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INTRODUCTION Nutmeg (Myristica fragrans Houtt.) originated in the Banda Islands of Indonesia1. It has many applications in the culinary, pharmaceutical, and cosmetic industries2. However, after the seeds and maces took, M. fragrans flesh was not utilized optimally and became waste. Therefore, M. fragrans flesh can be developed for functional drinks, which are sources rich in antioxidants good for preventing and treating diseases such as cancer and cardiovascular diseases3,4. The antioxidant activity of several parts of M. fragrans has been established by much research4-9. The antioxidant activity of M.

fragrans seed was the most reported that seed had the greatest reducing property and radical scavenging ability compared to other parts of M. fragrans4. However, Ginting et al.8 reported that extract of M. fragrans flesh had the most significant antioxidant activity compared to the seed. Antioxidant properties contributed by the variety of active phytochemicals from its essential oil1,8, including saponin, alkaloid, tannin, flavonoids, a-pinene, ß-pinene, myrcene, 1,8-cineole, carvacrol, terpinen-4-ol, sabinene, camphene, myristicin, elemicin, isoelemicin, eugenol, isoeugenol, methoxyeugenol, safrole, and lignan7,8,10-13. Myristica fragrans fruit contains tannin compounds that can cause a bitter and sour taste for the functional drinks to be developed.

Therefore, the level of tannins should be reduced by adding flocculant substances from egg white albumin14. Reduced tannins can cause a decrease in antioxidant activity because tannins could be a natural source as an effective natural antioxidant compound15-18. Antioxidant activity of plant extracts correlated with the number of their phenolic compounds that tannin is a phenolic compound19,20. Gupta and Rajpurohit1 reported that total phenolic content and antioxidant activity have a significant and positive correlation.

Besides that, analysis of the correlation with total flavonoid content in which it is the largest group of compounds in the phenolic group should be done. It had been reported that antiradical scavenging has a significantly high level of phenolic and flavonoid content21,22. Vivia et al.14 reported that egg white concentration significantly affected pH, titratable acidity, precipitate percentage, and organoleptic. This indicates that the correlation between tannin precipitation and antioxidant activity has not been studied. Therefore, the purpose of this study is to find out the comparison of antioxidant activity between the combination of M.

fragrans flesh extract with various concentrations of egg white consisting of 0, 1, 2, 3, 4, and 5%, as well as to correlate its antioxidant activities with tannin, total flavonoid, and total phenolic contents. MATERIALS AND METHODS Materials Myristica fragrans fruit (Figure 1) obtained from Sangihe Island of North Sulawesi, Indonesia and determination

carried out in a Biology Laboratory, Faculty of Math and Science, Universitas Sam Ratulangi with a certificate number of 70/LBD.11/IT/2021. The result of the determination states that the plants used in this study were Myristica fragrans Houtt from the family Myrtaceae.

This study also used chemical reagents such as 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS, Sigma-Aldrich), potassium persulfate (Sigma-Aldrich), ethanol p.a. (Merck), tannic acid (Merck), sodium tungstate (Sigma-Aldrich), phosphomolybdic acid (Sigma-Aldrich), phosphoric acid (Sigma-Aldrich), natrium carbonate (Merck), gallic acid (Sigma-Aldrich), Folin-Ciocalteu (Merck), quercetin (Sigma-Aldrich), natrium nitrite (Merck), natrium hydroxide (Merck), aquadest (Bratachem), and water (Danone). // a b Figure 1.

Myristica fragrans whole fruit (a) and its flesh (b) Methods Preparation of aqueous extract Myristica fragrans have been cut into small pieces extracted with water with a sample : solvent ratio of 1:3 w/v23. The resulting M. fragrans extract was heated at a temperature of 80°C and added egg white with a concentration of 0, 1, 2, 3, 4, and 5% while stirring. Then, it was silenced and extracted again to separate the white dregs eggs and M. fragrans extract14. The M. fragrans extract was obtained then dried using an oven at a temperature of 50°C until a constant weight was obtained. Determination of antioxidant activity with DPPH Antioxidant activity of DPPH radical of samples was performed based on Kikuzaki et al.24 with modified. In this assay, as much as 1 mL from each sample solution was briefly taken and added with 3 mL ethanol p.a., then 1 mL of 0.4 mM DPPH.

The mixture was shaken vigorously using vortex for 1 minute and was allowed to stand for 15 minutes at 25°C in the darkroom. The absorbance of each solution was measured using a spectrophotometer at 517 nm using ethanol as blank. The antioxidant activity was calculated as IC50 value. The IC50 value was obtained from a linear regression equation that plotted the percentage of inhibition with the concentration (µg/mL). The equation [1] calculated the percentage of inhibition: %????h??????????????= ?????? ????????????? - ?????? ??????????? ?????? ????????????? ×100% [1] Determination of antioxidant activity with ABTS Antioxidant activity of ABTS radical of samples was performed based on Aktumsek et al.25 with slightly modified. Briefly, ABTS radical cation was produced by reacting 28.406 mg of ABTS and 14 mg of potassium persulfate in 20 mL of aquadest.

The mixture was allowed to stand for 16 hours at room temperature and then added aquadest until 100 mL. As much as 1 mL from each sample solution was briefly taken and added with 3 mL of aquadest then 1 mL of ABTS. The mixture was shaken

vigorously using vortex for 1 minute and allowed to stand for 10 minutes at 25°C in a darkroom. The absorbance of each solution was measured using a spectrophotometer at 730 nm using aquadest as blank. The antioxidant activity was calculated as IC50 value. The IC50 value was obtained from a linear regression equation that plotted the percentage of inhibition with the concentration (µg/mL). The equation [1] calculated the percentage of inhibition.

Determination of tannin content The tannin content of samples was performed based on Pratama et al.26 As much as 2 mL from each sample solution was briefly taken and added with 2 mL Folin-Ciocalteu reagent. The mixture was stood for 3 minutes, then added 2 mL of 7,5% Na2CO3. The mixture stood for 40 minutes. The absorbance of the mixture was determined at 654 nm versus a blank containing all reagents except the samples. The absorption of standard tannin solutions was measured under the same conditions for making a calibration curve. All determinations were carried out in triplicates.

The number of tannin contents in samples was expressed as g tannic acid equivalents (TAE)/100 g of sample (%w/w TAE). Determination of total phenolic content Total phenolic content of samples was performed based on Chun et al.27 As much as 1 mL from each sample solution was briefly taken and added with 0.4 mL Folin-Ciocalteau reagent. The mixture was stood for 5 minutes, then added 4 mL of 7% Na2CO3 and aquadest until 10 mL. The mixture stood for 2 hours. The absorbance of the mixture was determined at 755 nm versus a blank containing all reagents except the samples. The absorption of standard phenolic solutions was measured under the same conditions for making a calibration curve.

All determinations were carried out in triplicates. The amount of total phenolic contents in samples was expressed as g gallic acid equivalents (GAE)/100 g of sample (%w/w GAE). Determination of total flavonoid content The total flavonoid content of samples was performed based on Zou et al.28 As much as 1 mL from each sample solution was briefly taken and added with 4 mL aquadest and 0.3 mL of 10% NaNO2. The mixture was stood for 6 minutes, then added with 0.3 mL of 10% AlCl3, 4 mL of 10% NaOH, and aquadest until 10 mL. The mixture stood for 15 minutes.

The absorbance of the mixture was determined at 495 nm versus a blank containing all reagents except the samples. The absorption of standard flavonoid solutions was measured under the same conditions for making a calibration curve. All determinations were carried out in triplicates. The number of total flavonoid contents in samples was expressed as g quercetin equivalents (QE)/100 g of sample (%w/w QE). Statistical analysis All data were presented as mean±standard deviation (SD) for at least three

replications for each sample.

Statistical analysis was performed based on the independent sample T-test level 95% using SPSS (SPSS Corporation, Chicago, IL) version 16.0 for Windows. Linear regression to correlate between total phenolics and total flavonoid with antioxidant activities was carried using Microsoft Excel 2010. RESULTS AND DISCUSSION Antioxidant activity of Myristica fragrans flesh extract Antioxidant activity of M. fragrans flesh extract (MfFE) can be measured by several methods, such as the capacity to scavenge the stable free radical (DPPH and ABTS), ferric reducing/antioxidant power assay (FRAP), chelating agent, inhibition of lipid peroxidation, inhibition of bleaching of ß-carotene, and other1,22. In this study, the antioxidant activity of MfFE was measured by the capacity to scavenge the stable free radical (DPPH and ABTS).

Using the DPPH method, the MfFE showed free radical scavenging activity based on its ability to donate hydrogen atoms29, reducing the purple color of the DPPH solution at a wavelength of 517 nm30. The decrease in absorbance value can be seen visually with changes in color from purple to yellow. In the ABTS method, the MfFE showed free radical scavenging activity based on stabilizing free radicals by donating proton radicals29. The antioxidant activity of MfFE using DPPH and ABTS radical was evaluated based on inhibitory concentration (IC50) parameters, as seen in Table I.

Table I showed the IC50 value of MfFE classified in the category of strong antioxidant31 in scavenging the stable free radical ABTS (0% MfFE), an intermediate antioxidant in scavenging the stable free radical DPPH (0% MfFE), and weak antioxidant for another sample in scavenging the stable free radical DPPH and ABTS31. The antioxidant activity of the samples was not better than ascorbic acid's antioxidant activity. However, the antioxidant activity of sample 0% MfFE in this research was more potent than the antioxidant activity reported by Assa et al.32, Ginting et al.8, and Selonni33.

This can be influenced by the different areas of the sample collected, the solvent, and the extraction method used. The sample obtained by Ginting et al.8 and Selonni33 comes from outside North Sulawesi. Ginting et al.8 reported that samples of MfFE were extracted using the water distillation method to produce essential oils. This caused the antioxidant activity to be influenced by the compounds contained in the essential oil. Selonni33 reported that samples of M. fragrans flesh extracted using maceration method with solvent ethanol 70% has better antioxidant activity (IC50 219.2 µg/mL) than Ginting et al.8 This can be influenced by the extracted components, not just the essential oil.

Antioxidant activity reported by Assa et al.32 (1372.91 µg/mL) is lower than all, even

though the samples were from the same province as the samples in this study. Assa et al.32 reported that samples dried before extraction, whereas this study used fresh samples. The sample drying process can damage compounds that contribute as antioxidants34. Table I. Antioxidant activity of M. fragrans flesh extract Sample _Antioxidant activity _ _ _DPPH - IC50 ± SD (µg/mL) _ABTS - IC50 ± SD (µg/mL) _ _0% MfFE* _105.669 ± 0.102 _89.980 ± 0.480 _ _1% MfFE** _252.668 ± 4.137 _252.163 ± 1.418 _ _2% MfFE _299.543 ± 1.487 _313.523 ± 2.956 _ _3% MfFE _365.568 ± 1.593 _348.755 ± 7.400 _ _4% MfFE _381.047 ± 2.417 _389.138 ± 6.886 _ _5% MfFE _387.536 ± 1.687 _481.778 ± 2.124 _ _Ascorbic acid _0.539 ± 0.001 _0.699 ± 0.004 _ _*M. fragrans flesh extract without egg white addition; **M.

fragrans flesh extract with 1% egg white addition, and so on Myristica fragrans flesh extract has different antioxidant activities based on two methods of DPPH and ABTS. The statistical test showed the mean of each group in the DPPH method was 2.5608 x 102, which was lower than ABTS with 2.6801 x 102 but not significantly different ($p > 0.05$). This can be caused by the radical's mechanism reaction. The DPPH and ABTS assays are classified as Single Electron Transfer (SET) reactions35. The different results reported by Martysiak-Zurowska and Wenta36, with the total antioxidant capacity of human milk determined by the ABTS method, were significantly higher than those reported in the DPPH assay.

It can be affected by the difference in the solvent used. Martysiak-Zurowska and Wenta36 used methanol to dissolve DPPH, but in this research used ethanol p.a. Methanol is a solvent with reactions in strong hydrogen-bonding, which interferes with the release of hydrogen atoms, thus enormously enhancing SET over Hydrogen Atom Transfer (HAT)37. According to Table I, the antioxidant activity of samples was decreased with the addition of egg white.

This situation can happen because polyphenols compound can affect interaction with protein from egg white by a covalent and non-covalent bond so that higher concentration of egg white added to the M. fragrans flesh extract caused stronger interaction between polyphenols and protein38-41. McRae and Kennedy42 reported that the protein's binding mechanism of polyphenols (tannins) is divided into three different stages, which occur by the presence of ionic bonds, hydrophobic interactions, and hydrogen bonds.

Ionic bonds occur on the cation side (as a protons compound receptor) from proteins (NH_3^+) and a negatively charged hydroxyl group (as a proton donor) from tannin compounds (O^-). Hydrophobic interactions occur between the aromatic ring of polyphenols with the pyrrolidine group, which is the hydrophobic side of the protein.

Hydrogen bonds occur between the hydroxyl (OH) groups of phenolic compounds (tannins) and the carboxyl group (COO) of the protein. The bond formed causes the polyphenols content to decrease when the egg white is filtered.

In this case, the content of tannins, which are polyphenol compounds, is expected to decrease to reduce the sour and bitter taste in the development of functional drinks. Correlation analysis of antioxidant activities with tannin, total phenolic, and total flavonoid contents of Myristica fragrans flesh extract This research showed that M. fragrans flesh extract contains condensed tannins. The condensed tannins precipitated using protein, such as egg white43. The higher concentration of egg white used causes more significant precipitation so that tannin, total phenolic, and total flavonoid contents of M.

fragrans flesh extract decreased, as shown in Table II. Tannin contents expressed in tannic acid equivalents (TAE) (Figure 2), total phenolic contents expressed in gallic acid equivalents (GAE) (Figure 3), and total flavonoid contents expressed in quercetin equivalents (QE) (Figure 4). Table II. Tannin, total phenolic, and total flavonoid contents of M. fragrans flesh extract Sample _Tannin content ± SD (%w/w TAE) _Total flavonoid content ± SD (%w/w QE) _Total phenolic content ± SD (%w/w GAE) _ _0% MfFE _14.034 ± 0.100 _26.929 ± 0.129 _53.164 ± 0.129 _ _1% MfFE _6.674 ± 0.093 _16.080 ± 0.146 _24.508 ± 0.020 _ _2% MfFE _4.934 ± 0.015 _12.985 ± 0.146 _17.483 ± 0.020 _ _3% MfFE _3.218 ± 0.007 _11.523 ± 0.080 _13.446 ± 0.017 _ _4% MfFE _3.142 ± 0.008 _10.684 ± 0.030 _12.448 ± 0.017 _ _5% MfFE _2.293 ± 0.018 _8.255 ± 0.064 _9.727 ± 0.052 _ _ / Figure 2. Calibration curve of standard tannic acid for determination of tannin content / Figure 3.

Calibration curve of standard gallic acid for determination of total phenolic content / Figure 4. Calibration curve of standard quercetin for determination of total flavonoid content Tannins are a flavonoid compound because its structure, which has two aromatic rings bonded by three carbon atoms44. Flavonoids are a class of phenolic secondary metabolite compounds characterized by a benzo-pyrone structure45. Reduced tannin contents caused the total phenolic and flavonoid contents to decrease.

Therefore, tannin, total flavonoid, and total phenolic contents of M. fragrans flesh extract often correlated with its antioxidant activity43,46. Its correlation could be seen in Figures 5 to 10 based on coefficient determination (R2) value. The antioxidant mechanism of tannic acid is still far from being fully understood; therefore, it requires further investigation47. Figures 5 and 6 indicated that tannin compounds contributed 97.63% toward the DPPH radical scavenging and 90.91% toward the ABTS radical scavenging in the evaluated M. fragrans flesh extract. Figures 7 and 8 showed a

correlation between radical scavenging activity and the total flavonoid contents of M. fragrans flesh extract, namely 96.68% for DPPH radical and 94.61% for ABTS radical.

The antioxidant mechanism of flavonoids compounds as radical scavenging influenced by hydroxylation of ring B and a C2–C3 double bond connected with a C-3 hydroxyl group and a C-4 carbonyl group, whereas hydroxylation of ring A also enhances the activity, as does the presence of gallate and galacturonate moieties as a substituent on the flavonoid skeleton48. The antioxidant mechanism of phenolic compounds neutralizes lipid free radicals and prevents the decomposition of hydroperoxides into free radicals21. Based on that mechanism was obtained, the results were in Figures 9 and 10. The correlation between radical scavenging activity of DPPH and ABTS with total phenolic contents of M. fragrans flesh extract was 95.91% and 89.23%, respectively. / Figure 5.

Correlation between radical scavenging activity of DPPH with tannin contents of M. fragrans flesh extract / Figure 6. Correlation between radical scavenging activity of ABTS with tannin contents of M. fragrans flesh extract / Figure 7. Correlation between radical scavenging activity of DPPH with total flavonoid contents of M. fragrans flesh extract / Figure 8. Correlation between radical scavenging activity of ABTS with total flavonoid contents of M. fragrans flesh extract / Figure 9. Correlation between radical scavenging activity of DPPH with total phenolic contents of M. fragrans flesh extract / Figure 10. Correlation between radical scavenging activity of ABTS with total phenolic contents of M.

fragrans flesh extract Tannin, total flavonoid, and total phenolic contents contribute to inhibiting DPPH radicals than ABTS radicals. A similar result was discovered by our previous research22 who had demonstrated that total flavonoid and total phenolic contents more contribute to the antioxidant activity by DPPH than the ABTS method. The antioxidant activity of M. fragrans flesh extract is influenced by tannin, total flavonoid, and total phenolic contents about 89.23-97.63%.

It indicates that the three compound's contribution is very high so that the antioxidant activity of samples decreased significantly from 105.669 ± 0.102 to 387.536 ± 1.687 µg/mL for the DPPH and 89.980 ± 0.480 to 481.778 ± 2.124 µg/mL for ABTS method. Besides that, the correlation report 2.37-10.68% antioxidant activities may also come from other antioxidant secondary metabolites in the M. fragrans flesh extracts49. CONCLUSION Myristica fragrans flesh extracts are classified as a strong antioxidant in scavenging the stable free radical ABTS (0% MfFE) and intermediate antioxidant in scavenging the stable free radical DPPH (0% MfFE).

The tannin, total flavonoid, and total phenolic contents strongly influence its antioxidant activity. Therefore, precipitation from the tannin - protein (egg white) bond caused antioxidant activities to decrease.

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