Journal of Health and Nutrition Research
Published by
Media Publikasi Cendekia Indonesia
https://www.journalmpci.com/index.php/jhnr/index
ISSN : 2829-9760 (Online - Elektronik)
ISSN 2829-9760

Original Research
Journal of Health and Nutrition Research, Vol. 1 No. 3 (2022): 178-184

Antioxidant Activity from Multiple Extraction of Kratom Leaf (Mitragyna speciosa) without Veins with Sonicator-Type Bath

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Abstract
Kratom is one of the export commodities from West Kalimantan, containing alkaloids that are beneficial to health. The export product currently in demand is extracted flour. So far, the most widely used extraction method is conventional single extraction, using maceration which is inefficient, long and consumes a lot of solvents. This study examined the process of alkaloid extraction using the technology of drying with a microwave and extraction using a sonicator. The study aimed to determine the microwave drying method which produces kratom leaf alkaloid levels and the type of solvent which produces the strongest IC-50 antioxidant properties from the bath-type sonicator multilevel extraction method. The research method was divided into 2 stages, namely, stage 1 drying with 2 ingredients, kratom leaves with veins and without veins, then tested for alkaloid levels. The leaves material with high alkaloids was continued for phase II of the IC-50 antioxidant property test from various solvents (hexane, methanol, ethanol) using multilevel extraction with a bath-type sonicator. Data analysis was carried out descriptively. The results showed that leaves without veins produced relatively higher levels of alkaloids than leaves with veins. The type of burner that produced IC-50 antioxidant activity was 0.3 ppm ethanol extract. Thus, these results can be a recommendation to stakeholders.

Keywords: Mitragyna speciosa, leaf vein, antioxidants, extraction, alkaloids

Key Messages:
- The highest extract yield from the sonication bath method was methanol extract while the strongest IC-50 antioxidant activity was 0.31 ppm ethanol extract

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Quick Response Code
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Received: 16 November 2022
Accepted: 30 November 2022
DOI: https://doi.org/10.56303/jhnresearch.v1i3.64

1. Introduction

Kratom (*Mitragyna speciosa*) is a medicinal plant that has been empirically used by people in Kapuas Hulu District, West Kalimantan. Kratom trees are classified as NTFPs (Non-Timber Forest Products) forestry plants because they originally lived in the forest and the leaves were harvested. This can be seen from several publications that mention kratom is included in NTFPs (1). Initially, kratom in the local wisdom of the people of Kapuas Hulu was consumed to increase energy vitality for farmers who often work in the hot sun, to be able to withstand thirst, so they don't get tired quickly due to hard work. In addition, kratom is also said to have an anti-cancer effect (2,3), antidiabetic (4), antimicrobial (5,6), and antioxidant (3). The bioactive metabolites that have

been widely reported and studied in kratom leaves are the alkaloids mitragynine and the polyphenol quercetin (7–10).

To extract bioactive compounds from plants requires a specific extraction method, but the preparation of the material before extraction is that the leaf material must have low water content, meaning that the water content has been reduced as low as possible. This aims to maximize the solubility of the solvent that will be carried out at the extraction stage. Conventional drying methods have often been carried out on kratom leaves starting from drying in the sun and room temperature, however this has never been done using a microwave.

Microwave drying has been widely used to help remove the moisture content of leaf material in food (11,12). The advantages of using a microwave include that it is more effective and does not damage the bioactive content unless the power used is very high (up to 1000 watts). For this reason, it is necessary to study whether with very high power it is still possible to dry the leaves and produce high quality. The shape of the leaf material varies, from having a thin to very thick texture, from having a small surface area to being very large so that the surface area is also getting higher. To find out whether there is an effect on the shape of the leaves, the leaves in the study were divided into kratom leaves without veins and kratom leaves with veins. This is in accordance with the form of the raw material, namely kratom leaves whose veins have been removed as an export material. It is suspected that the smaller the leaf surface area, the higher the damage to the bioactive contained, particularly when using high power, high temperature, and relatively long time with microwaves.

Extraction methods are very diverse from conventional methods and modern methods. Many conventional methods are inefficient because they require a lot of solvents and the extraction time is relatively long, even though this method is very simple, the results are inconsistent, and there are safety issues due to the large quantity of solvent involved (13). Modern methods are very efficient but not economical because generally the equipment costs are very expensive and the energy consumption is high, such as sonication, but the extract quality is very high. Ultrasonicators are non-destructive and non-invasive (14). Microwaves and ultrasonicators work through the working mechanism of sound waves. Various extractions of the main alkaloids of kratom (mitragynine) have been carried out including using water, ethanol, ethyl acetate, methanol, hexane, chloroform, dichloromethane, petroleum ether (15,16). Leaves are generally green because they contain chlorophyll while chlorophyll belongs to the lipid group and only dissolves in non-polar solvents such as hexane. In this study, only hexane, ethanol, and methanol solvents will be used.

The extraction method that has been carried out so far has focused on mitragynine, which is cold maceration at room temperature (17) and sonication (18). However, only a few publications mention the yield, total alkaloids, and quantity of mitragynine results in extraction, fraction, or isolation from kratom powder, it is rarely tested for functional properties such as antioxidants. Because the development of the kratom product industry is developing in West Kalimantan, it is necessary to try using technology because the demand for extracts is very high abroad, it is necessary to study whether modern methods can be applied to produce the best quality alkaloids.

2. Methods

Experimental research conducted in the Lab. Food Chemistry, Food Technology, and Food Design, Faculty of Agriculture; Faculty of Forestry, and Faculty of Mathematics and Natural Sciences, Tanjungpura University. The implementation was carried out from August-October 2022. The material used was fresh leaves from the kratom garden in Kubu Raya Regency were picked and grouped into 2 types, those without veins and leaves with veins. The chemicals used for analysis were DPPH, hexane, methanol, ethanol, HCl, acetic acid, BHT, and ammonium hydroxide (all spec. pro analysis). Equipment used: spectrophotometer, bath type sonicator, microwave oven, rotary evaporator, centrifuge extractor, vacuum filter including Whatman filter paper.

The leaves were dried using a commercial microwave for 7 minutes and analyzed for alkaloid levels (19). Leaves with high levels of alkaloids were selected to be raw materials for multilevel extraction using bath-type sonication for 1 hour at 40-50 °C. The ratio of ingredients to each solvent was 1:10. Stratified extraction was started using hexane, and the resulting hexane extract was precipitated using an extractor centrifuge, then the supernatant was concentrated with an evaporator (40 °C) until dry. The hexane residue was extracted using methanol in the same way as with hexane and the methanol residue was extracted using ethanol in the same way. The yield of evaporation was calculated (20). Then each extract was analyzed for IC-50 antioxidant activity (21,22).

Data analysis was carried out descriptively (mean ± standard deviation).

3. Results

Alkaloid levels

Our results showed that the dried kratom leaves with the initial preparation removed from the veins contain higher alkaloids (11.65%) than leaves with veins (9.41%). It is suspected that there are chemical compounds in the veins that can affect the alkaloid content of the overall material, causing a decrease. Alkaloid data can be seen in Figure 1.

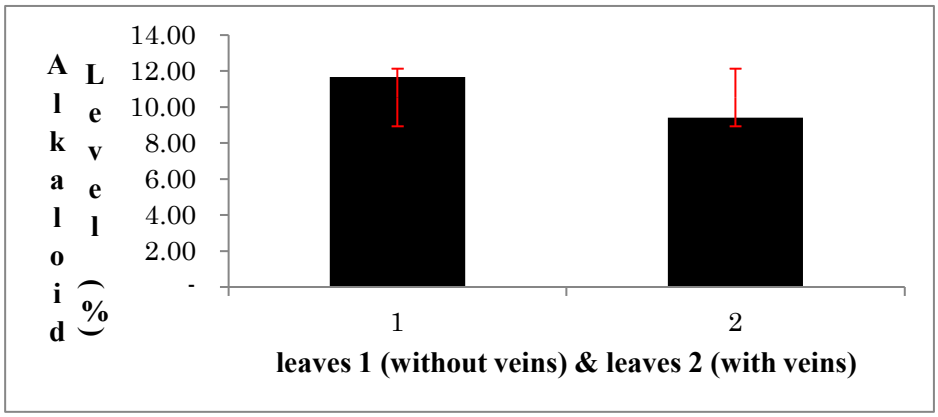

Figure 1. Alkaloid level in kratom leaves with veins and without veins

Extraction Yield

The yield calculated was based on samples with high alkaloid content found in leaves without veins and multilevel extraction was carried out starting from hexane, methanol, and ethanol. Extraction yield data can be seen in Figure 2. The highest extraction yield was derived from an extract using 11.78% methanol and the lowest was from 1.63% hexane extract

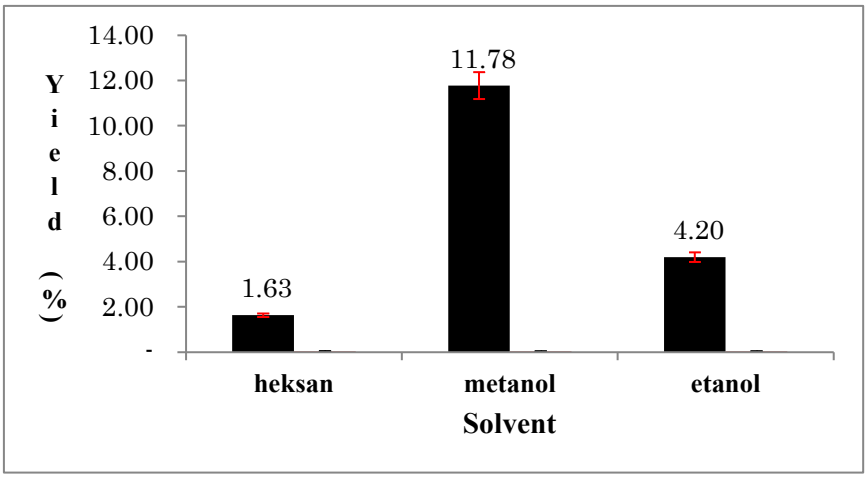

Figure 2. The yield of various extracts according to the solvent

ANTIOXIDANT ACTIVITY OF IC-50

To determine the IC-50, data on the relationship between the concentration of each extract and the BHT control and its antioxidant activity are needed, which can be seen in Figures 1-4. After getting the value from the regression equation, the y in the equation is replaced with a value of 50 to get the IC-50 value of the antioxidant activity. Each image is the result of an average of 3 times repetitions of its antioxidant activity.

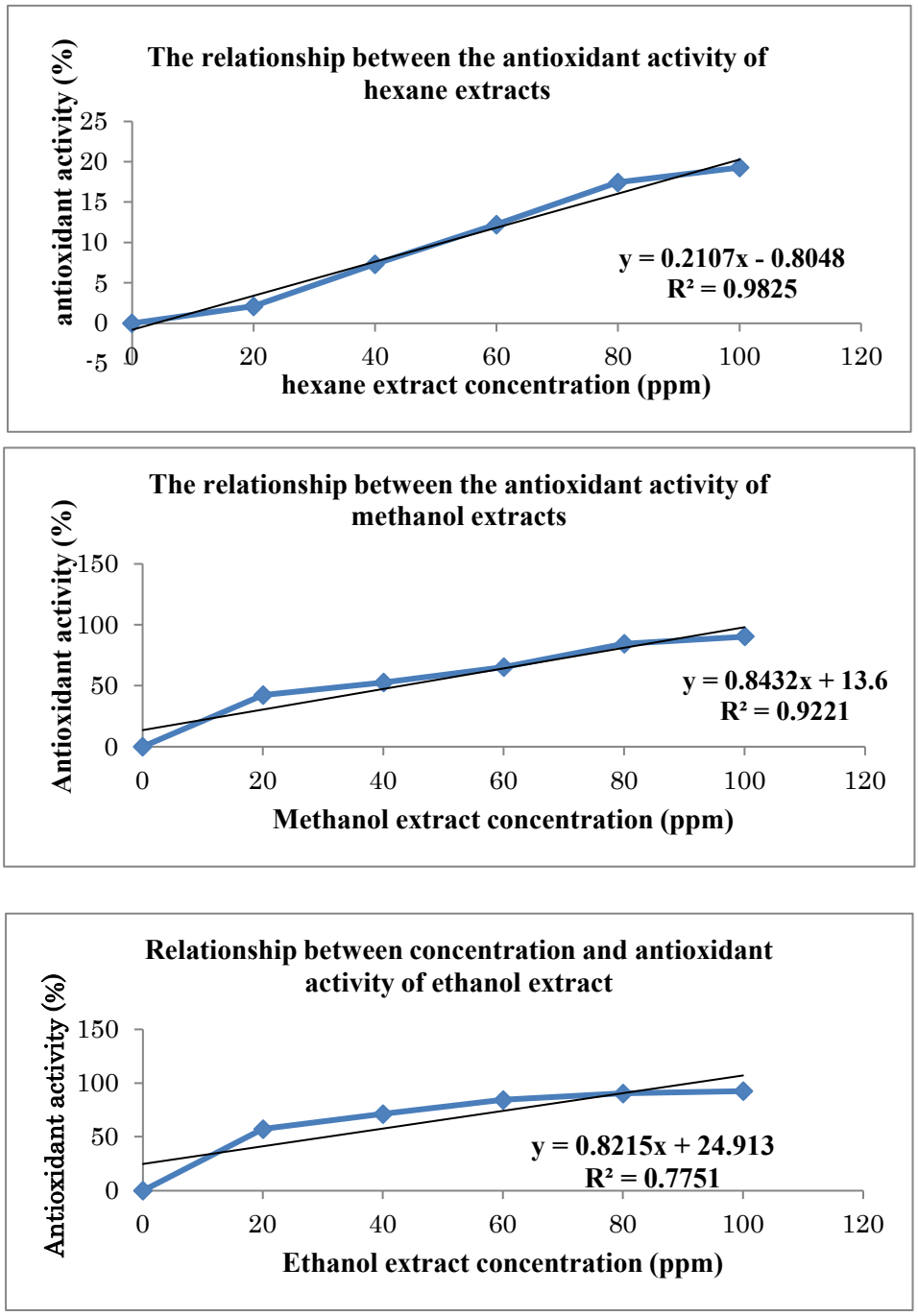

Figure 1-3. Graph of the relationship between concentration and antioxidant activity of the hexane, methanol, and ethanol extracts of kratom leaves

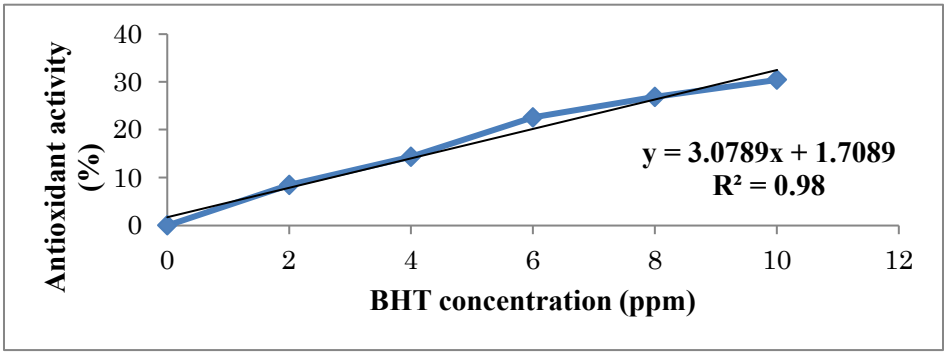

Figure 4. Relationship between Concentration and Control Antioxidant Activity

Table 4. IC-50 Value of Various Kratom Leaf Extracts

	Hexane extract	Methanol extract	Ethanol extract	BHT
Regression equation	Y= 0.2107X-0.8048 R^2= 0.98	Y= 0.8432X-13.6 R^2= 0.92	Y= 0.8215X-24.913 R^2= 0.76	Y=3.0789x+1.7089 R^2= 0.98
IC-50	241.12% = 2.4 ppm	43.17% = 0.43 ppm	30.54% = 0.31 ppm	15.69 % = 0.16 ppm

Note: n=3 repetitions

4. Discussion

In several popular articles, it is explained that leaf bones (veins) are not included in the processing of kratom leaf powder/flour. It is suspected that leaf bones can cause constipation (constipation) in the body when consumed (23,24). Thus from the results of this study scientifically the presence of veins proves that they do not increase the alkaloid content, in other words, veins are not useful related to the bioactive content of alkaloids. The yield of hexane extract is lowest because the alkaloid compounds of kratom leaves that dissolve in hexane include mitragynine alkaloids which can dissolve in non-polar solvents (25). The results of previous studies reported by (26) whom both extracted kratom leaves with hexane produced a slightly lower yield of each than the results of this study about 1.22%; 1.5%. (27) research results used a single extraction with the maceration method while Rinaldi used a multilevel extraction method (maceration) after extraction with ethanol. Thus multilevel extraction using a sonication bath is not much different to produce a hexane extract between 1.5% performed by Rinaldi and 1.64% from the results of this study.

The yield of methanol extract was the highest yield from this study. When compared with the results of the research reported by (17) 17.97% using multilevel extraction using only methanol and followed by dichloromethane, while the yield found by (28) produces a yield of 31.4%. The principal thing that distinguishes it is the different extraction methods, calculations in Luliana use the initial raw material for the total test leaves, whereas in the multilevel extraction of this research the raw material for methanol extraction comes from hexane residue (which is lower than the total leaf material used in the test). In research (28) using single extraction with maceration.

The yield of ethanol extract carried out by single extraction using maceration was higher than research, up to 31.14% (29), and after multilevel fractionation with hexane and ethanol resulted in a significant increase to 87.17%. The main difference in this study is the use of residues after hexane and methanol are extracted, so that the raw materials are much lower, with some of the bioactive already soluble in hexane and methanol. Research of (26) produced an ethanol yield of 19.34%, because it used raw materials that had not been extracted with other solvents so that the soluble bioactive had all dissolved in the ethanol. Based on Figure 1-3, it can be concluded of each IC-50 (Table 4). Based on Table 4, the lowest IC-50 value is derived from an ethanol extract of 0.31 ppm. According to (30), the IC-50 value of the antioxidant activity is very strong if the IC-50 value is less than 50 ppm, strong (50-100 ppm), moderate (100-150 ppm), and weak (151-200 ppm). The smaller the IC-50 value, the high the antioxidant activity.

When compared with the results of previous studies that have examined the IC-50 of various extracts from kratom leaves, the results of (31) also showed IC-50 of the ethanol extract of kratom leaves at 38.56 ppm; classified as very strong, but Yuniarti used the maceration extraction method for 5 days. Other research shows that the IC-50 ethanol extract of 91.86 ppm (strong) kratom leaves also uses the maceration extraction method for 3 days (22). Thus when compared with maceration extraction which is quite long and consumes a lot of solvents, the extraction method using a sonication bath is more efficient because it produces the most powerful IC-50. Even so, when kratom extract is compared to control BHT as a commercial antioxidant that is most widely applied to food products, the IC-50 of BHT is still 50% higher and stronger than ethanol extract.

5. Conclusion

Based on the drying method using a commercial microwave, the alkaloid content of leaves without veins is higher than those with veins. The highest extract yield from the sonication bath method was methanol extract while the strongest IC-50 antioxidant activity was 0.31 ppm ethanol extract. The high yield does not indicate that

it is directly proportional to the bioactive content and its functional properties.

Funding: Faculty of Agriculture, Universitas Tanjungpura.

Conflicts of Interest: The authors declare no conflict of interest.

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