THERMODYNAMIC STUDY ON POLYPHENOL THERMAL DEGRADATION FROM ORTHOSIPHON STAMINEUS

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**ABSTRACT**

*Orthosiphon stamineus* is an herbal medicine plant that is commonly used in daily life to treat diseases. It has many medical properties that are contributed by polyphenol component. However, the properties will decline by thermal degradation once the heat treatment is applied to preserve the plant.

Previous studies have been done more on identifying the component and properties of *O. stamineus* rather than studies on thermal degradation. Therefore, this study is aim to identify and investigate the effect of temperature on the thermal degradation of polyphenol from *Orthosiphon stamineus* leaves. Dry powdered leaves of *O. stamineus* will be extracted by solvent extraction using ethanol to produce crude extract and then the heat treatment is carried out between temperatures from 70°C to 120°C at varies time interval. The UPLC analysis and total flavonoid content (TFC) are conducted before and after the heat treatment to analyse the thermal kinetic changes and these changes will be explained using best kinetic model that fits the data. The polyphenol thermal degradation can be identified for the used temperature range and the kinetic modelling can be developed from the kinetic changes. From the result, it can be proved that active components degraded when temperature and residence time increased during heat treatment and sinensetin showed larger degradation compared to rosmarinic acid where it showed less degradation. The data obtained from the experiment fitted well by both Hinrichs-Rademacher and Arrhenius equation using Microsoft Excel Solver and degradation rate constant increases when heating temperature increased and activation energy decreased. Therefore, it can be conclude that, higher temperature and residence time will increase the thermal degradation of bioactive component in *Orthosiphon stamineus* and the both Hinrichs-Rademacher and Arrhenius equation fitted the data. Result from this work may be useful to preserve the polyphenol content in *Orthosiphon stamineus* during processing involving thermal exposure that can retain the quality of the final product.

*Keywords*: *Orthosiphon stamineus*, polyphenol, thermal degradation, kinetic model.
ABSTRAK

Orthosiphon stamineus merupakan tumbuhan perubatan herba yang biasanya digunakan dalam kehidupan sehari-hari untuk merawat penyakit. Ia mempunyai ciri-ciri perubatan yang disumbangkan oleh komponen polifenol. Walau bagaimanapun, sifat-sifat akan merosot disebabkan degradasi apabila rawatan haba digunakan untuk memelihara tumbuhan supaya jangka hayat tumbuhan ditingkatkan.

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LIST OF ABBREVIATIONS

TFC    Total Flavonoid Content
UPLC   Ultra Performance Liquid Chromatography
RA     Rosmarinic Acids
SIN    Sinensetine
1 INTRODUCTION

1.1 Background

Herbal medicine or herbalism becomes more popular and demanding nowadays due to its effectiveness, availability and often affordable than modern medicine. According to World Health Organization (WHO), 80% of Asians and Africans use the herbal medicine in their daily health care. A study was done by Matthews et al. (2010) stated that in many countries herbal medicine were used to treat pregnancy and related illness but there are lack of evidence for the safety. In Malaysia, the uses of herbal medicine are based on practical experiences, observation and ritual derived from socioreligious belief. It have been discovered that there are more than 2,000 herbal plants are available in Malaysia.

*Orthosiphon stamineus* is one of the natural herbs that are commonly used in the herbal medicine as an herbal tea. *Orthosiphon stamineus* is a wild tropical plant which is popularly known as Java tea and commonly referred as “Misai Kucing” which means cat whiskers because it has white or purple flowers that resembles cat whiskers. This plant is rich in therapeutic properties where it is used in treating urinary lithiasis, oedema, eruptive fever, influenza and so on.

Figure 1-1: *Orthosiphon stamineus* plant with white and purple flower which resemble cat whiskers.
All the properties of the *O. stamineus* plants ascribed to its bioactive component mainly by polyphenol. Polyphenol is macromolecule that composed of repeating unit of phenolic compound bonded by covalent bond which results from the secondary metabolism of plant. There are few phenolic compounds that can be found in this plant. For example, sinenstine, eupatorine, 3-hydroxyl-5, 6, 7, 4-tetramethoxyflavone (TMF) and so on (Loon et al., 2004).

Due to its medical values, *Orthosiphon stamineus* is very demanding among the society and many products are available in market in the form of tablets, capsules, tea sachets, drinks, raw herbs, dried leaves and extracts.

![Figure 1-2: Varies form of *Orthosiphon stamineus*’s product in market.](image)

In the production of these products, heat treatment such as drying will carried out which also makes it possible to change the composition of the component by thermal degradation. Thermal degradation can define as molecular deterioration where the component of the polymer separate from the long backbone chain and react with one another that will change the properties of the polymer. Hence, it is known that thermal degradation can modify the therapeutic properties of the polyphenol since polyphenol is highly unstable species in elevated temperature (V.Cheynier, 2005). To predict quality damage during heat process, the knowledge on kinetics behaviour and degradation reaction rate as a function of temperature is required. This kinetic behaviour can be explained by kinetic models such Arrhenius model, ball model, Hinrichs and Rademachier model, Dolan model and so on.
1.2 Motivation and Problem Statement

Previous studies primarily investigated on the constituents, pharmacological and phytotoxic properties of *Orthosiphon stamineus*. Thus, all the bioactive components and therapeutic properties of the plant such as antioxidant and anti-angiogenic properties were successfully identified. However, previous researches are mostly limited to the properties identification but the effect of thermal treatment on the bioactive component (polyphenol) and its properties is not well discovered compared to other plants. For example, the kinetics thermal degradation studies of polyphenol for red cabbage, blackberry, cocoa beans, and orange juice have been done before.

1.3 Objectives

This research work is carried out to investigate the effect of temperature on the thermal degradation of polyphenol from *Orthosiphon Stamineus* leaves with varies time interval.

1.4 Scope of this research

In order to achieve the objective of this work, the following scopes have been identified:

i. Ultrasonic extraction of *Orthosiphon stamineus* by using 50% Methanol

ii. Discover the bioactive constituent content in *Orthosiphon Stamineus* leaves using UPLC analysis.

iii. Determine the total flavonoid content (TFC) in *Orthosiphon Stamineus* leaves.

iv. Identify the kinetic models that fit the polyphenol thermal degradation.

1.5 Organisation of this thesis

The structure of the reminder of the thesis is outlined as follow:
Chapter 2 provides a description of the *Orthosiphon stamineus* plant and its therapeutic properties that help to treat various types of illness. This chapter also provides a brief description on the bioactive component that contributes to the properties of the *Orthosiphon stamineus* mainly the phenolic compound. The thermal degradation of the bioactive component is also discussed in this chapter. A summary of the previous experimental work on the thermal degradation of polyphenol from different plants is also presented. A brief discussion on the kinetic models to explain the thermal degradation activity is also provided.

Chapter 3 gives a review on the method of sample preparation of *Orthosiphon stamineus* including plant material preparation and microwave-assisted extraction. The heat treatment procedures and method to investigate the thermal degradation is also described in this chapter. Other than that, method of analysis after the thermal degradation such as UPLC analysis, total flavonoid content (TFC) analysis, and statistical and kinetic modelling analysis are also described.

Chapter 4 gives a review on the result obtained from the experiment. The result from the UPLC analysis and the TFC analysis were shown and discussed detailed here. The trend of thermal degradation after the heat treatment were identified and analysed in detail. In this chapter also, the kinetic modelling that fitted the kinetic changes due to thermal changes have been described using the Hinrichs-Rademacher and Arrhenius equation. The best modelling that fits the changes was identified and a brief discussion on the parameter of the models was also provided.

Chapter 5 gives a review on the overall conclusions that have been made from the experiment to achieve the objectives of this research study. It also draws together a summary of the thesis and outlines the future work which might be derived from the model developed in this work.
2 LITERATURE REVIEW

2.1 Overview

This chapter presents the experimental studies that have been carried out previously on Orthosiphon Stamineus and its properties. The bioactive component of Orthosiphon Stamineus was discussed. Other than that, the thermal degradation studies on polyphenol from various foods also have been explained with the kinetic model used.

2.2 Orthosiphon stamineus

Orthosiphon stamineus or Cat’s Whiskers (Misai Kucing) is a medicinal plants from a family of Lamiaceae that will grow to a height of 1.5 meter. This herbaceous shrub is native plant of South East Asia and currently cultivated in Indonesia (Tezuka et al., 2000). According to Siddig et al. (2011), this plant is widely used as an alternative medicine due to its huge properties in anti-inflammatory, anti-hypertensive, antiallergenic and diuretic effects. It is also posse’s antifungal and antibacterial activities. Due to its popularity and demand, many studies on these plant properties have been conducted. All the properties of O. stamineus were resulted from the bioactive component that contain in it.

Figure 2-1: Orthosiphon stamineus plant
2.2.1 Anti-angiogenic and anti-oxidant properties study

Shahib et al. (2009) discovered that the *O. stamineus* leaves extract exhibited anti-angiogenic effect which will inhibit the new blood vessels formation and this inhibiting could contribute to the anti-oxidant behaviour. In this study, the perturbation of new blood vessels ability of *Orthosiphon stamineus* was tested in rat's aorta. Different solvent extraction was used to extract and it was identified that the methanol extraction of *O. stamineus* has the highest anti-angiogenic activity compared to other solvent in the rat aorta.

2.2.2 Diuretic properties study

According to Adam et al. (2009), *Orthosiphon stamineus* extract gave dose-dependent diuretic properties. Adult male Sprague-Dawley rat was used in the study to test the content of urinary electrolyte. As a result, the plant extract given rat excreted urine contained increasing amount of potassium (K\(^+\)) ion but lower amount of sodium (Na\(^+\)) ion. This shows that the diuretic activity of *Orthosiphon stamineus* extract is mediated through a change in potassium transport. However, this effect was less potent than furosemide and hydrochlorothiazide which act as control.

2.2.3 Anti-diabetic properties study

A study was conducted by Mohamed et al. (2013) on anti-hyperglycaemia activity of a sub-fraction of chloroform extract from in normal and STZ-induced diabetic rats. From the study, it was identified that the blood glucose levels of the diabetic rats decrease significantly when dose of 1g/kg of extract was administrated but the plasma insulin level doesn't showed any difference and this effect was due to the extra pancreatic mechanism. Therefore, it showed that *Orthosiphon stamineus* extract posse's anti-diabetic effect.
2.2.4 Toxicity study

In year 2011, a study on genotoxicity of *Orthosiphon stamineus* was conducted by Muhammad et al. In this study, male and female Swiss Webster mice were used and it was discovered that there is no clinical sign of toxicity, no mutagenic and no catalytic activity in liver were noted in mice which were orally treated with the *Orthosiphon stamineus* extract. From this, it can be concluded that the *Orthosiphon stamineus* does not poses any genotoxicity.

2.2.5 Anti-hypertension study

Moreover, an anti-hypertension study was carried out in year 2012 by Cicero et al. This study was aimed to evaluate the anti-hypertension effect of *O. Stamineus* leaf extract combined with hydrochlo Rothiazide (HCTZ). A centre randomized- clinical trial was conducted. From the studies, a significant reduction of systolic, diastolic and pulse pressure reduction in hypertensive dyslipidaemic exerted by a combined nutraceutical containing *O. Stamineus*.
<table>
<thead>
<tr>
<th>Author</th>
<th>Journal</th>
<th>Properties study</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shahib et al. (2009)</td>
<td>Anti-angiogenic and anti-oxidant properties of <em>Orthosiphon stamineus</em> Benth methanolic leaves extract.</td>
<td>Anti-angiogenic and anti-oxidant properties study.</td>
<td>The study discovered that <em>O. stamineus</em> leaves extract exhibited anti-angiogenic effect.</td>
</tr>
<tr>
<td>Muhammad et al. (2011)</td>
<td>Evaluation of the genotoxicity of <em>Orthosiphon stamineus</em> aqueous extract.</td>
<td>Toxicity study.</td>
<td><em>Orthosiphon stamineus</em> does not contain any genotoxicity.</td>
</tr>
<tr>
<td>Arafat et al. (2008)</td>
<td>Studies on diuretic and hypouricemic effects of <em>Orthosiphon stamineus</em> methanol extracts in rats.</td>
<td>Diuretic and hypouricemic study.</td>
<td><em>Orthosiphon stamineus</em> towards diuretic properties and evident of hypouricemic properties in <em>Orthosiphon stamineus</em>.</td>
</tr>
<tr>
<td>Reference</td>
<td>Effect/Property Description</td>
<td>Result/Findings</td>
<td></td>
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<tr>
<td>----------------------------</td>
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<td></td>
</tr>
<tr>
<td>Doleckova et al. (2012)</td>
<td>Antiproliferative and antiangiogenic effects of flavone eupatorin, an active constituent of chloroform extract of <em>Orthosiphon stamineus</em> leaves.</td>
<td>The result showed that eupatorin exhibited cell growth inhibition and apoptosis induction in cancer cell.</td>
<td></td>
</tr>
<tr>
<td>Cicero et al. (2012)</td>
<td>Effect of a combined nutraceutical containing <em>Orthosiphon stamineus</em> effect on blood pressure and metabolic syndrome components in hypertensive patients</td>
<td>This study discovered that <em>Orthosiphon stamineus</em> have high anti-hypertension effect</td>
<td></td>
</tr>
</tbody>
</table>

Table 2-1: *Orthosiphon stamineus*’s properties review summary
2.3 Bioactive component of Orthosiphon stamineus

According to Tezuka et al. (2000), *O. stamineus* contains several classes of bioactive constituent and they included terpenoids, polyphenol, oleanolic acid and steroid. Among the mentioned classes, polyphenol is the most abundant component that possesses therapeutic properties.

![Figure 2-2: a) Structure of oleanolic acid, b) terpenoids and c) polyphenol](image)

2.3.1 Polyphenol component

Sumaryono et al. (1991) stated that twenty phenolic compound were extracted from *O. stamineus* which included nine lipophilic flavones, two flavonol glycosides and nine caffeic acid derivatives. From the review journal of Routray and Orsat (2012), there are many classes of flavonoids in food or plants such as flavones, isoflavones, flavanones, flavandiols, anthocyanins, proanthocyanidins, catechin and their chemical structures of the classes of flavonoids Flavonoid in the plants can be used to act as antioxidants and their role in the prevention of coronary heart diseases are the most important actions of
these compounds (Patel, 2008). The major phenolic component identified in this plant was caffeic acid such as rosmarinic acid and 2, 3-dicaffeoyltartaric acid. Other than that, component for example sinesetine, eupatorine and 3- hydroxyl-5, 6, 7, 4-tetramethoxyflavone (TMF) are main marker compound in *O. stamineus* (Sriyana et al, 2011).
<table>
<thead>
<tr>
<th>Classes of flavonoids</th>
<th>Basic chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonols</td>
<td><img src="image" alt="Flavonol Structure" /></td>
</tr>
<tr>
<td>Flavones</td>
<td><img src="image" alt="Flavone Structure" /></td>
</tr>
<tr>
<td>Catechins</td>
<td><img src="image" alt="Catechin Structure" /></td>
</tr>
<tr>
<td>Isoflavones</td>
<td><img src="image" alt="Isoflavin Structure" /></td>
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<tr>
<td>Flavanones</td>
<td><img src="image" alt="Flavanone Structure" /></td>
</tr>
<tr>
<td>Anthocyanins</td>
<td><img src="image" alt="Anthocyanin Structure" /></td>
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<tr>
<td>Proanthocyanidins</td>
<td><img src="image" alt="Proanthocyanidin Structure" /></td>
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<tr>
<td>Anthocyanidins</td>
<td><img src="image" alt="Anthocyanidin Structure" /></td>
</tr>
<tr>
<td>Chalcones</td>
<td><img src="image" alt="Chalcone Structure" /></td>
</tr>
</tbody>
</table>

Figure 2-3: Chemical structures of classes of Flavonoids
2.3.1.1 Sinesetine compound

Sinesetine component is one of the important polymethoxylated flavones which can be found in the extract of *O. stamineus* that has a wide range of biological activities such as antifungal, antibacterial, antitumor, anticancer, prostaglandin binding and antifeedant (Hossain and Ismail, 2012). According to Akowuah et al. (2004), it was discovered that the concentration of sinesetine in the *O. stamineus* was identified in the range of 0.22%- 1.67% when the methanolic leaf extraction is used.

![Image of Sinesetine](image)

Figure 2-4: Structure of sinesetine

2.3.1.2 Eupatorine compound

Eupatorine component is also a polymethoxylated flavones compound that has a molecular formula, **C₁₈H₁₆O₇**. This component exists in the *O. stamineus* extract with the concentration range between 0.34% - 3.37% (Akowuah et al., 2004).

![Image of Eupatorine](image)

Figure 2-5: Structure of eupatorine
2.3.1.3 3-hydroxyl-5, 6, 7, 4-tetramethoxyflavone (TMF) compound

Besides eupatorine and sinesetine, 3-hydroxyl-5, 6, 7, 4-tetramethoxyflavone (TMF) component is also one of the main polymethoxylated flavones compound found in *O. stamineus*. This component was identified in the range of 0.05% -0.69% concentration which is the least amount compared to eupatorine and sinesetine (Akowuah et al., 2004).

![3'-hydroxy-5,6,7,4'-tetramethoxyflavone (TMF)](image)

Figure 2-6: Structure 3- hydroxyl-5, 6, 7, 4-tetramethoxyflavone (TMF)

2.3.1.4 Rosmarinic acid compound

Rosmarinic acid which is the caffeic acid derivatives is the main component found in the *O. stamineus* that has molecular formula, C_{18}H_{16}O_{8}. From study carried out by Akowuah et al. (2004), the concentration of this acid was range from 5.1% -29.9% in the *O. stamineus* extract.

![Rosmarinic acid](image)

Figure 2-7: Structure rosmarinic acid
2.3.2 Therapeutic effect of polyphenol component

Since the amount of polyphenolic compound in *O. stamineus* is very huge compared to other compounds, many studies were conducted on the effect of polyphenol to the properties of *O. stamineus* and it were discovered that most of the properties of *O. stamineus* was contributed by the polyphenol component in it.

In year 2004, Akowuah et al. stated that polyphenol compound (sinesetine, eupatorine, 3-hydroxyl-5, 6, 7, 4-tetramethoxyflavone (TMF) and rosmarinic acid) extracted from this plant contributed anti-oxidant properties.

Moreover, polyphenol isolated from this plant was identified as anti-hyperglycemia which will reduce plasma glucose concentration in the body and this will be beneficial to diabetes mellitus patients. From a study, it was discovered that the total phenolic compound in 1g of plant extract contained $13.24 \pm 0.33$mg gallic acid and $1.73 \pm 0.147 \mu g$ flavonoid which may play a role in control hyperglycemia (Sriplang et al, 2007).

2.4 Thermal Degradation

In plant foodstuff, thermal treatment or heat treatment is the most widely used method to preserve and extend the shelf life of food (Kaiser and Hartmann 2013). During the heat process, thermal degradation of compounds will occurred when elevated temperature was used and this will causes deterioration of food quality (Vikram et al., 2005). As a result, studies on thermal stability of varies food product at different temperature have been conducted.

A study on thermal degradation of colour from red chilli puree was conducted by Ahmed et al. (1999). In this study, the red chilli puree was subjected to heat treatment at different temperature with selected time interval and the colour was analysed by using Hunterlab colorimeter. From the results, it was discovered that the total colour