extract (0.274 mg GAE/g extract).

In this study, soxhlet method has produced higher phenolic content compared to maceration method for all herbs. Soxhlet utilizes solvents at their boiling points, thus more phenolic compounds can be extracted. Furthermore, the type and polarity of solvent play important roles in extracting the more polar phenolic compounds. From this study, it can be concluded that the major compounds present in the herbal plants are more polar and hydrophilic compounds.

3.3 Effects of Solvent and Extraction Method on Antioxidant Capacity

The results of DPPH inhibition for the three extracts are shown in Figure 3. In general, 50% ethanol extracts exhibit the highest antioxidant capacity compared to other solvents. It can be seen, however, that 50% ethanol extract of *L. pumila* (stem) exhibits the highest DPPH (98 ± 0.001 %) followed by *L. pumila* (leaf) extract (97± 0.001 %), *O. stamineus* extract (95.95 ± 0.06%) and *P. niruri* extract (95 ± 0.063%). The ethanol extracts for the three plants gave the lowest percent of DPPH or antioxidant capacity. It can be deduced from the previous finding that the highest antioxidant capacity correlates with the highest total phenolic content of the extracts. The phenolic component structure, hydroxyl, flavone and carboxylic acid play important role in the antioxidant capacity.

This finding is similar to the results obtained[25], where the highest antioxidant capacity is found in the plant extracted by water. The study on *P. niruri* by Zahra et al. (2011) also showed the highest DPPH in the water extract (68.5 %). On the other hand[26], found ethanol extract of *P. niruri* to give 6.26 % DPPH inhibition for every 6.25 µg/ml[27] found O. stamineus extracted by water and

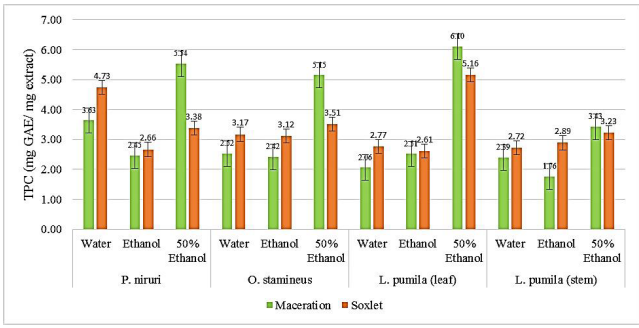


Figure 3. Effects of solvents and extraction methods on antioxidant capacity.

50% methanol gave the highest DPPH (85% and 90%, respectively). In their study, caffeic acid and rosmarinic acid were the major phenolic compounds in both extracts[28] found the DPPH inhibition of L. pumila methanol extract to be 51.3% and 42.2% for leaf and stem, respectively.

4. Conclusion

Choices of solvent and extraction method play important roles on maximizing extract yield and bioactivity. Soxhlet extraction is a preferable technique compared to cold maceration. In this study, 50% ethanol is the best solvent for achieving the highest extract yield, phenolic content and antioxidant capacity.

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