Quest Journals
Journal of Research in Agriculture and Animal Science
Volume 8 ~ Issue 6 (2021) pp: 34-39
ISSN(Online) : 2321-9459
www.questjournals.org

Research Paper

Effect Of Solvent Type And Maceration Time On Phytochemical Contents, Yield Precentage And Antibacterial Activity Of Red Onion Skin (Allium Cepa L.) Extract

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ABSTRACT: *Red onion skin is a waste generated from the food industry and households, most of which cannot be utilized. Red onion skin is known to contain chemical compounds that are antibacterial. Based on the antibacterial activity and the content of active compounds found in red onion skin, red onion skin has the potential to be used as a natural antibiotic growth promoter (AGP) for poultry. This study aims to determine the type of solvent and the best maceration time in the extraction of red onion skin in terms of phytochemical content, yield percentage and antibacterial activity. The types of solvents used are water, ethanol and methanol. The length of maceration in this study was 24 hours, 36 hours and 48 hours. The results showed that the best type of solvent in the extraction of red onion skin is ethanol with a maceration time of 36 hours, which contains phytochemical compounds such as phenolics (+++++), flavonoids (++), alkaloids (++++), steroids (++) and triterpenoids (+), with a yield percentage of 15.63% and able to inhibit the growth of Escherichia coli as indicated by an inhibition zone of 16.9 mm.*

KEYWORDS: *red onion skin, phytochemicals, yield precentage,antibacterial activity*

Received 01 June, 2021; Revised: 13 June, 2021; Accepted 15 June, 2021

I. INTRODUCTION

Indonesia is a storehouse of various types of plants with various bioactive substances and has the ability to act as antimicrobials, antifungals, antioxidants, immunomodulators and hypocholesterolemics so that they can be used as a natural antibiotic growth promoter (AGP). One of the plants that can be used as a natural antibiotic growth promoter (AGP) is red onion (*Allium cepa L*). Currently, red onion is the second largest medicinal plant and horticultural product after tomatoes (Arshad *et al*., 2017). However, the use of red onion is only limited to the tubers, while the skin is not used. This is because people often perceive red onion skin as waste generated from the food industry and households, most of which cannot be utilized. Skerget *et al.* (2009) stated that the phytochemical content in the red onion skin is higher than the tuber part. Manullang (2010) states that red onion skin contains many chemical compounds such as flavonoids, saponins, tannins, glycosides and steroids or triterpenoids. Furthermore, Rahayu *et al.* (2015) stated that the chemical compounds contained in the red onion skin in the water fraction consist of flavonoids, polyphenols, saponins, terpenoids and alkaloids, the ethyl acetate fraction contains flavonoids, polyphenols and alkaloids, and the n-hexane fraction contains saponins, steroids and terpenoids.

Research by Apriasari *et al.* (2013) stated that chemical compounds such as flavonoids, saponins and tannins have a bacteriostatic effect. Flavonoid memiliki kemampuan untuk mendenaturasi protein sehingga metabolisme sel bakteri terhenti. Saponins interact with bacterial cells that cause these cells to break or lysis (Poeloengan and Praptiwi, 2010). Octaviani *et al.* (2019) stated that the ethanol extract of red onion skin has activity in inhibiting the growth of Staphylococcus aureus, Staphylococcus epidermidis, Salmonella thypi and Eschericia coli bacteria. Based on the antibacterial activity and the content of active compounds found in red onion skin, red onion skin has the potential to be used as a natural antibiotic growth promoter (AGP) for poultry. In order for the active compounds found in red onion skin to be optimally utilized by poultry, it is necessary to process them with simple technology, and can be used easily by breeders, one of which is through the extraction method.

Extraction is the process of separating a substance from its mixture using a suitable solvent. During the extraction process, the active ingredient will be dissolved by the solvent according to its polarity properties. The solvent used must be able to extract the desired substance without dissolving other materials. In extracting the factors that will affect the extraction results are the extraction method (Dutra *et al*., 2008), extraction time (Kemit *et al*., 2016) and the type of solvent (Suryani *et al.*, 2016). The extraction method used in the research is maceration. Maceration is an extraction method that is carried out by immersing the simplicia in one or several solvent mixtures, so that the entire surface of the simplicia is completely immersed by the solvent. The advantages of the maceration method are low cost, easy to perform and without heating so that it does not damage phytochemical compounds (Cuppet *et al.,* 1954).

The effectiveness of the extraction of a compound by a solvent depends on the solubility of the compound in the solvent, according to the like dissolve like principle, which is that a compound will dissolve in a solvent with the same properties. The use of the solvent type or the ionic strength of the solvent can have an effect on the yield of the compound produced (Anggitha, 2012). The extraction time will also affect of the yield extract of a substance because it determines the duration of contact and the dissolution of the components into the solvent used. The maceration time that is too short will result in not all phytochemical compounds being dissolved in the solvent used, and if the extraction time is too long, the extracted phytochemical compounds will be damaged (Utami, 2009). Therefore we need the right type of solvent and maceration time to obtain the best red onion skin (*Allium cepa L.*) extract, in terms of phytochemical content, yield percentage, and antibacterial activity.

II. MATERIALS AND METHODS

2.1. Materials of Research

The materials used in this research are red onion skin, a solvent consisting of: water, ethanol, methanol, chemicals needed for qualitative analysis of phytochemical content, nutrient agar, mueller-hinton agar, mc farland solution of 0.5 and Physiological NaCl 0.85%. And the equipment used in this research is a blender, analytical scale, Erlenmeyer volume 250 ml, aluminum foil, filter paper, rotary evaporator and petridish.

2.2. Types of Research

This type of research is qualitative research with a laboratory experimental approach to determine the phytochemical content, yield percentage and antibacterial activity of red onion skin extract.

2.3. Implementation of Research

2.3.1. Sample Preparation

The red onion skin that has been collected is wet sorted, by removing the unnecessary parts, then chopping it by cutting the sample into smaller sizes, then drying it by aerating. Furthermore, the red onion skin is dry sorted to separate the skin of the red onion skin which is damaged by drying. Then mash the onion skin using a blender until it becomes flour.

2.3.2. Red Onion Skin Extraction

The red onion skin flour was extracted by maceration method using different solvents (water, ethanol and methanol). Maceration extraction of red onion skin flour was carried out by weighing 25 grams of red onion skin flour, and placed in 250 ml Erlenmeyer, then adding 250 ml of different solvents (water, ethanol and methanol) in a different Erlenmeyer for each solvent. The ratio of red onion skin flour to solvent is 1:10. Furthermore, it was macerated at room temperature according to different maceration time treatments (24, 36 and 48 hours) while stirring occasionally, then filtered using filter paper. After that, repeat the maceration process with the type of solvent and the length of maceration according to the treatment. The amount of solvent used is half of the amount of solvent in the first maceration (1: 5). The filtrate obtained in both macerations was evaporated using a rotary evaporator, so that a thick red onion skin extract was obtained from each solvent.

2.4. Observed variables

2.4.1. Phytochemical content

The phytochemical content of red onion skin extract was tested qualitatively based on the Harborne method (1987). The results of this qualitative test will be stated in negative or no compound (-), positive weak (+),positive moderate (++),positive strong (+++),positive is very strong (++++) and positive is very powerful once (+++++). The phytochemical content of red onion skin extract, including examination of phenolic compounds, flavonoids, alkaloids, saponins, steroids and triterpenoids.

2.4.2. Percentage of Yield

The yield measurement was obtained based on the method of Sudarmadji et al. (1997), the yield of red onion skin extract is the ratio of the weight of the extract produced to the weight of the extracted red onion skin, with the calculation:

$$\% \text{ Yield} = \frac{\text{Mass of red onion skin extract (g)}}{\text{Mass of red onion skin flour (g)}} \times 100\%$$

2.4.3. Antibacterial Activity

The test for the antibacterial activity of red onion skin extract was carried out using the disc diffusion method. Testing for antibacterial activity begins with nutrient agar preparation, Mueller-Hinton Agar preparation and Escherichia coli bacterial suspension preparation. Determination of the inhibition zone diameter was carried out by inserting the extract solution that had been made, into 150 µL petri dishes on each paper disc. Then incubated for 24 hours in the incubator. And after 24 hours the clear zone that appears is measured using a caliper.

III. RESULT AND DISCUSSION

3.1. Phytochemical Contents

The phytochemical content of red onion skin extract qualitatively in each treatment can be seen in table 1.

Table 1. Phytochemical content of red onion skin extract with different solvent and maceration time

Different solvent	Maceration time	Phytochemical content					
		Phenolik	Flavonoid	Alkaloid	Saponin	Steroid	Triterpenoid
Water	24 Hours	+++++	+	++++	-	-	-
	36 Hours	+++++	+	++++	-	-	-
	48 Hours	+++++	+	++++	-	-	-
Ethanol	24 Hours	+++++	++++	++++	-	++	+
	36 Hours	+++++	++	++++	-	++	+
	48 Hours	+++++	++	++++	-	++	+
Methanol	24 Hours	+++++	++++	++++	-	++	-
	36 Hours	+++++	++++	++++	-	++	-
	48 Hours	+++++	++	++++	-	++	-

Note : (-) = No compound, (+) = Positives weak, (++) = Positive moderate,
(+++) = Positive strong, (++++) = Positive is very strong, (+++++) = Positive is very powerful once

Phytochemical test results of red onion skin extract with water solvent is containing phenolic, flavonoid and alkaloid. Red onion skin extract with ethanol solvent is contain phenolic, flavonoid, alkaloid, steroid and triterpenoid. And red onion skin extract with methanol solvent contain is phenolic, flavonoid, alkaloid and steroid. In the red onion skin extract with water, ethanol and methanol as a solvent with a maceration time of 24, 36 and 48 hours, found very powerful once of phenolic compounds (+++++).This shows that the phenol content in the red onion skin extract with water, ethanol and methanol solvents with a maceration time of 24, 36 and 48 hours is very large. The high phenol content in the extract is due to the fact that phenol compounds tend to dissolve easily in polar solvents, because generally they often bind to sugars as glycosides.

The flavonoid compounds found in red onion skin extract using water solvent with a maceration time of 24, 36 and 48 hours are positive weak (+).In ethanol solvent with a 24 hour maceration time, a very strong flavonoid compound was found (++++), while with a maceration time of 36 and 48 hours it was found that the content of flavonoids was moderate (++).In the red onion skin extract using methanol solvent with a maceration time of 24 and 36 hours, a very strong flavonoid content was found (++++), while the maceration for 48 was moderate (++).The flavonoid content contained in the red onion skin extract is due to the fact that flavonoid compounds are polar compounds containing a number of bound sugars so that flavonoids are more likely to dissolve in polar solvents such as water, ethanol and methanol. Red onion skin extract with ethanol solvent with 24 hours maceration time and methanol solvent with 24 and 36 hours maceration time contains a lot of flavonoids. The high content of these flavonoids, and the low content of flavonoids in red onion skin extract with water solvent, are thought to be due to the ability and properties of the solvent to dissolve different flavonoid compounds, depending on the degree of polarity of the solvent and the extracted compound.

Harborne (1987) states that flavonoid compounds are divided into several types, each type of flavonoid has a different polarity depending on the number and position of the hydroxyl groups of each type of flavonoid so that this will affect the solubility of flavonoids in the solvent. The high content of flavonoids in red onion skin extract with ethanol and methanol solvents explains that the characteristics of the flavonoid compounds in red onion skin extract have the same polarity as ethanol and methanol, so that the red onion skin extract with ethanol and methanol solvents contains a lot of flavonoids. The flavonoid content in red onion skin extract with ethanol and methanol solvents decreased during the longer maceration time. This can be caused because it has passed the optimum point of maceration. Cikita et al., (2016) stated that the maceration time that passes the optimum time will damage the solutes in the material and have the potential to increase the process of losing compounds in the solution due to the evaporation process.

The alkaloid content found in red onion skin extract with water, ethanol and methanol solvents is very strong (++++). The high alkaloid content in red onion skin extract is due to the fact that alkaloid compounds are polar and will dissolve in polar solvents such as water, ethanol and methanol. Suratmo (2009) states that

alkaloids are more commonly found in polar solvents, because the alkaloid class of compounds that have the potential to act as antioxidants is a polar compound that will be extracted in polar solvents. Furthermore, Lenny (2006) states that alkaloids are generally soluble in organic solvents, while some of the psedoalkaloid and protoalkaloid groups are soluble in polar solvents.

In the red onion skin extract using water, ethanol and methanol as a solvent, no saponin compounds were found. This can occur because the saponin compounds found in red onion skin are non-polar, so they do not dissolve in polar solvents. According to Widyasari (2008) saponins contain compounds that are partly water-soluble (hydrophilic) and compounds that are soluble in non-polar (hydrophobic) solvents. Furthermore, Octaviani (2009) states that saponins are non-polar, because saponin compounds have hydrophobic groups, namely aglycones.

The steroid content in red onion skin extract with ethanol and methanol solvents is moderate (++), however, steroid compounds are not found in red onion skin extract using water solvent. Harborne (1987) states that steroids can be present in the form of glycosides. Glycosides are compounds consisting of sugars and aglycones. The presence of bound and polar sugars causes glycosides to be able to dissolve in polar solvents, so that steroids are found in red onion skin extract with ethanol and methanol solvents, however the streroids in red onion skin extract cannot dissolve in highly polar solvents such as water.

In the red onion skin extract with water and methanol solvent, triterpenoid compounds were not found, this could occur because triterpenoid compounds are non-polar, so it is thought that they can dissolve in non-polar solvents. However, in this study triterpenoid compounds were found in ethanol extracts with a weak or low content, this is thought to be caused by intermolecular forces, namely induced dipole-dipole forces and hydrogen bonds. A polar molecule that has a permanent dipole will induce a non-polar molecule that does not have a dipole, so there will be an electrostatic force between the two or what is called an induced dipole-dipole force (Effendi, 2006).

3.2. Yield Precentage

The yield precentage of red onion skin extract in each treatment can be seen in Figure 1.

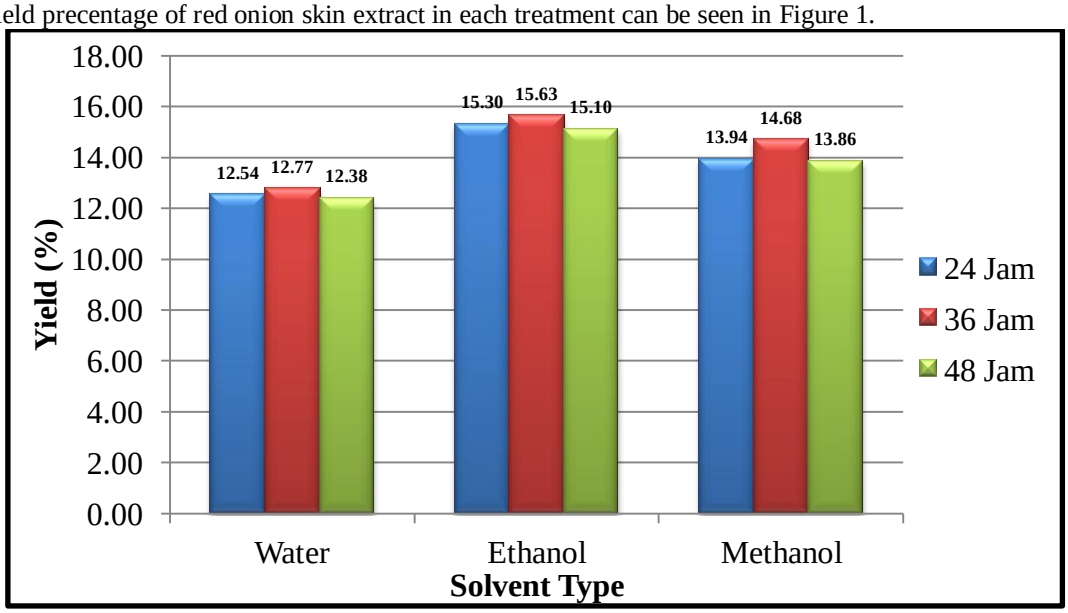

Figure 1.Yield Precentage of Red Onion Skin Extract (%)

Based on Figure 1, it can be seen that the yield of red onion skin extract ranges from 12.38% to 15.63%. The highest yield of red onion skin extract was found in the methanol solvent treatment with a maceration time of 36 hours, namely 15.63%, while the lowest yield was in the water solvent treatment with a maceration time of 48 hours, namely 12.38%. And from Figure 1 it can be seen that the difference in the type of solvent and the time of maceration affects the yield of the extract produced. Ethanol solvents produce higher yields than water and methanol solvents. This shows that the compounds in the red onion skin extract have a polarity close to ethanol, because the compounds obtained are based on the similarity of polarity to the solvent.

Kristian et al. (2016) stated that the longer the extraction time, the greater the opportunity for the material to come into contact with the solvent, so that the yield precentage obtained will be high until the saturation point of the solution, but the number of certain compounds will decrease after reaching the optimal length of time. And from this research, it can be seen that the optimum point in the extraction of red onion skin using water, ethanol and methanol as a solvent was reached at 36 hours of maceration time, so that the addition of maceration time of 48 hours is no longer effective to increase the extract yield. Cikita et al. (2016) stated that

the maceration time that passes the optimum time will damage the solutes in the material and have the potential to increase the process of losing compounds in the extracted solution due to evaporation.

3.3. Antibacterial Activity

Antibacterial activity of red onion skin extract in each treatment can be seen in Figure 2.

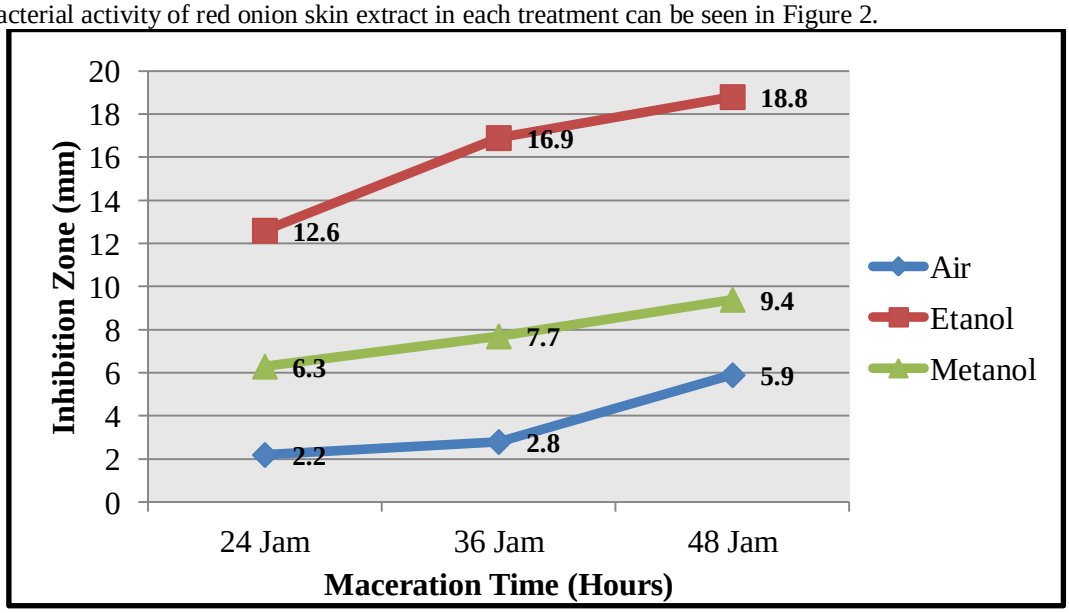

Figure 2. Antibacterial Activity of Red Onion Skin Extract

Based on Figure 2, it can be seen that the antibacterial activity of red onion skin extract against Escherichia coli bacteria ranges from 2,2 mm to 18,8 mm. The highest antibacterial activity of red onion skin extract was found in ethanol solvent treatment with a maceration time of 48 hours, namely 18,8 mm, while the lowest of antibacterial activity was found in water solvent treatment with a maceration time of 24 hours, namely 2,2 mm. And from Figure 2 it can be seen that the difference in the type of solvent and maceration time in the extraction of red onion skin affects the ability of the inhibition zone against Escherichia coli bacteria.

The inhibition zone of the Escherichia Coli bacteria is caused by chemical compounds contained in red onion skin extract. The size of the inhibition zone formed in various extracts is influenced by the solvent type and the maceration time. Lapornik et al., (2005) stated that the use of the right solvent and maceration time will affect the area of the narrow inhibition zone (antibacterial) that is formed. The inhibition zone formed is influenced by compounds that have antibacterial properties in the extract. And from Table 1 it can be seen that the red onion skin extract with ethanol solvent contains more phytochemical compounds that are antibacterial than water and methanol solvents. So that the red onion skin extract with ethanol solvent with a maceration time of 48 hours has the ability to have a greater antibacterial activity in inhibiting the growth of Escherichia coli bacteria.

IV. CONCLUSION

From the results of the study it can be concluded that the best type of solvent in the extraction of red onion skin is ethanol with a maceration time of 36 hours, which contains phytochemical compounds such as phenolics (+++++), flavonoids (++), alkaloids (++++), steroids (++) and triterpenoids (+), with a yield percentage of 15.63% and able to inhibit the growth of Escherichia coli as indicated by an inhibition zone of 16.99 mm.

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