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Original Article

Influence of Extraction on the Yield, Phytochemical, and LCMS Profile from Standardized Kemuning Leaf (*Murraya paniculata* (L.) Jack)

Elly Wardani[1], Yahdiana Harahap[2], Abdul Mun'im[3], Anton Bahtiar[4,*]

ABSTRACT

Introduction: Mahanimbine alkaloid is carbazole alkaloids found in kemuning (*Murraya paniculata* (L.) Jack) potentially as antihyperlipidemia. Chemical compounds from plants can be obtained by extraction. The aim of this study was to determine influence of extraction solvents on the yield, phytochemistry and LCMS profiles from standardized kemuning to produce safe and quality medicinal raw materials. **Materials and Methods:** The study was used kinds of ethanol concentration with different polarity as a solvent for extraction. Solvent 40%, 70% and 96% of ethanol were used for maceration of simplicia from Bogor, West Java. The extract obtained was evaluated for the content of alkaloid compounds for LCMS then extracts were selected to be proceed to standardized extract quality. **Results:** The LCMS results showed that all 40%, 70% and 96% ethanol extracts contained Candidate Mass 354,19014 compounds which were thought to be e alkaloids based on the m/z value. Phytochemical screening in all extracts contained alkaloid compounds, flavonoids, saponins, tannins and steroids/triterpenoids. 96% ethanolic extract contained non-specific parameter such as content of compounds soluble in water 22,73%, soluble in ethanol 17,37%, drying down 9,10%, water content 18,36%, total ash content 4,18%, the yield of extracts was 33,45%, microbial contamination <3,0.10^4 (7,0.10^4) colony/g and mold yeast number 0 colony/g. The total gravimetric alkaloid level was 1,031%. **Conclusions:** 96% ethanolic extract of kemuning contains mahanimbine alkaloids and has met the standard quality requirements of extracts.

Key words: Murraya paniculata, Pharmacognosy, Standardization of extracts, Specific parameters, Non-specific parameters.

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INTRODUCTION

Mahanimbine alkaloid are carbazole alkaloids as potential antihyperlipidemia.[1] Asia has been well known as "Land of spices" since ancient where places like Maluku Island, Indonesia (Spices Island), Sumatera (Spice Isle) and Melaka (Spice City) were popular as the spice markets.[2] In Indonesia and Malaysia, one of the most commonly used herbs in Indonesia cuisines is *Murraya paniculata* which is commonly known as "kemuning".[3] Mahanimbine found in *Murraya paniculata* (L.) Jack and *Murraya koenigii*.[4] *Murraya paniculata* (L.) Jack belongs to the family of Rutaceae.[5] *Murraya exotica* Linn. synonym *Murraya paniculata* Linn Jack locally known as Kamini.[6] *Murraya paniculata* (L.) Jack, commonly known as Orange Jessamine, is a tropical, evergreen plant with tiny, white, scented flowers, which is cultivated as an ornamental tree or hedge.[7] This plant is a small shrub, tropical, green, flowering small, white, and flavorful. *M. paniculata* often grows in southern China (Hainan, Guangdong, Fujian, and Guangxi), India, Thailand, Malaysia, and Australia.[5] There are many reports on pharmacological effects of the plant including antinociceptive, antioxidant and anti-diabetic, to antimicrobial and analgesic activities.[7,8] Kemuning also showed the decrease in triglyceride, total and LDL cholesterol levels on rats.[3] Kemuning leaves (*Murraya paniculata* (L.) Jack) contain chemical compounds which are secondary metabolites such as essential oils, indole alkaloids, flavonoids, saponins, coumarins, and tannins.[2,5,8]

The use of bioactive compounds in different commercial sectors such as pharmaceutical, food and chemical industries signifies the need of the most appropriate and standard method to extract these active components from plant materials. Extraction is the first step of any medicinal plant study, plays a significant and crucial role on the final result and outcome. The most common factors affecting extraction processes are matrix properties of the plant part, solvent, temperature, pressure and time.[9]

Extraction using solvents is one way to withdraw the active ingredients of an extract. The success of the extraction process is very closely related to the yield, quality and content of active compounds produced. Aquadest could dissolve alkaloid and glycoside compounds, but ethanol was effective to extract sterol, flavonoid, phenolic, and alkaloid.[10] Most alkaloids are readily soluble in alcohol and though they are sparingly soluble in water, their salts of are usually soluble.[11] Both free and salt alkaloids can be dissolved in methanol, ethanol, alcohol reflux, percolation or immersed can be used to extract them.[12] Ethanol is also used to obtain alkaloid components from *Murraya paniculata* leaf

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Figure 1: Kemuning leaf.

Figure 2: Kemuning leaf powder.

extract. Mahanimbine is a carbazole alkaloid and present in leaves, stem bark and root of *Murraya koenigii*. Most of the carbazole alkaloids have been isolated from taxanonomically related plants of the genus *Murraya*, *Glycosmic* and *Clausena*. The *Murraya* species has richest source of carbazole alkaloids.[13] One way to obtain the active ingredient of mahanimbine alkaloid on kemuning leaf through extraction by using ethanol solvent.

The purpose of this research is to choose the right type of solvent for purification of kemuning leaves extract. Extraction, by studying the non-specific and specific parameters of the resulting kemuning leaf extract.[14] The hypothesis proposed in this study is to know the best type of solvent used will affect the yield, quality and total content of alkaloids. The results of this study to give beneficial for practitioners in the field of making biopharmaca products in obtaining kemuning extract which contain high amounts of alkaloids. This research expected to get impact in development of the manufacturing industry of kemuning leaf extract as a raw material for medicine and the herbal product industry.

MATERIALS AND METHODS

Materials

Mahanimbine (powder) as reference standard was purchased from Sigma Aldrich (Germany). All other chemical for analysis including ethanol 96%, ethanol 40%, ethanol 70%, aqua dest, Mayer reagent, Dragendorf reagent, anhydrous acetic acid, sulfuric acid, $FeCl_3$, Mg metal, NaCl.

Maceration containers, blenders, analytical scales, glassware, stirring rods, flannel cloths, vacuum rotary evaporators, ovens, dropper pipettes, and LCMS/MS.

Collection and authentication of plant material

The plants material of kemuning (*Murraya paniculata*) leaves were collected from Bogor, West Java, Indonesia. *Murraya paniculata* was indentified by Indonesian Insitute of Sciences, Research Center for Biology with identification number 1246/IPH.1.01/If.07/VII/2018. The leaves were washed, dried, milled into powder, and then stored in a sealed container in cool, dry place for further use.

Macroscopic characteristics

Determination of the characteristics of simplicia is done by direct observation based on the characteristics of the fresh kemuning leaves. Observation was carried out on the kemuning leaf and organoleptic identification of the powder in the form of texture, color, aroma, and taste.

Microscopic characteristics

Microscopic description includes observations of the cross-section of the simplicia or the part of the simplicia and of the identification fragment of the simplicia powder.[14] Microscopic observations in this study were carried out on powder and cross-sectional simplisia of kemuning leaves. Observation of fragments of kemuning leaves was made. Kemuning powder and kemuning leaves are prepared on a glass plate and covered with a glass cover, 2 drops of distilled water is added and the preparation is observed under a microscope.

Preparation of extraction

Screening of solvent extraction of kemuning leaf was carried out to produce kemuning leaf extract which contains the highest levels of mahanimbin alkaloids. The solvents used were ethanol 40%, 70% and 96%. The extraction was done by maceration using ethanol 96%, 70% and 40% solvent. The maserate was dried with a rotary vacum evaporator and then continued to dried in an oven at 40 °C until a constant weight extract is obtained.

Phytochemical screening

Phytochemical Screening can be done in Table 1 below.

Evaluation of non-specific parameters of 96% ethanol extract from kemuning leaves14

Organoleptic examination (includes examine the shape, smell, taste and color), shrinkage drying, moisture content, total Ash, determination of the level of dissolved compounds in certain solvents (content of water soluble compounds and levels of compounds that are soluble in ethanol), microbial contamination test (bacteria and mold yeast), determination of Total Alkaloid Levels and testing the content of mahanimbine alkaloids (Mahanimbine alkaloid content was identified through LCMS at LIPI, Cibinong, Bogor).

RESULTS AND DISCUSSION

Plant determination results

The result of the determination of the kemuning leaf No. 1246 / IPH.1.01 / If.07 / VII / 2018 conducted at the LIPI Herbarium Bogoriense, Cibinong showed that the plants used in this study were the kemuning leaves (*Murraya paniculata* (L.) Jack) belongs to Rutaceae family. Determination is the first step to get the true identity of the plant to be studied, so that it can provide certainty about the truth of the plant. This is done so there is no error in the plants that will be used.

Macroscopic characterization results

Macroscopic testing is carried out by observing the physical and organoleptic shape of the kemuningish leaf simplicia. The observations made were as follows:

According to the Ministry of Health of the Republic of Indonesia (2008) kemuning leaves have long leaf-shaped or rounded egg-shaped leaves,

Table 1: Phytochemical screening tests of powder and kemuning leaf extract.[15]

Chemical Content	How to Identify	Library Results
Alkaloids	Samples 0.5 g + 1 ml HCl 2N + 9 ml aquadest, heated 2 minutes then cooled and filtered. The filtrate is divided into 3 savings. Tubes 1 in + 1-2 drops of Bouchardat LP, Tubes 2 in + 1-2 drops Mayer LP Tubes 3 in + 1-2 drops of Dragendorf LP	a. Black chocolate deposits in tube 1 (Bouchardat reagent) b. White or kemuning precipitate formed in tube 2 (Mayer reagent) which is soluble in methanol c. Brown orange precipitate formed in tube 3 (Dragendorf reagent)
Flavonoid	Samples 0.5 5 + ethanol 95% 1-2 ml, heated and filtered. Filtrate + HCl & Mg (1: 1)	Red or orange colors
Saponin	Samples 0.5 g + 10 ml hot water, cooled and then shaken strongly + 1 drop of 2N HCl F	Formed foam after being shaken as high as ± 3 cm. After adding HCl the froth does not disappear
Tannins	0.5 g sample was dissolved with 3 ml of 10% Gelatin solution	White sediment formed
Triterpenoid dan Steroid	0.5 ml sample + 5 ml ethanol, heated 25 minutes then cool and filtered. The filtrate is evaporated + 3 drops of ether + 3 drops of anhydrous acetic acid + 1 drop of concentrated H2SO4	a. Red or purple discoloration shows triterpenoids b. Formed in green indicates steroids

2 cm to 11 cm long, 1.5 cm to 5 cm wide, tapered leaf tips, tapered leaf base slightly rounded, leaf edge flat or slightly raised, the surface of the leaf is smooth and shiny, brownish green color, aromatic distinctive odor, spicy, bitter and kelat taste.[15]

Observations that have been made on the kemuning leaf powder green, weak odor. Based on the Ministry of Health of the Republic of Indonesia (2008) organoleptic kemuning or green kemuning leaf powder, distinctive odor, spicy, bitter and kelat taste.[15]

Microscopic characterization results

Microscopic testing is carried out on the cross section of leaves and kemuning leaf powder. Microscopic testing aims to determine the identification of fragments in the form of cells or tissue contained in the kemuning leaf simplisia. The results obtained in the cross section of the kemuning leaf are found in the upper epidermis without stomata, epidermis with palisade cells, mesophyll with kemuning oil cells or almost colorless as shown in Figure 3.a. The results obtained in kemuning leaf powder found anticlinal type cell walls (Figure 3.b), lower epidermis with hair covering (Figure 3.c), calcium oxalate crystals in rosette form (Figure 3.d) and many stomata. Round stomata, anomocytic type, are mostly found in the lower epidermis as shown in Figure 3.f. In the kemuning leaf powder with a magnification of 10 × 40 obtained cone-shaped hair fragments sometimes bent cone, there are at the edge of the leaf and lower epidermis near the edge of the leaf, the transport network with thickening like a ladder (Figure 3.e). Microscopis characterization can be seen in Figure 3 below.

Kemuning leaf powder extraction results

Kemuning leaf extract was obtained 8 kg of fresh kemuning leaf then washed and dried by aerated and 1.65 kg of dry kemuning leaves were obtained. The dried kemuning leaves are then pulverized to obtain 1.5 kg of dry powder and sifted with mesh no. 40 so that 1 kg of powder is obtained. The dry powder is then macerated with 96% ethanol solvent so that the maserate is concentrated by using a vacuum rotary evaporator. The results of the thick extract obtained were put in an oven at 40 °C. The extraction results can be seen in Tables 2 and 3 below.

The kemuning leaf separated from impurities such as soil, gravel, other plant parts, and other impurities that may also be carried away when taking plants. After that the plant is washed and then dried it. Drying is done by aerating it in a place that protected from direct sunlight for approximately 1 week. Drying aims to reduce the water content contained in the simplicia, so it can be stored for a longer time and can prevent the simplicia from being moldy.[14]

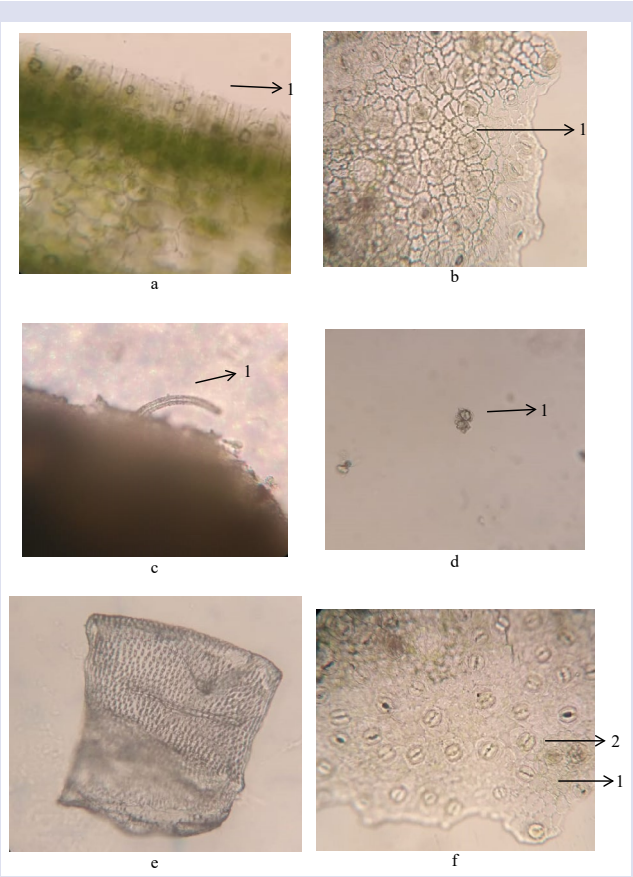

Figure 3: Microscopic results of kemuning leaf powder (a) 1. Upper epidermis with palisade cells, (b) Anticlinal cell walls, (c) Curved hair covering at the edges, (d) 1. Calcium calcium oxalate shaped prism, (e) Carrier network with ladder thickening, (f) 1. Lower epidermis, 2. Round anomocytic stomata are found in the lower epidermis.

Table 2: Results of 96% ethanol extract of kemuning leaves.

No	Result	Type
1	Fresh kemuning leaves	8 kg
2	Dry kemuning leaves	1,65 kg
3	Kemuning leaf powder	1 kg
4	Ethanol extract (liquid)	11,11 liter
5	Ethanol extract (thick)	344 g

Table 3: Simplisia yield extracts of simplisia leaves of kemuning.

No.	Extract Name	Colour	Yield extract (%)
1.	40% ethanol extract	Black	25,98%
2.	70% ethanol extract	Black	27,60%
3.	96% ethanol extract	Black	33,45%

Table 4: Phytochemical screening results of powder and ethanol extracts of kemuning leaves.

Types of Secondary Metabolites	Test Method	Result	Powder kemuning leaf	40% Ethanol Extracts	70% Ethanol Extracts	96% Ethanol Extracts
Alkaloid	Bouchardat reagent	Chocolate Deposition	+	+	+	+
	Mayer reagent	Yellow precipitate	+	+	+	+
	Dragendorff reagent	Brick red precipitate	+	+	+	+
Flavonoid	Etanol + Mg powder + HCl 2N + HCl(p)	Red brick color	+	+	+	+
Tannin	NaCl + Gelatin	White sediment formed	+	+	+	+
Saponin	Aquadest, then shaken + HCl	Formed foam that does not disappear	+	+	+	+
Steroid/ Triterpenoid	Kloroform + Acidum Asetat Anhidrat +H2SO4(p)	A brownish green ring is formed	+	+	+	+

Dry simplicia are sorted from the remaining impurities, such as soil or impurities that may be adhered to during drying. Simplisia that has been sorted from impurities then pollinated. Pollination is carried out to expand the surface of the simplicia so that the solvent can easily absorb into the simplicia so that the active compound which is attracted more optimally.[14]

Kemuning leaf which have became powder after that extracted. The extraction process aims to attract the chemical components contained in simplicia. In this study the extraction method used is the maceration method. The maceration method is a simple cold extraction method by immersing simplicia powder with a liquid solution. This method was chosen because the process is easy and simple. Extraction screening was carried out to determine the right ethanol solvent based on gravimetric measurement of total alkaloid levels, calculation of extract yield results and identification of mahanimbine alkaloid content through LCMS. When compared to the effect of the concentration of ethanol solvents on the levels of total active alkaloids, the 96% ethanol solvent capable of attracting higher alkaloids compared with 40% and 70% ethanol solvents. The lower the concentration of the solvent, the lower the total alkaloid level.

The yield shows the amount of chemical compounds contain in the extract. The extract yield obtained from ethanol extraction of 40% produces the lowest yield, followed by 70% ethanol. The yield obtained at 96% ethanol extraction showed the highest results. Ethanol is a versatile solvent that is good for preliminary extraction. The maserate obtained was then concentrated using a vaccum rotary evaporator at 50oC until a thick extract was obtained. Concentration aims to separate the solvent liquid with the active substance that is dissolved and reduce the remaining solvent that is still present in the extract. Vaccum rotary evaporator is an instrument that uses the principle of distillation (separation) based on differences in the boiling point of solvents.

Phytochemical screening of powder and kemuning leaf extract

In the thick ethanolic powder and extracts of 40%, 70% and 96% kemuning leaf, phytochemical screening tests were carried out which included alkaloid, flavonoid, saponin, tannin, and terpenoid/steroid tests. Based on these results, 40%, 70% ethanol and 96% positive kemuning leaves and ethanol extracts contain alkaloids, flavonoids, saponins, and triterpenoids. The test results are in accordance with the results of phytochemical testing of kemuning leaves according to the library of the Ministry of Health RI.[14]

Testing non-specific parameters of 96% ethanol extract kemuning leaves

To find out the characteristics of 96% ethanol extract and powder, organoleptic tests were carried out, water content, drying losses, total ash content, ethanol and water soluble compounds, microbial contamination test, total alkaloid content and identification of mahanimbin alkaloids. The results can be seen in Table 5 and table 6 below.

Extract parameter testing was performed on 96% ethanol extract of kemuning leaves. This test includes drying shrinkage, water content, total ash content, water soluble extract content, and ethanol soluble extract content. The results of testing the water content and total ash content of 96% ethanol extract of kemuning leaves showed that the extract had met the requirements based on the literature listed in Table 6. Determination of water content is carried out to determine the maximum value or allowable range value associated with purity and contamination.[14] Testing the total ash content is used to provide an overview of internal and external minerals from the initial process until the extract is formed. In testing the ash content, the extract is heated until the organic compounds and their derivatives are distructed and evaporate, so that only mineral and inorganic elements are left behind.[14]

Tests of water soluble extracts and ethanol soluble extracts were carried out to provide an initial picture of the amount of compounds that can be absorbed with water and ethanol solvents from a simplicia.[14] The test results showed that the water soluble extract of kemuning leaves had a value of 22.73%, while the ethanol soluble extract content of the kemuning leaf had a value of 17.37%. This

shows that the amount of polar compounds that can be dissolved in water, is greater than the number of non-polar and semi-polar compounds that can be dissolved in ethanol.[11] The results of this test meet the standard requirements in the literature listed in Table 6.

Testing for bacterial contamination is one of the tests for the purity of extracts. This test includes determining the number of microorganisms that are allowed and to show the absence of certain bacteria in the extract. In the extract there is no bacterial and mold contamination. This is below the maximum limit of microbes in food which is 10^4 colonies/g and the limit of mold contamination in food is 10^3 colonies /g. The low growth of this bacterium may be caused by the extract used is ethanol extract, where ethanol can inhibit the growth of bacteria or microbes in the fraction.[16]

Determination of total alkaloid levels

Gravimetric analysis or quantitative analysis based on weight is the process of isolating and weighing an element or a certain compound from that element, in the purest form possible. Most of the determinations in gravimetric analysis involve changes in the elements to be determined into a pure and stable compound, which can be easily converted into a form suitable for weighing can be seen in Table 7 below.

From the test results obtained total alkaloids in ethanol extract 96% of kemuning leaves as much as 1.031%. These results indicate that kemuning leaves have the main compound, namely alkaloids. According to Birari (2011), kemuning leaves have an alkaloid content, namely mahanimbine.[1]

Identification of mahanimbine alkaloid content

Based on the LCMS results in Figure 4, there were 5 compounds contained in 96% ethanol extract of kemuning leaf. Among the compounds obtained there were Candidate Mass 354,19014 compounds were thought to be mahanimbine based on a neutral mass value of 331.19361 with Adducts + Na Na. The Na + adduct is thought to originate from electrospray ions that are read on the detector. Adduct

Table 5: Organoleptic results of 96% ethanol extract kemuning leaves.

No.	Test	Result
1.	Form	Thick
2.	Smell	Aromatic
3.	Color	Black

Table 6: Result of physicochemical parameter of kemuning leaf extract.

No.	Test	Result (%)	Reference	Information
1.	Dry shrinkage	9,10 %	No more than 10%	qualified
2.	Water content	18,36	No more than 19 %	qualified
2.	Total Ash Content	4,18	No more than 10,5 %	qualified
3.	Water Soluble Sari Content	22,73	Not less than 5,3%	qualified
4.	Ethanol Soluble Sari Content	17,37	Not less than 2,0 %	qualified
5.	Total Plate Number Microbial	$<3,0.10^4$ ($7,0.10^4$) colonies/g	No more than 10^4 colonies/g	qualified
6.	Total Plate Number Yeast Mold	0 colonies/g	No more than 10^3 colonies/g	qualified

Table 7: Total alkaloid content in kemuning leaf extract.

Weight of Alkaloid	Alkaloid Levels (%) (%)
10 gram	1,031

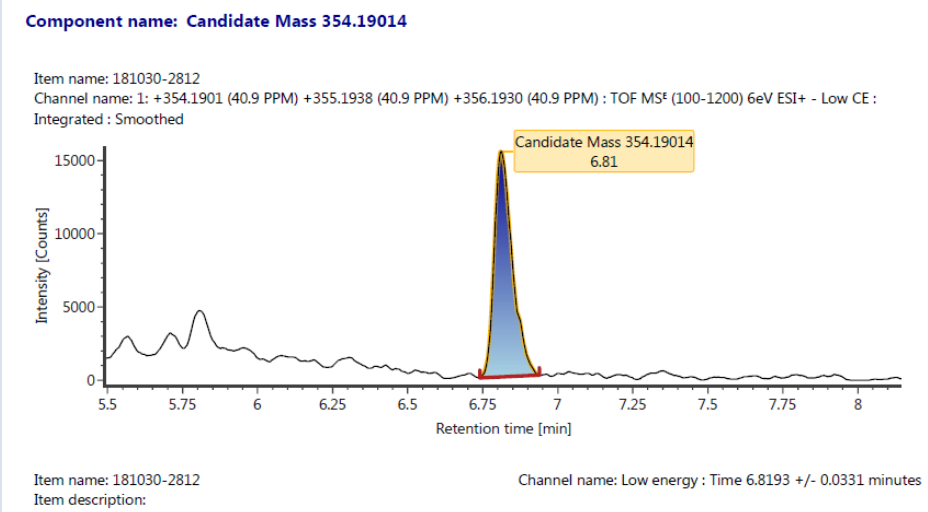

Figure 4: LCMS results of Mahanimbine alkaloid content.

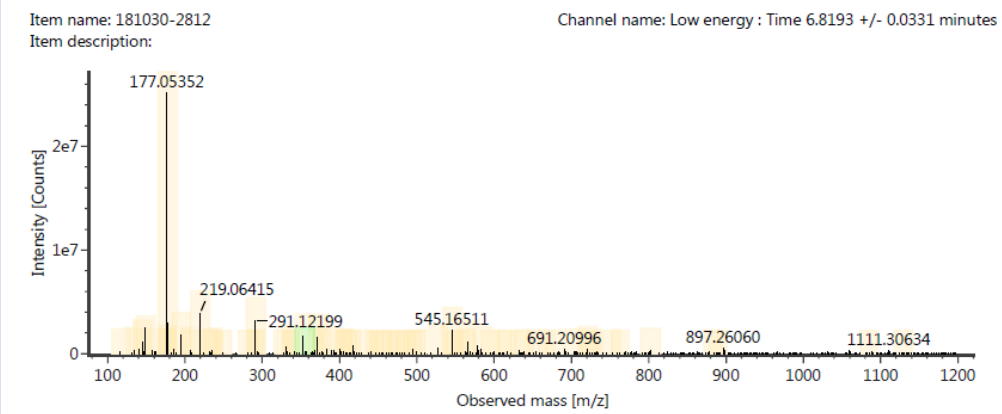

Figure 5: Mahanimbine alkaloid ion mass spectrum fragmentation.

Table 8: Results of the 96% ethanol extract containing kemuning leaves.

No	Component name	Identification status	Observed m/z	Neutral mass (Da)	Observed RT (min)	Detector counts	Adducts	Formula
1	3,5,6,7,8,3',4'-Heptemethoxyflavone	Identified	433.1484	432.14203	8.99	4618118	+H, +Na	C22H24O9
2	7-O-(3,3-Dimethylallyl)-scopoletin	Identified	261.1113	260.10486	8.46	3610329	+H, +Na	C15H16O4
3	Osthenol	Identified	231.1008	230.09429	6.65	3186323	+H	C14H14O3
4	Piperlongumine	Identified	318.1329	317.12632	8.03	4479917	+H	C17H19NO5
5	Candidate Mass 354.19014	Identified	354.1901	331.19361	6.82	9513	+Na	C23H25NO

(Addition product) is an addition product (addition of ions in LCMS terms) as a result of ionization of compounds in the sample. The addition product is the confirmation of the molecular weight of the compound present in the sample.

Figure 4. shows that the low energy will show the molecular weight of a compound contains in a kemuning leaf extract in the range of retention time of 6.8 minutes. In contrast to Figure 5 shows the high energy yield will show the m/z value of a compound. The green peak in the figure does not show numbers that indicate the neutral mass weight of a compound. The m/z value that is not visible may be due to its small intensity. There is no library data of mahanimbine compounds in the LCMS tool so that the content of the mahanimbine alkaloid as a marker of kemuning leaf extract is only limited to speculation because it has the same neutral mass of 331,19361.

CONCLUSION

96% ethanol extract of kemuning contains carbazole alkaloids and has met the standard quality requirements of extracts.

ACKNOWLEDGEMENT

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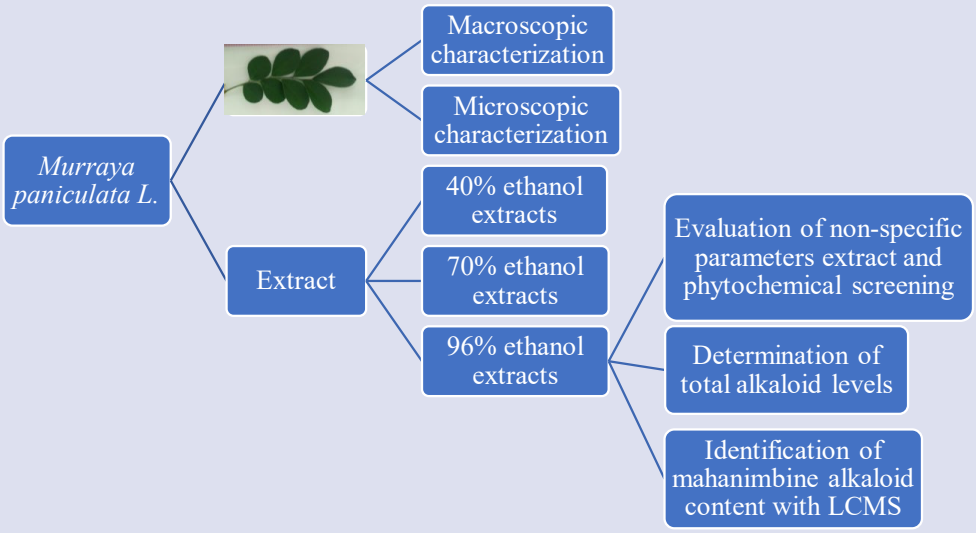
GRAPHICAL ABSTRACT



SUMMARY

Mahanimbine alkaloid is carbazole alkaloids found in kemuning (*Murraya paniculata* (L.) Jack). Determination of sample characteristics was done by direct observation based on the macroscopic and microscopic characteristics of the fresh kemuning leaves. Based on LCMS profile, 96% ethanol extracts contained Candidate Mass 354,19014 compounds which were thought to be alkaloids based on the m/z value. Phytochemical screening in all extracts contained alkaloid compounds, flavonoids, saponins, tannins and steroids/triterpenoids. 96% ethanolic extract contained non-specific parameter such as content of compounds soluble in water 22,73%, soluble in ethanol 17,37%, drying down 9,10%, water content 18,36%, total ash content 4,18%, the yield of extracts was 33,45%, microbial contamination <3,0.10^4 (7,0.10^4) colony/g and mold yeast number 0 colony/g. The total gravimetric alkaloid level was 1,031%. As conclusions, 96% ethanolic extract of kemuning contains mahanimbine alkaloids and has met the standard quality requirements of extracts.

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