Journal of Research in Pharmacy

Research Article

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Response surface methodology-aided maceration method optimization of quercetin-standardized purified extract of shallot skin (*Allium cepa L var. aggregatum*)

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Received: 08 December 2022 / Revised: 01 February 2023 / Accepted: 07 February 2023

ABSTRACT: Shallot skin has potential as a medicinal raw material because it contains many quercetin compounds. It is necessary to conduct a study on the optimization of extraction methods and the extracts standardization. The purpose of this study is the standardization of simplicia, optimization of extraction methods and standardization of shallot skin purified extract as standardized on quercetin. This study is aimed to support quality control of simplicia and shallot skin extract as a source of quercetin. The methods of the study include the collection of raw materials, preparation of simplicia, standardization of simplicia, and optimization of extraction methods with Response Surface Methodology (RSM) using Box-Behnken Design (BBD), standardization of standardized quercetin purified extracts. Based on the results obtained, shallot skin simplicia has a moisture content of 7.98 and a total ash content of 9.91%. Based on the analysis, the optimum point of the solvent concentration factor, time, and the solvent ratio on the yield of the purified extract were respectively 60.42%, 1.29 hours, and 24.45 mL/g. The yield average obtained was 5.16 ± 0.1125 % with a content of 5.947 ppm (0.005497 mg/g).

KEYWORDS: purified extract; quercetin; shallot skin; maceration; Response Surface Methodology (RSM).

1. INTRODUCTION

One of the Indonesian government programs in the pharmaceutical sector is the procurement of medicinal raw materials on their own efforts both from natural and synthetic materials. Many of Indonesia's natural ingredients are still not optimally used. One of the medicinal raw materials from natural materials is quercetin which is found in many plants [1]. Quercetin has pharmacological activities such as antioxidant [2], antiviral, immunomodulator, anticoagulant [3], antibacterial [4], anticancer [5], anti-inflammatory [6], antihypertensive, hepatoprotector, antiobesity, antidiabetic, Alzheimer's therapy [7,8], antiallergic [9], antidiabetic [10], and antimelanogenesis [11].

One of the potential plants as a source of quercetin is shallots (*Allium cepa* L. var. aggregatum) [12,13]. Quercetin compounds are reported to be mostly found in shallot skin [14]. Quercetin in free form is the dominant flavonoid in shallot skin, which is 83% of total quercetin [15]. The great potential of shallot skin as a source of quercetin triggered the studies on the optimization of the extraction method of the quercetin compound.

Several methods of shallot skin quercetin extract have been reported, such as maceration [15–18], reflux [19], socletation [20], ultrasound assisted extraction [21], microwave assisted extraction [22], supercritical fluid extraction [23]. In these studies, the quercetin obtained is still in the form of a crude extract. The extract still contains ballast substances such as lipids, sugars, dyes, and tannins [24,25], it needs to be purified in order to obtain an extract with a purer active substance content. Thus, it is necessary to develop methods that are more efficient, economical, simple techniques, and easy to be applied in the development of Indonesian herbal medicines. One of the simple methods is maceration, but it has several disadvantages such as requiring a long time and a lot of solvent. So, it is necessary to optimize the maceration method to obtain the optimal yield extract.

How to cite this article: Sapri S, Riswanto FDO, Wulandari ET. Response surface methodology-aided maceration method optimization of quercetin-standardized purified extract of shallot skin (*Allium cepa* L var. *aggregatum*). J Res Pharm. 2023; 27(4): 1414-1420.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.428

In obtaining high yields extract and quercetin levels, the optimization of shallot skin extraction methods is conducted with Response Surface Methodology (RSM) using Box-Behnken Design (BBD). The RSM-BBD technique is able to analyze the effects of interactions between variables [23] and more efficient research design with fewer experimental runs, especially three variables experiment [26]. The purpose of this study is to obtain the optimum extraction method of shallot skin extract on standardized quercetin.

2. RESULTS

The data results of the optimization of maceration and reflux methods towards the yield of purified shallot skin extract is presented in the Table 1. In this study, the extraction factors that were optimized were ethanol concentration, extraction time, and the ratio between solvent and simplicia.

Table 1. Factors and responses in the optimization of maceration method of shallot skins quercetin extraction

No	Ethanol concentration (%)	Time (hours)	Ratio (mL/g)	Yield (%)
1	50	1	20	4.89
2	90	1	20	5.19
3	50	3	20	5.04
4	90	3	20	5.13
5	50	2	10	4.03
6	90	2	10	3.19
7	50	2	30	3.99
8	90	2	30	6.06
9	70	1	10	1.99
10	70	3	10	3.42
11	70	1	30	5.31
12	70	3	30	2.43
13	70	2	20	4.39
14	70	2	20	4.40
15	70	2	20	4.55
16	70	2	20	6.33

Table 2. Analysis of variances of yield model using RSM

Terms	Df	Sum Sq	Mean Sq	F value	Pr(>F)
First order (X1, X2, X3)	3	3.8874	1.29582	2.5144	0.15513
Two Way Interaction (X1, X2, X3)	3	6.7721	2.25736	4.3801	0.05889
Pure Quadratic (X1, X2, X3)	3	7.8045	2.60149	5.0479	0.04432
Residuals	6	3.0922	0.51536		
Lack of fit	3	0.4159	0.13863	0.1554	0.91972
Pure error	3	2.6763	0.89209		

Analysis of variances of yield model using RSM was presented in Table 2. The analysis of the maceration method optimization showed that there was no significant difference indicated from a significance value (p-value) of 0.05337. The *lack-of-fit* value obtained was 0.91972 indicating insignificant error of the model. It showed that the maceration method optimization model was valid. The equation model obtained was:

$$\% \text{ Yield} = 4.9175 + 0.2025X1 - 0.1700X2 + 0.6450X3 - 0.0525X1X2 + 0.7275X1X3 - 1.0775X2X3 + 0.5875X12 - 0.4425X22 - 1.1875X32 \quad \text{.....(1)}$$

Notes:
X1 = Ethanol concentration (%)
X2 = Time (hours)
X3 = Simplicia solvent ratio (mL/g)

The relationship between these factors and the yield of purified shallot skin extract can be seen in Figure 1. The results of the spectrum produced by the sample of the purified extract have similarities with the spectrum produced by the quercetin standard (Figure 2).

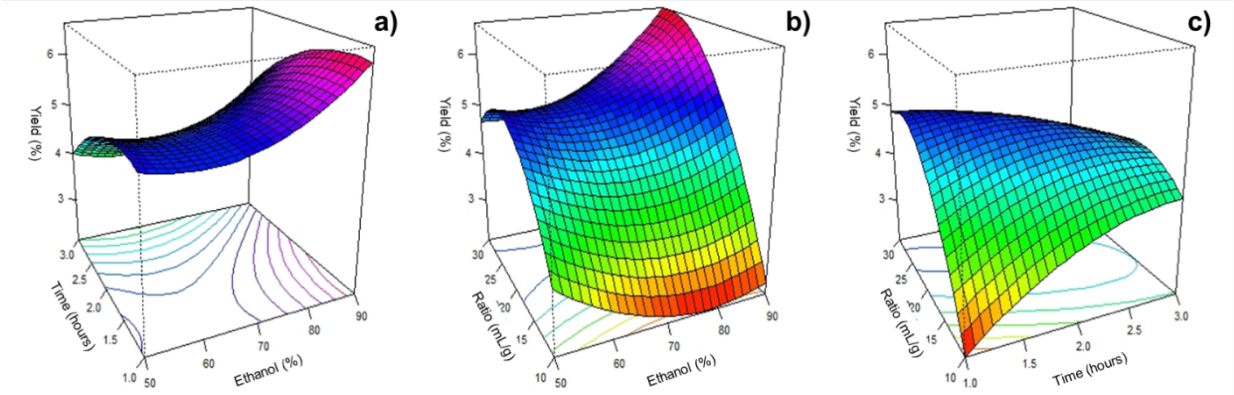

Figure 1. Response surface plot of ethanol concentration versus time (a), ethanol concentration versus simplicia solvent ratio (b), as well as time versus simplicia solvent ratio (c) on the yield of purified extract using maceration method.

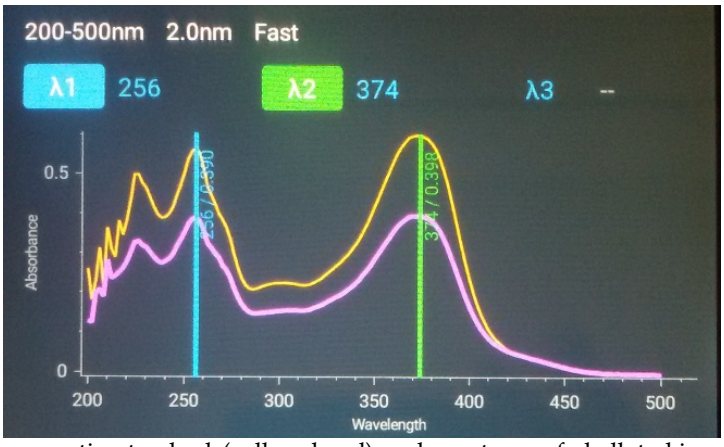

Figure 2. Spectrum of quercetin standard (yellow band) and spectrum of shallot skin purified extract (purple band).

3. DISCUSSION

Shallot skin simplicia powder has a brownish red color, the aroma was typically smells of shallots. The production of simplicia shallot skins powder was made from raw materials in total of 1000 g. After the drying process, it was resulted 853 g dried simplicia product. This result was processed further and resulted a total of 810 g of shallot skins simplicia powder. The yield of simplicia obtained was 81%. The testing average results of the moisture content of shallot skin simplicia powder was 7.98%. It was in accordance with the general requirements of the water content of simplicia, which was no more than 10% [27]. The moisture content determination is intended to find the amount of water in a material is. High water content can be a medium for the growth of microbes, molds, and microorganisms. It causes chemical changes in active compounds.

The total ash content of shallot skin simplicia powder was 9.19%. The ash content of a material gives an idea of the mineral content of the material [27]. The higher the ash content obtained, the higher the mineral content in the material. Minerals such as calcium, magnesium, phosphorus, sodium and potassium are needed by the body

In this study, purified extract of shallot skin was defined as the shallot skin extract after standardization process with the determined content of quercetin. The results of the study on the yield value of the shallot skin purified extract in Table 1 were used to determine the optimization model using analysis of variance (ANOVA). The ANOVA results showed a quadratic model that showed significant differences if the p value <0.05. Another parameter that can be used to determine the suitability of the model is the *lack-of-fit* value, if the p value> 0.05 then it shows a valid model for the optimization process.

Equation 1 shows that the ethanol concentration (X1) and the simplicia solvent ratio (X3) have a positive effect, while the time factor (X2) has a negative effect. The positive effect indicates an increasing number of responses, ethanol concentration and simplicia solvent ratio will affect the increasing number of the yield of shallot skins purified extract. Conversely, the negative effect by the extraction time factor will reduce the yield of the purified extract.

Based on the analysis obtained from the calculation of the R program, the optimum points of the solvent concentration, time, and solvent to solvent ratio factors on the yield of the purified extract were 60.42%, 1.29 hours, and 24.45 mL/g. From the optimum point of the independent variables, the optimum response value of yield was 5.08%. The verification was conducted six replications for the maceration method. The average yield obtained was 5.16% ± 0.1125. The verification results are within the significance range ($\alpha = 0.05$) so that the verification results can be accepted.

The determination of quercetin content was conducted with UV-Vis spectrophotometer. From the results of the standard series measurements, a curve was made between the concentration of quercetin standard solution with absorbance resulted to the linear regression equation y = 0.0642x + 0.0162 with R2 = 0.9728. Quercetin compounds produce two wavelengths in the maximum absorption at 256 nm and 374 nm [28]. In this analysis, the wavelength of 374 nm was used. The results of the determination of quercetin content in the sample of shallot skin purified extract obtained 5.947 ppm (0.005497 mg/g).

4. CONCLUSION

Based on the results obtained, it is known that shallot skin simplicia has a moisture content of 7.98% and a total ash content of 9.91%. Based on the analysis, the optimum point of the solvent concentration factor, time, and solvent ratio with respect to the yield of the purified extract were 60.42%, 1.29 hours, and 24.45 mL/g. The average yield obtained was 5.16% ± 0.1125. The results of the determination of quercetin content in the sample of shallot skin purified extract obtained 5.947 ppm (0.005497 mg/g).

5. MATERIALS AND METHODS

5.1. Material

Shallot skin (*Allium cepa L. var. aggregatum*), ethanol pro analysis, ethanol 96%, distilled water, magnesium powder, concentrated hydrochloric acid (HCl), aluminum chloride ($AlCl_3$), potassium acetate, standardized quercetin (Sigma), and toluene (Merck).

5.2. Tools

The tools used in this study were a set of UV-Vis (*OrionAquaMate 8100 ThermoScientific)* spectrophotometer instrument, micropipette (*Eppendorf*) in size of 20-200 µl, 100-1000 µl, and 500-5000 µl, a set of commonly used glassware in analysis laboratory, microscope (*Olympus*), UV 254 nm and 365 nm lamp, a set of macerator and a set of reflux equipment. UV-Vis spectrophotometer analysis and the optimization of extraction method were conducted at the Pharmaceutical Chemistry Laboratory, Faculty of Humanities and Health, Mulia University of Balikpapan. The analysis of water content and ash content was conducted at the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Mulawarman University.

5.3. Shallot Skin Collection

Skins of shallot (Taxonomy ID: 28911) were obtained from shallot farmers of Hikmah Farmer Group, in Gunung Bubukan village, Lamaru sub-district, East Balikpapan district, Balikpapan. The type of shallot that are grown is the *Bima Brebes* variety. Then, the samples were determined at the Herbarium Wanariset laboratory of the Natural Resources Conservation Technology of Research and Development Center (*Balitek KSDA*) Samboja to determine the correctness of the samples that are going to be used.

5.4. Shallot Skin Simplicia Production

Shallot skin samples are wet sorted, only the outer dry skin is selected, then washed with running water, which is then drained and dried without direct sunlight or aerated. Once the sample is dry, dry sorting is conducted and mashed with a grinder machine until simplicia powder with a mesh size of 40 is obtained.

5.5. Moisture Content Determination

The determination of water content is conducted by the distillation of toluene. The toluene used is saturated with water first. Then, the simplicia is carefully weighed as much as 5 g and put into a round bottom flask, then the saturated toluene is added. The flask was carefully heated for 15 minutes, after the toluene began to boil, the distillation was set at 2 drops/second, then 4 drops/second. Once all was distilled, heating was continued for 5 minutes. After that, the tube was cooled down to room temperature. The water volume was read after the toluene and water were completely separated [27].

5.6. Total Ash Content

A total of 2 g of simplicia, weighed carefully (W0), was put into the weighed kurs (W1). After that, the extract was put in a furnace at a temperature of ± 600℃ until the charcoal disappeared. Then, it was cooled down in a desiccator and weighed the weight of ash (W2) [27].

5.7 Optimization of Maceration Method for Shallot Skins Quercetin Extraction

The extraction method of quercetin compounds from shallot skin was conducted with a modified maceration method, namely with the help of stirring, digestion (maceration at 45° C). The research variables to be optimized were ethanol solvent concentration (50%, 70% and 90%), extraction time (1, 2 and 3 hours) and solvent ratio using sample (10, 20 and 30 mL/g). The independent variables of the optimization of maceration method of shallot skin quercetin extraction and its levels can be seen in Table 3.

Table 3. Independent variables of maceration method in various levels

Independent variables/Factors	Unit	Low level (-1)	Medium level (0)	High level (+1)
X1: Ethanol Concentration	%	50	70	90
X2: Time	hours	1	2	3
X3: Ratio	mL/g	10	20	30

5.8. Shallot Skin Purified Extract Production

The shallot skin extract from the results of the optimization method was collected, then evaporated to one-fifth of the initial volume. The concentrated extract was added with cold distilled water (1:1 ratio) and stored in a refrigerator at 4° C for 24 hours until a red precipitate was obtained. The precipitate obtained was filtered, then washed with distilled water. The precipitate was dried until red powder was obtained and then weighed so that the yield value was obtained.

The powder obtained was then dried, dissolved using ethanol p.a. solvent, and then filtered. The residue was then dissolved again, until yellow solution was no longer produced. The filtrate was collected, then evaporated to one-tenth of the initial volume. Cold distilled water was added and waited until a yellow precipitate was formed. The yellow powder obtained was dried, the yield was calculated and then the identification and determination of quercetin compound levels were conducted.

5.9. Determination of Quercetin Marker Compound

The determination of the maximum wavelength of quercetin was conducted by the running quercetin solution at a wavelength range of 200 - 400 nm. In this analysis, the wavelength of 374 nm was used for the detection. The maximum absorption wavelength is used to measure the absorbance of the shallot skin extraction sample. Weighed as much as 10 mg of quercetin standard and dissolved in 10 mL of ethanol. From the 1000 ppm quercetin standard solution, several concentrations were made, namely 5 ppm, 7.5 ppm, 10 ppm, 12.5 ppm and 15 ppm. The absorbance was determined using UV-Vis spectrophotometric method at the maximum absorption wavelength of 374 nm. Weighed 10 mg of extract, dissolved in 10 mL of ethanol, so that a concentration of 1000 ppm was obtained. From the solution, a concentration of 10 ppm was made. Absorbance was determined using UV-Vis spectrophotometric method at maximum absorption wavelengths of 374 nm. Samples were made in three replicates for each analysis and the average absorbance value was obtained.

5.10. Data Analysis

5.10.1. Extraction method optimization analysis.

The yield of the purified extract of each extract was processed using R software version 4.2.0 with the rsm package to obtain the analysis of variance (ANOVA) results, optimization equations, 3D surface graphs and process of optimization.

5.10.2. Analysis of quercetin content determination.

The results of the standard series measurements were made a curve between the concentration of quercetin standard solution and the absorbance obtained so that a linear regression equation would be produced. This linear regression equation was used to calculate the quercetin content (mg/g) by entering the absorbance of the extract as the Y value into the equation.

5.10.3. Verification of Optimum Conditions;

The shallot skin samples were macerated at optimum conditions obtained from R software, then continued with the purification process to obtain the standardized quercetin shallot skin purified extract. Verification of optimum conditions was carried out with six replications.

Acknowledgements: The authors would like to thank the Institute for Research and Community Service (LPPM) of Sanata Dharma University for awarding a Master-Doctoral research grant in 2022 (Contract number: 017 Penel./LPPM-USD/IV/2022) and all involved parties in the preparation of this article.

Author contributions: Concept – E.T.W.; Design – S.S., F.D.O.R.; Supervision – E.T.W.; Resources – S.S.; Materials – S.S..; Data Collection and/or Processing – S.S., F.D.O.R.; Analysis and/or Interpretation – S.S., F.D.O.R.; Literature Search – S.S.; Writing – S.S.; Critical Reviews – E.T.W., F.D.O.R.

Conflict of interest statement: The authors declared no conflict of interest.

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