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Comparison of different solvents on the extraction of *Melastoma malabathricum* leaves using Soxhlet extraction method

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ABSTRACT

This work aimed to investigate the effect of solvents on the extraction of some phythochemical from Melastoma malabathricum leaves. Melastoma malabathricum or locally known as Senduduk belongs tothe family of Melastomataceae and rich in flavonoid contents. The solvents tested were deionized water (H_2O), ethanol (EtOH), ethyl acetate (EA) and hexane (Hex) and six hour of extraction time using Soxhlet extraction (SOX). The main compounds (quercetin and rutin) of Melastoma malabathricum were identified by High Performance Liquid Chromatography (HPLC) in specific solvents of extraction such as H_2O,EtOH, EA and Hex. The results revealed that water was pronounced than the other solvents tested. The highest yield of extraction can be obtained during water extract(28.03 wt.%), followed by ethanol(26.61 wt.%), ethyl acetate (4.61 wt.%) and hexane (1.92 wt.%), respectively.In addition, Quercetin content was found to be higher in EtOH extract with 0.77 ppm, EA extract with 0.68 ppm, followed by Hex with 0.49 ppm. However, rutin content was found to be higher in EtOH extract (83.13 ppm), followed by EA extract (56.53 ppm) and Hex extract (0.66 ppm), respectively. The data generated in this work, provide an insight on phytochemical content of Melastomamalbathricum leaves at different solvents polarities.

Keyword: Solvent, *Melastoma malabathricum*, Extraction, Rutin, Quercetin

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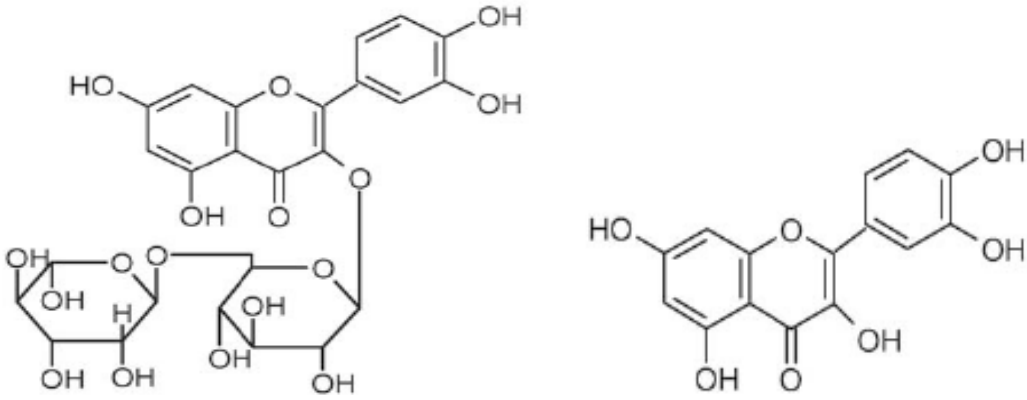
INTRODUCTION

Melastoma malabathricum or locally known as Senduduk belongs tothe family of Melastomataceae. It can be discovered liberally in Malaysia. It accompanies wonderful pink or purple blossoms and rich with flavonoid content [1&2]. In Malaysia, especially, the plant is exceptionally basic in the swamp and mountain, essentially in open spots. Various pharmacological studies and clinical practice have reported that different parts of *M. malabathricum* plant have biological activities, for example anti-oxidant and anti-cancer [1], anti-viral [3], anti-inflammatory, antinociceptive and anti-pyretic [5], and against ulcerogenic [6].

Flavonoids are the largest group of secondary metabolites that involved in many biological activities in plants. According to Atanassova and Bagdassarian (2009a) rutin and quercetin are two majors bioactive plant pigmentation (flavonoids) that present in substantial amount and found in various plants such as buckwheat, berries, citrus fruits and green tea [7]. The molecules structure of rutin and quercetin as shown in Figure I.



(a) Rutin (RU) **(b) Quercetin (QU)**

Figure I: Structure of rutin and quercetin in the extraction of *M. malabathricum*leaves

The point of this study is to research the higher yield, analyze the presence of bioactive compounds extract from *M. malabathricum* leaves with different polarities solvents extraction. Numerous scientists reported impact of various extraction solvents, methods on the phytochemical content of natural anti-oxidants in extracts [6 & 8]. However, the efficiency of solvents with different polarities and methods are strongly dependent on plant matrix used [8, 9, & 10].

MATERIALS AND METHODS

Sample preparation

M. Malabthricum leaves were continuously collected from Skudai, Johor, Malaysia. The fresh leaves of *M. Malabthricum* were cleaned and dried at 50°C. The samples were ground into fine powder (500 µm) by miller.

Extraction

Approximately ten gram of dried powder *M. Malabthricum* leaves were weighed and placed into a round bottom flask with 300 ml of the extracting solvent. The sample were extracted using soxhlet extraction method with different types of solvents including water (H_2O),ethanol (EtOH), ethyl acetate (EA) and hexane (Hex). Process duration of the extraction used was six hours and temperature of extraction based on the boiling point of solvents. The extract from *M. Malabthricum* was filtered through filter paper (Whatman No. 1) with Buchner filter under vacuum. The extract from H_2O were kept in freezer at -25°C (Model Sanyo biomedical, Japan) prior to freeze dry process and organic solvent extract stored at room temperature before solvent recovery process. Then, H_2O extract was freeze-dried in order to remove the solvent. However, the extract from EtOH, EA and Hex were recovered using rotary evaporator (Model Heidolph, germany) under vacuum. The evaporation process was conducted at 45°C to minimize any possible degradation of the phytochemicals in the samples. Extraction yield from both water and organic solvent were calculated using following equation [11]:

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

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.

Analysis

Chromatographic analysis was carried out using HPLC with water system (Milford, MA, USA) consisting of double pump and system controller (Model 2690), and automatic sampler and photo-diode array detector (Model 966) were used. The column configuration consisted of a reversed phase column (4.6 mm x 250 mm, 5 µ; Phenomemex, Torrance, CA, USA) and the mobile phase was methanol: acetonitrile: water (40:15:45, v/v/v) containing 1.0% acetic acid. Detection of Rutin (RU) andQuercetin (QU) wavelength were set at 257 nm and 368 nm. The flow rate

was 1.0 ml/min, respectively. The chromatographic peaks of analytes were confirmed by comparing their retention time and UV spectra with those of the reference standards. All operations were carried at ambient temperature.

RESULTS AND DISCUSSION

Figure II shows the comparison of yield of extraction of four different types of solvents including water (H_2O), ethanol (EtOH), ethyl acetate (EA) and hexane (Hex), separately. It was found that water gave highest yield of extraction among other solvents for the extracting *M.malabathricum* leaves. The extraction yield for water is 28.035 wt.%, trailed by ethanol, 26.61 wt.%, ethyl acetate , 4.61 wt.% and hexane, 1.92 wt.%, respectively.

The significant phytochemicals in *M.malabathricum* leaves are generally high soluble in H_2O solvent. Comparative discoveries are accounted for underway of Markom *et al*. (2007) and Pin *et al*. (2009) in which the most elevated yield was gotten while utilizing water as dissolvable as a part of the extraction of *Phyllanthisniruri* and Piper betel clears out [12 & 13]. Trabelsi*et al*. (2010) likewise reported that water yielded the most noteworthy measure of phenolic substance in extraction of *L. monopetalum* [14].

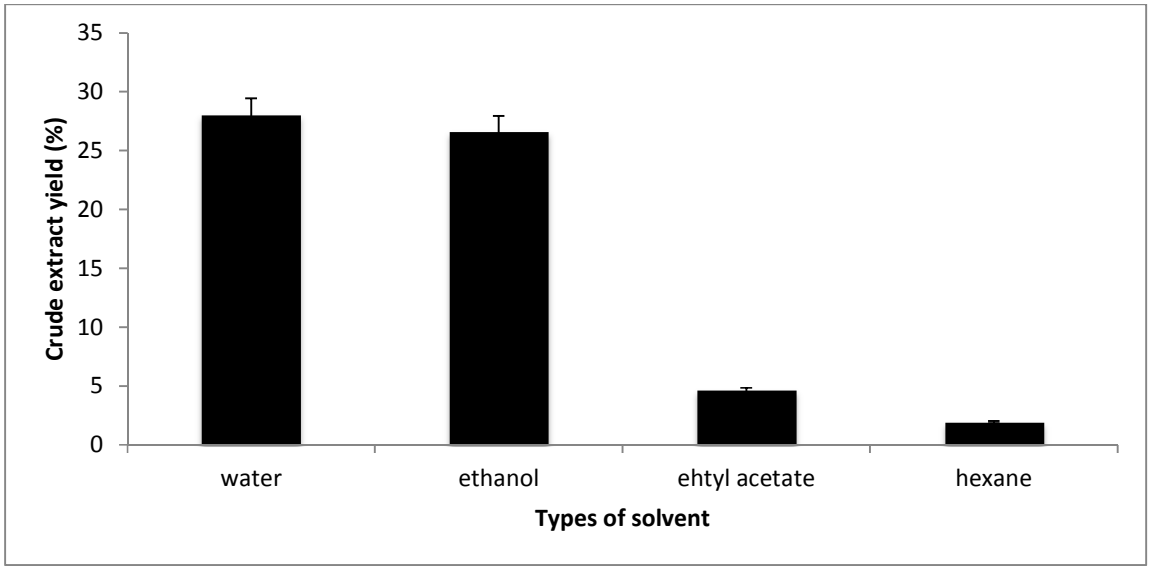


Figure II: Yield extraction of *M.malabathricum* leaves with different types of solvent

The phytochemical contents of extracts from four types of solvent were analyzed using HPLC. Figure III and Figure IV illustrate the concentrations of RU and QU for the extracts of *M.malabathricum* leaves. ANOVA analysis showed that the concentration of RU and QU in EtOH extract is significantly different ($p< 0.005$) compared to others. The order of increasing amount concentration of RU and QU in *M.malabathricum* leaves extracts were Hex < EA <EtOH. In this research, the phytochemical marker of RU and QU were not distinguished or not significant in water extract because this solvent not soluble for both chemicals marker. The combined use of water and organic solvent may facilitate the extraction of phytochemicalsthat are soluble in water and organic solvent. The yields extraction flavonoid contents of Limnophila aromatic are increases using 50% aqueous ethanol as a solvent[15].As expected, Hex, which has the lowest polarity among the solvents used, was clearly not effective in extracting of RU and QU content in this study. It can be consider that the solvents might play significant roles in increasing or decreasing the solubility in a solvent. The result of this study also confirm the work by others that in herbal extraction, aqueous solvents wee preferably used for the extraction of polyphenols [12, 13, & 16].

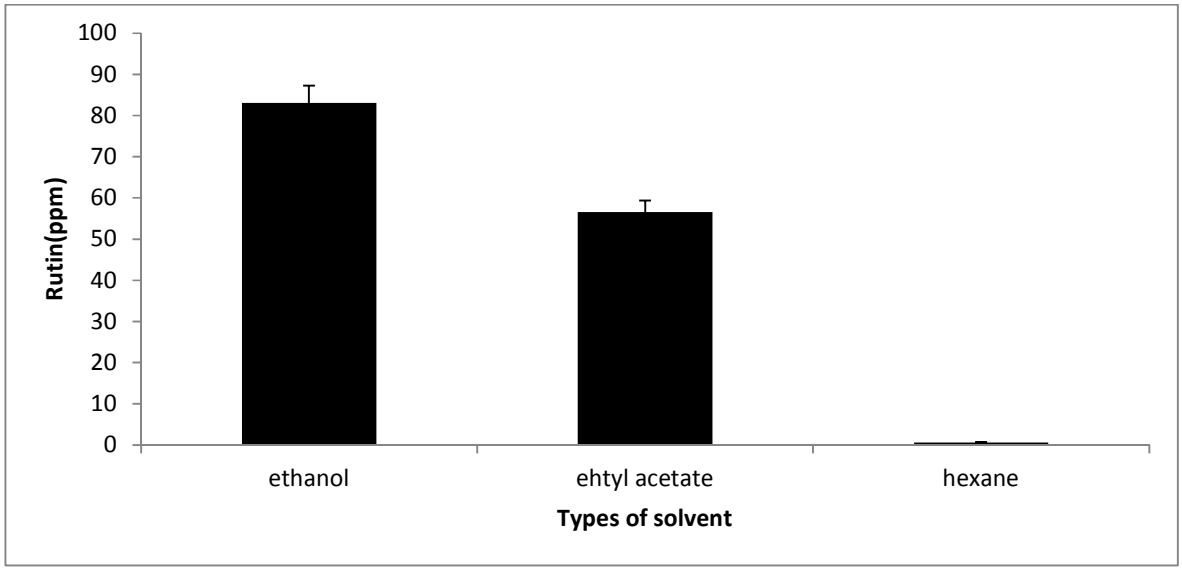


Figure III: Concentration of Rutin from *M.malabathricum* leaves with different types of solvent

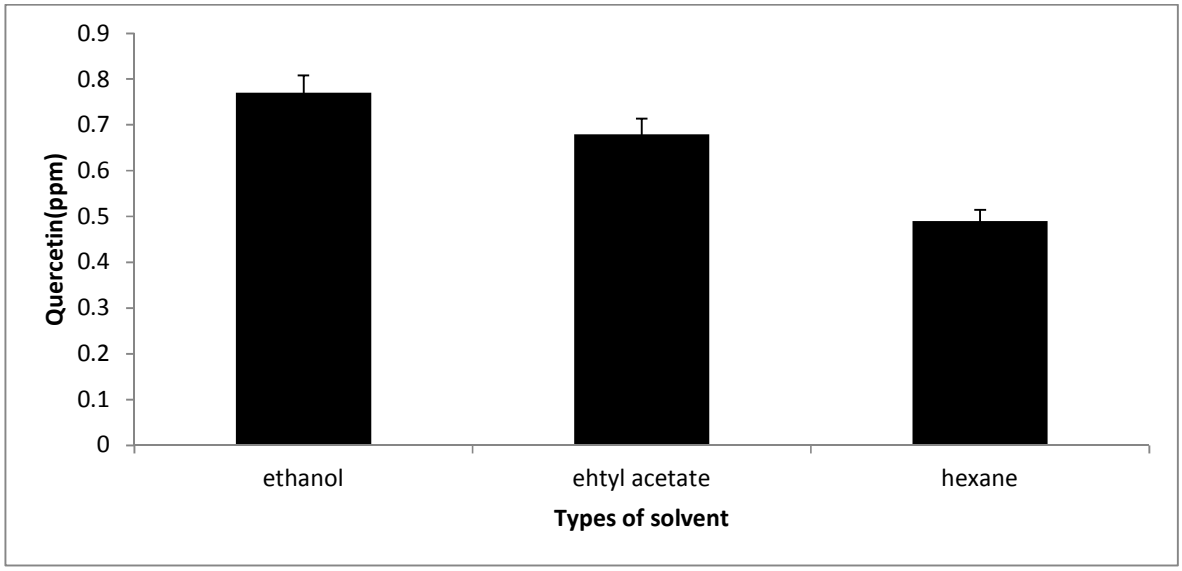


Figure IV: Concentration of Quercetin from *M.malabathricum* leaves with different types of solvent

CONCLUSION

The effects of the solvents on *M. malabathricum* leaves extracts using soxhlet extraction were successfully investigated. The extraction yields of four different types of solvents, such as water, EtOH, EA and Hex were calculated. The highest yield of the extraction of *M. malabathricum* leaves extracts was obtained using water as a solvent.The concentration of RU and QU were found to be highest in EtOH extract. The difference in the concentration of both RU and QU in the extracts was due to the polarity of the extraction solvents.

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