KINETICS OF ULTRASOUND-ASSISTED EXTRACTION AND CONCENTRATION OF ROSMARINIC ACID AS BIOACTIVE FRACTION

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AND CONCENTRATION OF ROSMARINIC ACID
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ABSTRACT

Malaysia's herbal industry is growing rapidly over the years. *Orthosiphon aristatus*, or locally named as "Misai Kucing", is one of the herbs in Malaysia that possesses remarkable therapeutic potentials. Rosmarinic acid is the biochemical marker of *O. aristatus* that is of interest in this study. This study was conducted to determine the effectiveness of ultrasound-assisted extraction in extracting rosmarinic acid, as well as solid-phase extraction in concentrating rosmarinic acid from *O. aristatus*. The aqueous extraction of rosmarinic acid assisted by ultrasound was performed for 30 minutes at temperatures ranged from 27℃ to 60℃, whereas extraction without ultrasound was performed at temperatures of 27℃ and 40℃. The samples were analysed for their yield, rosmarinic acid content, antioxidant, and anti-inflammatory activities. The aqueous extract from 27℃ produced high yield and rosmarinic acid content. The experimental data of rosmarinic acid extraction was fitted into seven kinetic models. The two-sites kinetic model was the best-fit kinetic model with the highest R^2 and the lowest RMSE values. The estimated rate constants were used to calculate the activation energy and the pre-exponential factor using the Arrhenius equation. The activation energies for extraction with ultrasound were 10.1065 kJ/mol for the washing stage and 0.7373 kJ/mol for the diffusion stage, whereas the pre-exponential factors for extraction with ultrasound were 12.1642 min^{-1} for the washing stage and 4.1371 min^{-1} for the diffusion stage. Consequently, the aqueous extract with high rosmarinic acid content at 27℃ was selected and subjected to solid-phase extraction with methanol. The eluted fractions were collected from the solid-phase extraction and analysed for their rosmarinic acid content, antioxidant, and anti-inflammatory activities. The solid-phase extraction succeeded in increasing rosmarinic acid yield from 1.36% in the crude extract to 11.14% in the methanol fraction. The antioxidant and anti-inflammatory activities of the extracts reduced after ultrasound-assisted extraction and solid-phase extraction due to the degradation and selective adsorption of phytochemicals. The findings for the antioxidant and anti-inflammatory activities were also affected by the immediate reaction of mixtures and the colour intensity of the extracts. This study concluded that the combination of ultrasound-assisted extraction and solid-phase extraction methods could increase the rosmarinic acid content in *O. aristatus* extract.

ABSTRAK

Industri herba Malaysia semakin berkembang saban tahun. *Orthosiphon aristatus*, atau nama tempatannya "Misai Kucing", adalah satu daripada herba-herba di Malaysia yang mempunyai potensi-potensi terapeutik yang luar biasa. Asid rosmarinik adalah penanda biokimia untuk *O. aristatus* yang menjadi tarikan dalam kajian ini. Kajian ini dilaksanakan bagi menilai keberkesanan pengekstrakan dengan bantuan ultrabunyi dalam mengekstrak asid rosmarinik, dan juga pengekstrakan fasa pepejal dalam memekatkan asid rosmarinik daripada *O. aristatus*. Pengesktrakan air bagi asid rosmarinik dengan bantuan ultrabunyi telah dilakukan selama 30 minit pada jarak suhu 27℃ hingga 60℃, manakala pengekstrakan tanpa ultrabunyi telah dilakukan pada suhu 27℃ dan 40℃. Sampel-sampel tersebut telah dianalisis untuk hasil, kandungan asid rosmarinik, aktiviti antioksida, dan aktiviti anti-radang. Ekstrak air daripada 27℃ menghasilkan hasil dan kandungan asid rosmarinik yang tinggi. Data eksperimen daripada pengekstrakan asid rosmarinik telah dimuatkan kedalam tujuh model kinetik. Model kinetik dua-tapak merupakan model kinetik yang terbaik dengan nilai R^2 yang tertinggi dan nilai RMSE yang terendah. Pemalar kadar yang dianggar telah digunakan untuk mengira tenaga pengaktifan dan faktor pra-eksponensial melalui persamaan Arrhenius. Tenaga pengaktifan untuk pengekstrakan dengan ultrabunyi adalah 10.1065 kJ/mol bagi peringkat basuhan dan 0.7373 kJ/mol bagi peringkat resapan, manakala faktor pra-eksponensial untuk pengekstrakan dengan ultrabunyi adalah 12.1642 min^{-1} bagi peringkat basuhan dan 4.1371 min^{-1} bagi peringkat resapan. Seterusnya, ekstrak air dengan kandungan asid rosmarinik yang tinggi pada 27℃ telah dipilih dan digunakan untuk pengekstrakan fasa pepejal dengan metanol. Ekstrak yang terhasil daripada pengekstrakan fasa pepejal telah dikumpulkan dan dianalisis untuk kandungan asid rosmarinik, aktiviti antioksida, dan aktiviti anti-radang. Pengekstrakan fasa pepejal berjaya dalam meningkatkan hasil asid rosmarinik daripada 1.36% dalam ekstrak mentah kepada 11.14% dalam pecahan metanol. Aktiviti antioksida dan aktiviti anti-radang menurun selepas pengekstrakan dengan bantuan ultrabunyi dan pengekstrakan fasa pepejal disebabkan oleh kehancuran dan penjerapan selektif fitokimia. Penemuan untuk aktiviti antioksida dan aktiviti anti-radang juga terkesan daripada reaksi campuran yang segera dan kepekatan warna ekstrak. Kajian ini merumuskan bahawa gabungan bagi kaedah pengekstrakan dengan bantuan ultrabunyi dan kaedah pengekstrakan fasa pepejal mampu untuk meningkatkan kandungan asid rosmarinik dalam ekstrak *O. aristatus*.

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LIST OF ABBREVIATIONS

DPPH	-	2,2-diphenyl-1-picrylhydrazyl
EST	-	Estimated Data
EXP	-	Experimental Data
FRIM	-	Forest Research Institute Malaysia
HPLC	-	High-Performance Liquid Chromatography
IUPAC	-	International Union of Pure and Applied Chemistry
NO	-	Nitric Oxide
R^2	-	Coefficient of Determination
RMSE	-	Root Mean Square Error
SPE	-	Solid-Phase Extraction
TMF	-	Tetramethoxyflavone
UV-Vis	-	Ultraviolet-Visual
WHO	-	World Health Organization
WUS	-	Without Ultrasound

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CHAPTER 1

INTRODUCTION

1.1 Background of the Problem

The herbal industry is growing rapidly worldwide. According to the World Bank Report, the global market value of the herbal industry experienced 7% annual growth and is expected to reach USD 5 trillion by the year 2050 (Ahmad & Othman, 2013). For Malaysia, the herbal industry is expected to reach a market value of RM 32 billion by 2020 (Bernama, 2019). Moreover, the export and import trends of Malaysian herbal-based products increased threefold from the year 2001 until 2016 (Noor Hazmira & Noor Azian, 2017). Many herbal products are introduced in the market, due to the increasing activities relating to the research and development of traditional medicine and ethnopharmacology (Nirmal et al., 2013). Examples of high-demand herbal products are functional foods, health supplements, traditional medicines, cosmetics, and personal care products (Ahmad & Othman, 2013). Furthermore, consumers have started to become more aware and concern about the ingredients in their products. Many consumers believed that herbal medicines have lower cost and more effective than modern medicines. The use of herbal products is largely influenced by cultural beliefs, medicinal practices, availability of native herbs, established policies, and distribution channels (Miroddi et al., 2013).

Herbal-based products are recognised as having one or more herbal materials and/or herbal extracts as the active principles that give them their intended functions. Herbal raw materials can be processed through several extraction methods (Aziz, 2003). Herbal extraction is defined as a process where the selective solvent is used to separate the soluble constituents from the herbal insoluble constituents and cellular matrix (WHO Expert Committee on Specifications for Pharmaceutical-Preparations, 2018). There are many herbal extraction methods available, each has its strengths, weaknesses, and applicability for various scales of extraction processes. The herbal constituents obtained right after the extraction process is known as crude herbal extracts. The crude herbal extracts are usually consisting of phytochemicals mixture available in the herbs, regardless of their functionality. Phytochemicals are defined as every natural chemical constituent present in the herbs and plants (Mendoza & Silva, 2018). Phytochemicals contribute to the colour, aroma, and defence mechanism of the herbs and plants against damages and diseases (Msomi & Simelane, 2019).

Nowadays, there is an increasing demand for phytochemical-rich herbal extracts. The therapeutic potentials of herbs, herbal extracts, and herbal products are largely contributed by the presence and amount of the phytochemicals (Guldiken et al., 2018). It is believed that the herbal extracts and products that have a significant content of phytochemicals can perform better and more efficacious than the crude ones. Most of the modern drugs and pharmaceuticals are also phytochemicals that originated from the herbs used in traditional and folk medicines (Yuan et al., 2016). Some phytochemicals are also selected as the biochemical markers for the quality control of herbal extracts and products (Chugh et al., 2018). To produce phytochemical-rich herbal extracts, the crude herbal extracts need to be concentrated and/or fractionated to remove the excess solvent and undesired constituents.

Malaysia is home to vast amounts of plants in which, around 1200 of them were identified as exhibiting potential therapeutic values (Farizah et al., 2015). *Orthosiphon aristatus*, or locally known as "Misai Kucing", is one of the most popular herbs in Malaysia. *O. aristatus* is traditionally used to treat diabetes, eruptive fever, gout, hepatitis, hypertension, influenza, jaundice, oedema, rheumatism, kidney, and bladder diseases (Chai et al., 2014). The leaves of *O. aristatus* exhibit characteristic odour and bitter taste (Global Information Hub On Integrated Medicine, n.d.). The commercial *O. aristatus* herbal drink, prepared by dipping the sachet in hot water for 5-10 minutes, has the same colour and taste as the ubiquitous tea (Darus, 2009). Rosmarinic acid is one of the remarkable biochemical markers of *O. aristatus* (Siddiqui & Ismail, 2011). Rosmarinic acid is widely known for its contribution to pharmacological effects such as anti-allergic, anticancer, anti-depressant, anti-inflammatory, anti-microbial, antioxidant, and immunity-enhancer (Bhatt et al., 2013).

1.2 Statement of the Problem

Many methods have been reported for rosmarinic acid extraction from *O. aristatus*. However, the extraction methods, namely reflux system and supercritical carbon dioxide, took a long time to complete. Thus, there is a need for a method that is fast but efficient in extracting rosmarinic acid from *O. aristatus*. Next, the studies for herbal extraction mostly focussed on the optimisation and the comparison of extraction methods. There is a lack of studies that cover the kinetics of herbal extraction. The kinetics of rosmarinic acid extraction from *O. aristatus* were reported for reflux system (Lau et al., 2014) and supercritical carbon dioxide (Abdul Aziz et al., 2020). Unfortunately, there is no kinetic study conducted for rosmarinic acid extraction from *O. aristatus* using ultrasound system. Previous kinetic studies for rosmarinic acid extraction from *O. aristatus* also did not cover the effect of temperature on the extraction kinetics. Lastly, the crude extract obtained after the ultrasound-assisted extraction contains many unwanted compounds. Thus, there is a need for a method that is fast but efficient in cleaning up and concentrating rosmarinic acid in the *O. aristatus* extract. The use of solid-phase extraction to clean up and concentrate samples from ultrasound-assisted extraction is also lacking.

1.3 Objectives of the Study

This study was conducted to determine the effectiveness of ultrasound-assisted extraction and solid-phase extraction in extracting and concentrating rosmarinic acid from *O. aristatus*, respectively. Specifically, the objectives of this study were:

1. To determine the best-fit kinetic model at different temperatures for rosmarinic acid extraction.
2. To concentrate rosmarinic acid from crude extract using solid-phase extraction.
3. To evaluate the total yield, rosmarinic acid content, antioxidant activity, and anti-inflammatory activity of *O. aristatus* extracts.

1.4 Scopes of the Study

To achieve the specified objectives, the following scopes were applied during this study:

1. Extraction of rosmarinic acid from *O. aristatus* with ultrasound at 27℃, 30℃, 40℃, 50℃, and 60℃; as well as without ultrasound at 27℃ and 40℃.

2. Determination of best-fit kinetic model and estimation of kinetic parameter values using Microsoft Excel's Solver function.

3. Determination of the effect of temperature on extraction kinetics using the Arrhenius equation.

4. Concentration of rosmarinic acid in *O. aristatus* extract using solid-phase extraction with 100% methanol.

5. Evaluation of total yield, rosmarinic acid content, antioxidant activity, and anti-inflammatory activity using dry weight, HPLC analysis, DPPH radical-scavenging assay, and NO radical-scavenging assay, respectively.

1.5 Significance of the Study

The ultrasound-assisted extraction and solid-phase extraction methods are considered environmental-friendly, fast, easy to operate, and low cost (Armenta et al., 2015). Next, this was the first study that carried out the kinetic modelling for ultrasound-assisted extraction of rosmarinic acid from *O. aristatus*. Other than that, this was the first study that determined the effect of temperature on the kinetics of rosmarinic acid extraction from *O. aristatus*. The findings are valuable for understanding the mechanism of ultrasound-assisted extraction. This study was also one of the few that used solid-phase extraction with methanol to concentrate rosmarinic acid. Hence, this study contributed to the existing works and literature by giving an insight into the potential and applicability of the methods in preparing the rosmarinic acid-rich *O. aristatus* extract.

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