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The Quercetin Extraction Of Bima Brebes Shallot Skin (Allium Cepa L. Var. Aggregatum) With Traditional Method (Maceration) And Green Method (Microwave Assisted Extraction)

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Abstract

There are many abundant shallot skins wastes available in Brebes, Indonesia which come from shallot production of Bima Brebes variety. A great number of Flavonoid compound (quercetin) can be found in shallot skin (Allium Cepa L). This study identifies flavonoid compound in shallot skin of Bima Brebes with maceration method by using 96% ethanol solvent, and MAE (Microwave Assisted Extraction) method. Flavonoid content test is performed with, a qualitative method, phytochemicals compound test. Meanwhile, the quantitative test is done by Spectrophotometer UV-VIS. The result of phytochemical data analysis shows that Bima Brebes shallot skin contain flavonoid compound within indications of colour changing into red and forming of yellow and black deposit. Quercetin level from maceration extraction result, by using 96% ethanol solvent, shows number of 56,455%, meanwhile the result of extraction of Bima brebes shallot skin by applying MAE (Microwave Assisted Extraction) method shows quercetin level of 88,540%.

Key words: flavonoid; quercetin; shallot skin; MAE; maceration

INTRODUCTION

According to The Food and Agriculture Organization (FAO), there is a 60% loss of waste as a result of total horticulture production. One of horticultural production that can produce many side productions is shallot. In the last decade, the production of shallot is increased to 98 million tons worldwide(1). Shallot is one of crops that can produce skin as its waste(2). Shallot skin is mostly gathered while it is peeled for industrial purpose(3). One of many agricultural wastes found in Brebes regency, Indonesia is shallot skin(4). Brebes regency of Indonesia contributes in shallot production as many as 18,5% of total national shallot production(5). Shallot varieties in Brebes is a local variety which is called Bima.

Side production of shallot is in the form of skin which can be as many as 550.000 ton every year that can cause number of biological issues related to environment(6). Shallot yield also produce waste in the form of skin and root about 0,5% of total production or as many as 1,9 x 106 kg/year(7). Shallot skin contains an active compound such as flavonoid and polyphenol that can be used as an antibacterial compound and also as additional herbal feed for antibiotics alternative, it can it can also be used as a painkiller(8)(4). Shallot skin contains substances which has precious bio functional use(9)(10). Biochemical compounds of shallot skin such as total flavonoid, total polyphenol, and quercetin are compounds that are very beneficial from its functional nature perspective(3). Flavonoid content in shallot skin is 2–10 g/kg (6 gram/kg average or 0,6 % b/b) which is higher than the consumable part that is as many as < 0,03 to 1 g/kg(11). Flavonoid content of shallot skin is 1,276 mg/g -169 mg/g which is higher than garlic skin (0,08 mg/g on average)(3). Shallot is tuber type plant that its outer part is called skin which still has food reserves of flavanol 3,82 mg/kg from flavonoid group, it has antioxidant activity(12). At least, there are 25 different flavanols identified in shallot, the most important of all shallot cultivar is derivative quercetin(13). The finding of phenolic, flavonoid, flavanol, anthocyanin, tannin, vanillic acid, and ferulic acid

from shallot skin indicates that it contains strong antioxidant and antimicrobial substances(14). Shallot is a kind of a kind of onion that has highest phytochemical compound with phenolic type compound of flavonoid(15).

Flavonoid content in shallot skin is as many as 2–10 g/kg (6 gram/kg average or 0,6 % b/b) which is higher than the consumable part, it is from < 0,03 to 1 g/kg(11). Flavonoid content of shallot skin is 1,276 mg/g -169 mg/g which is higher than garlic skin (0,08 mg/g on average)(3). The dominant flavonoid compound group in shallot skin is anthocyanin and flavanol(16). Flavanol is colourless flavonoid group which consist of kaempferol, myristine, derivative methylated (isorhamnetin), and quercetin as most dominant compound. Shallot is tuber type plant that its outer part is called skin which still has food reserves of flavanol 3,82 mg/kg from flavonoid group, it has antioxidant activity(12). At the minimum, there are 25 different flavanols identified in shallot, the most important of all shallot cultivar is derivative quercetin(17). Bioactive compound sampling from shallot skin is performed by extraction process. Extraction method and solvent used is very influential toward bio active compound especially the extracted flavonoid(18).

Nowadays, the method of extraction is improved from traditional to modern method. Traditional method uses simple tool, large quantity of solvent, and it takes long extraction time. Meanwhile, modern method use san expensive and advanced tool to reduce extraction time and it can be properly working on higher temperature(19). One of traditional extraction method which is commonly used is maceration method, it is a method with simple disbursement(20). Maceration is traditional extraction method by submersing the plant material in solvent for a long time, namely several hours or days. This method is simple and effective to extract components which are not heat resistant, but it has several weaknesses(21)(22).

In one hand, the modern method which is implemented in the study is MAE (Microwave Assisted Extraction). Green Extraction, nowadays, shows high demand especially among pharmacy, cosmetics, and other industries which automatically trigger increasing market demand, that is why the continuous production must be environment friendly(23). MAE which is one of Green Extraction method is an extraction that utilises the help of microwave to heat up sample in order to extract target compound, it shortens extraction time, increasing the result, and minimizing solvent consumption(24). MAE is proven to be an effective extraction technique for various bio active compounds(25).

This research is conducted to identify flavonoid compound in Bima Brebes shallot skin (Allium Cepa L. Var Agregatum) that is native to Brebes regency, Indonesia by using two different methods which are traditional and Green Extraction method. The traditional implements maceration method with 96% ethanol solvent dan Green Extraction applies MAE (Microwave Assisted Extraction) method in order to review the flavonoid content.

EXPERIMENTAL

Chemicals and Materials

This study was conducted in chemistry laboratory of Muhadi Setiabudi University from June to July of 2024. Tools used during research were analytical balance (And), Oven, grinder, 20 mesh sieve, vacuum evaporator, microwave (Philips), and laboratory glassware. Meanwhile, the materials used are 96% ethanol, HCl, HCN, $AlCl_3$, Sodium Acetate, and shallot skin.

Characterization Methods

Extraction is performed traditionally by using maceration method with ethanol solvent, and the modern option is using MAE (Microwave Assisted Extraction) method. Flavonoid content test is performed by qualitative, phytochemicals compound test. Meanwhile, the quantitative test is done by Spectrofotometer UV-Vis. Shallot skin sample preparation follows Farmakope Herbal Indonesia standard, Ministry of Health, Republic of Indonesia of 2017. Meanwhile the testing procedure follows manual from Official Methods of Analysis of AOAC International. The research procedures are, as follow:

a. Sample Preperation

Material used in this research is shallow skin which is directly gathered from waste of PT. Sinergi Brebes Inovatif in Brebes regency, Indonesia. Materials are cleaned up from debris, washed in running water, and sun-dried for 3 days(13). The dried shallot skin is then mashed by using blender, next it is sifted by using 20 mesh sieve(26). Lastly, the sample is stored in sealed container, protected from sunlight(27,28).

b. Shallot skin Extraction with Traditional Method (Maceration)

50 gram of dry shallot skin powder is put into macerator and added 500 mL of 95% ethanol solvent. As long as 1 hour, the solution is stirred and left for 3 days, it is stirred occasionally during that time. The filtrate is then evaporated using rotary evaporator until dense extraction is formed. The extract is then dried by using vacuum dryer and calculate the yield(29).

c. Shallot Skin Extraction by Using Green Method (Microwave Assisted Extraction)

50 gram of dry shallot skin powder is put into Erlenmeyer and added 500 mL of 95% ethanol solvent, and it is stored into microwave with 800 watt of power for 6 minutes. The solution is radiated inside microwave oven periodically (1 and 2 minutes of radiation is shut offs) in order to prevent temperature from reaching 85°C. Next, the solution is to be left in room temperature for filtering. The filtrate is evaporated using Rotary evaporator to form dense extraction. The extract is then dried by using vacuum dryer and calculate the yield(30,31).

d. Phytochemicals Test

Extract of 0,5 gram is dissolved into 5 mL of 95% ethanol. 2 mL of solution sample is taken, added 0,1 gram of Mg powder and 10 drops of concentrated HCl from side of the flask and it is slowly shaken. The red or orange colour appeared shows the existence of flavonoid, meanwhile yellow orange shows content of flavone, chalcone, and aurone(32).

e. Flavonoid Extract Value Determination

Weigh 10 mg of shallot skin extract and dissolve it into 10 ml of methanol, then fill it to the limit. Standard solution extract is taken as many as 0,5 ml and added into 2 ml of distilled water and 150 µL $NaNO_2$ 5%. Add 50 µL $AlCl_3$ 10% after 5 minutes. After 6 minutes, add 2 ml NaOH 1M distilled water until it reached 5 ml volume. The solution is shaken until it become homogeneous and measure the absorbance of maximum wave length obtained from standard quercetin curve(33).

RESULTS AND DISCUSSION

Shallot skin simplicia shaped in powder with brownish red and strong unique aromatic fume. The result of water content test of shallot skin powder is displayed in Table 1 below:

Table 1. Water Content of Shallot Skin Powder

Test	1	2	3	4	5	Average
Water Content (%)	9,8	10	10	9,9	10	9,9

The average water content result of Bima Brebes shallot skin powder is 9,9%, it is stated in accordance with simplicia general requirements that must not be more than 10%(34). Such water content determination is to reveal the amount of water substance inside shallot skin powder. It is related to chemical reaction potential toward active compound as the material can be growth media for microbes, mold, and other microorganisms which are sensitive to high concentration water level(35).

The yield obtained from traditional method extraction by maceration is 10,3%, meanwhile the yield obtained from MAE method is 8,63%. Such different numbers can be occurred because of time span used during maceration extraction method (3 x 24 hours) which is possible to draw more polar compound into solvent, thus it produces bigger number of yield than MAE extraction method(30). However, MAE extraction method will increase efficiency and effectiveness of active polar compound by delivering heat evenly, so that, it will not damage the active substance of the materials, and it also performs shorter time(31).

Early screening for Flavonoid phytochemicals content

Flavonoid identification applies qualitative method in order to find the content of flavonoid by observing color change appeared in each shallot skin extracts. The identification of flavonoid compound is done by color change test with HCl + Mg solution and HCl solution.

Table 2. Flavonoid Phytochemicals Content

Treatment	Result	Explanation
Extract of Shallot Skin from Maceration + 0,1 gram of Mg powder and 10 drops of concentrated HCl	+	Brownish Red
Extract of Shallot Skin from MAE + 0,1 gram of Mg powder and 10 drops of concentrated HCl	+	Brownish Red

Identification test by adding Mg powder and concentrated HCL create brownish red fluids. HCL addition is to hydrolyze flavonoid compound into aglycone flavonoid by applying aglycone hydrolysis. Meanwhile, the addition of Mg powder creates a complex compound in red. Magnesium ions will be linked to flavonoid compound which is found in shallot skin extract(32).

Quercetin Content in Shallot Skin Extract

Flavonoid content in shallot skin extract is done by using Spectrophotometer UV-Vis with 360 nm wavelength. The primary solution used is quercetin that is observed in 300 – 400 nm wave length by using Spectrophotometer UV-Vis in order to obtain maximum wavelength related to maximum sensitivity and absorbance.

Table 3. Maximum Wavelenth of Quercetin

Wavelength (nm)	Absorbance (A)
300	0,490
310	0,532
320	0,642
330	0,796
340	0,985
350	0,976
360	1,078
370	0,871
380	0,687
390	0,481
400	0,389

According to the table, it is shown that maximum wave length of quercetin primary solution is 360 nm within absorbance of 1,078 which is later used as curve of wavelength and absorbance relation.

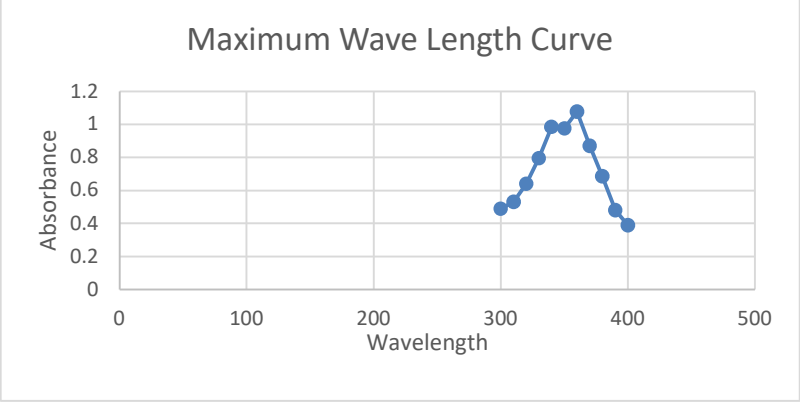

Figure 1. Maximum Wavelength Curve

Quercetin basic curve is implemented in order to reveal concentration relation between solution and its absorbance to find sample concentration. Some basic standard solution concentrations are 20,40,60, 80, and 100. The result of absorbance from several basic standard concentrations solutions are displayed in Table below:

Table 4. Absorbance Basic Standard

Concentration	Absorbance (A)			Average (A)
	1	2	3	
20	0,613	0,614	0,612	0,613
40	0,691	0,688	0,690	0,689
60	0,787	0,786	0,789	0,787
80	0,862	0,865	0,863	0,863

100	0,924	0,925	0,923	0,924

Flavonoid content is determined with equation of y = 0,0019x + 0,6204, with obtained correlation coefficient which is R2 = 0,3873. In accordance with equation above, it is concluded that the bigger concentration will result in bigger absorbance.

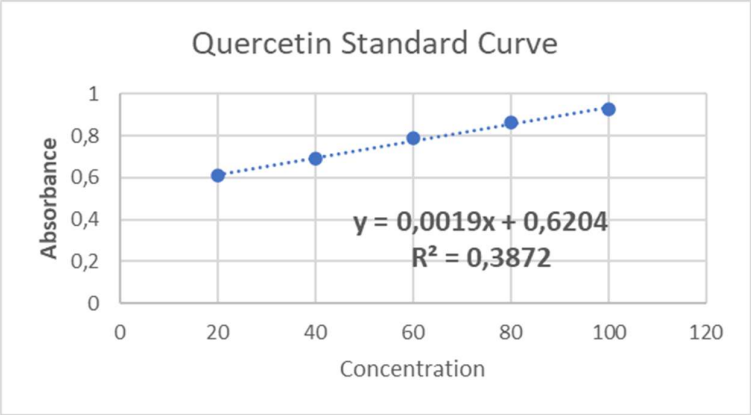

Figure 2. Quercetin Standard Curve

Flavonoid content measurement by using Spectrophotometer UV-Vis in 360 nm wave length is performed to find the absorbance. Here is total flavonoid content of extract result from maceration and MAE method.

Table 5. Total Flavonoid Content

Sample	Replication	Absorbance (A)	Flavonoid Content (%)	Flavonoid Content Average (%)
Maceration Extract	I	0,728	56,631	
	II	0,728	56,631	56,455
	III	0,727	56,105	
MAE Extraction	I	0,788	88,210	
	II	0,789	88,736	88,560
	III	0,789	88,736	

According to total flavonoid data result, it is shown that flavonoid content in Bima Brebes shallot skin from maceration (Traditional) method is 56,455 % and flavonoid content in shallot skin from Green Extraction approach with Microwave Assisted Extraction (MAE) method is 88,560 %. The data reveals that flavonoid content in shallot skin extract from maceration method is smaller compared to MAE method, thus it is predicted that MAE extraction method dissolve more active substances indicated by increasing porosity toward material compared to maceration extraction method(31). Extract production method, by maceration and MAE, undoubtedly affect flavonoid content of shallot skin. MAE method extraction uses electromagnetic wave that can penetrate simplicia wall cell and evenly excite water and fat molecule, it results in more effective extraction and it ensure the extraction does not only occur in surface level. Electromagnetic wave on MAE frequency of MAE 2.500 MHz (2,5GHz) is being absorbed by water, fat, and glucose which are contained in simplicial cell, thus it excites the atoms inside and creates heat. This process is quickly performed, so that the withdrawal compound of shallot skin can be done more effective and efficient. In maceration method, extraction process is performed by submerse technique in room temperature without heating process, so that the flavonoid content will be rather smaller than flavonoid content from MAE method extraction. MAE obtains higher result from expected compound as a result of shorter heating and cooling cycles(30,36,37).

Extraction by using maceration faces several limitations and challenges that can threaten effectivity and life sustainability(24). Maceration method has disadvantages namely large number of solvent usages, higher cost, and it has negative impact toward environment as it uses solvent that can negatively affect humans and environment(38,39). In the other hand, MAE extraction requires shorter time, it will significantly reduce extraction time into extremely shorter time, it took only several minutes compared to maceration extraction that requires days of research time(37). MAE extraction

is more efficient, compared to maceration method which is still a traditional method, by increasing extraction efficiency. Extraction with MAE method also improves bioavailability and stability of bioactive compound, phenolic compound, and flavonoid especially quercetin which is useful for functional food aplication(6).

CONCLUSION

Based on phytochemicals test result, it is concluded that flavonoid compound is found in Bima Brebes shallot skin, it is proven with red colour change and there is yellow and black sediment formed. Flavonoid content (quercetin) found during maceration extraction method with 96% ethanol is 56,455%. Meanwhile, the extraction result of Bima Brebes shallot skin with MAE (Microwave Assisted Extraction) method produces 88,540% quercetin. Maceration is a simple and affordable method, but it is limited to long extraction time and low efficiency. In other hand, extraction with help of microwave offers significant advantages with shorter extraction time, low solvent usage consumption, and higher extraction efficiency.

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