Media Farmasi: Jurnal Ilmu Farmasi (Journal of Pharmaceutical Science) e-ISSN 2503-5223
Vol. 20, No. 2, September 2023, pp. 44-50
http://journal.uad.ac.id/index.php/Media-Farmasi/index

The Potential of Sumbawa Honey (*Apis dorsata*) as A Natural Antioxidant

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ARTICLE INFO

Article history
Received: 03-01-2023
Revised: 02-04-2023
Accepted: 08-05-2023

Keywords
ABTS
Trolox
Freeze dry
Partition

ABSTRACT

Honey is a natural ingredient that Indonesian people widely use to maintain a healthy body. One of the benefits of honey is as an antioxidant. This antioxidant activity is influenced by the producing bee, the source plant, and the producing area. Antioxidants are chemicals that stop free radicals from oxidizing and causing harm or destruction. This research proposes to ascertain the antioxidant activity of Sumbawa honey from West Nusa Tenggara's ethanol extract, fractions of n-hexane, ethyl acetate, and water. Honey samples were collected and dried using freeze-drying, then macerated with 70% ethanol. The viscous extract obtained was then fractionated using a liquid-liquid extraction method using n-hexane, ethyl acetate, and ethanol-water as solvents so that the n-hexane (NF), ethyl acetate (EF), and water (WF) fractions were obtained. The ABTS technique was applied to assess the antioxidant activity of each sample, and Trolox was used as a reference. Antioxidant activity test using a UV-Vis spectrophotometer at a wavelength of 734 nm and operating time of 25th minutes. The results obtained were ethanol extract, NF, EF, and AF had IC_{50} values respectively 96.054; 106.953; 70.206; and 101.649 g/mL. That indicates that the ethyl acetate fraction has the strongest antioxidant activity. Comparatively, the antioxidant activity of Trolox has an IC_{50} value of 18.463 mg/ml. Trolox, a substance in the extremely strong category, has been able to outperform the antioxidant activity of the ethyl acetate fraction.

1. Introduction

One of the efforts made by the community to maintain their health is to consume foods that are beneficial to the body. The public consumes a lot of honey since it is a natural product with medicinal qualities and nutritional value. Honey is a natural substance that bees produce from flower nectar and plant secretions (Samarghandian et al., 2017). Indonesia has various types of honey products, one of which is famous for Sumbawa honey. This honey comes from forests in the Sumbawa archipelago, mainly produced by *Apis dorsata* bees, and has become one of the superior products of Sumbawa Regency, Indonesia (Rodiahwati et al., 2019). So far, Sumbawa honey is believed to have the efficacy of increasing stamina and increasing the immune system. Honey is an effective supplement traditionally used to treat several diseases. Antioxidant, antibacterial, anti-inflammatory, anti-proliferative, anti-cancer, and antimetastatic benefits have been linked to honey components (Samarghandian et al., 2017). The chemical constituents of the various forms of honey are responsible for the health advantages of honey. The content of various phytochemicals that act as antioxidants in honey depends on geographical and climatic conditions (Saputri & Putri, 2017). An antioxidant is a substance that is stable enough to provide an unchecked free radical an electron and neutralize it, reducing the radical's potential for harm. Free radical scavenging properties of these antioxidants are principally responsible for postponing or preventing cellular damage (Anwar et al., 2018; Arias et al., 2022; Hunyadi, 2019). Consuming antioxidants strengthens the immune system

and helps to stave off diseases (Arias et al., 2022; Ludfiyaningrum & Gustari, 2021; Roosdiana et al., 2019).

Sumbawa honey contains saponins and flavonoids (Yelin & Kuntadi, 2019). Honey also contains phenolic compounds, enzymes, and minerals. Honey's antioxidant properties are due to a phenolic component (Arias et al., 2022). Non-enzymatic antioxidant groups found in honey include phenolic acids, flavonoids (flavanones and flavanols), carotenoids, and organic acids. The total flavonoid content of honey extracted with microwave assistance and 30% propylene glycol was 519.05 g/mL (Rodiahwati et al., 2019). Previous research utilizing the DPPH method demonstrated the antioxidant properties of honey from diverse Sumbawa sub-districts with an IC_{50} value of 60.2 - 572.3 mg/mL (Saputri & Putri, 2017). Significant discrepancies resulted from dihydrochalcones and flavanones not reacting with the DPPH instead of the ABTS. The ABTS test should be used instead to evaluate the antioxidant ability of dihydrochalcone or flavanone-rich extracts (Platzer et al., 2021).

There haven't been many studies undertaken regarding Sumbawa honey's antioxidant capacity. Previous research has shown that honey from several sub-districts in Sumbawa has no activity because it has an IC_{50} value of more than 500 mg/mL and is known to have a high flavonoid content. This research attempts to separate the content of Sumbawa honey into several fractions based on the decrease in polarity from polar to non-polar and further evaluate the activity of these fractions as antioxidant agents. The ethanol extract and Sumbawa honey fraction were tested for their antioxidant capacity using the ABTS method, and Trolox was used as a comparison.

2. Materials and Methods

2.1. Preparation of samples

The ingredients used are Sumbawa forest honey (*Apis dorsata*) obtained from Lantung village, Lantung district, Sumbawa district, and West Nusa Tenggara. The solvents used were 70% ethanol, analytical grade ethanol, distilled water, n-hexane, and ethyl acetate. Other chemicals used are ABTS (2,2- Azinobis 3-Ethylbenzothaizoline-6- Sulfonic Acid), analytical grade (Merck), and Trolox (Sigma).

The tools used are a set of glassware (Iwaki pyrex), a set of maceration tools, a freeze dryer (Thermo Fisher Scientific), electric scales (Ohaus), rotary evaporator (Heidolph), clamps and stands, UV-Vis 1800 spectrophotometer (Shimadzu), as well as a micropipette.

2.2. Processes of Extract Making

Sumbawa honey was dried using the freeze-drying method at the Food Science laboratory at UNIKA Soegijapranata Semarang. Honey samples of as much as 1388.7 grams were dried for 48 hours at a temperature of -100°C. The maceration method extracted as much as 472.2 g of Sumbawa honey powder. The solvent used is 70% ethanol with a ratio of 1:10, so the total solvent used is 4.722 ml. The maceration process was carried out for three days, stirring twice daily. To produce a thick ethanolic extract of Sumbawa honey, the resulting macerate was subsequently concentrated using a rotary evaporator at a temperature of 50°C.

2.3. Extract Partitions

Extract fractionation was carried out by liquid-liquid partitioning using a separating funnel. Distilled water, n-hexane, and ethyl acetate were used as the solvents for the fractionation process. The thick extract was mixed using distilled water and then poured into a separate funnel with n-hexane added. The mixture is shaken and allowed to stand until it separates into 2 phases. The phases are separated, and the aqueous phase is returned to the separatory funnel. In a separating funnel, n-hexane solvent was added again, and the same method was carried out until the n-hexane phase obtained was clear. The remaining aqueous phase from the n-hexane fraction was then fractionated by adding ethyl acetate solvent, shaking again, and leaving until the separation occurred. This process is carried out until the ethyl acetate phase is clear, and then the ethyl acetate solvent and water are separated.

2.4. Determination of Antioxidant Activities

Testing of antioxidant capabilities starts by determining the maximal wavelength (λ) and operating time. Determination of the antioxidant activity of Trolox solution (5, 10, 15, 20, and 25 ppm) and fraction solution (25, 50, 75, 100, and 125 ppm), 1 mL of each pipette and 1 mL of ABTS solution

added, then allowed to stand in a place that is protected from light during the operating time. The absorption is measured with a UV-vis spectrophotometer at the maximum wavelength. Visualization of the methodology is presented in Figure 1.

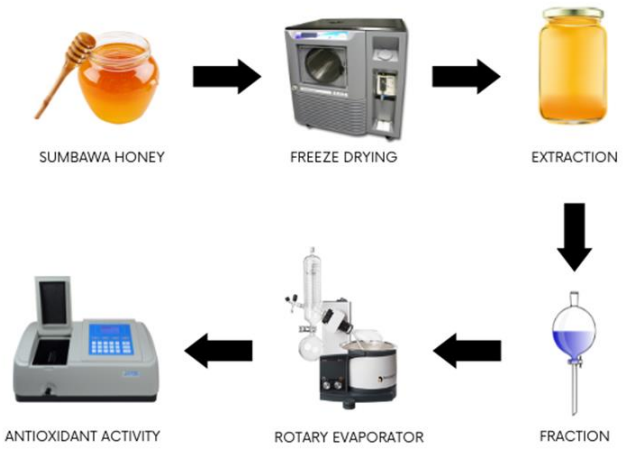

Fig. 1. Visualization of methodology

2.5. Data Analyzes

The data obtained on the determination of antioxidant activity in the form of absorbance values of the extract and the SHEE fraction were then analyzed using linear regression y = bx + a, between the concentration series (x) and % antioxidant activity (y). The IC_{50} value is obtained when the y value in the linear regression equation is 50. The amount of % antioxidant activity is calculated by the formula 1 (Winarsi, 2007) :

$$\% \text{ antioxidant activity} = \frac{\text{control absorbance- absorbance of test solution}}{\text{control absorbance}} \times 100\% \quad (1)$$

3. Results and Discussion

Drying Sumbawa honey by freeze drying or lyophilization method aims to reduce the water content in honey. The principle of freeze drying is drying food by removing the water content through the sublimation process; the water content that has become frozen is then converted into a gas (Nowak & Jakubczyk, 2020). 1388.7 g of Sumbawa honey produced 524.6 g of honey powder. The drying shrinkage obtained was 62.22%, and the honey yield was 37.77%. The Sumbawa honey extraction process uses the maceration method because this method can extract compounds that are efficacious and avoid damage to active substances due to excessive heating. The solvent used is 70% ethanol because the solvent is a universal solvent that can attract polar and non-polar compounds. Stirring in the extraction process aims to increase the contact between the sample and the solvent during the maceration process to attract compounds optimally. The extract of Sumbawa honey is light brown and can be seen in Figure 2.

Fig 2. Sumbawa Honey Extract

The Sumbawa honey extract was then gently fractionated using three solvents with various amounts of polarity. The solvents in the fractionation process are n-hexane, ethyl acetate, and water.

Various solvents enhance the withdrawal process by attracting the active ingredients in Sumbawa honey. The extract and partition yields can be seen in Table 1.

The water fraction has the largest yield compared to other fractions. Since the polyphenols are widely distributed in nature and are mostly found in glycosides, honey-producing bees can add these substances to honey by collecting plant nectar (Santos et al., 2021) . Glycosides contain aglycones bound to sugars, which are water-soluble molecules. It causes the yield of the water fraction to be greater than the other fractions.

ABTS method was used for antioxidant capabilities testing. Determining the maximum wavelength and operating time is the first step in the testing process. The maximum wavelength obtained in this study is 734 nm. The results follow the literature; the ABTS method's maximum wavelength is 414-417 nm and 730-734 nm (Ilyasov et al., 2020). The outcomes of the operating time measurement revealed that the 20-30 minute range showed a stable absorbance value of 0.723. Based on these results, it was determined that the operating time used was the 25th minute.

Table 2 shows that the ethyl acetate fraction has an IC_{50} value belonging to the strong category (Molyneux, 2004). Decreasing the IC_{50} value indicates increasing the antioxidant content in the sample. The ABTS method is better than the DPPH method in testing the antioxidant activity of foods containing hydrophilic and highly pigmented compounds. The ABTS cationic radical is soluble in organic and aqueous solutions, unlike the DPPH radical, which dissolves only in an organic medium. Thus, the ABTS test can screen lipophilic and hydrophilic substances (Sadeer et al., 2020; Gaber et al., 2023; Wołosiak et al., 2021). Table 2 displays the outcomes of the test for antioxidant activity.

The ABTS method for measuring antioxidant activity is efficient, straightforward, adaptable, and simple to repeat. ABTS, a radical with a nitrogen core and a recognizable turquoise blue hue, is reduced by antioxidants into a colorless non-radical state. This color change is brought about by giving hydrogen atoms from antioxidants to ABTS radicals. This type of testing aims to compare antioxidants' capacities for reducing ABTS free radicals. Usually, the ABTS radical is prepared the day before by mixing potassium persulfate and ABTS and then left overnight (12–16 hours). Potassium persulfate will oxidize ABTS stoichiometrically to form free radicals, easily observed with a color change (Ilyasov et al., 2020).

Phenolic acids and flavonoids are responsible for honey's well-known antioxidant effects. Some of the mechanisms of honey as an antioxidant are free radical scavenging, hydrogen transfer, metal ion chelation, the impact of flavonoid substrates for hydroxyl, and the activity of superoxide radicals (Ahmed et al., 2018). The n-hexane extract had the weakest antioxidant capabilities among the other fractions and was included in the medium category. The n-hexane fraction of Sumbawa honey contains non-polar flavonoids that are still bound to their glycoside groups. It prevents ABTS radicals from binding to these non-polar flavonoids and has a slight antioxidant effect. Due to steric hindrance, glycoside prevents flavonoids from transferring hydrogen and electrons to fight free radicals (Anwar et al., 2018; Arias et al., 2022). Methylated flavonoids can also result from other groups in the n-hexane fraction. Due to the reduction of the -H atom, a proton source for free radical scavenging, converting the -H atom into a methyl group (-CH3) during a methylation reaction can lessen antioxidant effectiveness (Hernández-Rodríguez et al., 2019; Sarian et al., 2017). The results of the ABTS technique were utilized for the antioxidant effect in this investigation, which was restricted to samples from a single location in Sumbawa. Implications of this research show that Sumbawa honey has many benefits. The form of utilization of these products was used as food ingredients, supplements, and medicines. The results of this study are expected to be useful and support future Sumbawa honey research.

Table 1. Extract and Partition Yields

Sample	Yield (gram)	Rendemen (%)
Extract	435.2	92.2
N-Heksan Fraction	12.3	4.4
Ethyl acetate Fraction	49	17.5
Water Fraction	256.1	91.5

Table 2. Outcomes of The Antioxidant Activity Test Using ABTS Method

Sample	Concentration (µg/mL)	Antioxidant Activity (%)	IC_{50} (µg/mL)
Trolox	5	11.835	18.463
	10	26.666	
	15	41.049	
	20	54.719	
	25	67.490	
Extract	25	14.082	96.054
	50	27.303	
	75	38.090	
	100	52.509	
	125	64.719	
N-Heksan Fraction	25	11.573	106.953
	50	23.296	
	75	36.067	
	100	46.741	
	125	57.940	
Ethyl acetate Fraction	25	37.266	70.206
	50	44.157	
	75	49.138	
	100	59.326	
	125	67.416	
Water Fraction	25	12.322	101.649
	50	24.831	
	75	36.367	
	100	50.524	
	125	60.749	

4. Conclusion

Sumbawa honey ethanol extract has antioxidant activity. The ethyl acetate fraction had the maximum antioxidant activity using the IC_{50} criterion, with a 70.206 g/mL value. The identification of natural flavonoids as a reliable and risk-free source of antioxidants opens up new avenues for the investigation of other flavonoid molecules, with an emphasis on novel structures and the application of novel scientific techniques and resources.

Author Contributions: The study was thought of and designed by Gharsina Ghaisani Yumni. Gharsina Ghaisani Yumni carried out every data analysis. The results were evaluated by Fina Listiana, Septi Puspita Sari, and Siti Nadhiroh and were revised by Gharsina Ghaisani Yumni and Sumantri. Writing the manuscript was Gharsina Ghaisani Yumni. The final manuscript was read and approved by all writers.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, Or not-for-profit sectors.

Competing Interests

The authors declare no conflict of interest.

Acknowledgment

Not available.

References

Ahmed, S., Sulaiman, S. A., Baig, A. A., Ibrahim, M., Liaqat, S., Fatima, S., Jabeen, S., Shamim, N., & Othman, N. H. (2018). Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. *Oxidative Medicine and Cellular Longevity*, *2018*, 1–19. https://doi.org/10.1155/2018/8367846.

Anwar, H., Hussain, G., & Mustafa, I. (2018). Antioxidants from natural sources. In *Antioxidants in Foods and Its Applications*. InTech. https://doi.org/10.5772/intechopen.75961.

Arias, A., Feijoo, G., & Moreira, M. T. (2022). Exploring the potential of antioxidants from fruits and vegetables and strategies for their recovery. *Innovative Food Science & Emerging Technologies*, *77*, 102974. https://doi.org/10.1016/j.ifset.2022.102974.

Gaber, N. B., El-Dahy, S. I., & Shalaby, E. A. (2023). Comparison of ABTS, DPPH, permanganate, and methylene blue assays for determining antioxidant potential of successive extracts from pomegranate and guava residues. *Biomass Conversion and Biorefinery*, *13*(5), 4011–4020. https://doi.org/10.1007/s13399-021-01386-0.

Hernández-Rodríguez, P., Baquero, L. P., & Larrota, H. R. (2019). Flavonoids. In *Bioactive Compounds* (pp. 265–288). Elsevier. https://doi.org/10.1016/B978-0-12-814774-0.00014-1.

Winarsi, H. (2007). *Antioksidan alami dan radikal bebas*. Kanisius : Yogyakarta.

Hunyadi, A. (2019). The mechanism(s) of action of antioxidants: From scavenging reactive oxygen/nitrogen species to redox signaling and the generation of bioactive secondary metabolites. *Medicinal Research Reviews*, *39*(6), 2505–2533. https://doi.org/10.1002/med.21592.

Ilyasov, I. R., Beloborodov, V. L., Selivanova, I. A., & Terekhov, R. P. (2020). ABTS/PP Decolorization Assay of Antioxidant Capacity Reaction Pathways. *International Journal of Molecular Sciences*, *21*(3), 1131. https://doi.org/10.3390/ijms21031131.

Ludfiyaningrum, S. D., & Gustari, S. (2021). Pengaruh penggunaan madu sumbawa dan madu olahan berbagai konsentrasi terhadap kualitas spermatozoa epididimis domba setelah penyimpanan dalam refrigerator. *Ovozoa Journal of Animal Reproduction*, *10*(1), 1. https://doi.org/10.20473/ovz.v10i1.2021.1-6.

Molyneux, P. (2004). The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, *26*(2), 211–219.

Nowak, D., & Jakubczyk, E. (2020). The freeze-drying of foods—the characteristic of the process course and the effect of its parameters on the physical properties of food materials. *Foods*, *9*(10), 1488. https://doi.org/10.3390/foods9101488.

Platzer, M., Kiese, S., Herfellner, T., Schweiggert-Weisz, U., Miesbauer, O., & Eisner, P. (2021). Common trends and differences in antioxidant activity analysis of phenolic substances using single electron transfer based assays. *Molecules*, *26*(5), 1244. https://doi.org/10.3390/molecules26051244.

Rodiahwati, W., Ariskanopitasari, A., & Saleh, I. K. (2019). Identification of total bioflavonoid compound of propolis extract from wild honey bee hives apis dorsata in Sumbawa region, Indonesia. *KMUTNB International Journal of Applied Science and Technology*, *12*(1). https://doi.org/10.14416/j.ijast.2019.01.007.

Roosdiana, A., Permata, F. S., & Graf, P. S. (2019). The Sumbawa forest honey as preventive agent for organ damaged caused by environmental pollution. *IOP Conference Series: Earth and Environmental Science*, *239*, 012031. https://doi.org/10.1088/1755-1315/239/1/012031.

Sadeer, N. B., Montesano, D., Albrizio, S., Zengin, G., & Mahomoodally, M. F. (2020). The

versatility of antioxidant assays in food science and safety—chemistry, applications, strengths, and limitations. *Antioxidants*, *9*(8), 709. https://doi.org/10.3390/antiox9080709.

Samarghandian, S., Farkhondeh, T., & Samini, F. (2017). Honey and health: a review of recent clinical research. *Pharmacognosy Research*, *9*(2), 121–127.

Santos, A. C. dos, Biluca, F. C., Braghini, F., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2021). Phenolic composition and biological activities of stingless bee honey: An overview based on its aglycone and glycoside compounds. *Food Research International*, *147*, 110553. https://doi.org/10.1016/j.foodres.2021.110553.

Saputri, D. S., & Putri, Y. E. (2017). Aktivitas antioksidan madu hutan di beberapa kecamatan di kabupaten Sumbawa Besar. *Jurnal Tambora*, *2*(3). https://doi.org/10.36761/jt.v2i3.170.

Wołosiak, R., Drużyńska, B., Derewiaka, D., Piecyk, M., Majewska, E., Ciecierska, M., Worobiej, E., & Pakosz, P. (2021). Verification of the conditions for determination of antioxidant activity by ABTS and DPPH Assays—A practical approach. *Molecules*, *27*(1), 50. https://doi.org/10.3390/molecules27010050.

Yelin, A., & Kuntadi. (2019). *Phytochemical identification of honey from several regions in Java and Sumbawa*. 080024. https://doi.org/10.1063/1.5115762.