Difference of Rutin Content in the Leaf of Male and Female *Carica papaya* Linn. using Microwave Assisted Extractive (MAE) Method

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ABSTRACT

The extraction of rutin from the leaf of male and female *Carica papaya* Linn. was conducted using Microwave Assisted Extractive (MAE) method. Box Behnken Design (BBD) was selected to design the experiment in order to achieve the objective of this paper. Response Surface Methodology (RSM) was chosen to monitor the relationship between extraction parameters and response. Parameters involved in this study were irradiation time, min (x_1), ethanol concentration, % (x_2), S/L ratio of papaya leaf/ethanol, wt./wt. (x_3), and particle size, μm (x_4). By using BBD and RSM, second order polynomial models were developed to calculate rutin yields based on conditions provided. High coefficient of determination (R^2) and adj-R^2 acquired indicating high accuracy of the models generated to calculate relative responses based on parameters inputted. Insignificant lack-of-fit at $p > 0.05$ further point to the high precision of the models. Extraction parameters were optimized with the goal of extracting highest yields of rutin under the most optimal conditions before comparing the result of male and female leaf. Higher concentration of rutin was observed to be extracted from 1 gram of female leaf compared to male leaf. As a conclusion, female leaf was determined to contain higher concentration of rutin for its reproductive effort.

Keywords: Rutin Extraction, Male *Carica papaya* Linn., Microwave Assisted Extractive (MAE) Method

1 Introduction

As one of the papaya distributor countries around the world, papaya plant is popular in Malaysia due to its juicy flesh and various active compounds it contains. However, the quantity of these active compounds can be varied between different gender of papaya plant given that there are reports documented higher concentration of active compounds in female plant than male plant [1]. Three different genders of papaya plant knowingly as male, female, and hermaphrodite can be found in papaya plantation. Hermaphrodite papaya plant is preferred in many region of the world for its high productivity of papaya fruit [2]. Consequently, male, and female papaya plant often eliminated during early stage of plantation as soon as the gender was identified. This resulted in waste of natural resources. By conducting this study, a better understanding on the biology of papaya plant could be achieved as well as obtaining a by-product from discarded waste.

To obtain targeted active compound from medicinal plants, solid-liquid extraction is often employed. This is a technique involving separating targeted compounds from solid particle with the present of extraction solvent [3]. Conventional extraction techniques such as maceration, decoction, and Soxhlet generally

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required longer extraction time and consumed larger volume of solvents are usually undesirable. Microwave Assisted Extractive (MAE) method that needed shorter extraction time, lesser solvent volumes, and has better selectivity compared to conventional method is therefore selected in this paper for the advantages mentioned [4]. In term of a suitable extraction solvent for rutin extraction, the combination of ethanol and water that are considered as green solvents by the United States Food and Drug Administration (US FDA) was selected for their lower to non-toxicity property [5]. On top of that, rutin is also reported to have better solubility in ethanol [6].

2 Materials and Methods

2.1 Chemicals

HPLC-grade methanol and analytical grade undenatured ethanol were procured from Fisher Scientific, rutin hydrate, purity ≥ 94% was purchased from Sigma-Aldrich, ultrapure water was obtained through Milli-Q ultrafiltration system.

2.2 Plant materials and preparation

Male and female *C. papaya* leaves were obtained through a local farm in Selangor, Malaysia. The leaves were then separated from their petioles and washed under running water to remove visible dusts and contaminants. Next, the leaves were oven dried until a constant weight is achieved. Dried papaya leaves were grounded using commercial juice blender to obtain fine powder and further categorized into different range of sizes between 355 μm and 710 μm [7]. Categorized leaves samples were stored in an air-tight container and under the temperature of 4 ^{o}C for future use.

2.3 Extraction of rutin

2.3.1 Microwave-Assisted Extractive (MAE) Method

A commercial microwave (Samsung, ME711K, South Korea) was equipped in this study. The mixture of plant sample and ethanol were placed in a Schott bottle at a pre-determined S/L ratio in which the bottle was capped before located in the microwave. Separate water bath was prepared by the side of microwave for cooling purposes of plant extracts after each extraction cycle and to condense trapped vapour within the bottle. Parameters involved in this study were irradiation time (x_1), S/L ratio (x_2), particle size (x_3), and ethanol concentration (x_4).

2.3.2 Design of experiment and statistical analysis

In order to form a constructive model for the yields of rutin from male and female papaya leaf, Box Behnken Design (BBD) was chosen for the design of experiment. The relationship between extraction parameters and the response involved was decided to be determined using response surface methodology (RSM). A total of 29 runs of experiments were each conducted based on a four-factors, three level (-1, 0, 1) of BBD for male and female papaya leaf. The range for parameters employed in this paper are between 0.5 to 9.5 min (x_1), 1:10 to 1:170 wt./wt. (x_2), 355 to 710 μm (x_3), and 20 to 80% (x_4).

The general secondary-order polynomial model developed in this paper to fit the experimental data and to determine the relationship between experimental terms can be expressed as Eq. (1). In addition, optimal conditions and calculated responses acquired were validated with experimental results.

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_{ii}^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

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where Y is the calculated response, which is yields of rutin in this current paper; β_0 is the constant coefficient; β_i is the linear effect; β_{ii} is the square effect; β_{ij} is the interactive effect, x_i and x_j are the independent variables, and ε is the error occurred.

2.4 Analytical Methods

2.4.1 Identification and quantification of rutin

Agilent 1200 series HPLC system were equipped in this study for rutin identification and quantification. Mobile phases employed in this study are HPLC grade methanol and ultrapure water produced by Mili-Q ultrafiltration system. Gradient elution was selected in this study. The flow rate was remained constant at 1.0 ml/min throughout the entire analysis whereas the injection volume of sample solution was fixed at 10 μL. The separation was detected by Ultra-Diode Array Detection (UV-DAD) at the wavelength of 360 nm Operating temperature of HPLC system was decided at 25 °C and the analysis column used in this study was Agilent Eclipse Plus C18, 5 μm and is 4.6 x 150 mm. Result obtained were compared against standard calibration curve to determine the exact rutin concentration in leaf extract.

2.4.2 Preparation of standard calibration curve

Rutin solution with its original concentration at 1.0 mg/mL was further diluted into different solution with lower concentrations in order to form a standard calibration curve. HPLC results of post-MAE leaf extracts were compared against obtained calibration curve to identify respective rutin concentration. The concentration of rutin in leaf extract was determined using Eq. (2).

$$\text{Yields of Rutin } \left(^{\text{mg}}/_{\text{g}}\right) = \frac{\text{Mass of rutin extracted (mg)}}{\text{Mass of papaya leaf(g)}} \quad (2)$$

3 Results and Discussion

3.1 Process optimization

29 experimental results based on different extraction conditions and genders were obtained and analysed. The significant level of the extraction variables and interaction effects between the variables were determined using analysis of variance (ANOVA). With reference to ANOVA analysis, both the models generated for rutin extraction using MAE from male and female papaya leaf were found to be significant with p-value, $p < 0.05$. In addition, significant experimental terms that could influence the yields of rutin are tabulated in Table 1. High coefficient of determination (R^2) and adj-R^2 that are greater than 80% further indicated the high accuracy of model generated. Moreover, insignificant lack-of-fit for both models again validated the precision of the model [8].

Table 1: *Significant experimental terms involved in this paper at the confidence level of 95%*

Male	**Female**
Model	Model
Linear effect	Linear effect
$\boldsymbol{x_4}$	x_2
Square effect	x_4
$\boldsymbol{x_3^2}$	2-way interaction
$\boldsymbol{x_4^2}$	x_2x_4
$\boldsymbol{x_2x_4}$	x_2x_3

3.2 Models fitting

Calculated rutin yields acquired using equation generated for extraction models involved were compared against observed yields of rutin. Regression plots were plotted to determine the accuracy of established models. High correlation coefficients (r^2) for both models that are greater than 0.90 were noted which indicating high correlation between calculated yields and observed yields of rutin.

3.3 Comparison of rutin content in the leaf of male and female *C. papaya* Linn.

Optimized conditions obtained through BBD and RSM to achieve optimal yields of rutin from the leaf of male and female *C. papaya* are showed in Table 2. This were verified experimentally and were carried out in triplicate.

Table 2: *Optimum conditions of rutin extraction from the leaf of male and female C. papaya Linn*

Parameters	Male	Female
x_1, min	6	7.5
x_2, wt/wt	1:70	1:170
x_3, µm	398	710
x_4, %	52	20

On top of validation results, deviation between calculated yields and observed yields was determined using Eq. (3) to monitor the accuracy and consistency of observed yields [9]. It was found to be lower than 7% which again suggested high reliability of the models. Low standard deviation (SD ≤ 0.02) was also noted in observed yields of rutin from the leaf of male and female *C. papaya* Linn.

$$\text{Deviation (\%)} = \left|\frac{y-\hat{y}}{\hat{y}}\right| \times 100\% \quad (3)$$

where y symbolises experimental yield and $\hat{y}$ represents calculated yield from RSM. Validated results for the yields of rutin were displayed in Figure 1. With reference to Figure 1, rutin content in the leaf of female *C. papaya* Linn. showed higher concentration than male leaf. Rutin concentration in female leaf was determined to be 49% higher than in male leaf. This observation is consistent with the study "Sexual Dimorphism in the Chemical Composition of Male and Female in the Dioecious Tree, Juniperus communis L., Growing under Different Nutritional Conditions" by Rabska et al. In this study, Rabska reported that female plant often contained higher concentration of resources than male plant for its reproductive effort [1].

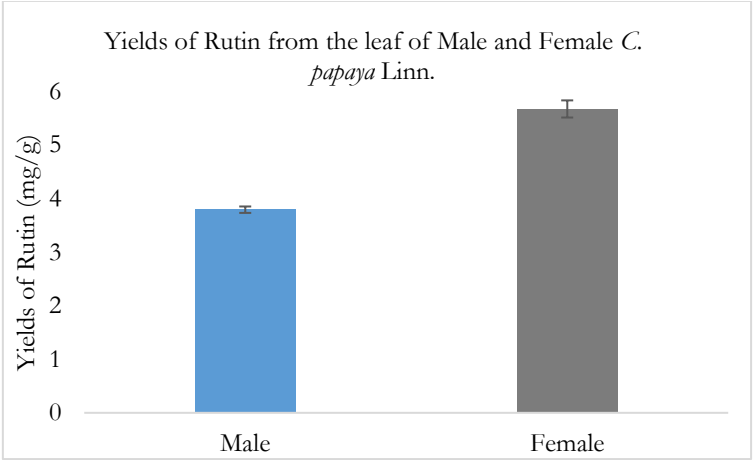

Figure 1: *The comparison of rutin yields from the leaf of male and female C. papaya Linn. using MAE*

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4 Conclusions

Rutin was successfully extracted from the leaf of male and female *C. papaya* Linn. using MAE method. The yields of rutin per gram of leaf were obtained and compared. The leaf of female *C. papaya* Linn. was determined to contain higher concentration of rutin compared to male leaf. This is believed due to the reproductive effort of female plant. The obtained result showed that the level of rutin concentration is vary between male and female leaf. Further study would be required by involving other parts of male and female plant in order to have a better understanding on the biology of papaya plant. Furthermore, the involvement of hermaphrodite papaya plant should also be considered for the same reason. On the other hand, the effect of extraction techniques on the yields of rutin should as well be investigated to form a more comprehensive conclusion. Energy study on rutin extraction from papaya plant could be consider in future study as sustainable extraction techniques are essential to protect and preserve the nature and resources for future generations.

5 Declarations

5.1 Study limitations

This paper presented only the differences of rutin concentration in the leaf of male and female *C. papaya* Linn. The concentration of natural resources in medicinal plants are not solely limited by the gender, it can also be influenced by the living environment as well as the nutrients that are available from the soil.

5.2 Acknowledgements

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5.4 Competing interest

The authors declare that the research was conducted without the presence of any commercial of financial relationships that could be construed as a potential conflict of interest.

5.5 Publisher's Note

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