

# Specific and Non-Specific Parameters Standardization of Ethanolic 96% Extract of Kersen Leaves (*Muntingia calabura* L.)

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## ABSTRACT

(*Muntingia calabura* L., commonly known as “Kersen” in Indonesia, which is a plant that grow in anywhere. Kersen leaves (*Muntingia calabura* L.) has been used empirically as a medicinal plant because it contains many potential compounds. This study aims is to standardize and Phytochemical screening the ethanolic 96% extract of kersen leaves (*Muntingia calabura* L.). Standardization of the ethanolic 96% extract of kersen leaves (*Muntingia calabura* L.) consist of two parameters that is specific and non specific. The specific parameters include organoleptic test, water and ethanol extractable material, identification of compound content. Whereas non specific parameter include water content, density extract, acid insoluble ash content, mold & yeast contamination, total ash content, microbial contamination, and metal contamination such as Pb and Cd. The result showed that the organoleptic properties of ethanolic 96% extract of kersen leaves (*Muntingia calabura* L.) are dark green color, distinctive smell and has a slightly bitter taste. Content of water solvent and ethanol solvent of the ethanolic 96% extract of kersen leaves is 60.67% and 12.1%. Phytochemical screening results of the ethanolic 96% extract of kersen leaves contains saponins, fenol, flavonoid, steroid. The non specific parameters of the ethanolic 96% extract of kersen leaves are water content of 8.88%; density extract of 0.815 gr/mL, total ash content of 2.27% ± 0.15; acid insoluble ash content 0.05% ± 0.04; mold and yeast contamination of <1.0 × 10<sup>0</sup> CFU/gr; microbial contamination of <1.0 × 10<sup>0</sup> CFU/gr; Pb level of 0.07 ppm ± 0.03 and Cd level of <0.001 ppm. Based on these result that the ethanolic 96% extract of kersen leaves (*Muntingia calabura* L.) has met the predetermined requirements.

**Keywords:** *Muntingia calabura* L. leaves, 96% Ethanolic extract, Standardization, Specific parameters, Non-specific parameters.

## INTRODUCTION

The use of herbs are medicine is the oldest form of healthcare known to humanity and has been used in all culter throughout history. Phytochemicals are the plant derived chemicals and have the capability of disease prevention, thus beneficial to human health<sup>1</sup>. Natural substances contained in plants can come from a parts of the plant, such as bark, roots, fruit, leaves, seeds, etc. That is, every part of the plant of the plant can contain the active substance. Generally, all medicines are synthetic or herbal, should fulfill the basic requirement of being safe and effective. According to WHO, standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects. The methods of harvesting, drying, storage, transportation, and processing (for example, mode of extraction and polarity of the extracting solvent, instability of constituents, etc.) also affect herbal quality.

*Muntingia calabura* L., commonly known as “Kersen” in Indonesia is a plant of the Elaeocarpaceae family encountered in almost all tropical regions due to its high adaptability, which is a plant that grow in anywhere<sup>2</sup>. Kersen leaves (*Muntingia calabura* L.) has been used empirically as a medicinal plant because it contains many potential compounds. There have been very many scientific studies on Kersen plants that have been done before, including the following such as the isolated fraction from methanol extract of *M. calabura* had antistaphylococcal activity<sup>3</sup>. *M*

*calabura* leaves ethanol extract can reduce glucose levels and increase blood insulin levels in rats<sup>4</sup>. Based on Aligita et al (2018)<sup>5</sup> study, *M. calabura* leaves water extract with dose 400 mg/kg,bw had the antidiabetic activities with mechanisms to lower blood glucose level, regenarte pancreatic β cells, and increase insulin sensitivity. The high phytochemical content of *M. calabura* leaf extract, especially flavonoids and polyphenols causes the leaf extract of *M. calabura* to have antioxidant activity<sup>6,7,8</sup>, antiproliferative<sup>6</sup>, antinociceptive<sup>7</sup>, anti-inflammatory<sup>8</sup>, and anticarcinogenic<sup>9</sup>.

Based on this, this study aims to standardize 96% ethanolic extract of *Muntingia calabura* leaves consist two parameters standard, such as specific and non specific standard. The specific parameters include organoleptic test, water and ethanol extractable material, identification of compound content. Whereas, non specific parameter can be determind based on water content, acid insoluble ash content, total ash content, mold & yeast contamination, density extract, microbial contamination, and metal contamination such as Pb and Cd.

## MATERIALS AND METHODS

### Material

The material used are filter paper, glass beaker, measuring cup, spatula, dropper, aluminium foil, rotary evaporator, analytical scale, vials, a stirring rod, oven, micropipette, blender, drip plate, erlenmeyer, Thin Layer Chromatogram (TLC).

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The ingredients used are 96% ethanol extract of Kersen (*Muntingia calabura*) leaves, ethanol from Merck (Germany), FeCl<sub>3</sub> from Merck (Germany), n-Hexan from Merck (Germany), chloroform from Merck (Germany), HCl 2 N, acetat anhidrat acid, aquadest, Mayer, Dragendorff, Wagner, Mg, NaCl.

### Preparation of 96% Ethanol Extract of Kersen (*Muntingia calabura*) Leaves

Kasturi (*Mangifera* Kasturi Kosterm.) leaves were collected from Banjarbaru, South Kalimantan. Kasturi leaves were taken as much of 5 kg and washed from dirt. Kasturi leaves were cut into small pieces and dried in the dryer. The dried simplicial are powdered and sieved with a sieve number of 40. Dry powder of Kasturi leaves was macerated using ethanol 96% in the macerator 3 X 24 hours while stirring occasionally. The filtrate was evaporated using rotary evaporator.

## STANDARDIZATION: SPECIFIC PARAMETERS

### Organoleptic Tested

The organoleptic test is to show the characteristics early, simply, and objectively by using the sense. The organoleptic parameters of Kasturi leaves extract were described include the shape, odour, colour, and taste. This test determined by observation after the material has been exposed to air for 15 minutes. The time of 15 minutes is calculated after opening the container containing no more than 25 g of material, then material is carried out after approximately 25 gr of the material is transferred to the 100 mL vaporizer plate after the material is exposed to air for 15 minutes<sup>10</sup>.

### Identification of Phytochemistry Content

#### Alkaloid Test

A number of Kasturi leaves extract was put into the test tube, added drops of HCl 2N then divided on the test tube. Each tube add reagent. In the addition of Mayer reagents, if the positive for alkaloids the sample form a white or yellow precipitate. In the addition of Wagner reagents, if the positive for alkaloids when deposits are formed. In the addition of Dragendorff reagents, positive for alkaloids if an orange precipitate is formed.

#### Flavonoid Test

A number of Kasturi extract was put into the test tube and was dissolved with 1 mL ethanol 96%, was added magnesium powder, then added hydrochloric acid. If it is formed orange, red or yellow, purple it means that it positively contains flavonoids.

#### Saponin Test

A number of Kasturi extract is put into a test tube, was dissolved 10 mL hot water, and was cooled down then shaken vertically with a little ether about 10 second. If a stable foam is formed for 10 minutes as high as 1 – 10 cm, it contains positive saponin compounds. With the addition of HCl 2N, the foam did not disappear.

#### Tanin Test

A number of Kasturi extract leaves was added into the test tube and dripped with FeCl<sub>3</sub>. If the colour turned into greenish black, it shows the presence of tannin class compounds.

#### Steroid and Terpenoid Test

The test tube containing Kasturi leaf extract was shaken and a little ether was added. Then the ether layer was taken which was then dropped on a petri dish and allowed to dried. After dried, two drops of anhydrous acetic acid and one drop concentrated sulfuric acid were added.

### Determination of Extract Compound Based on Solvent Water Soluble Compound Assay

1 gram of Kasturi leaf extract was macerated for 24 hours using 25 mL of water – chloroform in a plugged Erlenmeyer while shaking for the first 6 hours. Furthermore, allowed to stand for 18 hours, then filtered. The filtrate steam is dried in a cup. The residue was heated at 105°C until the weight was constant. Then calculated the percentage of water-soluble compounds to the weight of the initial extract<sup>11</sup>.

### Ethanol Soluble Compound Assay

1 gram of Kasturi leaf extract was macerated for 24 hours with 25 mL of ethanol (96%) in to a plugged Erlenmeyer while shaking for the first 6 hours then allowed to stand for 18 hours. It was filtered with filter paper quickly, then the filtrate was evaporated to dryness in a cup and the residue was heated at 105°C until it reached a constant weight<sup>11</sup>.

### Chromatogram (TLC) Profile Kasturi Leaves Extract

The extract of 96% Kasturi leaves was fractionated with solvent having different polarity (n-hexane, ethyl acetate, and water) using a separating funnel. The fraction and extract results were spotted on the silica layer, then eluted with mobile phase according to ratio. The result of the spot on silica layer can be seen through UV lamps of 254 and 366 nm and the Rf value is calculated.

### Standardization: Non Specific parameters

#### Water Content

Water content of ethanolic 96% extract of Kasturi (*Mangifera* Kasturi Kosterm.) using destillation azeotrop.

#### Density Extract

The density of the ethanolic extract was determine by dilution of 5% of extract in ethanol solvent with pycnometer. Pycnometer was dried and calibrated by assigning weights pycnometer and freshly boiled water on 25°C, then put into the pycnometer has been filled to a temperature of 25°C.

#### Total Ash Content

Determination of ash content is done by weighing 3 grams of ethanolic 96% extract Kasturi leaves, place on asbestos, flattened and heated until ash. Then the ash was weighed<sup>14</sup>

#### Acid Insoluble Ash Content

The ash obtained dissolved 25 mL chloride acid 10% v/v for 5 minute in porcelain cup then boiled. The obtained solution is filtered using a filter paper. The rest of the ash on filter paper is then washed with hot water. Filter paper and residual ash were heated until the weight is fixed.

#### Mold and Yeast Contamination

Extract from 10<sup>-4</sup> dilution pipetted with 1 mL sterile pipette, implanted in DG18 medium, then incubated at 25°C for 5 days. The number of colonies that grow for 5 days then observed and counted.

#### Total Plate Number

The test of total plate number of ethanolic 96% extract of Kasturi Leaves using count plate methods.

#### Metal Contamination (Pb and Cd)

Weighed 1 - 5 gram of the extract and fill into a 250 ml Erlenmeyer added 25 mL of HCl solution then heated to boiling and left in that state for 5 minutes. Cooled the solution and then transferred to a 50 milli

volumetric flask quantitatively diluted to mark the lines with distilled water then shaken and filtered through whatman filter No 1. A blank solution was made by adding the same reagent as the extract sample. Read the absorbance of the standard series solution of blanks and extract samples. A calibration curve is created with the Y as absorbance and the X as concentration. Then calculated the metal content in the extract sample.

## RESULTS AND DISCUSSION

Standardization is a system to ensure that every medicine, especially herbal that has been marketed has an active substances in accordance with the right level or dose and will produce a therapeutic effect<sup>12</sup>. This is an important step to maintain the consistency of biological activity, chemical profile, or simply a quality assurance of medicinal substance for the production and herbal medicine product<sup>3</sup>. Furthermore, extract standardization can also increase the economic value of herbal medicine producers<sup>11</sup>. Standardized extracts are high-quality extracts that have been used as raw materials for the manufacture of herbal medicines, that containing certain level of compounds and have passed quality control test from planting, harvesting, to the manufacture of herbal products. The specific parameter include organoleptic test, water soluble compound, ethanol soluble compound, and phytochemical screening. Organoleptic test on Kersen leaves extract is shown on table. The thick extract was obtained by the separation process and the solvent had evaporated completely. Bitter taste was caused by secondary metabolite compounds containing by the extract.

Determination of extract compound is aimed to estimated roughly the amount of polar compounds (water soluble) and the semi polar compounds or non polar compounds (soluble-ethanol). The result showed on Table 1. that the content of the ethanolic 96% extract of kersen leaves (*Muntingia calabura* L.) more soluble in water solutions was than ethanol solution. So the extraction process will be most favorable using polar solvent.

Phytochemical screening was aimed to provide an overview of the constituent compounds in the extract. The results of the chemical content showed that of the ethanolic 96% extract of kersen leaves (*Muntingia calabura* L.) was saponins, flavonoid, fenol, and steroid.

The non specific parameter include water content, density extract, total ash content, acid insoluble ash content, mold and yeast contamination, microbial contamination, and metal contamination shown in Table 2. The purpose of determining the water content in the extract is to provide a minimum limit for the amount of water content in the material (extract). The high water content of the extract will make it easier for fungi and molds to grow which can reduce the activity of the active substance in the extract of herbal. Based on the Indonesian Herbal Pharmacopoeia, the permitted watter content requirement is less than 10%. In this study, the water content of of the ethanolic 96% extract of kersen leaves (*Muntingia calabura* L.) by 8.88% thus they meet the standard quality requirements

The result of density the ethanolic 96% extract of kersen leaves (*Muntingia calabura* L.) was 0.815 gr/mL. These results illustrate the amount of unit of the volume to provide a boundary between liquid extracts and thick extracts, besides also specific gravity related to how to determine the purity of substance determined by its specific gravity.

Total ash aims to provide an overview of internal and external mineral content from the beginning to obtain the extract. The ash content test is a measure of the total amount of material remaining after burning and includes ash from the plant part itself and acid-insoluble ash. The residue obtained after boiling the total ash with dilute hydrochloric acid, and burning the remaining insoluble matter. The second procedure measures the amount of silica present, especially in the form of sand

**Table 1: The Result of Specific Parameter Standardization of the ethanolic 96% extract of kersen leaves (*Muntingia calabura* L.).**

No.	Assay	Result
1.	Organoleptic	Color: dark green Odor: distinctive odor Taste: Slightly bitter Shape: Thick in shape
2.	Water Soluble Compound Content	60.67%
3.	Ethanol Soluble Compound Content	12.1%
4.	Phytochemistry Content	Saponin, fenol, flavonoid, steroid

**Table 2: The Result of Non Specific Parameter Standardization of the ethanolic 96% extract of kersen leaves (*Muntingia calabura* L.).**

No.	Assay	Hasil Ekstrak Daun Kasturi
1.	Water Content (%)	8.88%
2.	Pb (mg/kg)	0.07 ± 0,03
3.	Cd (mg/kg)	<0.001
4.	Ash Content (%)	2.27 ± 0,15
5.	Acid Insoluble Ash Content (%)	0.05 ± 0,04
6.	Total Plate Number (colonies/gr)	<1.0 x 100
7.	Mold & Yeast Number (colonies/gr)	<1.0 x 100
8.	Density extract	0.815 gr/mL

and siliceous earth<sup>16</sup>. Total ash content of the ethanolic 96% extract of kersen leaves (*Muntingia calabura* L.) was 2.27% ± 0.15. According to Herbal Pharmacopoeia Indonesia, the requirement of extract total ash content maximum at value of 13.3%<sup>15</sup>.

Herbal medicinal plants can be associated with various kinds of microbial contaminants, namely bacteria, fungi and viruses. The existence of this microbiological growth depends on several factors, namely the environment, this has a very important effect on the overall quality of herbal preparations. Herbal medicines usually contain a bacteria and fungi which often come from the soil. Harvesting methods, wet sorting, dry sorting, and poor storage can also cause additional contamination, such as the possible growth of *Escherichia coli* or *Salmonella* spp. while a large of bacteria and fungi are derived from the natural microflora, it is only aerobic spore-forming bacteria that often predominate. Microbial contamination test was seen from the presence of bacteria (Total Plate Number) and yeast moulds (Yeast Fungi Number) in each sample. Table 2 shows the total plate number and mould yeast number of the ethanolic 96% extract of kersen leaves (*Muntingia calabura* L.) was <1.0 x 10<sup>0</sup> colonies/gr. According to WHO (2016) regarding limits of microbial contamination allow in herbal medicine is maksimum 1 x 10<sup>5</sup> colonies/gram. Thus, for maximum limit of mold and yest according to POM RI (2014) is max 1 x 10<sup>3</sup> colonies/gram. So that, it can be concloude that the ethanol 96% extract of the ethanolic 96% extract of kersen leaves (*Muntingia calabura* L.) has met the standard requirements for microbial contamination and mold yeast number.

Contamination by toxic metals can either be accidental or intentional. Contamination by heavy metals such us mercury, lead copper, cadmium, and arsenic ini herbal remedies can be attributed to many causes, including environmental pollution. The determination of heavy metal contamination was conducted to ensure that the extract didn't contained heavy metals exceeding limits that can be toxic to the body. Based on the study results, ethanol 96% extract of Kersen leaves contained Pb of 0.12 mg/kg ± 0.03 extract. This level was in accordance with the required maximum limit of <10.00 mg/kg<sup>17</sup>. The Cd content obtained of Cd <0.001 mg/kg extract, and fulfilled the required maximum limit of <0.3 mg/kg<sup>17</sup>.

## CONCLUSION

Based on the result of standardization of specific and non specific parameters that the ethanolic 96% extract of kersen leaves (*Muntingia calabura L.*) has met the specified requirements.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

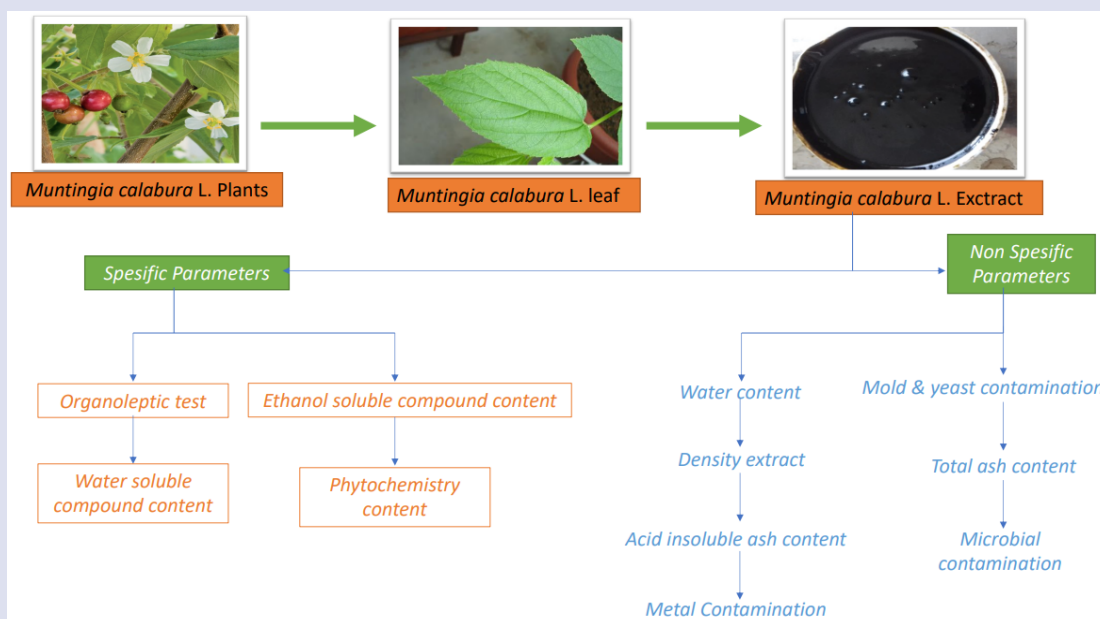
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## GRAPHICAL ABSTRACT





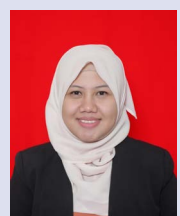
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