# Effect of Different Extraction Methods and Types of Solvent on Bintaro (*Cerbera manghas*) Leaf Extract

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**ABSTRACT**
Bintaro (*Cerbera manghas*) is a mangrove plant family that can be found growing on the beach in barren land. The Bintaro tree has excellent exploration potential because it contains many alkaloids, namely cerberin. Alkaloids are secondary metabolites that are widely used in the pharmaceutical, medical, and industrial fields. Cerberin is a natural pesticide because it has cytotoxic, antibacterial, and depressant properties. Alkaloids can be obtained through the extraction of non-thermal plant tissues such as maceration because they are not heat resistant using polar solvents such as distilled water and ethanol. In this study, two types of extraction were used, namely maceration and Ultrasonic Assisted Extraction (UAE) using three types of solvents, which are acetone, ethanol, and methanol. The purpose of this study is to determine the best combination of solvent use and extraction method in bintaro leaf extraction in terms of yield, refractive index value, and total alkaloid content. This research was conducted with a nested experimental design, namely the use of nested solvents in the use of extraction methods. Each treatment was repeated 3 times so that there were 18 experimental units. Statistical tests were carried out using normality, homogeneity, and ANOVA tests, and continued with the selection of the best treatment using the Zeleny Multiple Attribute method. The calculation of total alkaloid content was carried out using UV-VIS spectrophotometry so that a standard curve using caffeine compounds was needed. By making a caffeine standard curve, a line equation was created that is y = 0.0045 x + 0.0787 with a value of r2 =0,9547. The results of this study indicate that in the yield parameters, refractive index, and alkaloid content, the best treatment combination is the use of 70% methanol solvent with UAE method with an average of 2.374 ± 0.05 for the yield, 1.480 ± 0.03 for the refractive index value, and 3.827 ± 0.09 for the alkaloid content parameter. After calculating the best treatment, the best treatment was obtained using 70% methanol solvent with UAE extraction.

***Keywords:*** *Alkaloids, Bintaro, Cerbera manghas, maseration, Ultrasonic Assisted Extraction.*

## 1. INTRODUCTION

Bintaro (*Cerbera manghas*) is a type of mangrove plant that grows easily in various types of soil. This plant can grow in swamps, forests, beaches, and even on barren land because its nutritional requirements are not large, supported by Mudia [1]. Bintaro can be found easily in almost all regions in Indonesia. Generally, the use and cultivation of bintaro plants is limited to providing road shade, preventing coastal erosion, or cultivating dry plants as decoration, supported by Susanti [2]. This plant actually has great potential, such as it can be used as a pesticide because it contains toxic chemical compounds, supported by Asikin [3]. However, the lack of exploration of this commodity makes it have low economic value, supported by Iman et al. [4]. In fact, bintaro has a high content of alkaloid compounds, making it have high exploration potential.

Alkaloids are a type of secondary metabolite compound that can be found in both plant and animal tissues. Alkaloids are widely used in the pharmaceutical and medical fields because they can help treat several diseases such as diabetes, diarrhea, malaria, and have antimicrobial properties, supported by Wahyuni et al [5]. One example of a plant with high levels of alkaloids is the bintaro plant. All parts of the bintaro plant contain the alkaloid, namely cerberin, which has cytotoxic, antibacterial and depressant properties for the central nervous system, supported by


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Indah et al [6]. Cerberin can disrupt the heart rhythm and even make the heart stop beating because it can inhibit calcium ion channels. Therefore, cerberin is known as a compound that can increase the mortality of plant pests, so it is widely used as an organic pesticide for both insects and mice, supported by Kartini [7]. The method that can be used to obtain alkaloids from the bintaro plant is by extraction.

Extraction is a method used to separate a desired substance from the solvent. Extraction has various types and the selection must be adjusted to the characteristics of the material or substance to be extracted, such as particle size, boiling point, and others, supported by Zhang et al [8]. In this research, two extraction methods were used, namely maceration and Ultrasonic Assisted Extraction (UAE). Both are non-thermal extractions, which are extractions carried out without heating, supported by Handaratri et al [9]. These two extraction methods were chosen because alkaloids are not heat resistant, so the use of thermal extraction can damage these compounds, supported by Puspitasari [10].

Extraction of polar alkaloids can use several types of solvents such as methanol, ethanol, acetone and distilled water. The use of these solvents can produce different levels of alkaloids because each has its own characteristics. Chairunnisa et al. [11] said that the choice of solvent type in extraction is one of the factors that influences the extraction process and results. Therefore, it is necessary to know which solvent is best for extracting a compound. One method of testing the concentration of compounds in the extract is through the refractive index of the solution.

Refractive index is one of the parameters that can be used in testing the characteristics of a solution. The refractive index number is able to indicate the concentration of substances, purity and quality of an extract. The higher the refractive index number indicates the higher the concentration or content of the substance in a solution, supported by Fauziyah et al. [12]. Therefore, refractive index measurements can also be used to find out which method and solvent are best for attracting compounds in an extraction process.

The objectives of this research were to analyze the effect of maceration and Ultrasonic Assisted Extraction (UAE) extraction methods, solvent types, and treatment combination on Bintaro extract characteristics. The results would help to develop the potential use of Bintaro, such as a pesticide.

## 2. METHODS

This research was done in February 2023 till July 2023. The research took place at the Laboratory of Agroindustrial Process Engineering, Laboratory of Entrepreneurship, and Laboratory of Basic Chemical, Faculty of Agricultural Technology, Universitas Brawijaya.

### *2.1. Materials and Tools*

Materials used in this research were Bintaro Leaves from Universitas Brawijaya, aquadest, acetone, ethanol 70%, caffeine, phosphate buffer (pH 4 and 7), Bromocresol Green (BCG), and chloroform.

The tools used were Ultrasonic Assisted Extraction (UAE) for extraction, jars for maceration, analytical balance, filter paper, measuring pipette, volumetric flask, measuring cylinder, Erlenmeyer, test-tube, test tube rack, vortex, UV-Vis Spectrophotometer, refractometer, and quartz cuvette.

### *2.2. Sample Preparation*

Fresh bintaro (*Cerbera manghas*) leaves was washed using fresh water and drained. Then drying step was carried out using cabinet dryer at 70 ºC for 10 hours. Dried bintaro leaves was powdered using a blender and shieved through 40 mesh shieve. Bintaro powder then stored in a closed container before the extraction process conducted.

### *2.3. Maceration*

Thirty grams of bintaro leaves powder was placed into a jar. Solvent with a ratio of 1:10 (m/v) was added into the jar. The maceration process was conducted for 1 x 24 hours at room temperature. Filtrate and residue were separated through a separating process using filtration paper to get bintaro leaves extracted. Afterward, the extract was evaporated using a rotary vacuum evaporation at 50ºC and 65 rpm to get concentrated extract, supported by Effendi et al [13].

### *2.4. Ultrasonic Assited Extraction (UAE)*

Thirty grams of bintaro leaves powder was placed in a jar. Solvent with a ratio of 1:10 (m/v) was added into the jar. Ultrasonic assisted extraction process was conducted at 40 kHz frequency for 30 minutes, supported by Candraningsih et al [14]. Filtrate and residue were separated through a separating process using filtration paper to get bintaro leaves extracted. Afterward, the extract was evaporated using a rotary vacuum evaporation at 50ºC and 65 rpm to get concentrated extract, supported by Effendi et al [13].

### *2.5. Yield Calculation*

Yield was calculated using the formula below:

yield (%)= (a)/(b) x 100 % (1)

a : mass of concentrated extract

b : mass of raw materials

### *2.6. Refractive Index*

One milliliter extract was dropped on the prism of the refractometer then the result displayed was converted to get the refractive index value.

### *2.7. Total Alkaloid Content*

Testing for total alkaloid levels is carried out using UV-Vis spectrophotometry which will calculate the absorbance of the extract. This method begins with creating a standard curve which will be used as a reference for determining alkaloids using a regression equation with caffeine. The next analysis was carried out by determining the maximum wavelength that would be used to test the bintaro leaf extract. Calculation of alkaloid content from bintaro leaf extract was carried out using a linear regression equation obtained from the caffeine standard curve. If the concentration and absorbance values have been obtained, then both are entered into the formula:

$Y=Ax+b$, where Y is the absorbance and X is the concentration of the solution.

## 3. RESULT AND DISCUSSION

### *3.1. Yield extract*

Extract yield is a comparison between the weight of the thick extract produced and the simplicia powder used at the start of extraction, supported by Islami et al [15]. The yield calculation is related to the concentration of bioactive compounds obtained from the extraction process so that the yield can indicate how well the extraction process is going, supported byMohammad et al [16]. The results of the calculation of the extraction yield of Bintaro leaves have various values, namely between 0.383% to 2.443%. The yield value of bintaro leaf extract with variations in solvent use and methods can be seen in **Figure 1.**

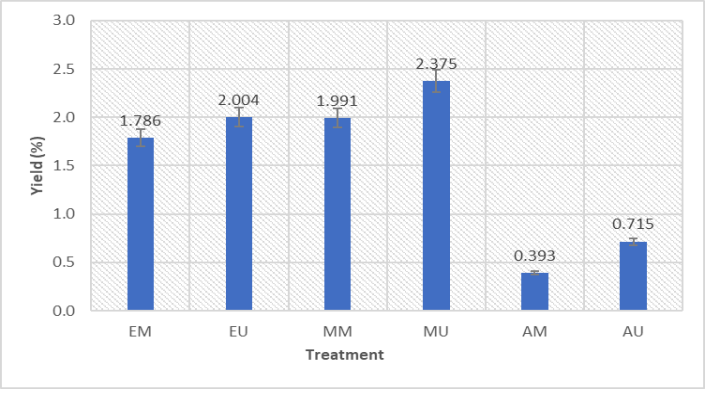


**Figure 1** Yield of Bintaro Leaves Extract

In this research, the results showed that the lowest extract yield was produced using the maceration extraction method with acetone solvent with an average of 0.393%, while the highest yield value was in the combination of using 70% methanol in the UAE, namely 2.375%. The use of the Ultrasonic Assisted Extraction (UAE) extraction method is capable of producing the largest yield of all solvents used. The use of the UAE method produces a higher yield because the use of this method is equipped with cavitation waves which are able to damage the sample cell walls, so that the solvent can enter more quickly and attract the compound you want to extract. Apart from that, the presence of ultrasonic wave vibrations can increase the rate of mass transfer in cells and accelerate solvent penetration through cell walls, so that bioactive compounds can be extracted optimally, supported by Mawarda et al [17]. In terms of time, the extraction method using UAE which is carried out for 30 minutes runs faster because it is able to produce a greater yield when compared to using the maceration method which lasts for 3 days.

Overall, 70% methanol solvent produced the greatest yield, both using the UAE and maceration methods, followed by the use of 70% ethanol solvent, and finally the use of acetone solvent. This result is related to the polarity of the solvent and the solute. Solutes tend to dissolve in solvents that have similar properties, or have a like-dissolve-like principle, so based on the data obtained it can be said that the compounds in bintaro leaves tend to have a polarity level that is more similar to the methanol solvent because it has the highest yield, supported by Sucipto et al [18].

The results of this study are also in accordance with research by Boeing et al., [19] which states that the best solvents to use to extract all secondary metabolite compounds from plant tissue are water and methanol, ethanol, and finally acetone. Even though it has a similar level of polarity, ethanol produces a lower yield than methanol due to the presence of longer ethyl radicals in ethanol, resulting in low solvation. The use of acetone solvent is considered the most inefficient because acetone is only a proton acceptor, while methanol and ethanol are also proton (H+) donors. Methanol and ethanol as polar protic solvents have hydrogen bonds, namely the greatest dipole force, so that the greater the dipole moment of a solution, it indicates that the solution is more polar because it has a high difference in electronegativity values, supported by Vinsiah et al [20].

The effectiveness of compound withdrawal by solvents can also be influenced by the dielectric constant, namely the ratio of the material's capacitance value to the capacitance value or electrical energy in a vacuum, supported by Aziz [21]. The dielectric constant can be used as a measure of solvent polarity. If the dielectric constant of a solvent is greater, the higher the polarity of the solvent, so it is considered better at extracting polar compounds. The results showed that extraction with methanol solvent produced the greatest yield, while acetone solvent had the smallest average yield. This is in accordance with the dielectric constant of each solvent, namely 32 for methanol, 24 for ethanol, and 21 for acetone, so that methanol is the most polar solvent and the best for extracting polar compounds such as alkaloids in bintaro leaf samples, supported by Rachmawati et al [22].

### *3.2. Refractive Index*

The refractive index is the ratio of the speed of propagation of light in a substance to that in a vacuum. The refractive index can be a determining parameter for the quality of an extract which shows the concentration of the extract, supported by Koohyar et al [23]. The higher the refractive index of an extract, the higher the long chain components and oxygen groups contained, so the density of the extract is higher and the quality of the extract is also better, supported by Hidayati et al [24]. In this study, the refractive index results of bintaro leaf extract had the lowest average value of 1.366%, namely when using the maceration method with acetone solvent, and had the highest average value when using the UAE extraction method with methanol solvent, namely 1.480% . The refractive index value of bintaro leaf extract can be seen in **Figure 2.**

The results of this study show that the use of the UAE method produces the largest refractive index value with the use of all types of solvents. This shows that using the UAE method is able to extract bioactive compounds better than using the maceration method. In this method, the dual effect through mechanical energy and kinetic energy helps extraction run more efficiently. Kinetic energy in the UAE method is produced from ultrasonic waves which will produce cavitation bubbles on the cell surface, thereby increasing mass transfer between the simplicia (solid) and solvent (liquid) surfaces. The mechanical energy that occurs results from increasing the solvent's ability to penetrate the simplicia cell membrane, so this will increase the mass transfer and cell release ability, supported by Wiranta et al [25].

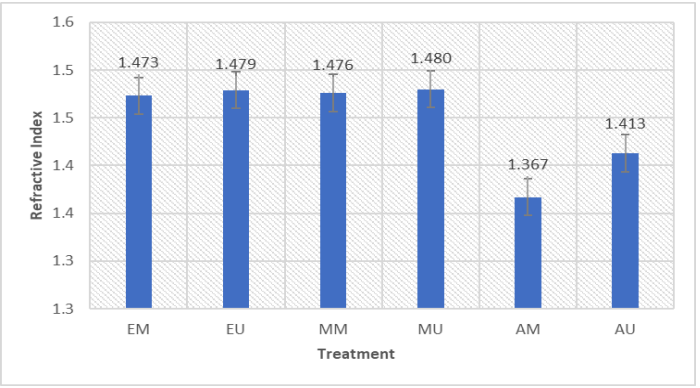


**Figure 2** Refractive Index of Bintaro Leaves Extract

The presence of kinetic and mechanical energy that appears during extraction can increase the binding ability of compounds to be better when compared to using the maceration method. The large number of compounds or bioactive components extracted produces a greater refractive index of the extract, so this indicates that the extract is thicker or has a higher concentration.

The use of 70% methanol solvent produces the highest refractive index when compared to the use of 70% ethanol and acetone solvents, either using UAE or maceration extraction methods. This shows that the use of 70% methanol is able to extract the bioactive components in bintaro leaves better, which can be caused by the similar properties between methanol and the bioactive components in bintaro leaves. Methanol is the most polar solvent when compared to the other two types of solvents. This can be seen from the dielectric constant values of the three solvents, where methanol has the largest value, namely 33.60, while acetone has the lowest value, namely 20.70, supported by Ariyani et al [26]. From the dielectric constant value, it is shown that methanol is the most polar solvent and is the best used to extract compounds from bintaro leaves.

Generally, the bioactive compounds in bintaro leaves such as alkaloids, flavonoids, tannins and saponins are polar, supported by Kurniawan [27]. Therefore, a polar solvent is used to extract these compounds. In accordance with the like-dissolve-like principle, the bioactive compounds in bintaro leaves will tend to dissolve in solvents that have the most similar properties to these compounds, namely methanol solvent. Apart from its polarity, methanol also has the property that it is not easily oxidized, so it does not degrade several bioactive compounds such as phenol and hydrogen peroxide because there are fewer free radicals formed in the sonication process when compared to ethanol and acetone. This causes methanol to be able to more optimally extract bioactive compounds from plant tissue. Previous research conducted by Handayani et al. [28]said that the refractive index value of Bintaro extract has an average value of 1.465%. When compared with this research, the values already have similar and close values.

### *3.3. Alkaloid Content*

Determination of total alkaloid content in bintaro leaf extract with each treatment can be seen in Figure 3. which shows that the use of the UAE extraction method and methanol solvent produced the largest alkaloid content, with an average of 3.827% from three repetitions. The use of the sonication extraction (UAE) method produces higher alkaloid levels compared to the maceration method when using different solvents. The UAE method is able to produce higher levels of alkaloids compared to the maceration method because of the cavitation waves which help break down the simplicia cell walls and accelerate mass transfer so that more bioactive compounds are bound. Apart from that, this method is also able to modify the cell structure so that it becomes more porous, supported by Aguilar-Hernandez [29]. The heating in the UAE method can also help the extraction process, namely by increasing the diffusion coefficient and increasing the solubility of substances, supported by Tlili et al [30]. The results of this research are in accordance with previous literature, which states that the UAE method is able to increase levels of bioactive compounds such as flavonoids, alkaloids and antioxidants by up to 20%, supported by Setyaningrum et al [31].

Overall, the use of methanol as a solvent produces the highest alkaloid levels, while the solvent that produces the lowest alkaloid levels is acetone. This shows that methanol provides the best results in extracting alkaloids from bintaro leaves. Alkaloids are polar compounds, so to extract them you need a solvent that has the highest polarity, namely methanol, which is the most polar solvent after water, supported by Andayani et al [32]. Truong et al. [33]stated that methanol is the best solvent for extracting bioactive compounds from plants, such as phenols, terpenoids, flavonoids and alkaloids compared to the use of distilled water and ethanol because of its high polarity level. This causes alkaloid compounds to dissolve more easily in methanol. When compared with previous literature, the results of this study produced greater alkaloid levels, namely 3.273%, while the alkaloid content in bintaro plants with maceration extraction using methanol solvent was only 0.28%, supported by Wong et al [34]. This difference can be caused by differences in drying methods and maceration time, which is only one day in stages. On the other hand, the results of the smallest alkaloid levels were when using acetone solvent. This can be caused by the less polar nature of acetone and the tendency of acetone to be better at extracting compounds that have a high molecular weight such as flavonoids, supported by Kumar et al [35].

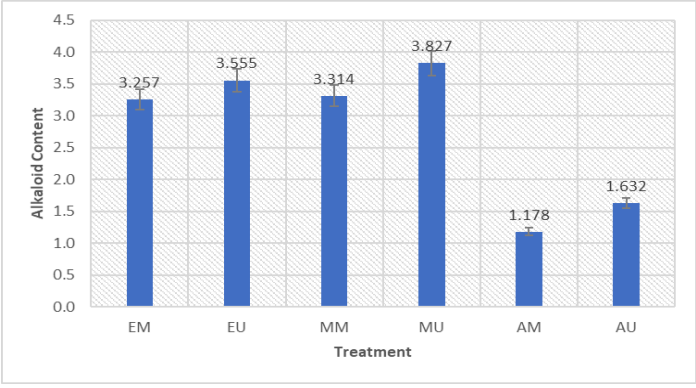


**Figure 3** Alkaloid Contents of Bintaro Leaves Extract

Determining the best treatment begins with determining the objective value for each parameter. In the yield test parameters, the maximum value approach was chosen which indicates that if the yield value is greater, then the treatment combination is considered the best. The largest yield value was in UAE extraction with methanol in the third repetition, namely 2.443%. Furthermore, for the refractive index parameter, the maximum value approach was chosen, that is, if the refractive index value is greater, then the treatment combination is considered the best. The largest refractive index value was in UAE extraction with methanol solvent in the second repetition, namely 1.4830%. Finally, for the total alkaloid content parameter, the maximum value approach was chosen, namely if the total alkaloid content is greater, then this combination is the best treatment. The best treatment value for this parameter was UAE extraction with methanol solvent in the second repetition, namely 3.947%. After doing the calculations, it was found that the first best treatment was UAE extraction with methanol solvent, and the second-best treatment was UAE

**Table 1.** Parameter Comparation between Experiment result and Lieratures.

extraction with ethanol solvent.

Because there is no standard regarding bintaro leaf extract, the yield parameters, refractive index and total alkaloid content were then compared with the literature to determine the suitability of the quality of the extract. When compared with previous research, the yield parameters have not reached the standard, namely 4%, supported by

Ramadhani et al [36]. This can be caused by the difference in the use of solvents with higher concentrations with the multilevel maceration method, so that all bioactive components can be extracted perfectly. Apart from that, in this research samples were used in the form of bintaro fruit and seeds which contain more bioactive compounds such as alkaloids in the form of cerberin, saponins, flavonoids and oils in greater quantities than other parts of the bintaro tree such as leaves, supported by Utami [37]. Regarding the refractive index parameter, the extract value in the best treatment was slightly greater than previous research, namely 1.465%, supported by Handayani [28]. In the total alkaloid content parameter, the best treatment produced a greater value than previous research which produced a total alkaloid content of 5.205 mg/g or 0.5205%, supported by Sahoo and Marar [38]. The quite high difference in alkaloid levels between the literature and research could be due to differences in methods, starting from sample preparation to evaporation. In the literature, the drying method used is airing at room temperature for 25 days so that it is possible to maintain water content in the simplicia. Furthermore, the extraction method used is soxhletation, namely thermal extraction which can damage several types of alkaloids. Evaporation carried out in this research was also limited until the extract volume reached one-eighth of the total extract volume, thus allowing for the presence of solvent to still be contained. A comparison of the values of each parameter with the literature can be seen in **Table 1.**

## 4. CONCLUSION

From the research that has been carried out, the results show that the difference between the use of maceration and Ultrasonic Assisted Extraction methods has an effect on the yield value, refractive index and total alkaloid content of bintaro leaf extract with the best results being the use of the Ultrasonic Assisted Extraction (UAE) method. Meanwhile, the differences in solvent use, namely 70% ethanol, 70% methanol, and acetone, have an effect on the yield value, refractive index, and total alkaloid content of bintaro leaf extract, with the best results being the use of 70% methanol solvent. The combination of using extraction methods and solvents can also influence the yield value, refractive index and total alkaloid content of bintaro leaf extract with the best combination being the use of the UAE method with 70% methanol solvent. So it can be concluded that the best treatment combination between the use of extraction methods and solvents was found in the use of the UAE method with 70% methanol solvent, with an average yield value of 2.374%, a refractive index value of 1.480, and a total alkaloid content of 3.827%.

## AUTHORS' CONTRIBUTIONS

Conceptualization, H.Y.S., B.S.D., and J.A.P.; methodology, H.Y.S., B.S.D., and J.A.P. software, J.A.P., D.U., and S.A.S.; validation, H.Y.S. and B.S.D.; visualization, D.U., and S.A.S.; supervision, H.Y.S. and B.S.D.; All authors have read and agreed to the published version of the manuscript.

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