OPTIMIZATION OF HYDRODISTILLATION EXTRACTION CONDITIONS OF ZERUMBONE FROM ZINGIBER ZERUMBET RHIZOME

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OPTIMIZATION OF HYDRODISTILLATION EXTRACTION CONDITIONS OF ZERUMBONE FROM ZINGIBER ZERUMBET RHIZOME

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A thesis submitted in fulfilment of the requirements for the award of the degree of Master of Engineering (Bioprocess)

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Dedicated to my beloved mother, Norogayah binti Abdul Aziz who means the world to me. To my brothers and sisters who supported me throughout the years, thank you very much.
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ABSTRACT

The optimization of zerumbone extraction from *Zingiber zerumbet* was investigated using a hydrodistillation extraction method. In the present work, the response surface methodology (RSM) based on a central composite design (CCD) was used to determine the optimum condition for the extraction of essential oil and zerumbone content from *Z. zerumbet* rhizome. Three evaluated process variables were the extraction time (60-120 min), raw material to solvent ratio (1:15-1:25) and particle size (250-2000 µm). The CCD consisted of 21 experimental points and three replications at the centre point. Data were analysed using Design Expert software 6.0.6. The optimal conditions suggested by the software for maximum yield of *Z. zerumbet* essential oil and zerumbone content were at 74.29 min, ratio of material to solvent of 1:18.76 and particle size of raw material of 2000 µm. From the experiment, the yield of *Z. zerumbet* essential oil was 4.10% slightly higher than the predicted value of the software (3.73%). Whereas for zerumbone content, it was observed that the amount obtained from the experiment analysis was 1.20% which was slightly lower compared with the predicted value of the Design Expert software (1.58%). The experimental values fell in a range between lower limit and upper limit of the predicted table indicating suitability of the model employed and the success of RSM in optimizing the extraction conditions. Hence, the CCD model can be used to predict the zerumbone content in essential oil extraction from *Z. zerumbet* in a hydrodistillation extraction system.
ABSTRAK

Pengoptimuman pengekstrakan zerumbon dari Zingiber zerumbet telah dikaji dengan menggunakan kaedah pengekstrakan penyulingan hidro. Dalam kajian ini, kaedah gerak balas permukaan (RSM) berdasarkan reka bentuk komposit pusat (CCD) telah digunakan untuk menentukan keadaan optimum bagi pengekstrakan minyak pati dan kandungan zerumbon di dalam rizom Z. zerumbet. Tiga pembolehubah proses yang dinilai adalah masa pengekstrakan (60-120 min), nisbah bahan mentah pelarut (1: 15-1: 25) dan saiz zarah (250-2000 µm). CCD ini terdiri daripada 21 eksperimen dan tiga ulangan pada titik pusat. Data eksperimen telah dianalisis dengan menggunakan perisian Design Expert 6.0.6. Keadaan optimum yang dicadangkan oleh perisian untuk hasil maksimum minyak pati Z. zerumbet dan kandungan zerumbon adalah pada 74.29 min, nisbah bahan pelarut 1: 18.76 dan saiz zarah bahan mentah 2000 µm. Dari eksperimen, hasil minyak pati Z. zerumbet adalah 4.10% lebih tinggi sedikit daripada nilai yang diramalkan daripada nilai yang diramalkan oleh perisian (3.73%). Manakala bagi kandungan zerumbon jumlah yang diperoleh daripada analisis eksperimen adalah 1.20% lebih rendah sedikit berbanding dengan nilai yang diramalkan oleh perisian Design Expert (1.58%). Nilai eksperimen berada dalam julat antara had bawah dan had atas di dalam jadual jangkaan bagi menunjukkan kesesuaian model yang digunakan dan kejayaan RSM dalam mengoptimumkan keadaan pengekstrakan. Oleh itu, model CCD boleh digunakan untuk meramalkan kandungan zerumbon dalam pengekstrakan minyak pati dari Z. zerumbet menggunakan sistem pengekstrakan penyulingan hidro.
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<td>Response Surface Method</td>
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<td>CCD</td>
<td>Central Composite Design</td>
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<td>GCFID</td>
<td>Gas Chromatography Flame Ionization Detector</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetre</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>millimetre</td>
</tr>
<tr>
<td>Df</td>
<td>Dilution factor</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
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<tr>
<td>MRM</td>
<td>Multiple Reaction Monitoring</td>
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<tr>
<td>UPLC-MS/MS</td>
<td>Ultra Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry</td>
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<tr>
<td>ppm</td>
<td>Part Per Million</td>
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<tr>
<td>LC-MS</td>
<td>Liquid Chromatography Mass Spectrometry</td>
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<td>SFE</td>
<td>Supercritical Fluid Extraction</td>
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<tr>
<td>MAD</td>
<td>Microwave Accelerated Distillatory</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon Dioxide</td>
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<tr>
<td>N₂</td>
<td>Nitrogen</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>df</td>
<td>Degrees of Freedom</td>
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<td>DOE</td>
<td>Design of Expert</td>
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<td>3D</td>
<td>Three Dimension</td>
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<td>2D</td>
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<td>µL</td>
<td>Microlitre</td>
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<td>Symbol</td>
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<tr>
<td>w/w</td>
<td>Weight Per Weight</td>
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<tr>
<td>$R^2$</td>
<td>Coefficient of Determination</td>
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<td>min</td>
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LIST OF SYMBOL

%   -   Percentage
°   -   Degree
µ   -   Micro
±   -   Plus Minus
-   -   Minus
α   -   Alpha
β   -   Beta
<   -   Less than
>   -   More than
Δ   -   Delta
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CHAPTER 1

INTRODUCTION

1.1 Research Background

Herbal remedies have played an enormous important role in the maintenance of human health throughout the history of mankind. Over 50% of modern clinical are sourced from various plants extracts and have been employed as supplements and nutraceuticals (Paper and Sivasubramanian, 2014; Bradley, 1992). There has been a revival of interest in herbal medicines. This is due to relatively lower incidence of adverse reactions to plant preparation compared to modern conventional pharmaceutical products (Schweiggert et al., 2005; Tang and Eisenbrand, 1992). The effects of plants extracts on the processing parameters have been studied by a very large number of researchers in different parts of the world. This worldwide interest in medicinal plants processing parameters reflects the quality and value of natural products in healthcare.
Zingiber zerumbet or locally known as ‘Lempoyang’ has caught increasing attention from researchers due to its potential active ingredient that can be beneficial to human healthcare such as anti-inflammatory, antitumor and antibacterial (Yob et al., 2011; Murakami et al., 2002; Nag et al., 2013). Z. zerumbet that belongs to Zingiberaceae family, is widely cultivated throughout the tropical and subtropical areas, especially in South East Asia, Hawaiian Islands and Puerto Rico (Rashid et al., 2005). It is used as traditional medicine for curing the swelling, sores and loss of appetite. The juice of the boiled rhizomes has also been used in indigenous medicine for worm infestation in children (Somchit and Nur Shukriah, 2003). This plant is reported to contain sesquiterpenoids, flavonoids, aromatic compounds, vanillin, kaempferol derivatives and other polyphenolic compounds (Nag et al., 2013).

Zerumbone, a predominant sesquiterpene from this plant, has been studied intensively for its use as an anti-inflammatory, and in chemoprevention and chemotherapy strategies (Calder et al., 2009; Surh, 2002; Unnikrishnan and Kuttan, 1988). Based on previous reports, zerumbone content in the essential oil from the rhizomes of Z. zerumbet was 46.83% (Bhuiyan et al., 2009). Zerumbone was further demonstrated to inhibit both azoxymethane-induced rat aberrant crypt foci and phorbol ester-induced papilloma formation in mouse skin a further indication of its efficacy to prevent colon and skin cancers (Murakami et al., 2004). This suggests that zerumbone is a phytochemical which has potential as both chemopreventive and chemotherapeutic strategies against cancer.

Essential oils are complex mixtures of volatile substances generally present at low concentrations. Before such substances can be analyzed, they have to be extracted from the plants. Various different methods can be used for that purpose, for example hydrodistillation, steam distillation, soxhlet extraction, and simultaneous distillation extraction (Lucchesi et al., 2004). The composition of the extracted oil may vary from one extraction method to another (Charles and Simon, 1990). These drawbacks have upsurge researchers in finding more effective and selective extraction methods in order to obtain a high yield of essential oil and its compounds. Commonly used traditional
extraction methods usually are time consuming, laborious, low selectivity and most of them resulting in low extraction yields (Gámiz and Luque, 2000). Moreover, these techniques employed large amounts of toxic solvents which is known to harm the environment.

Hydrodistillation is one of the oldest and common method of extracting essential oil since it is economically viable and safe (Golmakani and Rezaei, 2008). Although distilling equipment has gradually improved throughout the years, the method for extracting essential oil from the plant has hardly changed, especially in this Southeast region (Mohamed et al., 2004). Hydrodistillator can produce a similar composition of oil extracted from other methods that are more expensive such as supercritical fluid extraction (SFE) and microwave accelerated distillatory (MAD) (Ferhat et al., 2007; Khajeh et al., 2004). Numerous studies had shown that when a plant is being processed, the amount of chemical content was influenced by the processing parameter that is being used (Mohamed et al., 2004). Thus, the choice of processing parameters and methods are important in minimizing the nutrient losses during processing.

1.2 Problem Statement

Plant material continues to contribute in primary health care as a therapeutic remedies in many developing countries such as Malaysia. Herbs and medicinal plants are also act as exclusive sources of drugs for the majority of the world’s population. However, extraction of the bioactive plant constituents has always been a challenging task for the researchers. Z. zerumbet is known with high in zerumbone and α-caryophyllene content (Sabulal et al., 2006). However, numerous studies had shown that when a plant was being processed, the amount of the chemicals content in a plant were influenced by the processing parameter that being used. Therefore, it is essential to find out that the effect of these processing parameters towards the yield of essential oils and phytochemical compound.
To our knowledge, there is no clear guideline to prepare the plant extract according to the application of products as well as herbal medicines especially from Z. *zerumbet* by using hydrodistillation method (Wachtel and Benzie, 2011). Research into the quality, safety, molecular effects, and clinical efficacy of the numerous herbs in common usage is needed. Non standardized procedures of extraction may lead to the degradation of the phytochemical present in the plants and thus leading to the lack of reproducibility. Efforts should be made to produce batches with quality as consistent as possible.

Hydrodistillation on the other hand, has shown a great advantage in the extraction of Z. *zerumbet* essential oil due to its consistently which resulted the greatest number of constituents in an oil sample (Charles and Simon, 1990). In addition, based on previous study, the compositions of the hydrodistillation extracts were higher as compared to the SFE extracts of *Curcuma longa L* (Braga *et al*., 2003). 15 compounds were identified in hydrodistillated oil whereas only 10 compounds were identified using SFE. This method is suitable for screening compound. Up to recently, there is no report by previous study about the standardized processing method of *Z. zerumbet* by using hydrodistillator extractor. In this study, optimization of extraction of zerumbone content from *Z. zerumbet* rhizome using hydrodistillation was evaluated. The result obtained can be used for development and usage of zerumbone in *Z. zerumbet* rhizome in the future by providing a scientific experiment basis and improving the extraction efficacy of the product yield.

1.3 Objective of the Research

The objective of this study is to optimize the hydrodistillation extraction conditions for high essential oil yield and zerumbone content from *Zingiber zerumbet* rhizome.
1.4 Scopes of the Research

In order to achieve the objective, two scopes have been identified as listed below:

1) Identification of ideal conditions to produce maximum yield of Z. zerumbet oil and zerumbone content by hydrodistillation extraction method.

2) Optimization of hydrodistillation extraction conditions including particle size, raw material to solvent ratio and extraction time of zerumbone yield from Z. zerumbet rhizome by Response Surface Methodology (RSM).
REFERENCES


Muhammad A. M. S. (2009). A Study on Microwave-Assisted Extraction of Zingiber aromaticum. Faculty of Chemical and Natural Resources Engineering.


