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Research Article

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Effect of solvents on the extraction of Kacip Fatimah (*Labisia pumila*) leaves

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ABSTRACT

This study aimed to ascertain the effect of solvents on the extraction of some bioactive compound from Kacip Fatimah (Labisia pumila) leaves was investigated. The main compound identified using High Performance Liquid Chromatography was gallic acid. Thus, the solvents tested were water (H_2O), ethanol (EtOH), ethyl acetate (EA) and hexane (Hex) as the extraction solvents with 40 °C temperature and four hour extraction time using Solid Liquid Extraction (SLE). Result showed that water was the best solvent for extraction of Kacip Fatimah (Labisia pumila) gave higher yield (13.42 wt. %) followed by ethanol (5.96 wt. %), ethyl acetate (2.46 wt. %) and hexane (1.29 wt. %). This is believed to give good information for particular extraction processes in different polarities of solvents.

Keywords: Gallic acid; Phytochemical; Bioactivities; antioxidant; anti-inflammmatory.

INTRODUCTION

Labisia pumila (Myrsinaceae, also placed in the Primulaceae family) is known in Malaysia as *kacip fatimah*. It found in Southeast Asia and the *Labisia pumila* is a small genus of about seven species of sub-herbaceous perennials [10]. Others familiar names of *Kacip fatimah* is sometimes called *kunci fatimah*, which roughly translates into "Fatimah's key;" *selosoh fatimah* means "Fatimah's childbirth medicine;" and *rumput siti fatimah* is "grass of our lady Fatimah". It is traditionally used as a protective medicine, the decoction of whole plant of *Labisia pumila* is administered after childbirth, but also before birth to expedite delivery. The decoction is also used for the treatment of dysentery, intestinal gas, and dysmenorrhea, as well as for a condition described as "sickness in the bones" [3].

Gallic acid (3,5,7-trihydroxybenzoic acid) is important phytochemical found in *Labisia pumila* leaves and it is derived from the hydrolysis of tannins [8]. It is present most of the plants, such as green and black teas [15], pomegranate husk [6], oak [9] and grape [14]. It attracted considerable interest since it has been found to have many significant biological activities, such as anti-oxidant [1], anti-inflammatory [4], anti-fungal [14] and carcinogenic [8] properties. Gallic acid and its esters are important chemicals in industries that used in the food and pharmaceutical industry which are synthesis of propyl gallate and trimethoprim [14, 7]. The aim of this study is to investigate the highest yield, examine the presence of bioactive compound and the quality of the extract using different polarities in the extraction of the leaves of *Labisia pumila*. The information will be useful in preparing the herbal formulation for health supplements in market.

EXPERIMENTAL SECTION

Material

All the dried leaves of *Labisia pumila* were provided by Natural Product Division, Forest Research Institute Malaysia (FRIM) in order to ensure constant supply throughout the experiment. The dried leaves were recorded and stored in Raw Material Storage Room of Herbal Technology Centre, FRIM.

Methods

Extraction process: Four different types of solvents were used in the experiment including water (H_2O), ethanol (EtOH), ethyl acetate (EA) and hexane (Hex). The non-toxicity and the polarities of the solvents were looked into while selecting them [18, 17, 12]. According to Synder's polarity index, the order of the solvents in increasing polarities are Hex < EA < EtOH < H_2O. The extraction was carried out by using water bath (Model WNB29, Memmert, Germany). The ratio of solvent to solid selected is 10:1 (ml:g) [16]. Process duration of the extraction used was four hours, which were proven to be sufficient by Akowuah *et al*., (2004). A low temperature of 40 °C was selected to avoid degradation of phytochemicals. Ten grams of *Labisia pumila* dried leaves were weighted and was added in a round-bottom flask (500 ml). 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 then freeze-dried 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 [12]:

$$Y = \frac{W_d}{V_e} \; x \; R_{ss} \; x \; 100 \qquad (1)$$

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.

High performance liquid chromatography (HPLC): The dried extracts from H_2O (50.0 mg) were dissolved in 1.0 ml of 50 % methanol in water. The dried extracts (50.0 mg) from EtOH, EA and Hex were dissolved in 1.0 ml of methanol (CH_3OH). The solutions were then filtered using polytetrafluoroethylene PTFE syringe filter (diameter: 13 mm, 0.45 mm) before HPLC analysis. 30µL of samples were injected and the HPLC analysis was carried out with Waters system composed of a quaternary pump (Waters 600E), an autosampler (Water 717 plus) and a PDA detector (Waters 2996 PAD) scanning from 190-400 nm using a reversed-phase Phenomenex Luna C-18 column (4.6 i.d. x 250 mm, 5 µm). The mobile phase was in gradient mode and consisted of 0.1% orthophosphoric acid (H3PO4) and 100% CH3CN. The analysis was carried out following the procedure in [12]. The chromatographic profiles were obtained using a reversed-phase C-18 column at flow rate of 1.0 mL/min at room temperature and the extract was eluted with a gradient system of acetonitrile (A) and 0.1 % HCOOH (B). The elution profile was 5-20 % A in B (0-10 min), 20-100 % A in B (10-30 min) and isocratic 100 % A (30-55 min).

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 AND DISCUSSION

Solvent effect on extract yield

Figure 1 represents the comparison of extraction yield of four different types of solvents.The result obtained from this study suggest that water most suitable solvent for extracting *Labisia Pumila* leaves compared to other solvents. This was because it gave highest yield and the extract from water (13.42 wt.%) followed by ethanol (5.96 wt.%), ethyl acetate (2.46 wt.%) and hexane (1.29wt.%). According to the ANOVA analysis, the extraction yield of H_2O was significant ($p<0.05$) compared to the others. This shown that the major phytochemicals in *Labisia Pumila* leaves are mostly high in polarity and soluble in H_2O. The findings of the current study are consistent with those reported

in Markom *et al.*, 2007 and Pin *et al.*, 2009 in which the highest yield was obtained in H_2O in the extraction of *Phyllanthis niruri* and betel leaves. Different color of extract was observed in this study, it can be seen that color of water extract was dark brownish and brownish green in ethanol extract. However, the color of extract from non-polar solvents including hexane and ethyl acetate were observed to be yellowish green. A possible explanation for the green color might be that caused by the presence of chlorophylls in the extract.

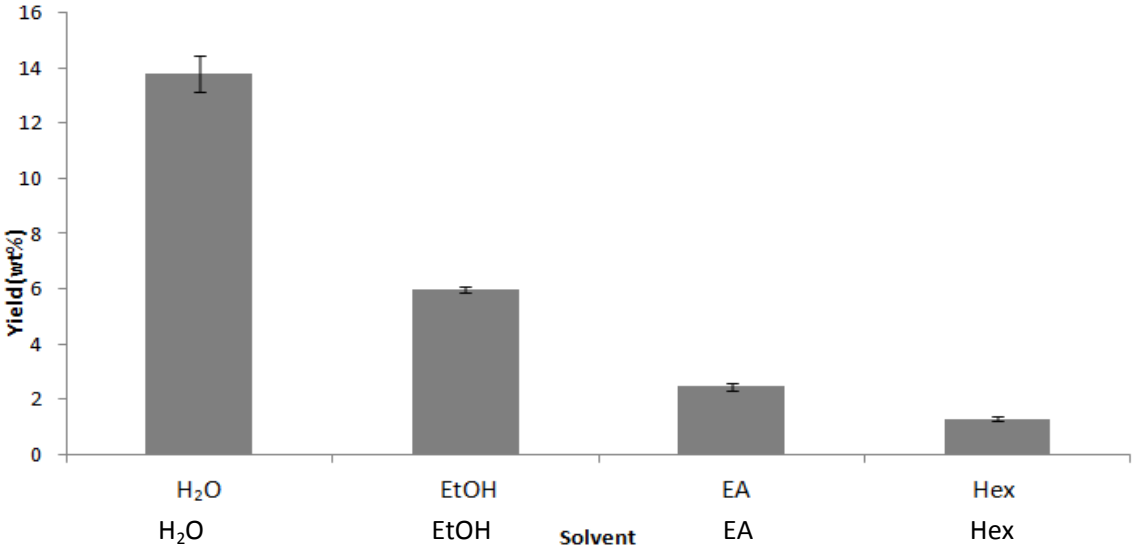

Figure 1:Yield of extraction from different solvents

It is interesting to note that solvent polarity plays an important role, the result shown extraction yields increase with the Synder's solvent polarity and dielectric constant. The polarities of solvents used are listed in Table 1. Snyder defines polarity as the relative ability of a molecule to engage in strong interactions with other "polar" molecules (not specifically the presence in a molecule of a large dipole moment).

Table 1: Properties of solvents

Extraction Solvent	Dipole Moment (Debye)[a]	Dielectric Constant[a]	Synder' Polarity Index[b]
Water, H_2O	1.87	78.36	9.0
Ethanol, EtOH	1.69	24.85	5.2
Ethyl acetate, EA	1.78	5.99	4.3
Hexane, Hex	0.08	1.88	0.0

[a]*Lide (1995)*
[b]*Synder (1974)*

This observation also implies that most components in *Labisia pumila* leaves are polar compounds, which are easier to be extracted compare to non-polar compounds. The presence of hydroxyl group in water and ethanol which could form hydrogen bonding with the solute, water is more effective in extracting the solute compared to ethanol because it has higher polarity and shorter chain [12]. The characteristics of water improved its capability to extract the polar compounds.

There was a significant difference observed between the two solvent H_2O and EtOH. The difference in yields might be due to other factors, such as phytochemical content in plants, extraction temperature, extraction time and solvent to solid ratio of solid liquid extraction.

Solvent effects on phytochemical content:

The presence of major component peaks was consistently detected in the HLPC chromatograms and some of the *Labisia pumila* leaves extract. The chromatograms of *Labisia pumila* leaves extract from different solvents as shown in Figure 2.

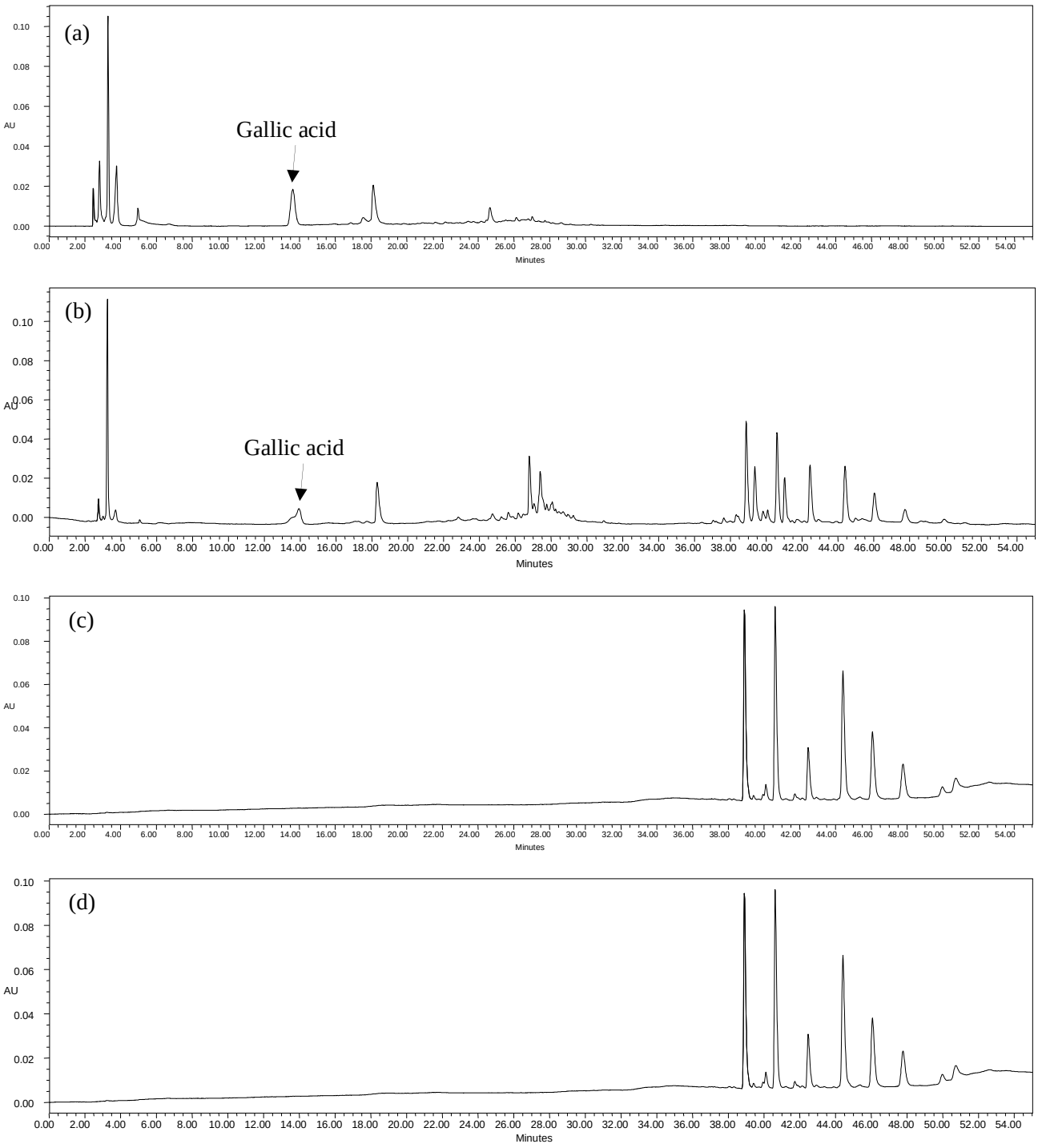

Figure 2: HPLC Chromatograms of (a) H_2O extract; (b) EtOH extract; (c) EA extract; (d) Hex extract at 275nm

Based on the HLPC result indicated that the selected compound in *Labisia pumila* leaves extracts was gallic acid by comparison with the external standard. The gallic acid only presented in H_2O and EtOH extracts as shown in Figure 3. Comparatively, gallic acid content was higher in water extract with 0.12 mg/ml and ethanol extract with 0.09 mg/ml. This was also supported by Markom *et al*., 2007 in the extraction of *P. niruri* where gallic acid contains carboxylic acid group and it was most soluble in water. Gallic acid contains four hydroxyl and a carboxylic groups and is more soluble in the high polar solvents such as water based on index of polarity. It can be deduced that the

hydroxyl and carboxylic groups in gallic acid are preferably extracted by H_2O through hydrogen bonding. The gallic acid are not present or might not significant in Hex and EA extracts because both of these solvents are non-polar solvent and non- soluble for gallic acid. In the herbal extraction, Hex and EA were used mainly for removing non-polar lipids and unwanted glycosides [2, 5].

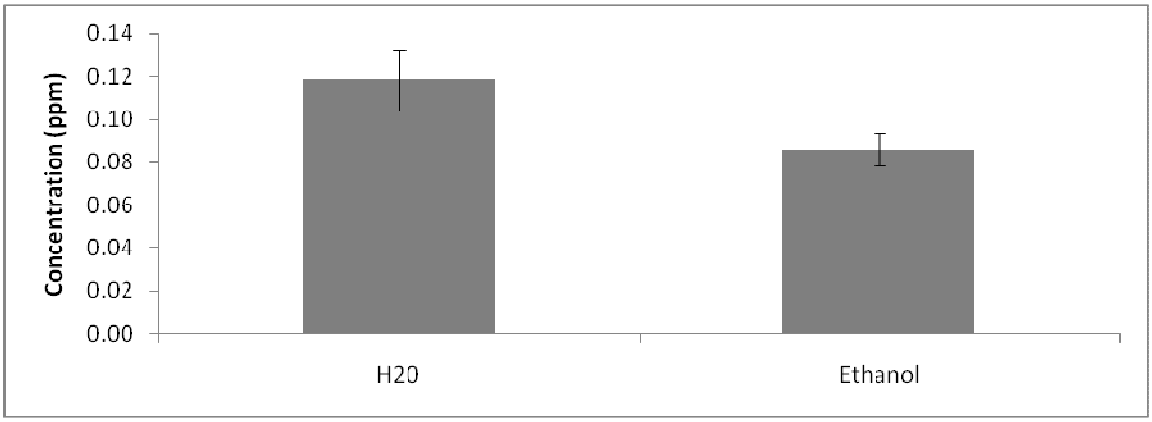

Figure 3: Recovery of Gallic acid in the extract from water and ethanol.

CONCLUSION

The result on the present of study showed that water gave the highest extraction yield and concentration of gallic acid among the selected solvents. Gallic acid is the compound that possesses beneficial activity including anti-oxidant and anti-inflammatory. Judging from the quantitative and qualitative results, water is the most suitable and effective solvent to obtain *Labisia pumila* leaves extracts.

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