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IDENTIFICATION OF ACTIVE COMPOUNDS IN RED ONION (*Allium ascalonicum* L.) PEEL EXTRACT BY LC-ESI-QTOF-MS/MS AND DETERMINATION OF ITS ANTIOXIDANT ACTIVITY

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ABSTRACT

Red peel onion is one of the foodstuffs that are often used by people in daily life, but their use is limited to the onion bulbs so that 10% of red onion cannot be consumed and only becomes waste, namely the peel of the red onion. Although the peel of the onion is a waste, the peel of the onion contains flavonol compounds from the flavonoid group that has the potential as antioxidants. So this study was conducted to determine the compounds in the red onion peel that acts as antioxidants. The extraction method used in this research is the maceration method with 80% ethanol solvent. The compound content test was carried out by testing phytochemical compounds by identifying flavonoid compounds, polyphenols, steroids and terpenoids, saponins, alkaloids, anthocyanins, antioxidant activity, and identification of compounds using LC-ESI-QTOF-MS/MS. The study results on qualitative testing of phytochemical compounds showed that the onion peel extract contained positive flavonoids, polyphenols, and saponins. Meanwhile, the identification of compound content of onion peel extract using LC-ESI-QTOF-MS/MS detected flavonoids (14 compounds), polyphenols (2 compound), saponins (2 compounds), and alkaloids (1 compound). The antioxidant activity of the ethanolic extract of the onion peel has obtained an average of 77%, and the total anthocyanin content was an average of 17.87 mg/L. It was found that the natural antioxidant of onion peel extract showed good primary oxidation inhibition ability. So it can be concluded that onion peel extract is effectively used as a natural antioxidant.

Keywords: Antioxidant; extraction; flavonoid; onion peel.

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INTRODUCTION

Red onion are food ingredients that are widely used in everyday life by the community. People usually only use the onion bulbs as food, but 10% of the onion cannot be consumed and only becomes waste, namely the peel of the red onion. (Panganku.org, 2018). According to the Central Statistics Agency (BPS), Indonesia's national shallot production in 2021 was 1.82 million tons. This number increased by 14.88% from the previous year at 1.58 million tons. Based on the province, the East Java region in 2020 became the second highest producer of shallots after Central Java at 454.58 thousand or 25.04% tons. According to Ristiawan (2013) onion peel waste produced by PT Indofood CBP is 5kg/day, and fried shallot waste is 200-300kg/day. This fact results in significant potential for waste, so it is necessary to handle the utilization of onion peel waste.

Previous research (Mardiah et al., 2017) resulted that onion peel extract has a antioxidant activity of 61.7543%. According to Soebagio et al. (2007a), onion peel contains phytochemical compounds that are potent

antioxidants, namely flavonoids. The onion peel extract contains flavonoid compounds, saponins, tannins glycosides, steroids, or triterpenoids (Manullang, 2010). In addition, other compounds contain anthocyanins which give red or purple colors to some varieties of as and flavonols (quercetin) which produce yellow and brown pigments (Ifesan, 2017). Recent Singh et al., (2009) onion peel extract ethyl acetate fraction was detected to contain quercetin and kaempeferol compounds. Total phenolic compounds (384.7 ± 5.0 mg GAE/g), flavonoids (165.2 ± 3.2 mg QE/g) and antioxidant activity (97.4 ± 7.6%). The high antioxidant activity of the onion peel is due to the presence of well-known antioxidant compounds (polyphenols) in large quantities. Meanwhile, in this study, onion peel extract used ethanol fraction and detected its compound content and antioxidant activity.

Eskilsson & Björklund (2001) reported that temperature was an important factor contributing to the increase in extraction yield. In addition, the use of solvents can affect the extract yield value and the total content of bioactive compounds (Santoso, dkk., 2012). The use of 80% ethanol solvent is based on research (S. Wang et al., 2017), where 80% ethanol solvent produces the most optimal extraction results in rosemary based on the antioxidant test parameters, yield, and phenol composition obtained. According to Viera et al. (2017), the results of optimizing flavonoid levels using conventional extraction methods with 80% ethanol solvent for 60 minutes resulted in levels of 40.35 mg/g dry weight of onion peel. This study used 80% ethanol as a solvent because ethanol is a semi-polar solvent and can attract most of the chemical content of simplisia. Ethanol is also known to attract more chemical compounds than methanol and water (Azizah & Salamah, 2013). In addition, ethanol is a universal solvent that can attract soluble compounds in non-polar and polar solvents and has a polarity index of 5.2 (Snyder, 1997). The use of ethanol as a solvent is based on Abdullah et al., (2021) statement which states that onion peel extract with ethanol fraction is able to produce a yield 63%.

LC-MS analysis has previously been carried out by Mangurana et al. (2019) to identify secondary metabolites in Sponge *Callyspongia aerizusa*, which showed 15 compounds at station I while station II had 13 compounds. Another study was also conducted by Sukandar et al. (2019) to identify the active compounds in edible mushroom, basil, manila sapodilla, namnam, honje, kawista, and pineapple using LC-MS analysis. The advantages of the analysis using the LC/MS method when compared to the HPLC method are fast analysis time, small sample volume required, high sensitivity, the column can be reused and can be used for organic and inorganic samples (Magiera et al., 2012; Rahim et al., 2014). In addition, according to Himawan (2010), another advantage of LC/MS is that it can analyze a broader range of components, such as thermally labile compounds, high polarity or high molecular mass, and even proteins. Therefore, the LC-ESI-QTOF-MS/MS analysis carried out in this study was needed to determine the characteristics of the compounds in the red onion peel so that they could determine the proper application of the onion peel extract. In addition, information regarding identifying the names of compounds in onion peel extract is also still limited. Therefore, this study focuses on the identity of the compounds and the links between antioxidant activity and its constituent compounds. Hoping that onion peel waste has no economic value in this community can be minimized and will become one of the wastes that are useful as natural antioxidants.

MATERIALS AND METHODS

Materials

Red onion peel was obtained from the waste of Pasar Besar Malang with the characteristics of choosing red and dry peel, ethanol p.a (Merck®), aquades, HCl p.a (Merck®), 2,2-difenil-1- pikrilhidrazil (DPPH) p.a (Merck®), biotin (Merck®), Chloramphenicol, aquabides p.a, methanol p.a (Merck®), and glacial acetic acid p.a (Merck®).

Tools

Measuring cup, stirrer, filter paper, test tube, blender, weighing scale, beaker glass, erlenmeyer, water bath shaker, funnel, dark bottle, filler, measuring pipette, bunsen, test tube rack, vacuum evaporator, vortex, dropper pipette, filter cloth, test tube, color reader, UV-Vis spectrophotometer, cuvette, pH meter and UPLC.

Methods

Red onion peel was extracted using 80% ethanol as solvent. Then the extract was tested for phytochemicals by identifying flavonoid compounds (Mustikasari & Ariyani, 2010), polyphenols (Rahayu et al., 2015), steroids, and terpenoids (Rahayu et al., 2015), saponins (Hanani, 2015), alkaloids (Mustikasari & Ariyani, 2015), anthocyanins (AOAC, 2005), antioxidant activity (Rahayu et al., 2015) with 3 replications on each test and identification of compounds using LC-ESI-QTOF-MS/MS.

Red Onion Peel Extraction

Red Onion Peel Extraction was estimated as described by S. Wang et al., (2017) the peel of the red onion is dried in the sun and covered with a black cloth until the peel dries, then it is ground to become powdered onion peel. Next, onion powder was soaked in 80% ethanol (1:20) and filtered. The use of 80% ethanol refers to research (Yu-Zhu et al., 2018) which shows the most optimal maceration extraction results based on the results of the DPPH test, yield and composition of phenol compounds obtained. Finally, the obtained filtrate was evaporated to remove the ethanol solvent.

Antioxidant Activity Test

According to Rahayu et al. (2015) analysis of antioxidant activity used the DPPH (2.2 diphenyl-1-picrylhydrazyl) method by mixing a solution of onion peel extract with DPPH using ethanol as a solvent. The solution mixture was allowed to stand for 30 minutes. After that, the test was carried out using a UV-Vis spectrophotometer with a wavelength of 517 nm.

Flavonoid Qualitative Test

The test was carried out by taking 2 ml of extract, then heated for 5 minutes. Then add 0.1 g of Mg metal and five drops of concentrated HCl. If each solution formed a yellow-orange to red color, then it was positive for flavonoids (Mustikasari & Ariyani, 2010).

Polyphenol Qualitative Test

To determine polyphenol compound according to Rahayu et al. (2015) a total of 1 ml of the extract was put into a test tube, then added with a few drops of 10% $FeCl_3$ solution. The formation of green, red, purple, blue, or black color indicates the presence of flavonoids.

Qualitative Test of Steroids and Terpenoids

A total of 2 ml of the extract was put into a test tube, then added ten drops of glacial acetic acid. Then the mixture is added with two drops of concentrated sulfuric acid and shaken. The presence of steroids is indicated by a blue or green color formation, while the presence of terpenoids is indicated by the appearance of red or purple color (Rahayu et al., 2015).

Saponin Qualitative Test

The extracted sample was weighed as much as 0.5 g, put into a test tube, added 10 ml of hot water, cooled, and then shaken vigorously for 10 seconds. The formation of a stable foam indicates a positive result for not less than 1 minute (Hanani, 2015).

Alkaloid Qualitative Test

According to Mustikasari & Ariyani (2010) the test was carried out by taking 2 mL of the extract into a test tube. After that, five drops of Dragendroff's reagent were added. If an orange precipitate is formed in the solution, it is positive that it contains alkaloids. Furthermore, using Mayer's reagent, Alkaloid testing was carried out by taking 2 mL of the extract into a different test tube. After that, three drops of concentrated hydrochloric acid and 5 drops of Mayer's reagent were added. If the solution forms a white precipitate, the sample is positive for alkaloids.

Anthocyanin Test

The sample was dissolved using acid methanol as a solvent (methanol+HCl) in a ratio (1:5) into a glass beaker, then homogenized in a test tube covered with aluminum foil. After that, the sample was macerated at 23℃ for 1 hour. Then 1 ml of each sample was put into the first and second test tubes. 9 ml of buffer solution one was put into the first test tube, while 9 ml of buffer solution 4.5 was put into the second test tube. Anthocyanin scanning was carried out with a wavelength range of 400 nm-550 nm in both sample solution buffers to determine the anthocyanidin wavelength possessed by the extracted sample. After that, the sample absorbance was measured, and the anthocyanin equivalent was calculated with cyaniding-3-glucoside **(AOAC, 2005)**.

Red Onion Peel Ethanol Extract Color Intensity

The color reader is turned on by pressing the power button. The sample is placed under a color-reading lens, then the color intensity values of L (Lightness), a*, b*, C (Chroma), and H (Hue) samples are read (Munsell, 1997).

Extract Compounds Identification Using LC-ESI-QTOF-MS/MS

The extract was weighed 0.5 g into a 10 ml volumetric flask, then added methanol/solvent according to ultrasonic for 30 minutes. Impress with methanol/solvent and homogenize. Filter with a 0.22 m GHP/PTFE membrane filter, then injects into the UPLC system. The screening process for active natural ingredients using LC-ESI-QTOF-MS/MS is carried out using the UNIFI software, which already has a mass spectrum library of natural active substances from the waters database. The extract was weighed 0.5 g into a 10 ml volumetric flask, then added methanol/solvent according to ultrasonic for 30 minutes. Impress with methanol/solvent and homogenize. Filter with 0.22 m GHP/PTFE membrane filter, then injected into the UPLC system. Filtering process The condition of the LC measurement instrument was carried out on column C18 with column temperature of 40°C, autosampler temperature of 15°C, injection volume of 10 L, mobile phase (A) 0.1% formic acid in acetonitrile and (B) 0.1% formic acid in aquabides, air rate 0.6 mL/min, Gradient. MS setting conditions were carried out with Tof MS^E operating mode, ESI (-)/ESI (+) ionization, and acquisition range 50-1200 Da. The screening process for active natural ingredients using LC-ESI-QTOF-MS/MS is carried out using the UNIFI software which includes a mass spectrum library of natural active substances from the waters database. UNIFI software can find out the mass spectrum of the compound in the sample which is then matched with the mass spectrum in the library. Criteria for active substances if they meet the requirements Mass error reads 5 ppm error, Isotope match MZ RMS PPM 6 ppm & Isotope match MZ RMS % 10%, analyte intensity 300, and there is one with brake < 4 in the Fragment match elucidation system of natural active ingredients using LC-ESI-QTOF-MS/MS is done using UNIFI software which already has a mass spectrum library of natural active substances from the aquatic database (Qiao, Lirui, et al. 2013).

RESULTS AND DISCUSSIONS

Onion Peel Ethanol Extract Compound (Qualitative analysis)

Phytochemical testing includes flavonoid test, polyphenol test, steroid test, saponin test, and alkaloid test. According to (Prabowo & Noer, 2020), qualitative phytochemical screening on dried onion peel extract (*Allium ascalonicum* L.) showed positive results for the content of alkaloids, saponins, and flavonoids (figure 2).

Flavonoids are secondary metabolites whose presence is influenced by photosynthesis in plants (Q. Wang et al., 2016). According to Dewi et al (2018), flavonoid compounds have different levels of polarity, but in general flavonoids are semipolar so they will be more effective if extracted with semipolar solvents, for example ethanol and methanol. The results of the qualitative test of flavonoids in the ethanolic onion peel extract in this study showed positive results containing flavonoids which were characterized by the presence of a

dark orange to reddish color in the ethanol extract. This result is in line with previous research (Dini, 2020) which stated that the results of qualitative testing of flavonoids in ethanol extracts showed positive results which were indicated by the presence of a thick orange color in the test sample. In addition, the results of other studies (Prabowo & Noer, 2020) also showed positive results, namely that the onion peel extract contained flavonoids in it which was marked by the appearance of a red color in the test sample (Figure 2).

According to Sandhiutami et al. (2012), the characteristics of polyphenolic compounds tend to be polar because polyphenolic compounds are known to have hydroxyl groups. Polyphenols are closely related to antioxidants; this is in line with the statement (Hermawan et al., 2018), which states that most of the antioxidants in plants are polyphenol compounds, which are phenolic compounds that can prevent oxidation events. This antioxidant activity is related to the content of polyphenol hydroxyl groups that can donate free radicals of hydrogen atoms to free radicals to neutralize their radical properties (Hermawan et al., 2018). The qualitative test results of polyphenols in the ethanolic extract of onion peel in this study showed positive results containing flavonoids indicated by the presence of red color in the tested ethanol extract. This result is in line with previous research (Rahayu et al., 2015), which stated that the results of the phytochemical screening test of the aqueous onion peel extract were positive for polyphenols. In previous studies, onion peel extract contains chemical compounds that are potential antioxidants, namely polyphenolic flavonoids that can prevent the development of free radicals in the body and repair damaged body cells (Soebagio et al., 2007). The flavonoid compound of the polyphenol group is the compound with the highest content in the onion peel, namely quercetin. Quercetin is one of the best flavonols. Quercetin can be found in many vegetables and fruits, and red onion (Dini, 2020). Quercetin is an antioxidant that has antiradical properties. According to research by Sibuea et al. (2008), quercetin has free radical scavenging activity dissolved in absolute ethanol than tocopherol, rutin, and BHT had more significant free radical scavenging activity.

According to Padmasari et al. (2013), generally, saponins are in the form of glycosides, so they are polar and are surface-active compounds that can cause foam when shaken in water. Prayoga. D.G.E et al. (2019) stated that saponin compounds have polar and nonpolar groups that are surface-active so that saponins are shaken with water, they will hydrolyze and form micelles. Therefore, the foam that appears in the final result has polar and nonpolar polar groups that will form micelles. These micelles cause the polar groups to face outward and the nonpolar groups to face inward, which looks like foam (Padmasari et al., 2013). Based on the qualitative test results of saponins in the ethanolic extract of the onion peel in this study, it was seen that there was foam in the test sample results, which meant that the content in the sample was positive for saponin compounds. Previous research (Elsyana et al., 2018) also stated that onion peel in suitable ethanol solvents contained secondary metabolites, one of which was saponins. According to Ali et al. (2012), saponins can reduce superoxide by forming hydroperoxide intermediates to prevent biomolecular damage by free radicals.

The results of the qualitative test of steroids on the ethanolic extract of the onion peel in this study were marked by the results of the red test sample, which indicated that the content in the sample was negative for steroid compounds and positive for triterpenoids. Steroid's qualitative identification is characterized by the formation of a blue or green color, while the presence of terpenoids is indicated by the formation of red or purple color (Rahayu et al., 2015). Dini et al. (2020) argued that the ethanolic extract of the onion peel was thought to contain triterpenoid compounds, while the n-hexane extract only contained steroid compounds. Samples on onion peel with ethanol extract showed negative results because the test samples did not show any color change in green rings. The test results showed a positive presence of triterpenoid compounds indicated by red color

in the ethanolic extract of onion peel because the triterpenoid group compounds are polar, so the compounds are more soluble in polar solvents (Harbone, 1987).

Testing for alkaloids was carried out using two types of reagents or reagents, namely Mayer's reagent and Dragendroff's reagent. The principle of this analytical method is the precipitation reaction that occurs due to ligand replacement. The nitrogen atom, which has a lone pair of electrons in the alkaloids, can replace the iodo ion in the reagents. Dragendorff's reagent contains bismuth nitrate and potassium iodide in a solution of glacial acetic acid (potassium tetraiodobismuthate(III)). At the same time, Mayer's reagent contains potassium iodide and mercury chloride (potassium tetraiodomercurate (II)). However, this method has a weakness, namely that these reagents can not only precipitate alkaloids but can also precipitate several types of compounds, including proteins, coumarins, -pyrons, hydroxy flavones, and tannins. This reaction is known as a "false positive" (Nuryanti & Pursitasari, 2014). Based on the qualitative test results of the ethanolic extract of the red onion peel alkaloids in this study, there was no orange precipitate in the Dragendroff reagent and no white precipitate in the Meyer reagent in the test sample results, which means that the content in the sample is negative for alkaloid compounds. Previous research, Elsyana et al. (2018), resulted that the qualitative test results of the phytochemical content of the ethanolic extract of the onion peel showed that the ethanolic extract of the onion peel showed negative results for alkaloids. In addition, it is different from the results of research (Rahayu et al., 2015), which is that the aqueous fraction of onion peel extract is positive for the presence of flavonoids, polyphenols, saponins, terpenoids, and alkaloids. This difference shows that the phytochemical components in the onion peel extract depend on the polarity of the solvent used (Elsyana et al., 2018).

Shallot Peel Ethanol Extract Color Intensity

Shallot peel shows a brightness value (L*) of 22.33. The high L* can be influenced by the length of the extract evaporation process to remove the solvent in the onion peel extract. Zhafira (2018) stated that the longer the heating process, the lower the brightness value of black garlic products. The reddish color analysis (a*) aims to determine the level of redness in the onion peel extract. The test results show the a* value with a positive result of 0.93. The presence of anthocyanins in it could cause the red color of the onion peel. Anthocyanins are used as pigments that give a purplish red color to onion peel extract (Figure 1) (Widjanarko, 2001). Meanwhile, the results of the color intensity test of the onion peel extract value b* showed a bluish color with a value of -0.16.

Table 1 Color Intensity Test of Red Onion Peel Ethanol Extract

Color Intensity Test	Result
L*	22,33 ± 0,90
a*	0,93 ± 0,25
b*	-0,16 ± 0,15

Figure 1 Shallot Peel Ethanol Extract

Onion Peel Extract Compounds

The results showed that 80% ethanol extract contained flavonoids, saponins, alkaloids, and polyphenols. Robinetin, 7-Hydroxy-1-methoxy-2-methoxyxanthone, Pedalitin, Quercetin-7-O-[β-D-glucopyranosyl(1→6)-β-D-glucopyranoside], Isoetin- 7-O-β-D- glucopyranosyl-2'-O-α-D-glucopyranoside, Isorhamnetin-3-O-β-gentiobioside, Kaempferol-3-glucuronide, Quercetin-3-O-β-D-glucopyranoside, Limocitrin , Quercimeritrin, 4′,5,7-Trihydroxy-8-methoxy-flavonol-3-O-β-D-glucopyranoside, Quercetin, 4,7,2′-Trihydroxy-4′-methoxyisoflavanol and Kaempferol-3-glucuronide is a flavonoid compound (Ahmad, 2012; Ahmed et al., 2016; Chen et al., 2016; Klimek-Szczykutowicz et al., 2020; Parwata, 2016; Putri, 2015; Tsamo et al., 2021). According to

(Gagola et al., 2014), phenolic compounds have antioxidant activity. The antioxidant properties of this compound are related to the presence of a phenolic group that can donate a hydrogen atom to a free radical so that the radical becomes unreactive. One of the compounds identified in onion peel extract is Quercetin, which is known to have antioxidant activity. Coşkun et al. (2018) stated that Quercetin is one of the flavonoid group compounds that can be an antioxidant; besides, this compound can generally be easily found in fruits, vegetables, and onions. In addition, it is also known that kaempferol compounds identified in onion peel extract have antioxidant abilities. According to (Novalinda Ginting & Chiuman, 2020), it is known that kaempferol compounds have antioxidant activity that can function to fight free radicals.

Neohecogenin-3-O-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl compounds (1→4)-β-D-galactopy-ranoside and Pennogenin-3-O-α-L-arabinofuranosyl(1 →4)[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside allegedly belongs to the class of saponins. According to (Gusungi et al., 2020), saponin compounds have an antioxidant effect by forming reactive species such as hydroperoxides and superoxides as antioxidants, thereby inhibiting the formation of lipid peroxides. In addition to the identified saponin compounds, onion peel extract was also identified as containing Stachydrine compounds which are thought to belong to the alkaloid group. According to (Nuraeni & Sembiring, 2018) it is known that Alkaloids can function as antioxidants. Many alkaloid compounds are found in polar solvents because the class of alkaloid compounds that may act as antioxidants are polar compounds that will be extracted in polar solvents (Sudirman, 2011). According to (Kurniati, 2013) the mechanism of alkaloids as antioxidants is by donating H atoms to free radicals, so this mechanism shows that alkaloids work as primary antioxidants.

Myricetin compounds and 1-Galloyl-glucose are thought to be included in the polyphenol group. According to (Qonitah & Ahwan, 2019), it has been explained that myricetin compounds act actively in antioxidant activity. Anggrawati & Ramadhania (2016) stated that Myricetin compounds have antioxidant activity after an in vitro activity test of the carotene bleaching method using a mixture of 0.2 mg beta carotene and 0.2 g bulk cooking oil with activity values antioxidants are 21.14% and 75.08%, respectively. In addition, Myricetin compounds can prevent the decay of the orange color of carotene due to oxidation in the cooking oil and -carotene emulsion system. Gallyl glucose, also known as 1-galyl-beta-D-glucose or beta-glucogallin, is a member of the class of compounds known as tannins. Tannins are chemical compounds classified as polyphenolic compounds (Ghamba et al., 2014). According to (Noer et al., 2018) tannins have a major biological role because of their function as protein precipitates and metal chelators. Therefore, tannins can be predicted to act as biological antioxidants.

Anthocyanin Content of Shallot Peel Extract

Anthocyanins are polyphenolic compounds responsible for cyanic colors ranging from salmon pink to red and purple to dark blue in most flowers, fruits, leaves, and stems. For example, according to Ilham (2020), onion peel extract contains anthocyanin compounds that give red-purple pigment to the onion peel.

Based on the analysis, the total anthocyanin content of the ethanolic extract of the onion peel is 17.87 mg/L. According to previous research (Fossen et al., 1998 in Krithika S. et al., 2020) Cyanidin 3 glucoside is the main part of anthocyanins in onion peel and has anthocyanin levels of 10.04 ± 0.90 mg/100g. Meanwhile, according to another study (Geetha et al., 2011), the anthocyanin levels in onion peel extract with 1% methanol HCl solvent contained 109 mg/g. This difference can be caused by several factors, including the sample preparation process, the conditions, and storage period of the sample, and the habitat of the shallot sample used. In the sample preparation process, during the evaporation process, the sample uses a relatively high temperature of 70-75°C, so

that in this process, it affects the stability of anthocyanins because it is heated continuously until a thick extract is obtained. According to Niendyah (2004), the stability of anthocyanins is influenced by several factors, including temperature, changes in pH, light, and oxygen, and other factors such as metal ions. In addition, the analysis process on the sample is not carried out immediately when the thick extract is ready so that the conditions and storage period of the sample cause anthocyanin compounds to degrade (Riyanti Widasari Putri & Choirun Nisa, 2015). Other acylated and non-acylated anthocyanins derivatives include cyanidin mono-dandiglucosides, peonidin mono-dan diglucosides petunidingglucoside, cyanidin 3-laminariobioside and 5-carboxypyrano-cyanidin 3-glucoside, are found in red onion (Sliesad et al., 2007).

Antioxidant Activity

The ethanolic extract of onion peel that was obtained was tested for antioxidant activity and anthocyanin content. Based on research (Rahayu et al., 2015), the aqueous fraction extract of the onion peel is positive for antioxidant compounds. Furthermore, Manullang (2010) concluded that the onion peel contains flavonoid compounds that function as antioxidant agents and contains many chemical compounds such as saponins, glycosides, and steroids or triterpenoids.

The percentage of inhibition of the ethanolic extract of the onion peel was an average of 77%, which was classified as a potent antioxidant activity. According to previous research (Suwardi et al., 2019), the percentage of inhibition of maceration extract of shallot peel with concentrations of 100, 200, 400, and 600 respectively was 66.16%, 76.57%, 80.91%, and 87.85%. The factor causing the decrease in antioxidant capacity is because the compounds contained in the onion peel extract are still not pure, so at high concentrations, the active substances contained in the onion peel will compete for solubility with other substances. Manullang (2010) concluded that the onion peel contains flavonoid compounds that function as antioxidant agents and many chemical compounds such as saponins, glycosides, and steroids or triterpenoids, which act as antibacterial agents. Another study conducted by (Rahayu et al., 2015) showed that the phytochemical results of onion peel using the water fraction showed that it contained secondary metabolites of flavonoids, polyphenols, saponins, terpenoids, and alkaloids. So it is not entirely with a high concentration of solubility of the active substance as an antioxidant agent is also high. Red onion have antioxidant properties because they contain flavonoid compounds that can ward off free radicals (Rohmawati et al., 2017). Factors affecting antioxidant activity are when these antioxidants are easily oxidized and degraded by air and heat. Materials that have potential antioxidant activity processed by heat and exposed to direct air will damage the chemical content to affect antioxidant activity (Kurniawati & Sianturi, 2016). Before being used as a sample, it was known that the onion peel was obtained through the drying process, this will also affect the active compounds contained in the sample. The antioxidant activity of onion peel extract is due to the content of secondary metabolites, namely flavonoids, polyphenols, and saponins. The qualitative test results of flavonoids in the ethanolic extract of the onion peel in this study showed positive results containing flavonoids characterized by the presence of dark orange to reddish color in the ethanol extract. So it can be seen that the % inhibition obtained in this study is in line with the presence of flavonoids in the onion peel extract. The activity of antioxidant content in flavonoid compounds is because these compounds are phenolic compounds, namely compounds with -OH groups bonded to aromatic carbons. The strength of flavonoid antioxidant activity depends on the number and position of the OH group in the flavonoid molecule. The more OH groups in the flavonoid molecule, the higher the antiradical activity (Suwardi, 2019). In addition, in previous studies, onion peel extract contains chemical compounds that can also have potential as antioxidants, namely polyphenols that can prevent the development of free radicals in the body and repair damaged body cells (Soebagio et al., 2007). Polyphenols are phenol derivative compounds that have

antioxidant activity. Phenolic antioxidants are commonly used to prevent damage caused by oxidation reactions in food, cosmetics, pharmaceuticals, and plastics. The function of polyphenols is as scavengers and scavengers of free radicals from damaging metal ions (Ayucitra et al., 2011). Saponin compounds also have an antioxidant effect by forming reactive species such as hydroperoxides and superoxides as antioxidants, thereby inhibiting the formation of lipid peroxides (Gusungi et al., 2020).

Based on the compounds identification test results, some compounds act as antioxidants such as Kaempferol, Robinetin, Isoetin, Isorhamnetin, Quercetin, and Myricetin. (Kurniawati & Sianturi, 2016) reported that Kaempferol is a compound with a role as an antioxidant; a similar case on Sumartini & Ikrawan (2020) demonstrated that Kaempferol is a flavonoid flavonol group with potential as an antioxidant. In addition, the Robinetin compound, according to (Sumartini & Ikrawan, 2020), Robinetin is a flavonoid component compound with antioxidant potential and cellular protection. According to (Mai et al., 2012), the Isoetin compound has the antioxidant activity to capture free radicals. Meanwhile, the Isohamnetin compound acts actively in antioxidant activity (Qonitah & Ahwan, 2019). In addition, it is known that Quercetin compounds, namely pigments from plants (flavonoids), are believed to have the ability as antioxidants by showing the ability to prevent the oxidation process of Low-Density Lipoproteins (LDL) by capturing free radicals (Sumartini & Ikrawan, 2020). Meanwhile (Narwanto et al., 2018) reported that Myricetin compounds have potential as antioxidants.

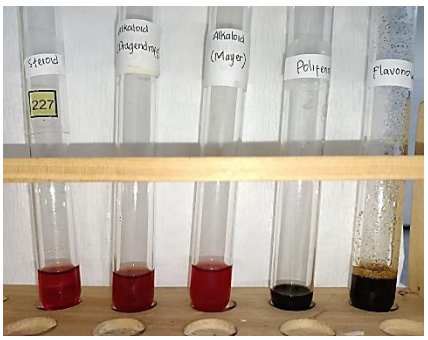

Figure 2 Qualitative Test Results of Steroids, Alkaloids (Drangendroff), Alkaloids (Mayer), Polyphenols and Flavonoids Ethanol Extract of Shallot Peel

Table 2 Qualitative Test of Onion Peel Ethanol Extract

Qualitative Test of Extract	Result
Flavonoids	+
Polyphenol	+
Steroids	-
Saponins	+
Alkaloids	-

Table 3 Identification of Red Onion Peel Ethanol Extract Compounds Using LC-ESI-QTOF-MS/MS

Component name	Formula	Measured m/z	Observed RT (min)	Mass error (ppm)	Total Fragments Found	Isotope Match Mz RMS PPM	Isotope Match Intensity RMS Percent	Response	Adducs
Flavonoids									
Kaempferol-3-glucuronide	$C_{21}H_{20}O_{12}$	465.1028	8.75	-2.7	4	0.86	2.14	9756	-H
Robinetin	$C_{15}H_{10}O_7$	303.0496	12.78	0.3	18	0.45	2.81	564059	+H
7-Hydroxy-1-methoxy-2-methoxyxanthone	$C_{15}H_{10}O_6$	287.0549	14.68	-0.1	4	1.44	2.43	37990	+H
Pedalitin	$C_{16}H_{12}O_7$	317.0656	14.75	0.4	13	1.18	1.38	85008	+H
Quercetin-7-O-[β-D-glucopyranosyl (1−6)-β-D-glucopyranoside]	$C_{27}H_{30}O_{17}$	625.1396	7.21	-2.3	7	1.08	3.43	19328	-H
Isoetin-7-O-β-D-glucopyranosyl-2'-O-α-D-glucopyranoside	$C_{27}H_{30}O_{17}$	625.1410	7.56	0.0	14	1.51	3.19	49076	-H
Quercetin-4′-glucoside	$C_{21}H_{20}O_{12}$	465.1033	7.73	1.3	14	1.15	4.46	41023	+H
Isorhamnetin-3-O-β-gentiobioside	$C_{28}H_{32}O_{17}$	639.1557	8.16	-1.3	4	0.75	4.03	16651	-H
Quercetin-3-O-β-D-glucopyranoside	$C_{21}H_{20}O_{12}$	463.0867	9.19	-3.3	9	1.30	9.92	8004	-H
Limocitrin	$C_{17}H_{14}O_8$	347.0762	10.04	-8.6	2	5.80	6.28	725	-H
Quercimeritrin	$C_{21}H_{20}O_{12}$	463.0883	10.13	0.2	15	2.35	3.82	194628	-H
4′,5,7-Trihydroxy-8-methoxy-flavonol-3-O-β-D-glucopyranoside	$C_{22}H_{22}O_{12}$	477.1034	11.00	-0.9	14	1.53	3.74	73761	-H
Quercetin	$C_{15}H_{10}O_7$	301.0348	12.49	-2.0	18	1.64	2.92	370899	-H
4,7,2′-Trihydroxy-4′-methoxyisoflavanol	$C_{16}H_{16}O_5$	287.0907	15.93	-6.4	5	3.04	9.42	13356	-H
Saponins									
Neohecogenin-3- O-β-D-glucopyranosyl (1−2)-β-D-glucopyranosyl (1−4)-β-D-galactopy-ranoside	$C_{45}H_{72}O_{18}$	901.4764	11.07	-1.8	26	2.33	0.74	64555	+H
Pennogenin-3-O- α-L-arabinofuranosyl (1−4)[α-L-rhamnopyranosyl (1−2)]-β-D-Glucopyranoside	$C_{44}H_{70}O_{17}$	871.4670	11.36	-0.2	39	0.93	7.51	131074	+H
Alkaloids									
Stachydrine	$C_7H_{13}NO_2$	138.0548	0.49	-2.9	12	2.81	4.87	4026	+H
Polyphenol									
Myricetin	$C_{15}H_{10}O_8$	317.0292	6.80	-3.4	17	1.33	4.41	49653	-H
1-Galloyl-glucose	$C_{13}H_{16}O_{10}$	331.0643	1.92	-8.3	8	5.04	9.25	1335	-H

Table 4 Quantitative Test of Ethanol Extract of Red Onion Peel

Extract Quantitative Test	Result
Anthocyanins	17,87 mg/L
Antioxidant	77%

CONCLUSIONS

The LC-ESI-QTOF-MS/MS method was successfully applied to onion peel extract to identify the compounds contained in the onion peel extract, with a total of 19 compounds identified, including flavonoids (14 compounds), polyphenols (2 compounds), saponins (2 compounds), and alkaloids (1 compound). Onion peel extract was qualitatively positive for containing flavonoid compounds, polyphenols, and saponins. Based on the antioxidant activity of the ethanolic extract of the onion peel, an average of 77% was obtained, and the total anthocyanin content was an average of 17.87 mg/L.

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